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Features of memory on novel situation and avoidance response: evidence from comparisons between open-field behavior and step-through task

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AIM: To study the features of memory on novel situation and avoidance response in mice. **METHODS:** Open-field activity and step-through latency were used to determine the memory of mice on a new environment and avoidance, respectively. **RESULTS:** The open-field memory was only observed 24 and 48 h after acquisition session. The avoidance memory still existed 72 and 96 h after training session. On d 1 mice were allowed to remain on open-field and step-through for 0.5, 1, 3, and 5 min. On d 2 the retention latencies in 0.5- and 1-min groups were shorter than those in 3- and 5-min groups, while the recall activities in open-field were indifferent between these groups. Scopolamine (1 mg·kg^{-1}) and caffeine (200 mg·kg^{-1}) injected ip 15 min before the first session inhibited the avoidance response and the adaptation to open-field environment in mice. Chlorpromazine, promethazine, picrotoxin and pentobarbital impaired the avoidance memory, but not impaired the open-field memory. **CONCLUSION:** These results supported the hypothesis that the adaptation of mice on open-field was a short or medium term memory.

KEY WORDS animal behavior; avoidance learning; memory; scopolamine; chlorpromazine; promethazine; picrotoxin; caffeine; pentobarbital; propranolol

Open-field test and step-through task have popularly been used to measure drug action on emotion[1,2] and memory[3,4] of animals using 2-session paradigms. The latency from safe compartment to electrified compartment at the retention session was increased in step-through task and the ambulation at the recall session was reduced in open-field behavioral test.

When mice were placed in a chamber in which they had previously received an electric footshock they exhibited a markedly reduced motility *vs* control[5,6]. However, the exploratory ambulation on d 2 was less than that on d 1 in mice without footshock in open-field[7]. The suppression of motility on d 2 in this test was antagonized by pretreatment with scoplamine at the acquisition session. It has therefore been hypothesized that the reduced motility on d 2 was resulted from the memory of mice to a novel environment. The present work studied the different memory features in mice to open-field chamber without footshock and step-through task with footshock.

MATERIALS AND METHODS

Kunming strain mice, ♀, weighing 28±*s* 3 g, were purchased from the China Academy of Traditional Chinese Medicine (Beijing). Each cage housed 10 mice under natural light-dark cycle (light from 06:00 to 19:00). Having adapted for at least 7 d, the mice were subjected to open-field and step-through tests. Experiments were carried out between 09:00 and 12:00.

Scopolamine (Scop, E Merck, Darmstadt), picrotoxin (L Linght Co), pentobarbital and caffeine (China Medicinal Co, Beijing), chlorpromazine

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(Huaihai pharmaceutical Factory, Shanghai), promethazine (Yongkang Pharmaceutical Factory, Beijing). All drugs were dissolved in normal saline and injected ip 15 min before or 60 min after the acquisition session. Control mice received an equivalent volume of saline.

Open-field behavior Mice were tested for 2 successive days in a 32 cm×21 cm×15 cm field. The d 1 and d 2 were respectively called acquidition session and recall session. The ambulation was automatically recorded using an activity meter (MK-ANIMEX, Japan). The defecation scores were quantified by counting the number of boluses laid on the open-field.

Step-through task Mice were tested immediately after the open-field behavioral test. Memory errors in training session were indicated by the number of mouse entrances to the dark compartment to receive footshock. Retention session was given 24 h after the training session. If a mouse did not enter the dark room within 10 min the retention test was ended and the mouse was assigned a score of 600 s. The memory errors and the retention scores were expressed as the learning and memory capabilities, respectively.

RESULTS

Comparison of various intervals between 2 sessions During the first session each mouse was allowed to have open-field or step-through for 5 min. The recall and retention session tests were conducted 24, 48, 72, or 96 h afterwards. The activities and latencies of 4 groups in the first session were not significantly different between them. But during recall session the ambulation in the groups of interval 24 and 48 h was declined, while that in the groups of interval 72 and 96 h was not, *vs* in the acquisition session. The retention latencies in all the 4 groups were significantly longer than the training latencies (Tab 1).

Comparison of various test times on d 1 Mice were randomly divided into 4 groups. On d 1 mice were individually placed on open-field and step-through for a period of 0.5, 1, 3, or 5 min. On d 2 the open-field behavior during 3 min and avoidance response during 10 min were recorded. The locomotor acitivity and defecation in recall period were not significantly different between the 4 groups. While 0.5- and 1-min groups led to a significant decrease in step-through latencies during the retention session (Tab 2).

Comparison of drug action In open-field test Scop and pentobarbital produced a locomotor stimulation during acquisition session. But the locomotor stimulation during recall session was only seen in the mice receiving Scop and caffeine. Promethazine, chlorpromazine, picrotoxin, and caffeine reduced the locomotor activity in acquisition session. Drugs inhibiting the defecation in acquisition session were Scop, chlorpromazine, and promethazine. The defecation did not show any difference between the mice received saline and drugs in recall session.

Tab 1. Comparisons of recall or retention session at various intervals after acquisition or training session. $n=10$ mice. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* acquisition or training session. $^dP>0.05$, $^eP<0.05$, *vs* interval 24 h group.

Interval the 1st and the 2nd sessions	Open-field test				Step-through test Latencies (s)	
	Ambulation		Defecation			
	Acquisition	Recall	Acquisition	Recall	Training	Retention
24 h	563±155	396±183[b]	4.4±3.2	4.2±2.3[e]	16±11	558±98[c]
48 h	570±88	441±155[bd]	4.4±2.2	3.9±2.9[ed]	18±10	578±85[cd]
72 h	583±54	476±246[ad]	4.4±2.6	2.3±1.9[ed]	21±9	471±150[cd]
96 h	571±95	570±77[ad]	4.8±1.9	5.0±1.4[ed]	20±12	381±253[cd]

Tab 2. Comparisons of various test times in acquisition or training session on open-field behavior in recall session or step-through latency in retention session. $n=12$ mice, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* 3-min group

Test time in acquisition or training	Open-field test		Step-through test	
	Ambulation in receall	Defecation in recall	Errors in training	Latencies (s) in retention
0.5 min	225±101[a]	2.9±2.3[a]	1±0[c]	162±206[c]
1 min	168±84[a]	2.5±2.0[a]	1.25±0.62[a]	294±240[c]
3 min	170±98	3.4±2.4	1.75±0.75	547±122
5 min	188±107[a]	3.1±2.4[a]	1.83±0.58[a]	500±107[a]

In step-through task high memory errors and poor retention scores were simultaneously shown in mice treated with Scop and pentobarbital. The mice injected chlorpromazine, promethazine, picrotoxin, and caffeine only exhibited retention deficits. Propranolol had no effects on open-field behavior and step-through task (Tab 3).

Tab 3. Drug actions on open-field behavior and step-through task. $n=12-14$ mice, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline.

Gruops	Dose mg·kg^{-1}	Open-field test				Step-through test	
		Ambulation		Defecation		Errors in training	Latencies (s) in retention
		Acquisition	Recall	Acquisition	Recall		
M receptor antagonist							
Saline		213±44	137±65	1.5±2.0	1.6±1.7	1.08±0.51	543±131
Scop	0.1	249±35[b]	165±63[a]	0.20±0.56[c]	2.1±2.1[a]	3.0±1.5[c]	232±217[c]
Scop	1	289±37[c]	256±29[c]	0±0[c]	2.5±2.0[a]	5.9±2.2[c]	39±45[c]
β-receptor antagonist							
Saline		217±57	132±60	2.0±1.9	2.7±1.2	1.86±0.77	504±103
Propranolol	5	234±46[a]	122±70[a]	2.0±1.0[a]	2.5±1.9[a]	2.5±1.0[a]	486±204[a]
Propranolol	20	248±30[a]	100±75[a]	1.5±1.4[a]	1.9±1.6[a]	2.1±1.4[a]	444±213[a]
DA receptor antagonist							
Saline		212±58	118±71	2.0±1.7	2.7±1.2	1.30±0.67	484±130
Chlorpromazine	1	164±69[a]	134±109[a]	1.3±1.3[a]	2.8±1.7[a]	1.50±0.53[a]	228±176[c]
Chlorpromazine	4	68±50[c]	140±61[a]	0.14±0.53[c]	3.5±1.4[a]	1.14±0.36[a]	225±238[c]
H_1 receptor antagonist							
Saline		256±29	124±65	1.0±1.0	1.5±1.6	2.1±0.9	509±151
Promethazine	2	242±38[a]	169±58[a]	0.8±1.3[a]	2.2±1.1[a]	1.9±0.8[a]	373±233[a]
Promethazine	20	172±47[c]	163±87[a]	0.11±0.33[c]	1.9±0.6[a]	2.0±1.5[a]	199±150[c]
GABA receptor antagonist							
Saline		246±27	144±85	1.8±2.0	2.3±2.6	1.8±0.6	531±135
Picrotoxin	1	223±64[a]	151±65[a]	0.7±0.9[a]	2.3±2.2[a]	1.6±0.5[a]	251±250[c]
Picrotoxin	5	57±38[c]	140±76[a]	0.9±1.0[a]	2.6±1.9[a]	1±0[c]	54±60[c]
CNS stimulant							
Saline		238±49	165±53	1.1±0.5	2.3±1.7	1.6±0.5	438±204
Caffeine	50	214±37[a]	134±54[a]	1.0±0.7[a]	2.4±1.3[a]	2.5±0.9[a]	149±183[c]
Caffeine	200	79±45[c]	239±43[c]	0.4±0.5[a]	2.8±2.0[a]	1.6±0.9[a]	21±38[c]
CNS inhibitor							
Saline		259±31	178±75	1.2±1.2	2.6±1.7	1.5±0.7	553±58
Pentobarbital	10	294±42[b]	144±60[a]	1.4±1.7[a]	1.7±1.5[a]	3.8±0.9[c]	514±156[a]
Pentobarbital	20	352±29[c]	151±82[a]	0.8±1.5[a]	1.5±1.8[a]	5.9±2.5[c]	236±272[c]

The drugs were injected ip at 15 min before or 60 min after the acquisition session. When injected before acquisition session the drugs influenced the recall activities in open-field or the retention latencies in step-through task. But injected after acquisition session they did not influence the mouse behavior and memory on d 2. (Results were not shown here.)

Comparison of behavior and memory

The 100 mice receiving saline in the above experiment were used to study the relation between the behavior, elimination, and activity, in open-field and avoidance memory in step-through. Results showed that the number of boluses was increased 82 % but the activity was depressed 39 % ($P<0.01$) at the recall session *vs* those at the acquisition session. Out of the 100 mice, 61 showed avoidance response for >600 s at the retention session in step-through test. The changes of elimination between the d 1 and d 2 did not relate to the latency on d 2. But a higher percent (91 %) of mice with >600-s latencies on d 2 was seen in those with ambulation unchanged (Tab 4).

Tab 4. Comparison between ambulation defecation in open-field recall session and latency in step-through retention session in 100 mice. *N*, *n*=mice.

Compared with acquisition session	*N*	Latencies were >600 s *n*	% of *N*
Ambulation in recall			
Increased	2	1	50
Decreased	87	50	58
Not changed	11	10	91
Defecation in recall			
Increased	59	34	58
Decreased	22	17	77
Not changed	19	10	53

DISCUSSION

The state of center and autonomic nervous system might be respectively represented by the activities and boluses dropped when mouse was placed in the open-field. The higher ambulation on d 1 and lower ambulation on d 2 might relate to a higher and a lower center excitation, respectively. Generally speaking, excitation in the center nervous system (CNS) plays an important role in inhibiting the eliminatory system. Thus, the lower activity and higher boluse in the second session was a result of weakening in center excitation. This function is generally regarded as representing adaptation and/or memory on the novel chamber.

Although locomotor activity was initiated by injection of picrotoxin into substantia innominata or subpallidal region[8,9], and the effects of caffeine on psychomotor and cognitive performance are more complex and relate to the doses used[10,11], they reduced the exploratory activity when given ip. This might be due to the drug-induced stereotype. Pentobarbital, promethazine and chlorpromazine are known to act as inhibitors on CNS. But smaller doses of pentobarbital can produce overt ambulation instesd of sedation.

The intelligence of mouse on step-through task included at least the adaptation to this box environment and memory on the footshock. Therefore, the process of avoidance response was more complex than that of open-field behavior and was easily impaired by drugs. For example, chlorpromazine, picrotoxin and caffeine significantly inhibited the avoidance memory at doses which did not influence the open-field behavior.

According to the classical models of memory processes, memory function can be divided into 3 stages, ie, short-term memory, storage processes, and retrieval processes[12]. These findings in this study suggested that the adaptation of mouse to open-field probably belonged to a short-term memory. This memory was easy acquisition, easy decline and was not

easily impaired by drugs *vs* avoidance memory, a long-term memory. However, it was not found that the mouse showing good adaptation and/or memory on open-field had good memory on step-through task at the same time.

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小鼠对新环境和回避反应的记忆特征：开阔行为和避暗实验比较

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目的： 研究小鼠对新环境和回避反应记忆特点. **方法**：用开阔和避暗实验. **结果**：小鼠对开阔环境记忆仅保持48 h，对回避反应记忆持续96 h 以上. 在开阔和回避反应实验中，小鼠获得记忆所需的学习时间分别为0.5和2 min. 东莨菪碱、氯丙嗪，异丙嗪，印防己毒素，咖啡因和戊巴比妥能抑制小鼠回避反应. 但小鼠对开阔环境的记忆仅被东莨菪碱和咖啡因阻断. **结论**：与回避反应相比，小鼠对新环境的记忆表现为易获得，易消退，不易被药物阻断.

关键词 动物行为；回避学习；记忆；东莨菪碱；氯丙嗪；异丙嗪；印防己毒素；咖啡因；戊巴比妥；普萘洛尔