Novel-Object-Recognition:
# Characteristics, Considerations, and Challenges

What is the Novel Object Recognition Task?

The Novel-Object-Recognition (NOR) test [Ennaceur & Delacour, 1987] is commonly used to evaluate the ability to discriminate (and remember) familiar and unfamiliar objects as they are presented, providing insight into cognitive and memory functioning. NOR has been used to help identify impairments or improvements in learning and memory, serve as indicators of disease processes and progress, identify relevant brain regions and mechanisms required for the task, and point to potential therapeutic or drug interventions.

What is the standard NOR protocol?

There are 4 general phases in traditional NOR tests, although the experimental setup varies widely across studies. All steps are typically performed by hand:

1. *Habituation*: experimenter introduces the animal into the testing environment (typically an open field square or circle, the size depending on the species tested) to acclimate, become familiar with the testing apparatus, and ideally reduce stress levels associated with the new, open environment.
2. *Training*: during training, the experimenter places the animal back into the open arena where the animal now encounters two objects (which vary widely across the literature) placed within the testing apparatus. They explore these objects, establishing a baseline level of familiarity with each.

Novel object recognition

3. *Testing*: after a delay period (or inter-trial-interval, ITI), one of the familiar objects is replaced with a novel, different object. The ITI between training and testing can be manipulated to explore retention intervals of differing lengths, and varies from seconds to days. Animals are reintroduced to the apparatus for testing, and their exploratory behavior is documented.
4. *Analysis*: a key measure from the NOR task is the amount of time an animal spends "exploring" each object. Increased exploration of the novel object (presented during the testing phase) compared to the familiar one indicates normal recognition memory functioning, whereas no difference in exploration of both objects can indicate a memory deficit.
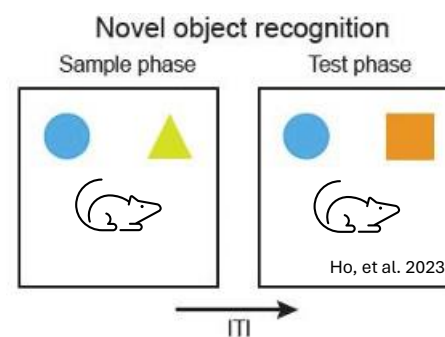
What are the challenges of NOR testing?

Although NOR is a widely used and valuable tool, it is not without considerable challenges, including:

Manual Testing and Handling. NOR testing is almost always conducted manually (unless you are using a **smart-Kage**!). Manual testing can introduce stressors to the animal from handling and potential experimenter bias, and by nature requires experimenter effort and expertise in behavioural testing practices. Prior handling of animals for several days/weeks before testing is important to reduce stress in the NOR task.

Separate Testing Environment. NOR is typically conducted in a separate, open arena environment, rather than a familiar homecage, which can induce stress in animals especially for certain models/strains. Room characteristics like lighting, sounds, odors can all influence behaviour and task performance.

Behavioural Variability. Group size for NOR is often larger than other tasks in order to achieve sufficient statistical power, given the variability in exploration across animals and likelihood for exclusion due to noncompliance in the task (i.e. no exploration).

Protocol Variability:

Object Selection. Objects used in NOR vary widely in the literature – from Legos to common household items to toys, to name a few. Careful selection of objects is necessary to ensure objects are interesting enough to explore (but not too interesting to be preferred!) and discriminable (but not too different!). Pilot tests should be conducted to ensure that objects are discriminable and object preference is balanced.

Object Use. Multiple identical objects and object counterbalancing (which one is novel, for example) should be used across training and testing phases to control for odor cues (as well as thorough cleaning).

Object Placement. Object location within the arena should be counterbalanced across sides/locations.

Defining Exploration. How exploration is defined and how behaviour is scored across studies often varies. Exploration has been defined as any or all of the following, and is scored via human observation and/or via software:
- instances when animals orient (look at, turn head towards) the object, usually within a defined proximity (2-3cm, varies) of the object, sometimes involves a duration threshold (i.e. >2s)
- instances when animals sniff (vibrissae movement) the object, usually within a defined proximity (2-3cm, varies) of the object, usually done by human scoring, sometimes involves a duration threshold (i.e. >2s)
- when the animal enters a defined threshold zone around the object (entry = head or body or both required to enter the zone, varies and is dependent on tracking nose, body, tail po ints), sometimes involves a frequency/duration threshold (i.e. points must cross X times or for Xs)
- when direct contact with the object occurs using the snout/nose or paws, can involve frequency/duration threshold
- climbing on, sitting on, moving, or chewing on objects should not counted as exploration

Outcomes and Interpretation. In addition to the above, other factors can influence the outcomes in NOR (i.e. exploration time, retention interval tolerance, etc) and should be considered in experimental design and interpretation, including animal strain, model, sex, age, and disease states/progression (timing).

The benefits and the future of NOR

NOR capitalizes on the innate tendency of rodents to exhibit increased exploratory behaviour toward new stimuli in their environment and thus requires fewer sessions compared to other tasks. NOR does not involve explicit experimental contingencies (reinforcement or punishment) to motivate behaviour. Compared to other rodent tasks, NOR also more closely emulates cognitive tests conducted with humans and is thus considered a more translatable and ecologically valid measure.

Did you know?

The combination of benefits and challenges described led to the creation of automated, hands-off, homecage NOR testing within our **smart-Kage.** Read more about smart-Kage and smart-NOR **here**!