Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'

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Rats and mice have a tendency to interact more with a novel object than with a familiar object. This tendency has been used by behavioral pharmacologists and neuroscientists to study learning and memory. A popular protocol for such research is the object-recognition task. Animals are first placed in an apparatus and allowed to explore an object. After a prescribed interval, the animal is returned to the apparatus, which now contains the familiar object and a novel object. Object recognition is distinguished by more time spent interacting with the novel object. Although the exact processes that underlie this 'recognition memory' requires further elucidation, this method has been used to study mutant mice, aging deficits, early developmental influences, nootropic manipulations, teratological drug exposure and novelty seeking.

INTRODUCTION

In 1950, Berlyne[1] reported that rats spend more time exploring a novel object than a previously explored (i.e., familiar) object. With a few exceptions — from researchers interested in curiosity, novelty and drive reduction[2] — there was little empirical research investigating this result until Ennaceur and Delacour[3] re-introduced it to the scientific community as a method for studying 'object recognition'. In general, object-recognition tasks consist of two phases. In the first phase, the animal is presented with the to-be-familiarized object (commonly referred to as the 'sample object') in a distinct environment that typically differs from the housing cage. Following sample-object exposure, the animal is returned to the home cage/colony for a retention period. In the second phase, the animal is returned to the environment and presented with two objects: the previously experienced sample object and a novel object. In this novel-object test, object recognition is distinguished by the animal spending more time exploring the novel object. This object-recognition procedure takes advantage of an animal's tendency to approach and explore novelty, does not require extensive preliminary training, can be conducted in one session (i.e., high-throughput potential), does not require exposure to aversive stimuli, does not require food or water restriction, and has been replicated in many laboratories using a variety of apparatus designs and objects in mice and rats. Together, these factors have contributed to the growing popularity of this procedure.

Although this protocol has been widely used in recent years, there is yet to be consensus of the exact processes under study[4,5]. Regardless, for the animal to discriminate between a familiar and a novel object indicates 'recognition' of the previously experienced object, as well as detection of differences between the objects. This recognition, by definition, requires intact memory of the previously experienced object. As such, this task is a useful tool for assessing the behavioral and neural processes mediating storage and/or subsequent recall of the features that comprise the familiar object. This task may be considered to be a 'non-matching-to-sample' learning task because evidence for object recognition involves greater interaction with the novel object compared with the sample object. Within this context, previous studies have used object-recognition tasks to examine memory function after pharmacological challenges[6,7], after lesions of specific brain areas or targeted alterations in brain neurochemicals[5,8–10], as a function of aging and development[11,12], and during drug withdrawal[13].

Regardless of the level of analysis, to insure proper interpretation of the results, all variables (see later) should be counterbalanced as much as the sample size allows. Such variables include which object serves as the sample versus novel object (Steps 4A and 4B), which environment the sample object first occurs (Step 4B), which sample object (left or right) in the familiarization phase of Step 4A will serve as the familiar objects in testing, and/or which corner (right or left; Step 4A) or environment (first or second; Step 4B) the novel object is placed. Finally, most of the novel-object research has used a between-subjects design with an animal only receiving one level of the independent variable (e.g., antagonist dose, time with object, etc.). Although not commonly used, a within-subjects protocol using Step 4B has been developed[6]. To repeatedly test the same animal requires the identification of discriminable object pairs in which each object in the pair controls similar object interaction to its counterpart, as well as comparable levels of object recognition across object pairs.

MATERIALS

REAGENTS

- Rats (e.g., Sprague Dawley, Long Evans or Wistar) or mice (e.g., C57BL/6J, DBA/2, BALB/c or Swiss) ! CAUTION Experimenters must follow Institutional Guidelines regarding use of experimental animals.

EQUIPMENT

- Cart for transporting animals
- Stopwatch for timing sessions
- Disposable towels to clean the apparatus
- 40–70% isopropyl to clean the apparatus

EQUIPMENT SETUP

Apparatus Depending on which procedure is selected (Step 4A or 4B; see later), a different apparatus will be needed. Step 4A requires a rectangular chamber. In research using rats, the floor space of the apparatus tends to be 40 × 40 cm (l × w), with the height of the walls differing across studies (e.g., 25–60 cm). In general, when selecting or constructing the chamber, consider that a less anxiety-provoking environment (e.g., higher walls, dark walls) will result in greater object exploration and hence more stable object recognition. Additionally, if the walls are too short or a latched lid is not used with short walls, some rats may jump out of the apparatus, thereby resulting in a loss of subjects. Published research with mice typically use an apparatus of similar dimensions as for rats, but those mouse studies tend to include more habituation sessions to allow the mice to become adequately familiar with the relatively larger environment (see PROCEDURE). Although this is a reasonable strategy, we recommend a smaller chamber be used with mice (e.g., 25 × 25 cm; l × w).

Step 4B requires an apparatus that can be divided into at least two separate environments. For rat studies, we have utilized a three-compartment apparatus (dimensions of the two end compartments: 31 × 24 × 45.5 cm (l × w × h); dimensions of the center compartment: 15 × 24 × 45.5 cm (l × w × h))[6]. The inside walls of the center compartment, when raised, allow the animal to move freely between the end compartments on novel-object test days (see **Supplementary Video** online). These barriers are lowered during the sample-object exposure phase to confine the animal to an end compartment. Again, we recommend a smaller apparatus for mice. Although the compartments of our apparatus differ along several stimulus dimensions (e.g., visual (black vs. white walls), tactile (rod vs. mesh floor)), this feature is not necessary for the object-recognition task. There should, however, be no difference in rats' preference for either end compartment. Sources for the apparatus include behavioral equipment manufacturers that custom build equipment (e.g., Med Associates), local cabinet and machine shops that build to specification, and already available behavioral equipment used for other tasks (e.g., a place-conditioning apparatus).

There are additional construction considerations:

- Do not make the apparatus too large (e.g., avoid open fields). Larger environments take longer to explore and evoke anxiety and stress-related behaviors (e.g., thigmotaxis) that compete with object exploration.
- We recommend that no bedding be placed in the apparatus. Some strains of mice and rats are more prone to defensive burying; the presence of bedding will also introduce other competing behaviors (e.g., digging, nest building, etc.).
- Use an apparatus that is built from washable materials such as Plexiglas, polycarbonate, aluminum, stainless steel or painted wood.
- Avoid an apparatus that has edges and/or materials that encourage chewing. For example, a step-down center compartment should be constructed of metal (see **Supplementary Video**) rather than wood.

Objects At least two distinct objects will be needed. Several features should be carefully considered when selecting objects to be used in this task. First, it is critical that animals spend a similar amount of time interacting with each object. To determine comparable object interactions, the experimenter must measure object interaction (see PROCEDURE) under the conditions that will be used for the actual experiment. The degree of object interaction can be influenced by the manipulability and the complexity of the object. Objects that can be manipulated will induce greater object interaction. For example, if the animal can drag or carry the object (e.g., marble), then that object will induce greater object interaction than a comparable object that cannot be displaced (e.g., a metal weight). Object interaction typically increases with the complexity of the object. That is, an object that has several different features (e.g., holes, multiple textures) will induce greater interaction than a less complex object (e.g., smooth ceramic). Second, the objects must be different enough from each other so that the animal can discriminate between them. The objects used in this task have varied widely (e.g., a paint roller, Lego structure, ceramic object, PVC pipe, etc.)[3,6,14].

Video equipment We recommend that the sample-object exposure phase and the novel-object test be videotaped. Doing so will provide a complete archive of the experiment and allow for analysis of other behaviors as well as object interaction during testing (see PROCEDURE, Step 6). Mount the video camera above the apparatus or on a tripod with the camera angled so that the entire arena can be captured and the object (or object area) is fully visible (see **Supplementary Video**).

Computer program to score time interacting with objects from video For this latter item, there are numerous suitable shareware programs available for download on a computer. One that we have recently tested is XNote Stopwatch. This shareware program will allow the experimenter to have two timers each with a 'hotkey'. The hotkey can be almost any key on the keyboard. Thus, the object on the left part of the video screen can be assigned a letter on the left side of the keyboard (e.g., 'x') and the object on the right can have a hotkey on the right side (e.g., 'm').

PROCEDURE

Pre-training

1| Following arrival in the colony, allow the animals to acclimatize for 3–7 d.

2| Stress and novelty of handling and transport procedures can aversely affect object interaction and hence object recognition. To minimize this disruption, handle the animals during the acclimation period (Step 1). Handling should be daily for at least 1 min per day for 3 d before the start of Step 3. If the animal colony is not near the procedure room (e.g., an elevator ride), then we recommend handling and exposure to the transport routine.

3| Familiarity with the testing environment can also affect object recognition[15]. If the experimental plan includes a relatively limited time with the object (see Step 4), the experimenter should give serious consideration to providing the animal with some time in the environment (e.g., 10 min) before object exposure so that environmental exploration does not interfere with object interaction[16]. To insure effectiveness of familiarization, we recommend that this exposure occurs within 24 h of Step 4.

Object recognition (training and testing)

4| Object recognition can be performed using Option A or Option B.

(A) Two sample objects with one environment

(i) Place the 'identical' to-be-familiarized (sample) objects in the back left and right corners of the apparatus (see **Fig. 1**).

(ii) Turn on the video recording equipment and place a card with the animal's identification number within the recording field or state the number when placing the animal.

(iii) Place the animal at the mid-point of the wall opposite the sample objects. When placed, its body should be parallel to the side walls and its nose pointing away from the objects. This orientation prevents any unintentional bias in placing the animal such that it is oriented more towards a particular side/object.

(iv) The experimenter should leave the procedure room/area so as not to serve as a cue for the animal or to introduce unintentional bias into the study (e.g., always standing to the left).

(v) After the planned sample-object exposure time (e.g., 10 min), remove the animal from the apparatus and return to the colony for the prescribed training-to-testing interval. A commonly used training-to-testing interval for robust object recognition is 1 h. If a pharmacological manipulation is involved in the sample-object exposure phase, then 24 h or more is recommended (i.e., allow enough time for the ligand to be metabolized). Too long an interval will result in loss of object recognition (see ANTICIPATED RESULTS).

(vi) To test for object recognition after the training-to-testing interval, place one of the familiar sample objects in one back corner of the apparatus; place the novel object in the other back corner.

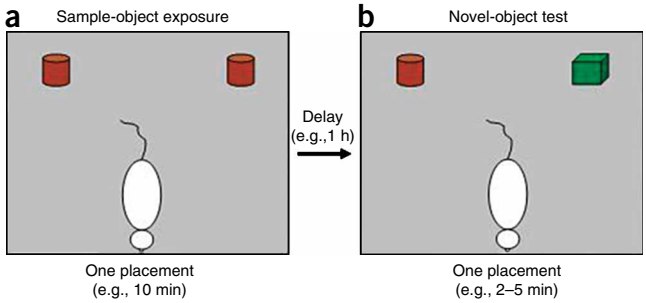

Figure 1 | A cartoon image of the sample-object exposure phase (**a**) and novel-object test (**b**) for Step 4A[3].

(vii) Turn on video recording equipment and place a card with the animal's identification number within the recording field or state the number when placing the animal.

(viii) Place the animal as described in Step 4A(iii).

(ix) Leave the room for the duration of the test. When designing the experiment, we recommend a relatively short test session (e.g., 2–5 min). Longer test sessions can decrease the object-recognition effect because the novel object becomes increasingly familiar as time passes in the test session.

(x) Remove the animal and return it to the colony.

(B) One sample object with two environments

(i) Place the to-be-familiarized (sample) object against the far end wall of one of the environments. Do not allow the animal to access the second environment (see **Fig. 2**).

(ii) Turn on video recording equipment and place a card with the animal's identification number within the recording field or state the number when placing the animal.

(iii) Place the animal against the opposite end wall such that its side is parallel to the end wall. The experimenter should leave the procedure room so as not to serve as a cue for the animal or introduce unintentional bias into the experiment.

(iv) After the planned sample-object exposure time (e.g., 5 min), remove the object and animal from the environment.

(v) Immediately after removal, place the same sample object against the far end wall of the other environment and then place the animal against the opposite end wall, as described in Step 4B(iii).

(vi) Again, leave the experimental room for the prescribed sample-object exposure time; this placement time should be the same as first placement (e.g., 5 min) to control for time in each environment with the object. Note that total time to access the sample object in this example is 10 min (5 min per placement). In Step 4A, the total time is the same (10 min), but this is accomplished in a single placement with simultaneous access to two presumed 'identical' objects.

(vii) Remove the animal from the apparatus and return to the colony for the prescribed training-to-testing interval. As in Step 4A, a commonly used training-to-testing interval for robust object recognition is 1 h. If a pharmacological manipulation is involved in the sample-object exposure phase, then 24 h or more is recommended to allow time for the ligand to be metabolized. Too long an interval will result in loss of object recognition (see ANTICIPATED RESULTS).

(viii) To test for object recognition after the training-to-testing interval, place the familiar sample object against the far end wall of one environment and the novel object against the end wall of the other environment. Remove the barrier between environments so that the animal has unrestricted access to both environments/objects.

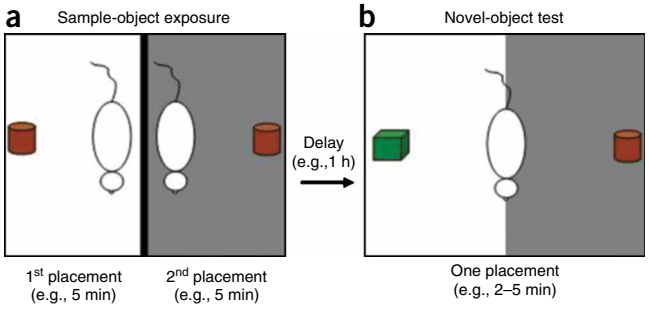

Figure 2 | A cartoon image of the sample-object exposure phase (**a**) and novel-object test (**b**) for Step 4B[6].

(ix) Turn on video recording equipment and place a card with the animal's identification number within the recording field or state the number when placing the animal.

(x) Place the animal at the mid-point between the two objects — presumably where the barrier was located or in a smaller third placement area (see EQUIPMENT SETUP section and **Supplementary Video**).

(xi) Leave the room for the duration of the test. When designing the experiment, we recommend a relatively short test session (e.g., 2–5 min). Longer test sessions can decrease the object-recognition effect because the novel object becomes increasingly familiar as time passes in the test session.

(xii) Remove the animal and return it to the colony.

? TROUBLESHOOTING

Between-animal precautions

5| Wipe the apparatus with a disposable towel that has been dampened with 40–70% isopropyl alcohol after each animal has been assessed.

▲ CRITICAL STEP Ensures that odor trails from the previous animal have been dissipated and spread in a nonsystematic fashion.

Scoring video

6| Object-related behavior can be scored using scoring Option A or B. These options are similar except for the operational definition of object interaction.

▲ CRITICAL STEP The observer needs to be naïve to the experimental conditions of the animals.

(A) Directed contacts scoring

(i) Assign a timer to each object.

(ii) Start appropriate timer when animal comes in 'directed' contact with the object. Stop timer when animal stops contacting the object. Repeat for the duration of the test and for each object. Directed contacts include any contact with mouth, nose or paw; they should not include contacts that are judged to be accidental, such as backing into the object or bumping the object as it passes. Also, any contact in which the animal is standing or leaning on the object as a way of exploring other aspects of the chamber is not included in this definition and thus is not scored as directed contact with the object[13].

(iii) The reliability of the primary observer's scoring should be assessed. To do so, take a random sample of all the animals and have a second observer, also naïve to experimental conditions, score object interaction. The more animals scored by the second observer the better from a statistical assessment perspective. The reliability between observers can be assessed and reported with a Pearson's product-moment coefficient of correlation (i.e., Pearson's *r*)[17].

(B) Within object area scoring

(i) An animal is scored as 'interacting' with the object when its nose is in contact with the object or directed at the object within some minimally defined distance ($\leq$2 cm is the most common). Standing, sitting or leaning on the object is not scored as object interaction[3]. Because this definition requires a clearly delineated area around the object (i.e., an 'object area'), this option requires that each object be fixed in a location either by weight or by attachment to the apparatus floor.

(ii) As in Step 6A(iii), the reliability of the primary observer should be assessed.

7| Recording sample-object exposure and object-recognition testing allows the experimenter to score other behaviors. Scoring these behaviors allows for assessment of motor-related explanations of pharmacological, neuroanatomical and genetic alterations of object recognition (see ANTICIPATED RESULTS). The following is a partial list: latency to make contact with first and second object; number of object contacts, which allows calculation of the duration of each contact; rearing defined as both front paws off the floor (some researchers include leaning on the walls); number of entries into each environment (Step 4B); and number of times that the animal crosses a line that bisects the apparatus (Step 4A).

● TIMING

The experimenter should refer to the PROCEDURE section for specific recommendations on the duration of components within each Step.

Steps 1–2, 1 min per animal for 3–7 d (longer per animal if transport exposure is required)

Step 3, 10–20 min per animal for 1–2 d

Step 4, 5–15 min per animal for 1–2 d depending on the familiarization-to-testing interval

Step 5, 1 min per animal

Step 6, 2–5 min per animal if only object interaction is scored on the test day using 2 timers

Step 7, varies depending on which other behaviors are scored

? TROUBLESHOOTING

See **Table 1** for troubleshooting advice.

TABLE 1 | Troubleshooting guide for object recognition task in rats and mice.

STEP	PROBLEM	POSSIBLE REASON	POTENTIAL SOLUTION
4	Sample-object interaction very low (i.e., only a few seconds)	Pre-training procedures insufficient to decrease any stress or novelty associated with the task	Increase handling experience Increase transport experience Allow time to settle in experimental room if transporting long distance Increase environmental familiarization

TABLE 1 | Troubleshooting table (continued).

4	Sample-object interaction very low (i.e., only a few seconds)	Equipment design or location interferes with object interaction	Decrease size of apparatus to decrease competition for exploration Remove apparatus from high traffic area (a small dedicated room is best) Decrease extra-environment cues by having solid rather than clear walls
4	Sample-object interaction very low (i.e., only a few seconds)	Object size or features inappropriate for animal	Increase size so that it takes longer to explore Increase number of sensory features of the object For mice, too large an object might induce an avoidance response
4A	Interaction focused on only one of the two sample objects	Protocol not conducive to sampling objects	Increase environmental familiarization Increase sample-object exposure time Decrease distance between objects Decrease apparatus size
4	Does not discriminate between familiar and novel object	Pre-training or sample-object exposure procedures interfere with discrimination	See earlier solutions for insufficient procedures to reduce stress Increase time with sample object to insure sufficient object and environment familiarity
4	Does not discriminate between familiar and novel object	Object selection not conducive to discrimination	Increase discriminability of the objects (see text for recommendations) Check that each object controls similar levels of interaction when both are novel and both are familiar
4	First few animals on a given day behave differently from the remaining animals	When assaying many animals, odors in environment for the early animal differ from the later ones	Sequentially place a few (e.g., 2) 'smeller' animals into the apparatus (10 min each) before starting each day[16]
	New mouse or rat strain is being tested and problem from above occurs	Experimental parameters allowing for object recognition differ from 'benchmark' strain*	Strain more sensitive to stressors so modify pre-training protocol Strain has motor differences so modify procedures to equate time spent interacting with sample object Consider perceptual rather than memory differences as an explanation Conduct parametric research on potentially important variables: e.g., sample-object exposure time; environmental familiarization time; time in object-recognition test

*The general list of problems and solutions apply for mice and rat strains that have not been tested in an object-recognition task. However, in labs that have repeatedly demonstrated novel-object recognition, we recommend considering the ideas described in the potential solutions before making broad conclusions about memory deficits in a new strain.

ANTICIPATED RESULTS

There are several different ways in which the results from object-recognition studies are handled for analysis and graphing (see **Table 2**). For example, commonly used measures include time with familiar object versus novel object, a difference score (novel object interaction – familiar object interaction), a discrimination ratio (novel object interaction/total interaction with both objects). Object recognition in these measures is reflected by more time interacting with the novel than familiar object, a positive difference score or a discrimination ratio above 0.5, respectively. As noted earlier, there are supplemental measures that can aid in the interpretation of the primary measure of object recognition. Total object interaction during the sample-object exposure phase and the novel-object test can serve as an assessment of motor activity. During the novel-object test, line crosses (Step 4A) or environment entries (Step 4B) can also provide an index of motor activity that is separate, albeit not completely independent, of object interaction. Line crosses during sample-object exposure can also be used to assay locomotor activity. These supplemental analyses are particularly important to examine when the experimental treatment (e.g., lesion, strain, drug, etc.) alters object recognition. That is, a failure to discriminate between the objects may be a function of

TABLE 2 | Results from an object-recognition experiment.

Rat ID	Novel (s)	Sample (s)	Difference score	Discrimination ratio
317	13.45	6.37	7.08	0.679
318	15.82	4.18	11.64	0.791
319	5.11	1.43	3.68	0.781
320	15.66	10.16	5.50	0.607
321	8.51	11.43	−2.92	0.427
322	10.16	4.18	5.98	0.709
323	8.85	7.69	1.16	0.535
338	22.74	6.81	15.93	0.770
339	19.17	13.56	5.61	0.586
342	22.30	19.17	3.13	0.538
343	23.46	8.07	15.39	0.744
346	12.20	30.81	−18.61	0.284
347	27.86	15.70	12.16	0.640
350	19.61	28.78	−9.17	0.405
351	19.50	19.34	0.16	0.502
354	22.74	9.56	13.18	0.704
355	9.06	6.98	2.08	0.565
358	21.70	8.85	12.85	0.710
359	21.26	8.90	12.36	0.705
Mean	**16.80***	**11.68**	**5.12**†	**0.615**‡

This experiment used Step 4A with male Sprague Dawley rats. There was 2 min of environmental habituation on Day 1. On Day 2, there was 2 min of exposure to the sample objects 1 h before the test. *Significantly different from sample time, $t(18)=2.54$, $p=0.02$. †Significantly different from a hypothetical 0, $t(18)=2.54$, $p=0.02$. ‡Significantly different from a hypothetical 0.50, $t(18)=3.56$, $p=0.002$.

compromised motor ability. Related to this latter point, we urge experimenters not to make premature conclusions concerning memory deficits until sufficient parametric research has been conducted and alternative explanations have been assessed (see **Table 1** for some suggestions). For instance, a new mutant mouse strain may be found not to discriminate between a novel and familiar object under the exact same experimental conditions in which a 'standard' strain in the same laboratory shows object recognition. Although this new mutant may have a memory/retention deficit, the lack of discrimination could be due to such factors as differences in efficiency to explore object or environment, perceptual processes or stress reactivity.

Note: Supplementary information is available via the HTML version of this article.

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