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Classic Labyrinth Test for Neurobehavioral Evaluation in Wistar Rats

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[Abstract] The Classic Labyrinth Test (CLT) is a simple way to evaluate behaviors in rodents such as learning ability, memory, and anxiety. The protocol presented here describes the procedure for use with rats, but the protocol can also be adapted for use in mice if a smaller device is used. In short, the CLT uses a square-shaped maze with a starting point and a stopping point. After the animal is trained, the animal is allowed to view and explore the labyrinth freely for 10 min. During this time, all of the animal's vertical and horizontal movements within the labyrinth are recorded. This is a very challenging task because it requires the animal to remember the quickest path between the starting points and the end. In cases where the labyrinth is designed so that the animal only needs to walk forward, it is quite easy for healthy rats, but for rats exposed to neuro-xenobiotics (drugs, pesticides) there will be disturbances in their path. Researchers use many different versions of this test and the procedure for each version can vary significantly. Here, we present a working protocol that enables the detection of traces of some toxic substances that may be exposed to individuals over a long period and in very small amounts under specific conditions such as drugs, medicines and pesticides.
Keywords: Classic labyrinth, Behavior, Learning, Drugs, Pesticides, Rats

[Background] We set out to elucidate how animals memorize and learn when they are challenged with stress utilizing the classical labyrinth test. The CLT is performed in a square-shaped plastic enclosure (125 x 125 x 40 cm) with several labyrinth passages of identical width and height (25 x 35 cm), but with variable length (Figure 1). Usually, this labyrinth is placed on a table 90 cm high. Control rats can quickly move through the labyrinth between the starting point and the ending point. When the animal explores the maze, it increases the time it spends in the maze's passages, which will be considered as aversive or anxiety-provoking for the stressed animal; while the leak behavior will be observed when the animal spends more time in the starting point or corners, which will be associated with a refuge (Leo and Pamplona, 2014; Gasmi *et al.*, 2017b). There are many tests similar to this test, but this test is unique because it is concerned with measuring the impact of environmental pollutants on the behavior and psychology of animals, especially rats (Serchov *et al.*, 2016). Some advantages of this approach are that this test is inexpensive, and it is quick; the test usually takes about 10 min, and after cleaning the labyrinth with 70% ethanol, it can be used to test the next animal (Gasmi *et al.*, 2017a and 2018).

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Materials and Reagents

1. Paper towels
2. Laboratory rats (3 months old and weight between 200 and 240 g)
   The rats are housed in groups of 4 per cage and kept in an environment with temperature (23 ± 2 °C) and humidity control. The animals have a 12-12 h light-dark cycle and food and water ad libitum.
3. Ethanol (70%) for cleaning (CID: 702)
4. Plastic sheets (PVC wall panel) (Dacheng, model: DCb-008)
5. Adhesive glue for plastics (SG300-05)

Equipment

1. Home cage (Tecniplast, catalog number: 1291H001, EUROSTANDARD TYPE III H, 425 x 266 x 185 mm)
2. Classic Labyrinth for rats [a square shaped plastic enclosure (125 x 125 x 40 cm) with several labyrinth passages of identical width and height (25 x 35 cm) but variable length according to the crossing. This Labyrinth can be assembled manually as shown in Figure 1, where it is made of white plastic sheets. These plates are smooth and solid and should be easy to clean and unbreakable or damaged by the animal. The maze is formed at 125 x 125 x 40 cm by welding the plastic sheets together with adhesive glue. According to Figure 1, after the maze is made, it is placed on a wooden table 90 cm high on the ground (Figure 1)]
3. USB Camera Lenses (ITEM: 60528/Lens for 60516, Vari-focal, 2.8-12 mm)
   Note: It was placed directly in the middle above the Labyrinth at the height of 1.20 m so that only the labyrinth was exposed.
4. Chronometer (LCD Digital Portátil Reloj Alarma Temporizador by SYMTOP)

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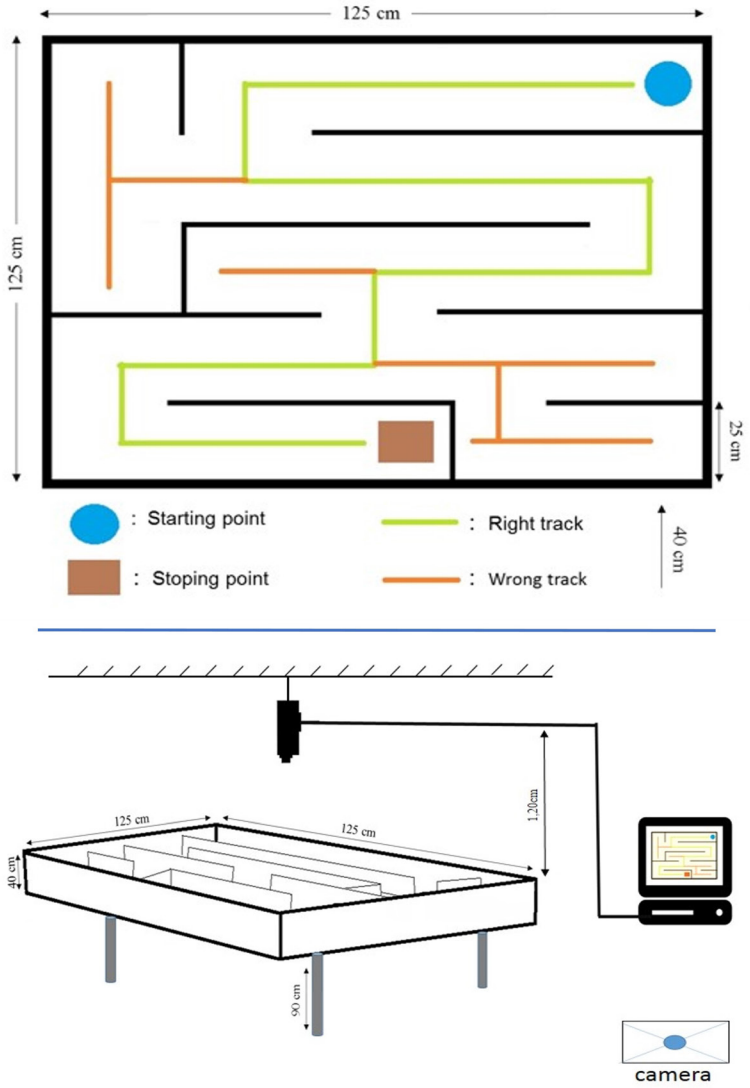

Figure 1. Classic Labyrinth and its dimensions

Software

1. Ethovision video tracking system software (version 10, Noldus Information Technology, France)
2. Microsoft Excel (Microsoft Corp® 2016, USA)
3. Minitab (Minitab® version 17.1, USA)

Procedure

1. Several exercises are performed for the rat (about four to five times as needed) before the final data are recorded, Animals should be allowed at least one week for habituation to the home cage, as they tend to present high anxiety levels upon arrival from the animal breeder facility (Leo *et al.*, 2014), During the training period, rewards with an odor cue (bread, grease, cheese, processed food, *etc.*) are used to attract the rats and help them quickly reach the end of the

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maze (the target). However, on the test day, nothing is left at leaves nothing at the stopping point. We do this because it eliminates external factors and variables (*e.g.*, odor) between experiments (Gasmi *et al*., 2017b).

2. The apparatus (labyrinth) is placed in an isolated room away from any external interferences and noises with a low-intensity white light source.
3. The experimenter must avoid making any excessive noises during the test and from wearing products with a strong smell because it could act as an anxiogenic stimulus for the mice.
4. The illumination in the lit chamber must be 200-400 lux.
5. The rats are transferred in their home cages to the behavioral testing room for at least 60 min before the experiment.
6. Clean both compartments of the apparatus with 70% ethanol (Serchov *et al.*, 2016).
7. Turn on the camera and place the first rat in the starting point of labyrinth.
8. The experimenter stays as far away as possible from the box and out of sight of the test animal.
9. The rats are allowed to move freely within the labyrinth for 10 min (between the starting point and the stopping), but in exceptional cases 10 min is added to the test for a total of 20 min.
10. After each trial, all urine and fecal matter are removed, and the maze is cleaned with 70% ethanol.
11. The recorded videos can be analyzed by automated computer software (Ethovision video tracking system software, Spink *et al*., 2001) or manually with the aid of a chronometer.
12. The duration of time required for the animal to move from the starting point to the stopping point (Arrival Time) is recorded (Figure 2). If the animal takes significantly longer to arrive at the stopping point, this delay might be explained by external factors that lead to decreased cognition. The arrival time is determined by when the rat reaches the stop point and then stays there for at least 10 sec.

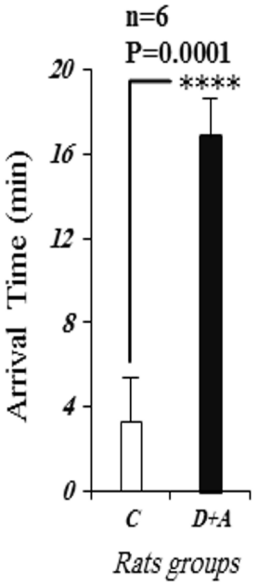

Figure 2. Representative data (mean ± SD) of the Arrival Time of control rats in the

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classical labyrinth test (*C*) and rats treated with a mixture of pesticides (*D+A*)

Data analysis

The results obtained were expressed as the average of six repetitions (mean ± standard deviation), and the data were visualized using Excel 2013 to represent these results in the form of graphs and histograms. Statistical analysis was performed using the Minitab® 17.1 software. The significance of difference between the control and the treated lots is verified using the Student's *t*-test, and the comparison result as follows:

$P > 0.05$ = The difference is not significant.

(*) $0.05 > P > 0.01$ = The difference is significant.

(**) $0.01 > P > 0.001$ = The difference is highly significant.

(***) $P < 0.001$ = The difference is very highly significant.

Notes

1. Testing this maze is one of the easiest and most effective tests.
2. Experiment does not consume a lot of equipment, which makes it cost effective.
3. We have used this test in our laboratory to study the negative impact of many chemicals on the neurological health of animals.
4. There can be a significant difference between the sexes. We recommend using male or female rats only. We do not recommend using both sexes in one test.
5. Failure to clean the maze well between tests and using a poorly prepared room can lead to bad/unreliable results.
6. Mice can be used in this type of test (maze test). However, in this protocol only the *Wister* rats were used. To evaluate if a rat is ready for the final test, we expect the rat to pass all of the initial training classes during the first five days of training.
7. Generally, over-training is not harmful and may last up to five days, but all groups of rats must undergo the same experimental conditions and the same duration of training. As any difference in the duration or number of training sessions will have a negative impact on the results of the test.
8. If the rat reaches the stop point and immediately departs (10 sec before it stops), do not record the result. The experiment will continue normally and the arrival time will be recorded only when the rat stops more than 10 sec at the stop. If the first ten minutes are completed without the rat reaching the breakpoint, another 10 min of the test will be added and the entire period (20 min) will be counted.
9. The average duration of the experiment is 10 min. If the animal does not reach the stopping point, another 10 min will be added. The total possible time is 20 min. When the rat reaches the stop point during this period, the test is successful. If the rat does not reach the stopped point

during this period (20 min), the test is considered to be a failure (the test failed) and is returned after three hours.

10. A simple and possible analysis has been provided by hand. If the Ethovision video tracking system software is used to analyze and present the results such as learning curve, error rate, memory ability, neurological state, *etc*.
11. Daily training of the rats for four or five days will help them remember the shortest path in the maze to reach the stopping point, but when these animals are exposed to neurotoxic drugs or environmental pollutants it becomes difficult for them to identify the right path in the Labyrinth.
12. In this study, the statistical test "t" is indicated because it is the basic test that the novice researcher can perform, but this does not preclude the use of the other types of statistical tests according to the type of study and the researcher's need. As for the results presented, it is the mean + SD.

Acknowledgments

This protocol has been used in our published works (Gasmi *et al.*, 2017b and 2018).
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Competing interests

I declare there are no competing interests.

Ethics

This study was conducted in accordance with the EU Commission Directive 2010/63/EU for animal experiments, after approval of the Scientific Committee of Applied Biology Faculty - University of Tebessa, which issued a permit No. 247SNV/12/2014.

References

1. Gasmi, S., Kebieche, M., Rouabhi, R., Touahria, C., Lahouel, A., Lakroun, Z., Henine, S. and Soulimani, R. (2017a). Alteration of membrane integrity and respiratory function of brain mitochondria in the rats chronically exposed to a low dose of acetamiprid. *Environ Sci Pollut Res Int* 24(28): 22258-22264.
2. Gasmi, S., Rouabhi, R., Kebieche, M., Boussekine, S., Salmi, A., Toualbia, N., Taib, C., Bouteraa, Z., Chenikher, H., Henine, S. and Djabri, B. (2017b). Effects of Deltamethrin on

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striatum and hippocampus mitochondrial integrity and the protective role of Quercetin in rats. *Environ Sci Pollut Res Int* 24(19): 16440-16457.

3. Gasmi, S., Rouabhi, R., Kebieche, M. and Menaceur, F. (2018). Neurotoxicité des pesticides chez les rats. *EUE* 224.
4. Leo, L. M and Pamplona, F. A. (2014). Elevated plus maze test to assess anxiety-like behavior in the mouse. *Bio-protocol* 4(16): e1211.
5. Serchov, T., van Calker, D. and Biber, K. (2016). Light/Dark transition test to assess anxiety-like behavior in mice. *Bio-protocol* 6(19): e1957.
6. Spink, A. J., Tegelenbosch, R. A., Buma, M. O. and Noldus, L. P. (2001). The EthoVision video tracking system-a tool for behavioral phenotyping of transgenic mice. *Physiol Behav* 73(5):731-744.