The Novel Object Recognition Test in Rodents: Which Are the Essential Methodological Aspects?

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Introduction

The Novel Object Recognition test (NOR) has been introduced by Ennanceur and Delacour in 1988 [1] and can be regarded as a spontaneous Delayed-Non-Matching-to-Sample (DNMS) [2] test. The test is based on a spontaneous behaviour: the main assumption at the base of this test is that access to novelty (e.g. an object or an environment) can elicit approach behaviours in animals. This apparent 'unconditioned preference' for novelty has been used in the NOR test in order to study memory functions, assessing the ability of animals to recognize a novel object in a familiar environment, because they maintain a representation of those is more familiar stored in memory

The Novel Object Recognition (NOR) test has been used in different variants, but typically consists in two trials. In the first trial (T1) the animal is exposed to one or two identical objects (sample object). Following the sample object exposure, the animal is returned to his home cage for a retention period. In the second trial (T2), which follows the retention time, the animal is returned to the environment (arena) and presented with a familiar (sample) and a novel object. When the subject 'remembers' the previous exposure to the familiar object, it will explore the novel object to a greater degree than that of the familiar one.

NOR test doesn't involve reference memory components (e.g. explicit rule learning), thus it can be considered a "pure" recognition memory test and a valid task to assess working memory. Finally the test doesn't involve positive or negative reinforces (e.g. food, electric shocks) and this makes NOR comparable to memory tests currently used in humans. All these advantages make NOR test quick and simple to be implemented and, therefore, it has been widely used for assessing mild cognitive impairment in pre-clinical research.

Analysis of available literature

Over the past 30 years or so, several hundred published papers report the use of the NOR test. Given NOR test wide use, the available literature on NOR test is abundant and there are quite lot differences in target aim of using this memory task, in the methodological approaches, and how results are interpreted, both in Academia and Industry. A comprehensive PubMed search on NOR test applied in Rodents from 1988 to 2010 retrieves 1389 papers. We restricted our in-depth analysis on published studies that explicitly and accurately describe the methodological aspects of the NOR task and the following parameters were identified, collected and compared:

- Animals - Species, strain, age, sex.
- Apparatus – large variety in shapes (circular, rectangular, Y and T maze) and in dimensions.
- Objects – large variety of shapes, materials, colours and odours associated.
- Time schedules – variety in durations.
- Behavioural measures – variety of data presentation.
- Other variables – habituation to test arena, handling, light-dark cycle, background noises, illumination of apparatus.

The aim of this exhaustive and detailed analysis of the available literature is to possibly provide guidelines on how NOR test could be better conduced in Rodents (rats and mice) in order to generate reliable and ethologically significant data, which can be easily compared between different laboratories. In addition, we intend to discuss what methodological aspects should be mentioned in NOR's papers and the reasons why they cannot be omitted.

Methodological aspects

1. **Apparatus** - There is a large heterogeneity among the analyzed literature for what concern the apparatus used in NOR task. The floor space of apparatus used were largely different across experiments in the different laboratories even if they had similar dimensions for rats and mice. The NOR task is generally performed in square or rectangular arenas or open fields and only few papers reported circular apparatus. It emerges that some variables of the apparatus are important for a successful NOR test: use age-appropriate apparatus size; allow sufficient environmental familiarization in order to minimize anxiety and stress related behaviors and decrease competition for environmental exploration; decrease more as possible extra-environment cues; the presence of bedding in the apparatus should be avoided to reduce distractor factors; the apparatus has to be built in washable materials to remove olfactory cues between test's pahses.

2. **Objects** - In the majority of studies analyzed, objects to be discriminated were made of odorless and easy to clean materials and there is a particular attention in changing them with identical copies when starting the choice phase, so that they could not readily be distinguished by olfactory cues. On the contrary there is a large variability between research groups on the characteristics of objects used, and they differ for shape, size, color, brightness, material, texture and combination of these variables. Within the NOR test, the researchers agree in using similar sized objects both in sample and choice phases and, as for the apparatus, also object sizes should be age-appropriate. First and foremost, the objects need to have no ethological significance for the animals and it is worth checking for any intrinsic preference for one of the two objects and its complexity (i.e. must be evaluated that animals spend similar amount of time interacting with both objects).

3. **Time schedules**

    - Sample phase: The evidences from reviewed literature suggest that a minimal amount of sample objects investigation is necessary to develop a novel object preference. It has to be taken into account that sample phase duration affects the length of subsequent retention, thus the use of a fixed duration is preferable over a limited cut-off duration, which doesn't always ensure a sufficient familiarization with the objects. On the other hand, sample objects are explored mostly in the first minutes of this phase; therefore a long sample phase (i.e. more than 10 min) may not be useful.

    - Retention phase: The length of the delay phase largely depends upon the type and aim of the experiment. Delay times have to be chosen in relation to the sample phase duration, keeping in mind that a shorter familiarization has been found to be related to a shorter retention period; it is worth not to choose delay over 24 hours and to avoid excessively short delays, which may cause a pavement effect.

    - Choice phase: The time of exposure more frequently used in choice phase is 1-3 minutes for rats and 3-10 minutes for mice, generally the same duration of the sample phase is maintained. Since it has been observed that preference for novelty in rodents is typically stronger during the first 2 minutes of the choice phase, but gradually diminishes during the last minutes, it is recommended to analyze the trial minute by minute during this phase.

4. **Other variables**: Most of the studies analyzed do not mention if the animals are undergone the NOR task during their light or dark period. Since rodents are nocturnal animals, they exhibit highest level of activity during the dark phase and, for this reason, we support papers which conduct the NOR test during the animals' dark/active phase, possibly using an inverted light/dark cycle. Considering that for

the NOR task a variety of habituation protocols has been used, it is recommended to increase environmental and apparatus familiarization in order to minimize the stress and novelty confounding factors, increasing as a consequence objects interaction and discrimination.

References

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