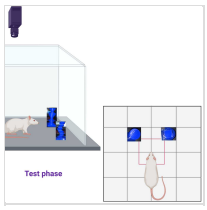

Oct 19, 2022

Novel Object Recognition test for rats, AJA, 10/18/22

Alicia Avelar[1]

[1]UCSD, Scripps

In Development Share

This protocol is published without a DOI.

Alicia Avelar
UCSD, Scripps

DISCLAIMER

This protocol requires additional troubleshooting with naive rats to ensure interpretable data. If you have suggestions for improvement please comment or email: aavelar@health.uscd.edu

ABSTRACT

Purpose: to study animal memory using recognition of a familiar object vs. a novel object

PROTOCOL CITATION

Alicia Avelar 2022. Novel Object Recognition test for rats, AJA, 10/18/22. **protocols.io** https://protocols.io/view/novel-object-recognition-test-for-rats-aja-10-18-2-ch34t8qw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

References: **Cruz-Sanchez A**, Wilkin J, Arruda-Carvalho M. Ontogeny of spontaneous recognition memory in rodents. Neurobiol Learn Mem. 2021 Jan;177:107361. doi: 10.1016/j.nlm.2020.107361. Epub 2020 Dec 8. PMID: 33307181. **Danışman B**, Akçay G, Gökçek-Saraç Ç, Kantar D, Aslan M, Derin N. The Role of Acetylcholine on the Effects of Different Doses of Sulfite in Learning and Memory. Neurochem Res. 2022 Jul 27. doi: 10.1007/s11064-022-03684-z. Epub ahead of print. PMID: 35895153. **Ennaceur A, Delacour J.** A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res. 1988 Nov 1;31(1):47-59. doi: 10.1016/0166-4328(88)90157-x. PMID: 3228475. **George lab** ethanol vapor binge grant **Inayat M**, Cruz-Sanchez A, Thorpe HHA, Frie JA, Richards BA, Khokhar JY, Arruda-Carvalho M. Promoting and Optimizing the Use of 3D-Printed Objects in Spontaneous Recognition Memory Tasks in Rodents: A Method for Improving Rigor and Reproducibility. eNeuro. 2021 Sep 30;8(5):ENEURO.0319-21.2021. doi: 10.1523/ENEURO.0319-21.2021. PMID: 34503967; PMCID: PMC8489023.
https://www.noldus.com/ethovision-xt/resources

KEYWORDS

Novel object recognition, NOR, memory, rat, behavior

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GUIDELINES

Ideas for future experimental troubleshooting/optimizing:
Ennaceur & Delacour, 1988 BehavBrainRes- excluded NOR data from rats with no exploratory behavior of objects during the tests
need to counterbalance objects used for familiar and novel and position in open field to account for object or location of object biases that could confound results
rat placed on side of chamber opposite the objects and facing away from the objects
white noise 70 dB above human threshhold and 40 lux light from one lightbulb above the apparatus

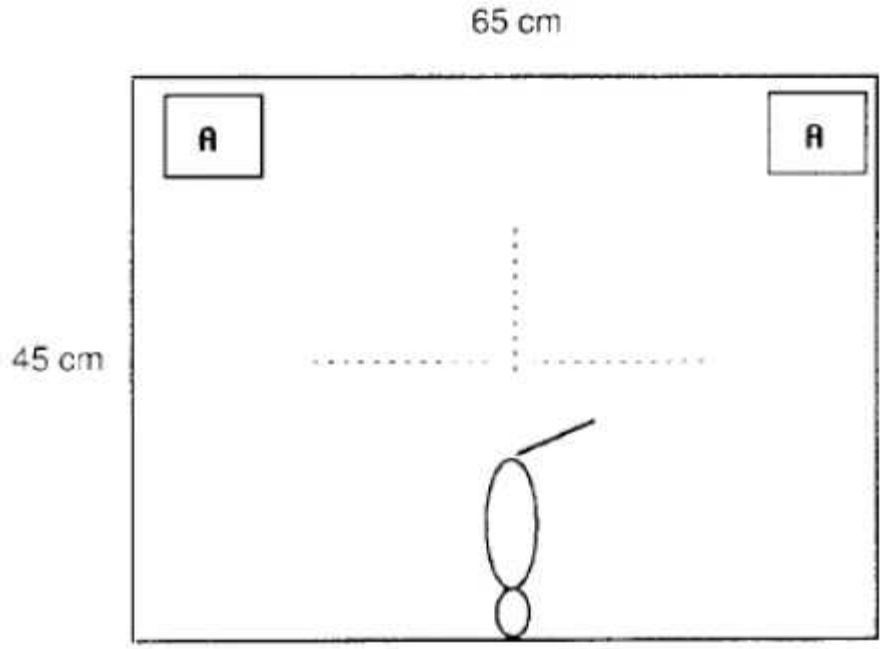


Fig. 4. Representation of the experimental conditions in T1, Expt. 3.

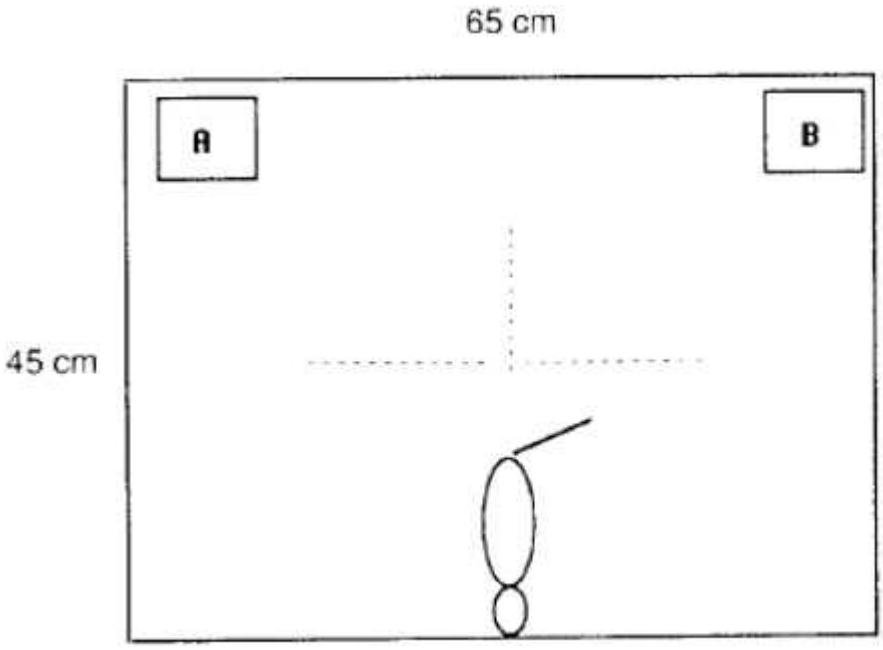


Fig. 5. Representation of experimental conditions in T2.

MATERIALS TEXT

Facilities and supplies needed:

A quiet room where the test will be performed (MTF behavior room at UCSD was the location of these tests)

Access to a 3D printer, software, and filament (USCD Geisel library digital media lab was used by

Alicia Avelar)
Red light (red light bulbs in metal lamps on tripods were used)
Open field chambers (dimensions: 42.5 width X 42.5 length X 30 cm wall height)
Multiple sets of identical objects (3D printed objects similar in size to adult Wistar rats.Objects chosen based on Inayat et al., 2021 using files found on thingiverse.com from the Khokhar lab.)
Velcrow tape/adhesives to attach objects to floor of open field chamber
Cleaning supplies (Airx44 disinfectant cleaner and paper towels)
Worksheet for tallying rat behavior during the test. (created in excel by Alicia Avelar)
Video camera for recording the tests (Zohulu 4K video camera with infrared night vision was used- recordings done in red lighted room using infrared night vision mode)
Very long video camera power cord to reach wall outlets (not always easily accessible)
Camera mount to secure camera to the ceiling directly above the open field chamber where NOR testing occurs.
Memory cards for collecting and transferring videos (32 and 64 GB SD cards were used.64 GB SD cards are better since videos don't have to be transferred as frequently throughout a testing day)
A safe, stable ladder to climb up to allow for mounting of the video camera and insertion and retrieval of the memory card each test day.
a computer that has a SD memory card slot or a SD memory card to USB adaptor (which is what we needed to transfer videos to the computer for anymaze analysis)
Anymaze software for tracking and data analysis of the videos
Excel for further data analysis of human tallied and anymaze data.
Graphpad prism for graphing the data and statistical analysis.
Computer(s) to run the necessary software on for data collection with anymaze and data analysis, graphing, and statistical analysis.
Worksheet for human tallied data collection (made in excel by Alicia Avelar).

Rat	Group	nose touch/sniffing		paw touch		climb		bites	
1	A								
2	A								
3	A								
4	A								
5	B								
6	B								
7	B								
8	B								
9	naïve								
10	naïve								
11	naïve								
12	naïve								
13	naïve								
14	naïve								
15	naïve								
16	naïve								
17	bonus								
18	bonus								
19	A								
20	A								
21	A								
22	A								
23	B								
24	B								
25	B								
26	B								
27	naïve								
28	naïve								
29	naïve								
30	naïve								
31	naïve								
32	naïve								
33	naïve								
34	naïve								
35	bonus								
36	bonus								
NOR test score sheet									
phase (sample or test)									
retention interval (1 min and 24 hr)									

DISCLAIMER:

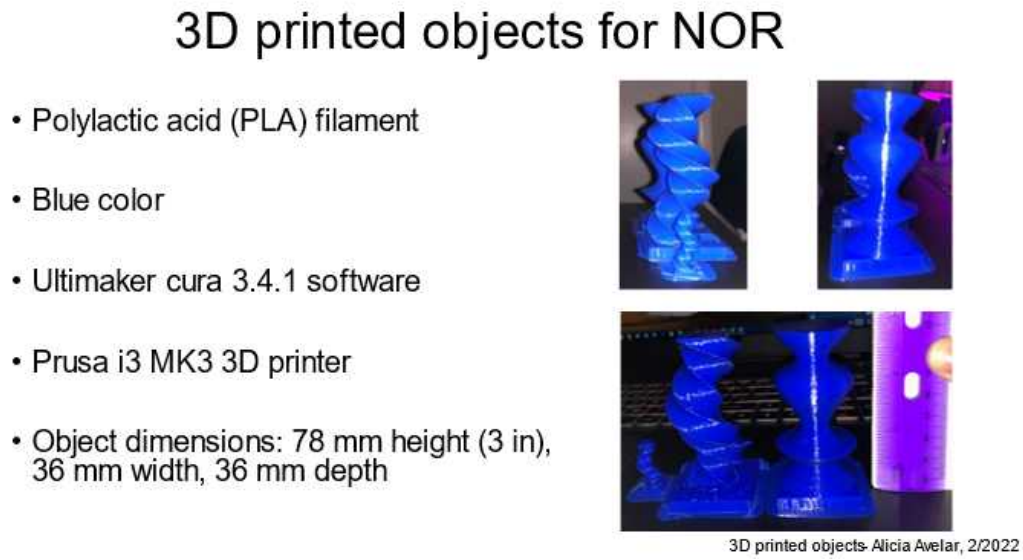
> This protocol requires additional troubleshooting with naive rats to ensure interpretable data. If you have suggestions for improvement please comment or email: aavelar@health.uscd.edu

BEFORE STARTING

Need to establish that protocol fully works with naive animals. Potentially accounting for side of chamber and object shape biases and use a larger room, with less clutter, and more soundproof walls will help the test work better with easier-to-interpret results.

3D printing

1 Objects were printed on Prusa 3D printers using ultimaker cura software at the UCSD digital media lab in the Geisel library.

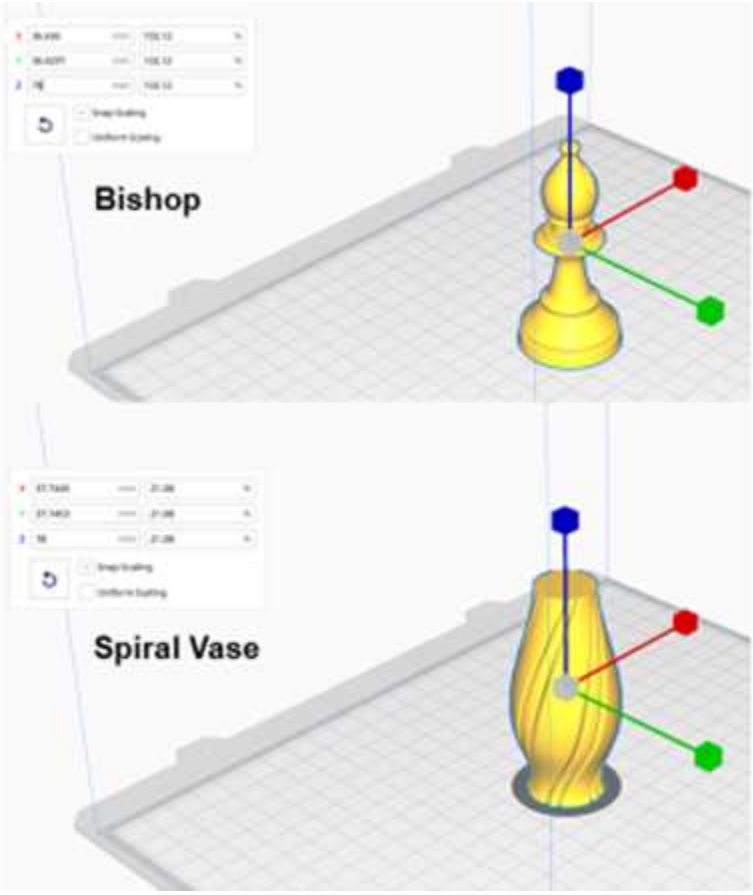


3D printed objects- Alicia Avelar, 2/2022

Printing took about 1-3 hours per object.

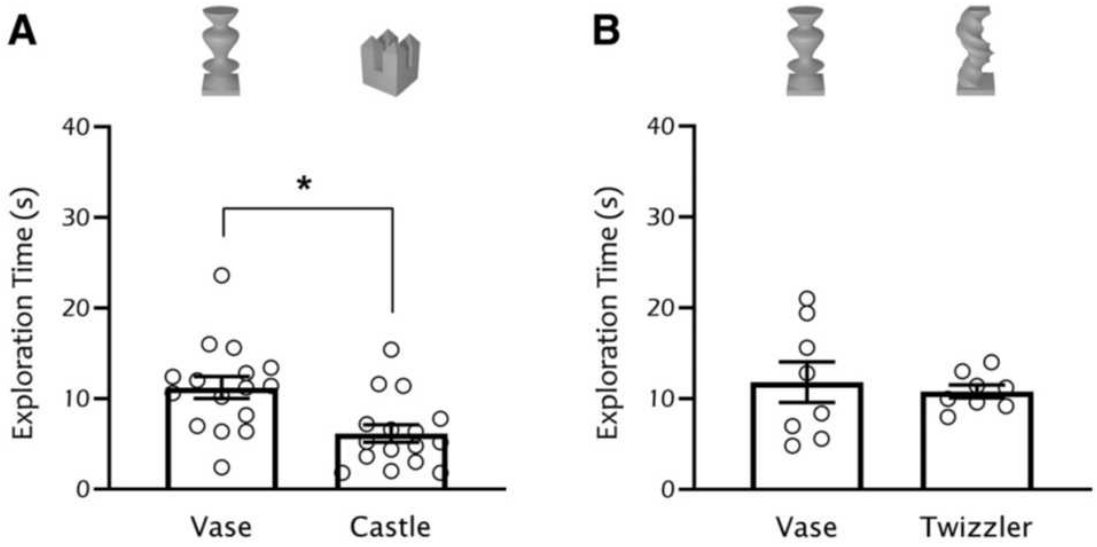
3D printed object (vase) compared to adult Wistar rat size. (Pictures and 3D prints by Alicia Avelar)

2 The object sizes were standardized as much as possible depending on the objects shape.



Bishop: https://www.thingiverse.com/thing:40605, Spiral Vase: https://www.thingiverse.com/thing:2421704

3 2 of the object shapes were from Inayat et al., 2021 publication that supported rats would interact with those 3D printed shapes.



Sprague-Dawley rat exploration of 3D printed objects. Rats preferred interacting with the vase and twizzler objects closer to their height than with the smaller castle object. (Inayat et al., 2021)

4 Additional object shapes were chosen based on having differing shapes and being printable in similar dimensions to the published objects for the NOR test.

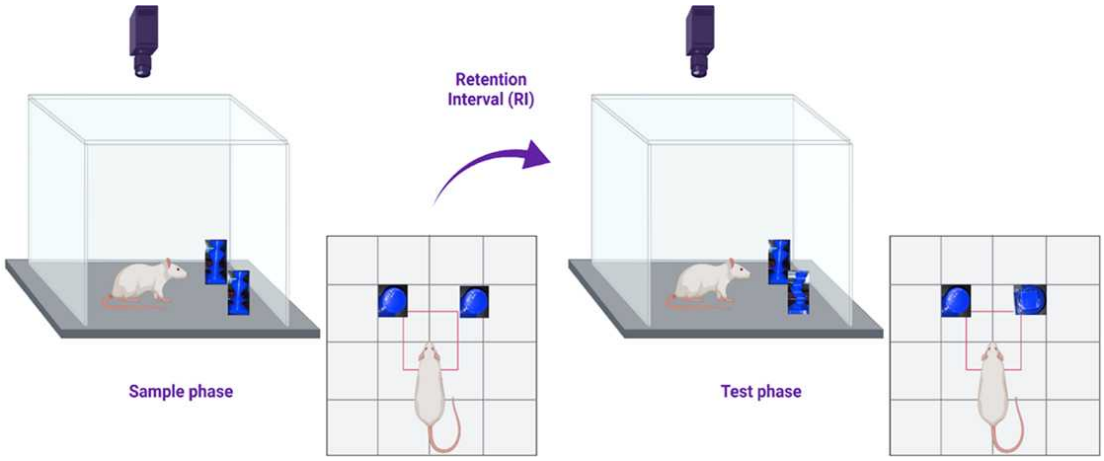
5 Multiple sets of objects (5-7 copies each shape) were printed to allow for testing multiple retention intervals (RIs- the time between the sample and test phases of the NOR test)

6 Object shapes 3D printed are vase (Inayat et al., 2021), twizzler (Inayat et al., 2021), spiral vase, bishop, king, plumbob. All 3D files used were found on thingiverse.com. https://www.thingiverse.com/thing:4964541

Set up

7 Open field chambers were set on a work table in MTF behavior room.

8 Velcro squares were applied to the floor of each open field chamber towards the back wall. (12 cm from the back and side walls so that objects adhered to the Velcro would be equidistant from each other and leave space around the object for the rats to explore)



NOR set up image created in BioRender (2/15/22) by Alicia Avelar

9 a video camera was mounted to the ceiling above the open field chamber and a long power cord was used to reach the wall outlet.

10 The power cord was taped in place to the wall near the outlet and the ceiling near the video camera to reduce shaking of the camera during recording from cord weight.

NOR test procedure 1h 10m

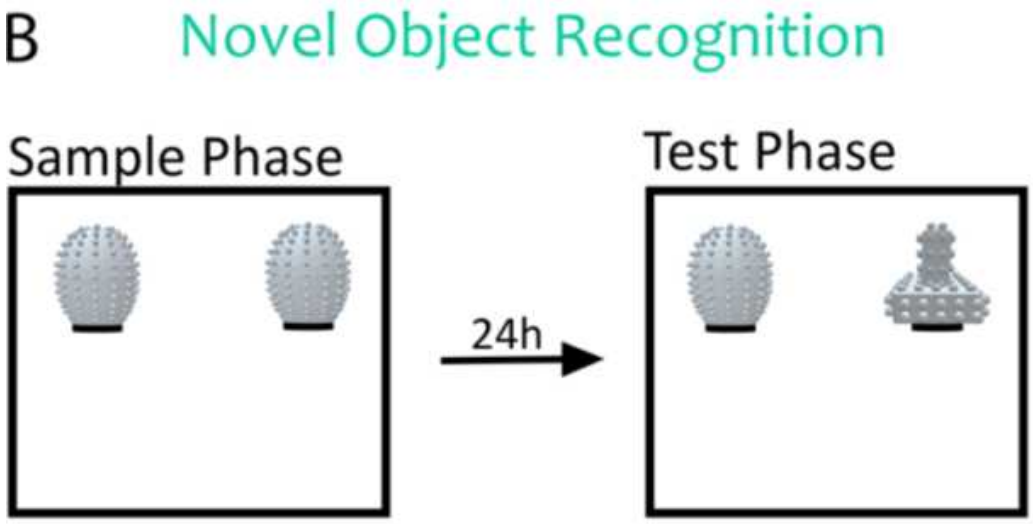
11 All rats were handled prior to tests.

12 Rats were brought into behavior room and given 1 hr habituation time in the room in home cages. For collection of NOR test intoxication data rats spent at least 3 hours in the ethanol vapor self-administration (EVSA) chambers immediately before NOR testing began. 1h

13 2 identical objects are placed in the open field chamber, attached to the Velcro on the chamber floor.

14 First rat is placed in the chamber, centered and close to the front wall (opposite the objects), facing the objects.

15 This is the sample phase and the rat is given 10 minutes to freely explore the open field chamber and objects. Vase shaped objects were used for the sample phase. 10m



NOR test experimental design. (Cruz-Sanchez et al., 2020)

16 Next, is the retention interval (RI). The retention interval is the time between the end of the sample phase and the beginning of the test phase. We have used 2 retention intervals for the NOR test: 1 minute (to test short term memory) and 24 hours (to test long term memory).

17 At the end of the sample phase, the rat is removed from the open field chamber.

18 The objects are replaced with one object identical to those present in the sample phase (left side) and a novel object (right side). One vase and one twizzler object were used during the RI 1 minute test phase. One vase and one plumbob object were used during the RI 24 hours test phase.

19 After the RI, the rat is placed into the open field chamber for the test phase. There is one familiar object and one novel object in the chamber during the test phase. The test phase lasts for 10 minutes.

20 When the test phase ends the rat is place back into their home cage and testing continues with other rats in the cohort until complete for each RI.

21 Objects and open field chambers were cleaned and dried with Airx44 disinfectant cleaner and paper towels between tests and sexes.

Data processing

22 Use anymaze for video behavior tracking and data collection. (I made an anymaze protocol to analyze NOR data from videos)

23 Use excel to create an electronic record of human tallied data and anymaze data and do further data analysis.

24 Use graphpad prism for graphing and statistical tests.

25 Noldus ebook behavior basics has information on NOR test data to graph.

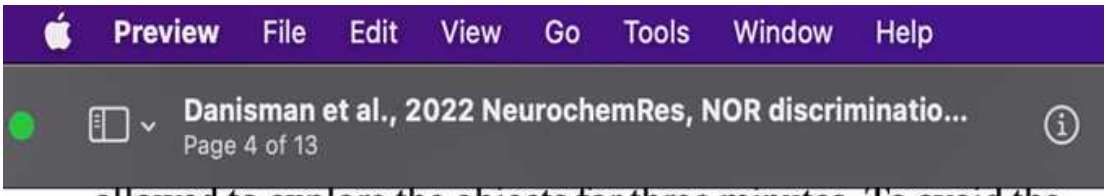
25.1 “The most important results from the novel object or novel location test include the exploration times of the objects and the frequency of each object exploration. These are generally expressed as the percentage of exploration time spent on novel object:

25.2 Time spent exploring novel object (x 100)/ Time spent exploring any object

25.3 The preference for novelty is a positive value if there is preference for novelty, and is zero if there is no preference:

25.4 Time novel - Time familiar (x 100)/ Time novel + Time familiar”

26 Danışman B, Akçay G, Gökçek-Saraç Ç, Kantar D, Aslan M, Derin N. The Role of Acetylcholine on the Effects of Different Doses of Sulfite in Learning and Memory. Neurochem Res. 2022 Jul 27. doi: 10.1007/s11064-022-03684-z. Epub ahead of print. PMID: 35895153.



allowed to explore the objects for three minutes. To avoid the presence of olfactory cues, objects were thoroughly cleaned with 70% ethanol between rats. After 24 h, the test phase was conducted for 3 min. During the test phase, one of the objects was replaced with another object of different shape and size, and the time spent exploring each object and the total time spent exploring both objects were recorded for 3 min. The nose of the animal was taken as a reference so that the animals approaching the objects more than 1 cm could be recorded as the time spent exploring and measured by the software program (Noldus Ethovision XT System, The Netherlands) [28]. The discrimination index, which is used to analyze cognitive performance, was calculated. Calculation of the discrimination index is as follows:

$$\text{Discrimination index} = \frac{(\text{Novel object exploration time} - \text{Familiar object exploration time})}{(\text{Novel object exploration time} + \text{Familiar object exploration time})}$$

27 Example data presentation from George lab ethanol vapor grant.

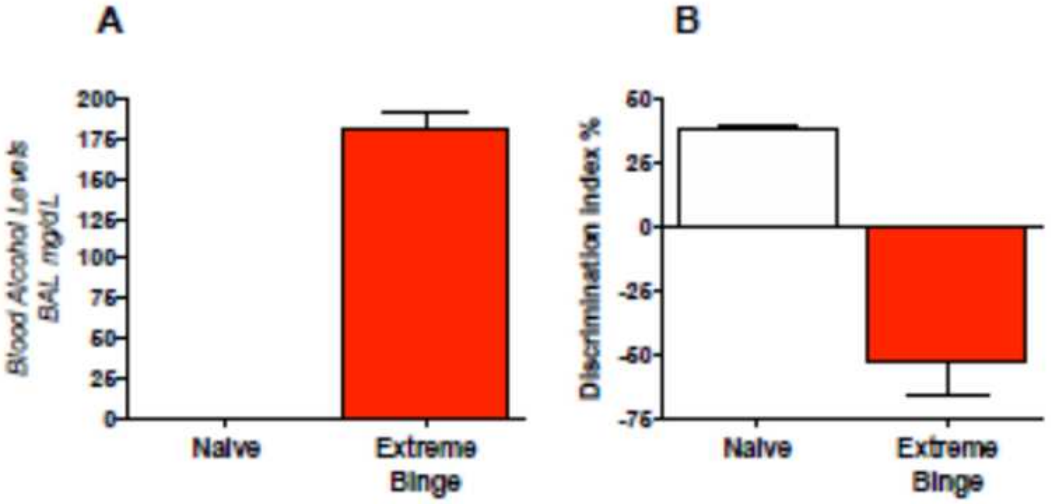


Figure 8. Alcohol-induced blackouts in Wistar rats. (A) BALs reached during passive alcohol exposure in the EVSA apparatus. (B) Discrimination index during the retention test in the novel object recognition test.