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SHORT COMMUNICATION

EFFECT OF METHYLPHENIDATE ON ENHANCEMENT OF SPATIAL LEARNING BY NOVEL ALTERNATED DUAL TASK

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Abstract : The novel alternated dual task (ADT) arranged rats to learn T-maze spontaneous alternation task and radial arm maze (RAM) task alternatively, and by doing ADT, rats could acquire the tasks more easily than non alternated dual task (NADT) group. Also retention capacity of ADT group was significantly more and ADT help to learn a complex task faster than learning it in isolation from other tasks. In the present study effect of methylphenidate (MPD), a mood elevator, known to enhance learning and memory, on ADT procedure is assessed. Also effect of ADT procedure and MPD on spatial learning and memory are compared. Different groups were assigned by administering MPD (intraperitoneal injection at a dose of 3 mg/kg body weight) during different phases of behavioural experiments, and control groups received saline injection. MPD administration increased both acquisition and retention capacities. The amelioration attained for retention of complex task by ADT procedure, could be achieved by NADT rats only by administration of MPD. The influence of ADT procedure on acquisition and retention of TM and RAM tasks were similar to the effects of MPD, especially for the RAM task. MPD at low dose is found to enhance the learning and memory capacity in rats, than deteriorating it, supporting the use of MPD as a drug to treat attention deficit hyperactive disorder. The recent reports suggesting the effect of MPD only on retention and not on acquisition could not be confirmed, as enhancement for both acquisition and retention was found in this study.

Key words : alternated dual task, methylphenidate, radial arm maze, T-maze

INTRODUCTION

Does learning two things different from learning one thing alone? Within that, learning of two things together (by alternating) better than learning one by one, and do learning of one task influence other? These are the different questions assessed in our lab for the past few years. So in our lab we designed a novel alternated dual task

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(ADT) to clarify whether learning two things different from learning one thing alone? Rats were made to learn both T-maze (TM) spontaneous alternation task and radial arm maze (RAM) task alternatively. Another group of rats were made to learn both the task separately without any alternation, and control group of rats were assigned to learn only one type of task. It was found that the group of rats performing ADT could acquire the tasks more easily than the single tasked groups and non alternated dual task (NADT) groups. This enhancement of acquisition was associated only with the complex task (RAM task) among the dual tasks. More over their retention (memory) ability was very significantly enhanced for both the tasks in dual tasks (1, 2).

It is a well known fact that RAM and TM induces activation of memory formation processes involved in the hippocampus. Hippocampus has one of the densest inputs of adrenergic terminals in brain, supporting the hypothesis that nor epinephrine (NE) and epinephrine play a role in learning and memory (3). In the present study for assessing the involvement of adrenergic system and hippocampus in ADT the administration of methylphenidate (MPD) was used.

MPD shows similar pharmacological properties as amphetamine and cocaine (4, 5). It increases synaptic levels of NE and dopamine (6). MPD can facilitate various aspects of cognition including memory formation, through their actions on noradrenergic and dopaminergic systems (7, 8). But higher NE and dopamine concentration in synapses decrease working memory performance of prefrontal cortex, decreasing short and long term memory storage (6). Neural circuit involving hippocampus and prefrontal cortex is a part through which spatial information acquired before a delay is used, subsequently to locate food on a RAM. In contrast, foraging in the absence of information obtained before a delay appears to depend on a direct interaction between hippocampus and lateral striatum (nucleus accumbens), and there does not appear to be a role for the prefrontal cortex (9). So the involvement of hippocampus, prefrontal cortex and adrenergic system in ADT is aimed to be assessed by administration of MPD in the present study.

NE release in hippocampus increased during spontaneous alternation behavior (SAB) testing, supporting role of NE in SAB (10). It has been shown that optimal dopamine is required for SAB (11). Hence it is believed that NE and dopamine may also be involved in ADT. MPD increases cortical and hippocampal acetylcholine release and may contribute for improvement of performance in RAM test (12). MPD increases histamine release in prefrontal cortex, so keeps the rat vigilant and wakeful, resulting in better performance (13). Oral administration of low dose MPD (3 mg/kg) improves spatial learning and memory in RAM test (14). But at high doses (10 - 18 mg/kg) MPD impairs memory formation independent of attention (15). In the present study a low dose (3 mg/kg) of MPD given as intraperitoneal injection was used, and its effect on acquisition and retention was studied. Also comparison between effect of MPD and effect of ADT procedure on acquisition and retention of RAM and TM tasks were done.

MATERIALS AND METHODS

Subjects

A total of 72 male Wistar albino rats were used for this study. They were housed in groups, in propylene cages in an acclimatized (25–27°C) room and were maintained on a 12 hr light/dark cycle. Food and water was given *ad libitum* until they aged 60 days at the beginning of the experiment. Body weight of the rats was between 150-200 g. They were randomly grouped into twelve groups as T maze (TM) alone group, TM alone with methylphenidate (MPD) during acquisition group, TM alone with MPD during retention group, radial arm maze (RAM) alone group, RAM alone with MPD during acquisition group, RAM alone with MPD during retention group, alternated dual task (ADT) group, ADT with MPD during acquisition group, ADT with MPD during retention group, non alternated dual task (NADT) group, NADT with MPD during acquisition group, and NADT with MPD during retention group, with six rats in each group.

Drug

Inspiral®-10 SR (sustained release) tablets manufactured by Ipca laboratories limited were used. Each tablet contained methylphenidate hydrochloride USP 10 mg. The tablets were powdered and mixed with sterile 0.9% w/v normal saline. MPD was administered to the rats as intraperitoneal injection at a dose of 3 mg/kg.

Experimental design

All the behavioural experiments were carried out in three phases viz; orientation and training session, learning performance test (acquisition test) and memory performance test (retention test). The rats were semi starved for 48 hrs before the start of behavioural experiments. The body weight was maintained at 85% of the original body weight, through out one session of behavioural experiment.

During various phases of behavioural procedure all the rats received either saline, or MPD injection, intraperitonealy, once every day, 30 minutes prior to the start of behavioural experiments, either during acquisition or retention phase depending on the group. Neither saline nor MPD was administered during the gap days between the phases. Saline injection was given at the rate of 5 ml/kg body weight of rat, to all the control groups and to all other groups during the phases where MPD was not injected. MPD was injected at a dose of 3 mg/kg body weight of rat, only during the phases where it was assigned depending on the group.

The behavioural experiments included were T-maze spontaneous alternation task and radial arm maze task. The details of procedure and apparatus used are same as described in our previous papers (1, 2). In ADT rats were trained to learn two tasks viz; TM task and RAM task. In ADT the rats were given the TM trial first then followed by RAM trial with an interval of one minute between them. The task was alternatively given with six trials (3 T-maze trials and 3 RAM trials) per day. The interval between one coupled TM-RAM trial to the next one was one hour. NADT group of rats were also given dual task, but the tasks were learned separately without alternating, i.e., the rats

learned TM task first by giving six trials/ day with an inter trial interval of one hour, and after attaining the learning criteria, the RAM task was learned also by giving six trials/day with an inter trial interval of one hour. 10 days after acquisition of both tasks retention test was carried out until attaining learning criteria. For details of the procedure for ADT and NADT also refer our previous papers (1, 2).

Statistical analysis

Statistical analysis was performed using SPSS version 10.0.1 for Windows. The statistical procedures used in the present data analysis are mentioned along with results. Significance was accepted at $P<0.05$. Means and standard deviations are reported.

RESULTS

Mean number of trials to criteria for TM and RAM tasks by different saline treated groups (control) and respective methylphenidate (MPD) treated groups are shown in Table I. One way ANOVA comparison between these groups showed a significant difference, $F(23, 120) = 47.176$, $P<0.001$. Least significant difference (LSD) post test results indicate that in all the groups MPD influenced positively the spatial learning and memory.

To see whether the influence of MPD on all groups are uniform or not, a 6 (behavioural tasks, viz; TM alone, TM task of ADT, TM task of NADT, RAM alone, RAM task of ADT and RAM task of NADT) x 2 (drug, viz; saline and MPD) factorial ANOVA was done separately during acquisition and retention. The non significant interaction between behavioral task x drug during acquisition $F(5, 60) = 0.575$, $P = 0.719$, and a significant interaction during retention $F(5, 60) = 6.031$, $P<0.001$, but with a very low Eta squared value of 0.334, indicates that MPD have similar effect in all cases. That is

TABLE I: Summary of results showing effects of methylphenidate on acquisition and retention of! maze task, radial arm maze task, alternated dual task, and non alternated dual task groups.

Groups		*Mean number of trials required for*	
		Acquisition	*Retention*
TM Group (Control)		16.33±2.1602	11.5±1.871
TM with MPD during acquisition		12.50±1.049	11.33±0.816
TM with MPD during retention		16.33±1.033	8.17±0.753
RAM Group (Control)		21±2.2804	16±1.789
RAM with MPD during acquisition		17.50±1.049	16.50±0.837
RAM with MPD during retention		21.17±1.169	9.83±0.983
ADT control	RAM	16.17±2.317	8.33±1.966
	TM	15.67±1.966	8.50±1.378
	RAM	11.50±2.074	8.83±1.169
ADT with MPD during acquisition			
	TM	10.17±1.169	8.33±1.033
	RAM	16.33±1.366	6.50±1.049
ADT with MPD during retention			
	TM	15.67±1.033	6.1 7±0.753
	RAM	21.83±1.941	16.33±1.862
NADT control			
	TM	16.00±1.414	10.67±1.751
	RAM	17.67±1.211	16.50±1.049
NADT with MPD during acquisition			
	TM	12.33±1.033	10.83±1.472
	RAM	2 1.67±1.506	10.00±1.265
NADT with MPD during retention			
	TM	15.83±1.472	8.50±1.049

Results are Mean±SD. MPD = methylphenidate, TM = T maze task, RAM = radial arm maze task, ADT = alternated dual task group, NADT = non alternated dual task group.

to say that either by adopting ADT or NADT procedures the influence of MPD have no change.

Various statistical analyses showed differential influence of MPD and ADT on acquisition and retention of TM and RAM tasks. A one way ANOVA comparison between acquisitions of TM task among the three groups viz; TM alone group, TM alone group with MPD treatment during acquisition and TM task of ADT group, showed a significant difference, $F(2, 15) = 7.837$, $P = 0.005$. But LSD post test revealed that significant difference was present between TM alone group and MPD treated TM alone group ($P = 0.002$), and not between TM alone group and TM task of ADT group ($P - 0.529$). Also significant difference was present between MPD treated TM alone group and TM task of ADT group ($P = 0.008$). This result indicates that during acquisition of TM task enhancement was caused only by MPD and not by ADT procedure. During retention of TM task a similar one way ANOVA comparison showed a significant difference, $F(2, 15) = 10.168$, $P = 0.002$. LSD post test showed a significant difference between the three groups, except between MPD treated TM alone group and TM task of ADT group ($P = 0.668$). This indicates that both MPD and ADT produced enhancement, and also the enhancement was similar. In the case of RAM task similar comparison also showed significant difference during acquisition, $F(2, 15) = 9.614$, $P - 0.002$, and retention, $F(2, 15) = 36.992$, $P<0.001$. But interestingly in LSD post test there was no significant difference between MPD treated RAM alone group and RAM task of ADT group during acquisition ($P = 0.26$) and retention ($P = 0.133$). These results indicate that for RAM task the enhancements caused by MPD and ADT procedure are similar. That is to say that for acquisition and retention of RAM task ADT procedure was very effective, but in the case of TM task it was effective only during retention.

DISCUSSION

It is a well known fact that RAM and T maze induces activation of memory formation processes involved in the hippocampus. It has been recently shown that neural circuit involving hippocampus and prefrontal cortex is a part through which spatial information acquired before a delay is used, subsequently to locate food on a RAM (9). In this study also, both ADT and NADT rats probably use this pathway to solve both RAM and T maze tasks. Some studies have shown that at higher doses MPD can impair prefrontal cortex dependent memory formation (6). But in the present study any impairment in this regard could not be established, as performance was enhanced in both ADT and NADT rats by using MPD. This is probably because of a low dose of MPD used in this study. Many reasons have been suggested by previous workers for this enhancement, including, an increase in histamine release in prefrontal cortex by MPD, so keeping the rat vigilant and wakeful, resulting in better performance (13); an increase in synaptic levels of dopamine and nor epinephrine (NE) by MPD can increase overall attention and may contribute towards better performance (6); and, an increase in cortical and hippocampal acetylcholine release by MPD significantly improves performance in RAM and TM (12). Some existing evidence indicates that MPD may reduce rats' preference for novelty (16, 17). However, no

indication of such an effect was found in the present experiments.

NE release in hippocampus increased during spontaneous alternation behavior (SAB) testing, which supports the role of NE in SAB (10). Also it is believed that optimal dopamine is required for SAB (11). In the present study also the SAB testing procedure have been used as a learned alternation procedure for T maze task. As such ADT involves a higher degree of alternation procedure and it can be assumed that, when MPD increased NE and dopamine release, improvement in spatial learning of ADT rats is due to this factor. When compared to NADT the complexity of alternation is more in ADT and the amelioration caused by MPD is thus more in ADT groups. The fact that ADT is more ameliorated by MPD is clear from the result that shows a higher number of trials to criteria for NADT groups than ADT groups for acquisition when MPD was administered during acquisition.

Adrenergic signaling is critical for the retrieval of intermediate-term spatial and contextual memories but not for retrieval of emotional memories in general (18). In Morris water maze, knockout rats for NE, exhibit a deficit in retaining spatial memory two days after last training. But no deficit was found when it was after two hours (19). Studies have also shown that spatial memory consolidation (retention) using aversive stimuli depend on adrenergic signaling, but acquisition does not depend on adrenergic signaling (19). But in the present study both acquisition and retention has been enhanced by MPD. So it may be assumed that NE increase caused by MPD might have ameliorated the retention capacity and enhancement in acquisition may be due to increase in histamine release (13) or acetylcholine release (12) or due to some other factors like increased attention or increased locomotor activity that is normally seen associated with MPD administration (20).

In 2007 Ning Zhu et al (14) showed improvement in spatial learning and memory by oral methylphenidate administration. But in their experiment number of days to criteria for a RAM task did not show a change, which is in contrast to the present study, where number of trials to criteria in RAM test has decreased significantly by MPD administration. The probable reason for this difference may be due to the consideration between number of days to criteria and number of trials to criteria.

In conclusion it may be stated that the amelioration attained for retention of complex task by ADT procedure, could be achieved by NADT rats only by administration of drugs like MPD. The influence of ADT on acquisition and retention of TM and RAM tasks were similar to the effects of MPD, especially for the RAM task. MPD at low dose is found to enhance the learning and memory capacity in rats, than deteriorating it, supporting the use of MPD as a drug to treat attention deficit hyperactive disorder (6). The recent reports (19) suggesting the effect of MPD only on retention and not on acquisition could not be confirmed, as enhancement for both acquisition and retention was found in this study.

REFERENCES

1. Praveen KV, Mukkadan JK. Evaluation of allocentric spatial learning in rats using a novel - alternated dual task". *Ind J Physiol Pharmacol* 2009; 53: 235–242.
2. Praveen KV, Mukkadan JK. Effects of scopolamine on a novel alternated dual task. Biomedicine 2009; 29: 315–321.
3. Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, Blakely RD. Immunolocalization of the cocaine-and antidepressant-sensitive I-norepinephrine transporter. *J Comp Neurol* 2000; 420: 211–232.
4. Solanto MV. Clinical psychopharmacology of AD/HD: Implications for animal models. *Neurosci and Behav Rev* 2000; 24: 27–30.
5. Volkow ND, Ding Y, Fowler JS, Wang GJ, Logan J, Gatley JS, et al. Is Methylpheindate like cocaine ? Studies on their pharmacokinetics and distribution in the human brain. *Arch Gen Psychiatry* 1995; 52: 456–463.
6. Arnsten AFT. Dopaminergic and noradrenergic influences on cognitive functions mediated by the prefrontal cortex. In. Solanto MV, Arnsten AFT and Castellanos FX (Eds.), Stimulant Drugs and ADHD: Basic and Clinical Neuroscience. New York: Oxford University Press 2001; 185–208.
7. Mehta MA, Sahakian BJ, Robbins TW (2001). Comparative psychopharmacology of methylphenidate and related drugs in human volunteers, patients with ADHD, and experimental animals. In. Solanto MV, Arnsten AFT and Castellanos FX (Eds.), Stimulant Drugs and ADHD: Basic and Clinical Neuroscience. New York: Oxford University Press 2001; 303–331.
8. Quartermain D. The role of catecholamines in memory processing. In Deutsch JA (Ed.) The physiological basis of memory. New York: Academic Press 1983; 387–423.
9. Stan BF, Jeremy KS, Anthony GP. Selective roles for hippocampal, prefrontal cortical and ventral striatal circuits in radial-arm maze tasks with or without a delay. *J Neurosci* 1997; 17: 1880–1890.
10. Men D, McCarty R, Gold PE. Enhanced release of norepinephrine in rat hippocampus during spontaneous alternation tests. Neurobiol Learn Mem 1999; 71: 289–300.
11. Lalonde R. The neurobiological basis of spontaneous alternation. Neurosci Biobehav Rev 2002; 26: 91–104.
12. Tzavara ET, Bymaster FP, Overshiner CD, Davis RJ, Perry KW, Wolff M, McKinzie DL, Witkin JM and Nomikos GG. Procholinergic and memory enhancing properties of the selective norepinephrine uptake inhibitor atomoxetine. *Mol Psychiatry* 2006; 11: 187–195.
13. Horner WE, Johnson DE, Schmidt AW, Rollema H. Methylphenidate and atomoxetine increase histamine release in rat prefrontal cortex. *Eur J Pharmacol* 2007; 558: 96–97.
14. Ning Z, Jeremy W, Dow-Edwards DL. Oral methylphenidate improves spatial learning and memory in pre- and periadolescent rats. *Behav Neurosci* 2007; 121: 1272–1279.
15. Yadvinder SC and Harald KT. Impairment of single-trial memory formation by oral methylphenidate in the rat. *Neurobiol Learn Mem* 2006; 85: 125–131.
16. Dyne LJ, Hughes RN. Effects of methylphenidate on activity and reactions to novelty in rats. *Psychonomic Sci* 1970; 19: 267–268.
17. Hughes RN, Syme LA. The role of social isolation and sex in determining effects of chlordiazepoxide and methylphenidate on exploratory behaviour. *Psychopharmacologia (Berl.)* 1972; 27: 359–366.
18. Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA. A distinct role for norepinephrine in memory retrieval. Cell 2004; 117: 131–143.
19. Thomas SA, Palmiter RD. Disruption of the dopamine beta-hydroxylase gene in mice suggests roles for norepinephrine in motor function, learning, and memory. *Behav Neurosci* 1997; 111: 579–589.
20. McDougall SA, Collins RL, Karper PE, Watson JB, Crawford CA. Effects of repeated methylphenidate treatment in the young rat: Sensitization of both locomotor activity and stereotyped sniffing. *Exp Clin Psychopharmacol* 1999; 7: 208–218.