Pharmacology Biochemistry and Behavior, Vol. 44, pp. 805-807, 1993
Printed in the U S.A.

0091-3057/93 $6.00 + .00

Effect of Age and Strain on Working Memory in Mice as Measured by Win-Shift Paradigm

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Received 5 June 1992

RITZMANN, R. F., A. KLING, C. L. MELCHIOR AND A. J. GLASKY. *Effect of age and strain on working memory in mice as measured by win-shift paradigm.* PHARMACOL BIOCHEM BEHAV 44(4) 805-807, 1993. – Working memory is disrupted in Alzheimer's disease and stroke; therefore, any therapeutic drug should restore deficits in working memory. The win-shift foraging paradigm has been demonstrated to be a model of working memory in rats. In the present study, this paradigm was adapted to mice because of the greater ease and economy of testing potential drugs in mice and the wider availability of strains of aged mice with naturally occurring working memory deficits. This study has demonstrated strain differences in the working memory trace and that age induces a deficit that can be detected at 11 months of age in mice. Tacrine and physostigmine enhance the memory trace in normal mice and physostigmine can reverse age-induced working memory deficits in subjects with mild and moderate deficits but not in subjects with severe deficits.

Working memory Mice Age Strain Memory deficit Physostigmine Tacrine

BASED upon a growing body of empirical evidence, memory has been divided into several subtypes (3,6,7,10). One of these subtypes, working memory, is in particular disrupted in humans suffering from pathologies that produce dementias such as Alzheimer's disease and stroke. It has been proposed that working memory consists of the remembering of an event in the absence of specific sensory cues (5). This is contrasted with dispositional memory, where a set of specific cues are present and a choice between a correct and incorrect cue(s) is required. Ordy et al. (5) reported that in rats the win-shift paradigm specifically tests working memory. In this paradigm, an animal is placed in a T-maze with food in both goal boxes. On the first trial (reference run), the animal will randomly choose one of the two arms, enter the goal box, and consume all the food. On the next trial, if the animal remembers which arm it had entered on the previous run it will go to the opposite arm. By increasing the time interval between runs, the length of time that an animal can remember where it was on the previous trial, that is, the duration of the memory trace, can be determined.

The neuronal circuits that are involved in working memory have been proposed to include circuits in the multisensory association areas of the cerebral cortex, the nucleus basalis of Meynert, the hippocampus, and possibly other cortical and subcortical structures (3,4,8,11,12). The win-shift model has been utilized to demonstrate deficits in memory that occur due to specific lesions in these brain structures, as well as in experimentally produced ischemia and in aged rats. The win-shift paradigm has a number of important attributes that include a) utilizing a naturally occurring foraging behavior, b) not employing aversive or disruptive stimuli, and c) being a rapid and reliable experimental method for testing working memory. These attributes make it in particular relevant for use in studies on the memory deficits associated with aging and Alzheimer's disease. Because there are many more strains of aged mice than rats available for investigation, it was of interest to determine if the win-shift paradigm could be adapted for use in mice. To validate the model, we tested the ability of mice to perform in this model at various intertrial delays. We also tested two drugs, physostigmine (PHY) and tacrine (THA), which have been shown to improve memory either in rats using this model (5) or in mice using other memory tests (2). In addition, we examined the ability of this model to detect age-induced memory deficits. Finally, we tested the ability of the drugs to improve any age-induced memory deficit.

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TABLE 1

EFFECT OF INTERTRIAL INTERVAL ON PERFORMANCE OF 6 MONTH OLD SWISS WEBSTER MICE

	Intertrial Interval(s)		
	30	60	90
Correct responses*	75%†	75%†	50%
Latency time‡	10.93	11.45	10.44
Running time‡	8.92	12.71	10.08

*Percent correct responses, chance = 50%.
†$p < 0.05$ (ANOVA); $N = 20$/group.
‡Time in seconds.

TABLE 2

EFFECT OF THA AND PHY ON PERFORMANCE OF 6 MONTH OLD SWISS WEBSTER MICE

	Treatment		
Type of test	Control (Saline)	THA (1.25 mg/kg)	PHY (30 0 mg/kg)
Correct responses	46%	71%*	65%*
Latency time†	2.68	8.22*	7.82
Running time†	1.95	3.65*	2.65
Number of subjects	40	17	17

*$p < 0.05$ (ANOVA).
†Time in seconds.

METHOD

Male Swiss Webster mice 6 (adult) and 11 (old) months of age and male 6-month (adult) C57BL/6 mice were maintained in individual cages on a 12 L : 12 D cycle with continuous access to water. Food was limited so that mice were at 80% of free-feeding weight. Mice were weighed and handled daily for 1 week. Thereafter, they were shaped in a standard T-maze, with the stem and each arm measuring 35 × 5 × 5 cm. Shaping consisted of placing the mouse in the maze for 3 min with all doors open and milk in both goal boxes. This process was repeated until the mouse drank the milk.

On the first run, milk was present in both goal boxes. Thereafter, the mice were run for 10 trials with milk present only in the goal box opposite the one entered on the previous trial. During tests of memory, the latency time to leave the start box was recorded as an index of motivation. The time to traverse the maze was recorded as a performance measure, and the arm of the maze entered was recorded. At short intertrial delays, that is, 30 or 60 s, mice with intact memory would enter the side of the maze opposite that which they had entered on the previous trial. Thus, entering the opposite side was designated as correct (win-shift). At the longer delays, 90 s (Swiss Webster strain) or 150 s (C57BL/6 strain), untreated mice performed at chance levels, that is, they chose the opposite side only 50% of the time. The selection of the correct side resulted in subjects receiving a reward of 0.5 cc milk, which the subject was allowed to consume.

When testing a drug, IP injections were made 1 h prior to the start of testing unless otherwise indicated. Drugs were dissolved in saline and prepared fresh daily. All injections were made at a volume of 0.1 cc/10 g of body weight. Control subjects received a similar injection of saline (vehicle).

The data was analyzed by an analysis of variance (ANOVA). Significant F-ratios were further analyzed by a Dunnett's multiple-range test. Arc sign transformations were performed on percentage data and latency scores were transformed to reciprocal or speed scores. Nonparametric data was analyzed by Fisher's exact probability test.

RESULTS

Control 6-month Swiss Webster mice tested in this model were able to perform significantly above chance (75%) at intertrial delays of 30 and 60 s. However, at delays of 90 s their performance fell to chance (50%) (Table 1). Both PHY (0.125 mg/kg) and THA (1.25 mg/kg) improved performance at the 90-s delay (Table 2). At the doses tested, PHY did not have any effect on either latency time to leave the start box or time to traverse the maze (running time). In contrast, THA produced an increase in both latency and running time.

When the win-shift paradigm was evaluated in the C57BL/6 strain of mice, the intertrial interval over which subjects could remember the correct response was 120 s, compared to 60 s in the Swiss Webster strain (Table 3). In subjects treated with PHY, the intertrial interval during which the memory was maintained (duration of memory trace) increased to 180 s.

TABLE 3

DURATION OF THE MEMORY TRACE IN 6 MONTH OLD C57BL/6 MICE

	Treatment Groups			
	Control (saline)		Physostigmine (0.125 mg/kg)	
Intertrial Intervals(s)	No Above Chance/Total No.*	Percent Correct†	No Above Chance/Total No.	Percent Correct†
30	3/5	70 ± 11†‡		
60	3/5	70 ± 16†‡		
90	4/5	70 ± 6†‡		
120	4/5	78 ± 16†‡		
150	1/5	56 ± 10		
180	2/7	58 ± 12	3/6	65 ± 16‡
210			1/6	53 ± 9

*No. animals scoring above chance (>60% correct)/total no. animals tested.
†Mean ± SD.
† $p<0.01$, ‡$p < 0.05$ (t-test against chance).

TABLE 4

EFFECT OF PHYSOSTIGMINE ON THE DURATION OF MEMORY TRACE IN SWISS WEBSTER MICE WITH AGE-INDUCED DEFICITS

	Degree of Memory Impairment					
	Severe		Moderate		Mild	
Treatment	No. Above Chance/Total No *	Intertrial Interval (s)	No. Above Chance/Total No.	Intertrial Interval (s)	No. Above Chance/Total No.	Intertrial Interval (s)
Untreated	0/10	<10	0/6	10	0/6	30
Physostigmine	1/10	<10	6/6†	10	6/6‡	60
			1/6	30	1/5	90
			0/1	60		

*No. animals scoring above chance (>60% correct)/total no. animals tested.
†$p < 0.01$, ‡$p < 0.03$ (Fisher's exact probability test).

In tests of memory in aged mice, it was found that Swiss Webster mice could be grouped into three categories: a) those that could not perform at the 10-s delay (severe deficit), b) those that could perform at a 10-s delay but not at 30 s (moderate deficit), and c) those that could perform at 30 s but not at 60 s (mild deficit). The percentage of 11-month-old mice in each category was: severe 10%, moderate 70%, mild 20%. The effect of PHY to improve memory in some mice from each group is presented in Table 4. PHY produced a significant improvement in the mild and moderate deficit groups. However, there was no effect in severely deficit animals.

DISCUSSION

These results indicate that two strains of mice (Swiss Webster and C57BL/6) perform well in the win-shift memory testing paradigm. Mice are able to recall which arm of the maze they entered on the previous trial and could enter the opposite arm at delays up to 60 (Swiss Webster) or 120 (C57BL/6) s. At longer delays, that is, 90 s in the Swiss Webster strain, mice perform at chance. PHY is an anticholinesterase drug that has been demonstrated to have memory-enhancing activity in animals (1,5) and has been shown to be effective in the win-shift paradigm in rats (5). THA is a drug that has memory enhancing activity (2) and may possibly act through a cholinergic mechanism (9). PHY and THA were found to also improve memory in the win-shift paradigm in mice. In addition, this test of working memory was able to detect age-induced deficits in mice. The data indicate that 70% of the 11-month-old Swiss Webster mice tested had a moderate memory deficit compared to the younger group, while 20% had a mild deficit. The remaining 10% were found to have a severe memory deficit. Drug treatment was found to have a slight effect in the moderate deficit group, while having a more robust effect in the mild deficit group. The drug treatment failed to produce any improvement in the severe deficit group. These data indicate that the win-shift memory test in mice may be a useful tool in screening drugs for cognitive enhancing properties. In addition, this model can detect varying degrees of memory deficit in aged mice. This may provide a mechanism to compare the efficacy of drug relative to the degree of impairment.

ACKNOWLEDGEMENTS

This work was supported by NIA Grant 09911, NIAAA Grant 08709, and Research Services of the Veterans Administration.

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