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Optimizing Object Recognition Task: A Practical Study in Neurobiology by using C57BL/6 normal mice.

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Abstract

Rats and mice have a tendency to interact more with a novel object than with a familiar object. This tendency has been used by behavioural 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, drug exposure . This study has been conducted to optimize this method by using different objects at different times. The results are shown by using small objects in different colures and shapes in 4 hours better than large ones in other times, which I recommend to Object Recognition Task.

Key wards: Recognition memory, Object Recognition Task

Introduction

Exploration of novel objects has to be rodents feature more than any other animals [1,2]. This behaviour can be easily quantified and utilized to study simple recognition memory and episodic memory. In spite of food-rewarding maze learning tasks, object recognition test does not require spatial learning, food or water deprivation and the application of reinforcing stimuli. It is, therefore, suited to test the effects of drugs and genetic manipulations as knock in or knock out of a gene specially those have interference effect with food intake or the metabolism [3, 4].

It has been reported that various challenges to the neurological work are the interference of sensory, motor and pain effects. One advantage of the object

recognition test over other learning tests is clearly distinguishing between the effect of drugs or genetic manipulations on sensory/motor abilities rather than on the memory particles by the delay in time spent exploring a novel and familiar object [4].

The role of hippocampus information of object recognition memory had been investigated by many researchers [5,6,7]. By means of NMDR-Receptor genetic manipulation or pharmacological effect, the research tries to reveal this connection.As widely spread genetic background, C57BL/6 mice showed impaired object recognition task after a 24 hours but not after 5-min delay; when their neuronal activity had been inactivated in the dorsal hippocampus by means of lidocaine infusion [8]. However, this is depending on the intensity of damage to the temporal loop tissues [7]. Recently, it has proposed that the exploration of novel objects facilitates the induction of Long Term Depression(LTD) and hinders the induction of Long Term Potentiation(LTP) [9]. Surprisingly, pre- but not post-acquisition trail i.p. injections of ethanol impaired object recognition task in C57BL/6 mice after 24 hours retention interval. However, ethanol inhibits NMDA-R dependent hippocampal LTP and LTD by stimulating Hippocampal GABA-Rs. It is assumed that ethanol impaired the encoding of the object information rather than memory processes [10]. While, Neuronal activity measurement studies showed no response to novel objects [11], the role of hippocampus in object recognition test is still infixed.

Colour, shape, size and type of materials of objects have an important consideration in the object recognition task. Therefore, it is essential to make sure that objects used can be easily discriminated by the animals but also should not be differentially preferred. One of many aims of this study is to know the effects of shape, architecture and size that may be preferred by the animal than the other or type of material that may be discriminated by olfactory cues. In the mean time, most of the researchers in the field of neurobiology are using an object recognition test to identify the temporally or episodic memory formation, study an effect of drugs or evaluate the genetic manipulation effectiveness. But each of them used a different acquisition time or different objects. Nobody tries to find an optimizing method for this important task. So, in order to arrive at this aim, I had done this research.

Material and methods

Experimental design

To study the effect of the size of object in the test, I distribute firstly two main groups that depend on the type of objects that will be used (small and large object). Each group will be subdivided into two subdivision groups upon the number of object through using one or two objects in the acquisition phase of Object recognition test. All the groups will be habituated to the arena on the first day. Then, the acquisition phase on the second day with one or two objects will be conducted. Finally, the retention phase when the animal is submitted to the arena with a new novel object after delay time of 4 hours and 24 hours. Therefore, there are four groups (n=6) as fellow (fig1):

Group A: used two large objects at 10 min. as acquisition phase.

Group B: used one large object at 10 min. as acquisition phase.

Group C: used one small object at 10min. as acquisition phase.

Group D: used two small objects at 10min. as acquisition phase.

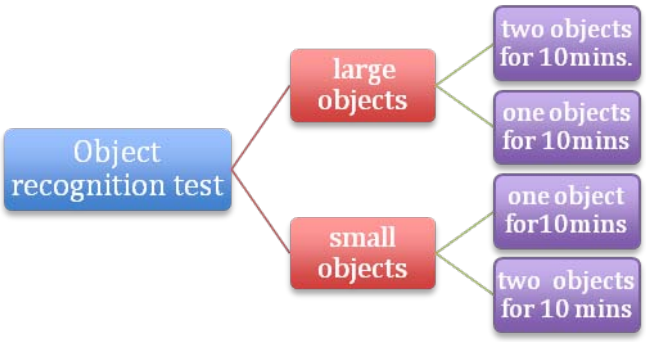

Fig.1 Experimental design diagram

Animals

24 males C57BL/6 at age of three months, were obtained from Harlan Institute, UK are weighing between 24 and 27 g. The mice were divided into groups (n=6). The mice were housed in a room with 12:12 light /dark cycle at 23C. Animals received food *ad libitum*. All training and testing were performed in the behavioural testing facility; which is adjacent to the animal housing area. Each animal was individually housed prior to training. A C57BL/6 mice were used because this is a strain widely used to study the neurobiology of learning and memory and provides the major genetic background for gene targeting studies in mice [12].

Screening procedure

Before starting the tests, the general health of mice and their normative responses to stimuli were determined [12]. Measures included: the general appearance of mice (clean fur, posture and muscle tone)and the normal behaviour (fur grooming, nest building, climbing) and reflexes (blinking to cotton swab placed close to eye, ear twitch when cotton swab is gently placed on ear). Only mice that were used showed a good health status in terms of body weight, fur appearance and intact eyes.

Animal handling

To habituate mice to handling, mice were picked up for a 1.5 min/day for a five consecutive days and exposed to novel stimuli on days 1-4 (new clean cage, weighing scale, moving to the procedure room, novel open field testing chamber (which was utilize in subsequent behavioural tasks that are described below)[13].

Open field

On day Four of handling training course, all the groups submitted to open field study for 10 minuets. Also this procedure was considered as a part of habituation time for Object recognition test. By using overhead video-camera connected to computer with video tracking analyzing software(Bio-signal Group Interactive Tracking system, ITS. Version 1.1) the activity of peripheral and central zones were recorded. Also, the same programme had recorded the frequency of contact with the objects in determining zones, the speed and travelling Distance. While, the climbing (number of exploring with forepaws on the wall of arena) and rearing (number of standing on legs with noise in the air for exploration) and grooming (number of times cleaning the fur and skin) had been measured manually at the same time (14). After each trail, the apparatus was cleaned with 75% ethanol.

Object recognition test

Objects

Two different objects were used (Large and Small). The larger objects made of plastic that are different in terms of height, colour, shape and surface texture in size of $5cm^3$ roughly. Smaller objects are the dice and the marbles; also they are different in terms of height, colour, shape, surface texture and the type of materials.Picture.2.

Procedure

The object recognition test, a test of non-spatial reference memory, was conducted in an open field circle arena (60 cm in diameter and 30cm-high wall), constructed of white laminate and located in quite room. This same open field was utilized in the habituation and open field procedures. A digital camera was mounted on the ceiling above the testing arena and connected to a computer with a video-tracking system that objectively monitored and quantified animal movement (Bio-signal Group Interactive Tracking system, ITS. Version1.1). Testing, which takes advantage of the natural affinity of mice for novelty, was conducted as described previously (3, 15,16,17,).

This task, based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one, was performed during three days. The general procedure consisted of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the first day (habituation phase), mice were individually subjected to a single familiarization session of 10 or 30 min depending on the schedule above, during which they were introduced into the arena, in order to become familiar with the apparatus. On the second day (acquisition phase), the animals were subjected to a single 10-min session, during which one or two floor-fixed objects (A or/and B) were placed in a symmetric position from the centre of the arena, 15 cm from each other and 8 cm from the nearest wall. The two objects made of the different materials with a different colour and shape but identical in size per each group. Mice were allowed to explore the objects in the open field. A recognition index for each mouse was expressed as a ratio of the amount of time spent exploring object A (TA*100)/ (TA + TB), where TA and TB are the time spent exploring object A and object B, respectively. It is acceptable if the recognition index of A object is a round 40-60%. On the 3rd day (retention phase), mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index, calculated for each mouse, was expressed as the ratio (TC *100)/ (TA + TC), where TA and TC are the time spent during the retention phase on object A and object C, respectively. The time spent exploring the object (nose pointing toward the object at a distance$\leq$ 4 cm to the large objects and at a distance$\leq$ 3 cm to small objects) was recorded by exploring analyzer programme in the same software. After each trail, the apparatus was cleaned with 75% ethanol. In cases of one object recognition we compare the exploration time between the acquisition phase and the retention phase after 4 and 24 hours retention intervals.

Statistical analysis

By using PRISM computing system, the results of ORT have been conducted to T-test for comparing the groups of normal mice, in order to explain the favorite object (small with different shapes and colors) or (Large with different shapes and colors).

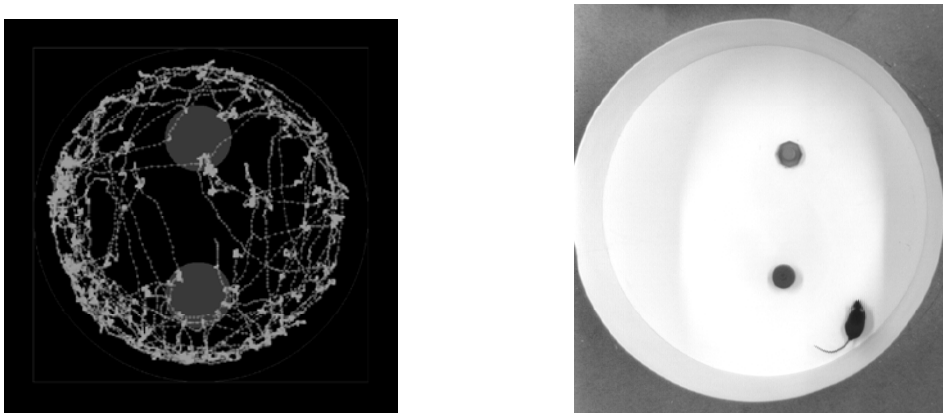

Fig.2 Shown is the arena with two objects and a mouse (Right). A sample track is shown on the left. Visible are two surrounding areas in the arena used by the software to analyse the object exploration events. n=12 per group.

Results

By working with the normal mice C57BL/6 , and in order to start our work with neurobiology, I have done this study. The Recognition index(RI) of object recognition experiment have mentioned a significant value ($p \geq 0.05$) by using large objects at 4 hours as a retention intervals, Fig. 1. In contrast, and by using the same large objects at 24 hours, we have found that there is no significant difference($P \geq 0.05$) in RI, Fig.2. While, there is a clear difference-high significant-($P \geq 0.05$) between the acquisition trails period RI and retention trails RI in case of using small objects at 4 hours as retention intervals, Fig.3. On the other hand, there is no difference($P \geq 0.05$) by using the same small objects at 24 hours as a retention interval, Fig.4.

Statistically, there are no significant differences between the groups of the mice in the level of open field parameters . Nearly all the groups have a similar results of speed, peripheral/central movement value, grooming , line crossing , and rearing, data not shown.

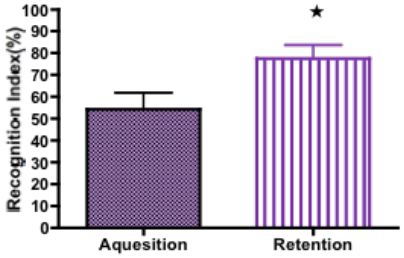

figure (1) explain the OBT results by using large objects, 4 hours retention intervals. A Significant differences has been occured(P≤0.05)

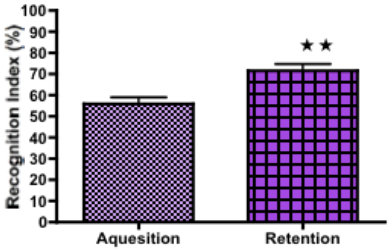

Fig.3. Show the ORT results by using small objects with 4 hours retention intervales.A high significant differences have been occured (P≤ 0.05)

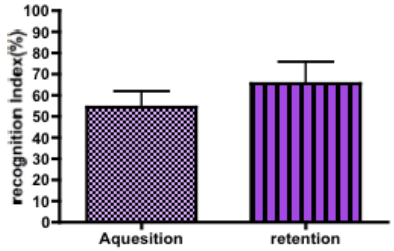

Fig.2. Explain the ORT results by using large objects with 24 hours retention intervale.Non signifcant Differences have been noticed (P≤0.05)

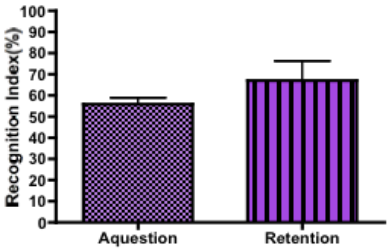

Fig.4. Explain the ORT results by using small objects with 24 hours retention intervales Non significant differences have been noticed (P≤0.05)

Discussion

Object recognition task is a recent indicator for memory status, has been used widely to discriminate between the normal and abnormal cases of neuronal disorders. A lot of scientists have used this task, But they used different methods by objects, time intervals, arenas, and distribution pattern of objects. Also, I found differences in the equation that is use to calculate the recognition Index(RI). Therefore, I suggested an experiment to find out the optimal conditions to this experiment in the lab depending on all the information available from other experiences advanced in this field of studies. Opject recognition test measures spontaneous behaviour. This task consists of the acquisition phase during which rodents explore two equal object followed by a delay retention phase; in which a novel object is presented together with one familiar object already presented during the acquisition phase(5,18). The researchers have used various retention intervals ranging from minutes to several hours up to days. Poor learning performance is usually interpreted as the delay between the acquisition phase and retention phase of the test increases (19). There are no differences between the test and control groups when the delay interval is in 5 minutes but significant differences after 3 hours (short-term memory scale)(20). Furthermore, the deterioration in object recognition indexes after 24 hours (long-term memory scale) seams to be experimental intervention rather than memory impairment (20).It has been inferred that animals can be tested repeatedly after week rest, but interestingly object memory can last for several weeks after sufficient training (22,23).Thus, Object recognition paradigm could be used as model to study the effect of hippocampus associated memory.

The results of the experiments in my work explained that using small objects is better than large objects Fig.1,3. It may be due to architecture of four legs bodies where the head not standing in upright angle, where the eyes should be found to recognize the objects and take a complete picture. A novel object should be recognized more than familiar ones normally at the same conditions . It has been found all the experiments in this study work well. The animals recognized the

novels more than familiars but not significantly Fig.2,4. So, I recommend to use small objects at 4 hours in mice experiments of object recognition task.

References

[1] Berlyne, D.E. Novelty and curiosity as determinants of exploratory behaviour. *Br. J. Psychol*. **41**, 68-80 (1950).

[2] Aggleton, J.P. One-trial object recognition by rats. *Quarterly Journal of Experimental Psychology* **37**, 279–294.(1985)

[3] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav Brain Res* ;**31**:47–59(1988).

[4] Dere E, Huston JP, and De Souza Silva MA . The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev* **31**:673-704 ,(2007).

[5]Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., and Lehmann, H., Hippocampal damage and exploratory preferences in rats memory for objects, places, and contexts. *Learning & Memory* **9**, 49–57, (2002).

[6] Clark, R.E., Zola, S.M., and Squire, L.R. Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience* **20**, 8853–8860,(2000)

[7] Broadbent, N.J., Squire, L.R., and Clark, R.E. Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **101**,14515–14520,(2004)

[8] Hammond, R.S., Tull, L.E., and Stackman, R.W. On the delay dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory* **82**, 26–34,(2004)

[9]Kemp, A., and Manahan-Vaughan, D. Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 8192–8197,(2004).

[10]Ryabinin AE,and Miller MN, Durrant S. Effects of acute alcohol administration on object recognition learning in C57BL/6J mice. *Pharmacol Biochem Behav* ;**71**:307–12(2002)

[11] Zhu, X.O., Brown, M.W., McCabe, B.J.,and Aggleton, J.P. Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience* **69**, 821–829(1995).

[12] Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L.,and Hill, J. M. Social approach behaviors in oxytocin knockout mice: Comparison of two independent lines tested in different laboratory environments. *Neuropeptides*, **41**, 145–163(2007).

[13] Frye, C. A., Sumida, K., Dudek, B. C., Harney, J. P., Lydon, J. P., and O'Malley, B. W., *l*. Progesterone's effects to reduce anxiety behavior of aged mice do not require actions via intracellular progestin receptors. *Psychopharmacology*, **186**, 312– 322,(2006).

[14] Lalonde, R.. Cerebellar contributions to instrumental learning. *Neurosci. Biobehav. Rev.* **18:** 161–170,(1994).

[15] Luine, V. N., Jacome, L. F., & MacLusky, N. J. Rapid enhancement of visual and place memory by estrogens in rats. *Endocrinology*, **144**, 2836–2844,(2003)

[16] Walf, A.A., Rhodes, M.E., and Frye, C.A. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormoneprimed rats. *Neurobiology of Learning and Memory* **, 86**, 35–46,(2006)

[17] Walf,A.A.; Koonce,C.J.; and Frye,C.A. Estradiol or diaryl propionitrile administration to wild type , but not estrogen receptor beta knockout mice enhances performances in the object recognition and object placement tasks *Neurobiology of learning and memory* **; 89**,513-521.(2008).

[18] Dere, E., Huston, J.P., and De Souza Silva, M.A.. Integrated memoryfor objects, places, and temporal order, evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory*, **84,** 214– 221,(2005)

[19] Obinu, M.C., Reibaud, M., Miquet, J.M., Pasquet, M., and Rooney, T. Brain-Selective stimulation of nicotinic receptors by TC-1734 enhances ACh transmission from frontoparietal cortex and memory in rodents. *Progress in Neuropsychopharmacology and Biological Psychiatry* ,**26**, 913–918(2002).

[20] Baker, K.B., and Kim, J.J., 2002. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learning & Memory* **9**, 58–65,(2002).

[21] De Lima, M.N., Laranja, D.C., Caldana, F., Bromberg, E., Roesler, R., and Schroder,N. Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats.*Behavioural Brain Research* ,**156**, 139–143,(2005).

[22] Mumby, D.G., Astur, R.S., and Weisend, M.P., Sutherland, R.J. Retrograde amnesia and selective damage to the hippocampal formation, memory for places and object discriminations. *Behavioural Brain Research* **106**, 97–107,(1999).

[23] Mumby, D.G., Glenn, M.J., Nesbitt, C., and Kyriazis, D.A. Dissociation in retrograde memory for object discriminations and object recognition in rats with peripheral cortex damage. *Behavioural Brain Research,* **132**; 215–226.(2002).

الخلاصة:

تميل الجرذان والفئران مع الهدف الجديد بشكل أكثر من الهدف المألوف أو المعتاد؛ وهذا الميل استخدم من قبل علماء السلوك الدوائي والعصبي لدراسة التعلم والذاكرة. أن الصيغة المعروفة لهذا النوع من البحوث هو اختبار تمييز الهدف Object recognition task . أولا، توضع حيوانات التجربة في آلة الاختبار ويسمح لها باستكشاف الهدف ، وبعد فترة زمنية موصوفة للاسترجاع، تعاد حيوانات التجربة إلى آلة الاختبار التي تحتوي الهدف المألوف أو الذي شاهدته الحيوانات سابقا مع هدف جديد لم تستكشفه حيوانات التجربة سابقا. دالة تمييز الهدف تعرف من خلال أكثر فترة لبقاء حيوانات التجربة مستكشفة ومتفاعلة مع الهدف الجديد. بالرغم من أن العمليات الدقيقة التي توضح ذاكرة التميز تتطلب شرحا أكثر؛ نجد الانتشار الواسع لهذه الطريقة في مجال دراسات الفئران المطفره جينيا، واختبارات التعرض للأدوية . أجريت هذه الدراسة لتقويض هذه الطريقة وتثقيفها باستخدام أهداف مختلفة بأوقات مختلفة . أظهرت نتائج البحث أن استخدام الأهداف الصغيرة بأشكال وألوان مختلفة بفترة استرجاع أربع ساعات هي أفضل من استخدام الأهداف الكبيرة بأوقات استرجاع مختلفة.