

Enhancement and impairment of cognitive behaviour in Morris water maze test by methylphenidate to rats

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Abstract: Methylphenidate (MPD), a psycho-stimulant is a prescription medicine for the treatment of Attention deficit hyperactivity disorder (ADHD). The drug is also being increasingly used by general population for enhancing cognition. Only few preclinical studies have been carried out on the effects of MPD on cognition and these studies show either an enhancement or impairment of memory following the administration of MPD. The present study was designed to evaluate the effects of different doses of methylphenidate on acquisition and retention of memory in Morris water-maze test. Twenty four male Albino Wistar rats (weighing 180-220gm) were randomly assigned to four groups: (1) Control (2) 0.5mg/kg (3) 2.5mg/kg (4) 5 mg/kg methylphenidate. Animals received drug or water orally before training phase. Memory acquisition was monitored 2hrs post drug administration while memory retention was determined next day. It was found that the clinically relevant doses of methylphenidate (0.5mg/kg and 2.5mg/kg) improved memory acquisition and its retention but higher dose (5mg/kg) impaired both. We suggest that MPD-induced increase of catecholamine neurotransmission may have a role in the improvement of water maze performance while agonist activity of the drug for 5HT-1A receptor in the impaired performance at high doses. Food intake and body weight changes were not affected by MPD administration due to short-term administration of the drug. Results may help in improving pharmaco-therapeutic use of MPD for ADHD.

Keywords: Methylphenidate, Morris Water maze test, memory acquisition, retention.

INTRODUCTION

Methylphenidate (MPD) is the most prescribed and commonly used psychostimulant in the treatment of attention deficit hyperactivity disorder (Greydanus *et al.*, 2007). It increases extra cellular concentration of dopamine and nor-epinephrine by blocking the uptake of these two neurotransmitters (Kuczenski and Segal, 2002). ADHD is a developmental disorder characterized by severe and persistent impulsiveness, inattention and hyperactivity (Newcorn and Halperin, 2000). Center for Disease Control reported that prescribed use of MPD to treat symptoms associated with ADHD has dramatically increased in the recent years (Pastor and Reuben, 2008). There is a growing trend to use MPD as 'cognitive enhancer' for studying or recreational purposes which later results intolerance (Steiner and Van Waes, 2012). Intranasal abuse produces effects rapidly that are quite comparable with the effects of cocaine (Dupont *et al.*, 2008). Psychostimulant abuse largely produces psychiatric symptoms as reported in clinical data (Morton and Stockton, 2000).

Studies on the effect of MPD on memory function have reported mixed results depending upon dosage and route of administration (Scherer *et al.*, 2010; Gerasimov *et al.*, 2000). Acute doses of methylphenidate have been reported to improve attention, learning memory while

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reducing impulsivity in a variety of tasks (Britton, 2012). Moreover, studies have used doses significantly higher (2-15mg/kg i.v. or 10-50mg/kg i.p.) to check their effect on long course of administration (Gerasimov *et al.*, 2000). Different route of drug administration may provide different behavioural and neurochemical effects. In majority of animal studies, MPD treatment have been achieved through sub-cutaneous or intra-peritoneal injections across a broad range of doses (0.5-80.0mg/kg) that surpass the recommended low therapeutically oral doses (0.3-1.0mg/kg) in humans (Yang *et al.*, 2006). On the other hand, repeated methylphenidate administration has been observed to function as a reinforcer and enhance its abuse potential in laboratory settings (Hiranita *et al.*, 2009; Rush and Baker, 2001).

Morris water maze (MWM) test belongs to the frequently applied behavioural test to monitor learning and memory or functional deficits in rodents (Morris *et al.*, 1982). Results obtained by pharmacological or genetical interventions illustrated that learning and memory in the MWM explained the mechanisms at cellular and/or molecular level with long-term potentiation (D'Hooze and De Deyn, 2001).

The biochemical action of MPD is well characterized (Somkumar *et al.*, 2016). The dopamine transporter (DAT) and norepinephrine transporter (NET) are blocked by MPD, resulting in elevated concentration of dopamine

and norepinephrine at synapses (Hannestad *et al.*, 2010). In contrast, the mechanisms by which therapeutic dose of MPD acutely improves cognitive functions and overdose of it induces psychosis are still not clear (Cheng *et al.*, 2014).

Only few studies have been preferred on the effects of clinically relevant doses of oral methylphenidate on cognition (Haleem *et al.*, 2015). These studies are not consistent and have reported mixed results i.e. both enhancement and suppression of working memory is reported to occur following various doses of MPD (Urban *et al.*, 2013). So the aim of study is to monitor the effect of clinically relevant doses (0.5mg/kg and 2.5mg/kg) as well as higher dose of MPD (5mg/kg) for single dose administration to examine its effect on cognitive functions. Water Maze test was used to monitor MPD effects on memory acquisition and its retention on a single day trial of MPD.

MATERIALS AND METHODS

Animals

Animals were housed and handled according to the strict guidelines of 'Guide for the care and use of laboratory animals', The National Academies Press, Washington D.C, USA and the Institutional Animal Ethics Committee (IAEC; Animal study protocol no. 2015-0014). Albino-Wistar rats (weighing 180-200gm) provided by animal house, ICCBS, University of Karachi were housed individually and kept under 12 hours light dark cycle and controlled room temperature ($24\pm 2^\circ\text{C}$) with free access to tap water and cubes of standard rodent diet 3 days before the start of experiment.

Drug and doses

Methylphenidate Hydrochloride; pulverized and suspended in distilled water, was given orally at doses of 0.5mg/kg, 2.5mg/kg and 5mg/kg to the respective animals. Drug suspension was prepared freshly every time before use.

Water maze test

The water maze used in the present study was a white circular pool, 90 cm in diameter and 60 cm high. The pool was made up of white plastic and filled with opaque milky water ($22\pm 2^\circ\text{C}$) to a depth of 30cm. It was positioned in a room surrounded by invariable visual cues (window, cabinets, equipments etc) which were not changed along with water maze till the completion of experiment. The water maze was divided virtually into four equal quadrants (north, south, east and west). In the center of north quadrant a square platform ($10 \times 10\text{cm}$) was placed at 2cm beneath the surface of water.

Experimental protocol

Twenty four locally bred Albino Wistar rats (150-200gm) were randomly divided into four groups (1) Control (2) 0.5 mg/kg (3) 2.5mg/kg (4) 5mg/kg treatment groups.

Acquisition and retention of memory was assessed as the latency time to locate the hidden platform. The procedure consisted of two phases: the training phase and the test phase. Rats were subjected to two separate training phases after 30 minutes of the oral administration of methylphenidate via oral gavage. First in the training phase, platform was placed in center of the water maze and rats were entered from each quadrant. One minute was given for each animal to find and mount onto the escape platform. Afterwards, the platform was placed in north quadrant and animals entered from a single position to locate the hidden platform. Cut-off time was 2 min for each session. If the rat succeeded, it was allowed to stay on it for 10 sec and if failed, was guided towards the platform. Three successive trials of second training session were provided to each animal. Acquisition of memory was assessed after two hours of the training sessions. Immediately after monitoring memory acquisition, drug or water was re-administered at respective doses. After 20 hours of drug administration, retention of memory was assessed (long-term memory) with the same procedure as followed for short-term memory.

Determining food intake and body weight changes

Cumulative food intake (g) was monitored by taking the difference of food given on first day, between 9:00 and 10:00 h, and food left next day (between 9:00 and 10:00 h). Body weights were also assessed simultaneously and changes in body weight were calculated (body weight on monitoring day/body weight on preceding day) $\times 100$ as reported previously (Haque *et al.*, 2013).

STATISTICAL ANALYSIS

All results are specified as means \pm S.D. Analysis of the data was performed by SPSS software (version 17.0) using one-way ANOVA (analysis of variance). Tukey's post-hoc test was used for comparisons. Results with p values $p < 0.05$ were considered statistically significant.

RESULTS

Fig. 1 shows the effect of methylphenidate on acquisition learning in Morris water maze test. One-way ANOVA showed that the effects ($F=66.057$; $df=3,20$; $p < 0.01$) were significant. Post-hoc test showed that low doses (0.5mg/kg, 2.5mg/kg) methylphenidate improved learning acquisition but higher dose (5mg/kg) impaired it. Increase in learning acquisition was greater at dose of 2.5mg/kg than 0.5mg/kg.

Fig. 2 illustrates the effect of methylphenidate on memory retention in Morris water maze test (20 hours after drug administration). One-way ANOVA showed that the effects ($F=39.135$; $df= 3,20$; $p < 0.01$) were significant. Post-hoc analysis showed that low doses (0.5mg/kg, 2.5

mg/kg) methylphenidate increased memory retention. Conversely, high dose (5mg/kg) of methylphenidate increased the time to reach the platform. Thus, low doses of methylphenidate enhanced memory while high dose impaired it. Unlike memory acquisition, the effects of 0.5 mg/kg and 2.5mg/kg of methylphenidate on memory retention were comparable.

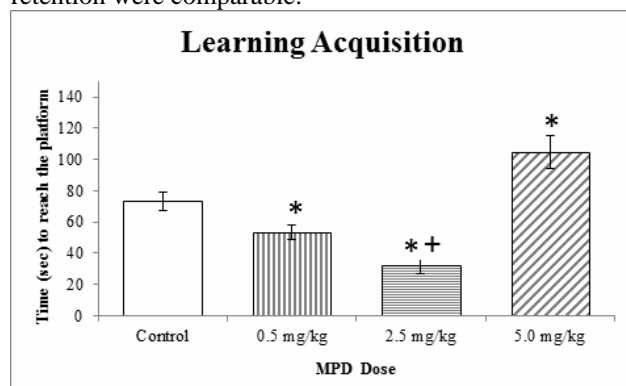


Fig. 1: The effect of methylphenidate on learning acquisition. Values are mean \pm SD (n=6). Significant differences by Tukey's test: *p<0.01 from control group; +p<0.01 from animal given 0.5 mg/kg, following One-way ANOVA.

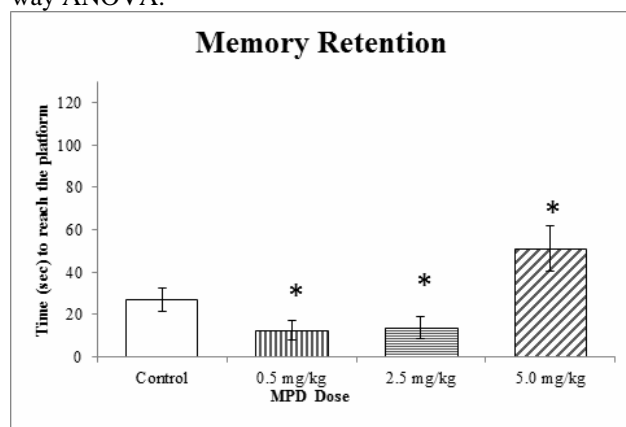


Fig. 2: The effect of methylphenidate on memory retention. Values are mean \pm SD (n=6). Significant differences by Tukey's test: *p<0.01 from control group following One-way ANOVA.

Effects of different doses (0.5, 2.5 and 5 mg/Kg) of methylphenidate on food intake of animals are shown in figure 3. One-way ANOVA showed that the effect of methylphenidate doses on food consumption were not significant. (F=0.292; df=3,20).

Data on growth rate analyzed by one-way ANOVA also showed that the effects of methylphenidate (F=1.398; df=3,20) on body weight changes were not significant.

DISCUSSION

The present study shows that the low doses (0.5 and 2.5 mg/kg) of methylphenidate administered orally to rats before training improved learning acquisition and

retention of memory in Morris water maze test. In contrast, high dose (5mg/kg) of MPD impaired it. Low doses of MPD used in the present study are clinically relevant. The present findings therefore tend to suggest that clinically recommended doses would improve performance in ADHD patients.

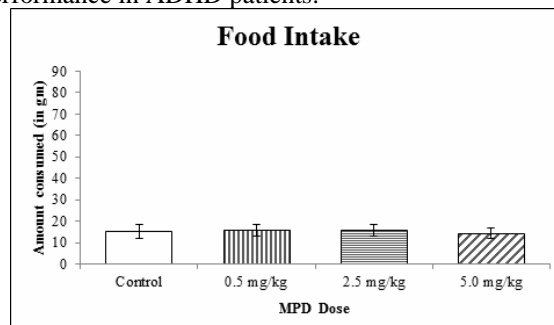


Fig. 3: The effect of methylphenidate on food intake. Values are mean \pm SD (n=6). Post-hoc analysis by Tukey's test showed that the difference between the groups were not significant.

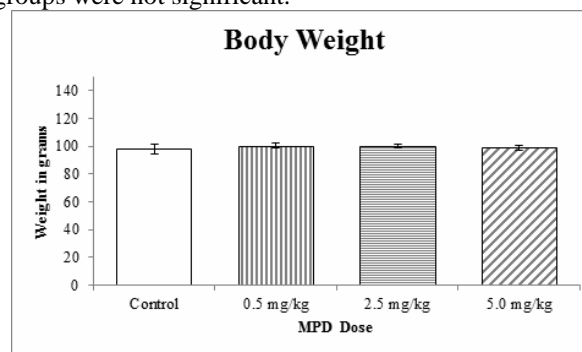


Fig. 4: The effect of methylphenidate on changes in body weight. Values are mean \pm SD (n=6). Post-hoc analysis for body weight showed that the difference between the groups were not significant.

In the current study, 7-week old rats were used and monitored effects of single oral administration of MPD on memory using a range of doses (0.5, 2.5 and 5mg/Kg). Morris water maze test was used to monitor the effects on memory acquisition (expression of memory 2 hours after training) and memory retention (expression of memory next day). The Morris water maze (MWM) is an apparatus well suited for monitoring spatial learning and memory (Morris, 1991). MWM offers numerous advantages in studying learning and memory over any other behavioural method especially regarding its capacity to dissociate deficits in the formation of memory and retrieval processes (D'Hooge and Deyn, 2001).

Other authors reported that oral administration of MPD at a dose of 3mg/kg from postnatal day 22 to 40 improved performance in radial arm maze (Zhu *et al.*, 2007). Intra-peritoneal administration of low doses of MPD enhanced Pavlovian fear learning but high dose impaired it (Carmack *et al.*, 2014). Transient effects on recognition of a novel object were observed when MPD was

administered orally at a dose of 5 mg/kg for seven weeks (Bethancourt *et al.*, 2009). Administration of MPD is also reported to reduce impulsivity in various tasks (Britton, 2012). Dose-response curves of MPD on water-maze were reported previously but with varied results. Lower doses (0.25-1.0 mg/kg) methylphenidate given orally increased memory acquisition and retention in Morris water maze test (Haleem *et al.*, 2015) but sensitization was produced at a dose of 1.0 mg/kg, while use of higher doses should be avoided. It was also reported that single administration of 40 mg/kg MPD sub-cutaneously produced no effect on water maze performance (Carrey *et al.*, 2000).

Methylphenidate is high affinity reuptake inhibitor of dopamine (DA) and nor-epinephrine (NE) (Han and Gu, 2006). It binds to DA transporter and inhibits its uptake with potency similar to cocaine (Chen *et al.*, 2005). MPD has also been shown to bind with NE transporter and found to be an effective in vitro inhibitor of NE uptake and might increase extra cellular NE (Vanicek *et al.*, 2014). The promising memory effects of MPD on learning and memory are possibly due to its ability to increase DA and/or NA transmission (Goodman *et al.*, 2006; Juarez & Han, 2016).

If an increase in DA and NA neurotransmission following administration of low doses of MPD enhances memory (Fig 1,2) the effects of high doses on impairment of memory cannot be explained on the same line because higher doses are expected to produce a greater increase in DA and NA neurotransmission. It is however, possible that activity of MPD reported for 5HT-1A receptors (Markowitz *et al.*, 2009) is involved in the inhibition of responses and modulates firing of dorsal raphe serotonergic neurons (Kharas *et al.*, 2017). Indeed 5HT-1A receptors have been reported to modulate DA neurotransmission in various brain regions (Haleem, 2013, 2015) and bupirone, an agonist of 5HT-1A receptors impairs memory (Leong *et al.*, 2012). The greater synaptic increase of DA at higher doses of MPD may impair working, as also suggested in other studies (Casey, 2008; Urban *et al.*, 2013).

MPD acts on brain dopamine and increases DA neurotransmission while drugs that increase DA neurotransmission suppress eating (Geiger *et al.*, 2009). It has been reported previously that the use of MPD for a short period decreases the food intake (Rada *et al.*, 2005; Goldfield *et al.*, 2007). The current study shows that short term administration has no anorectic effect.

CONCLUSION

The present study shows that low dose of MPD which are also clinically relevant for improving memory and may be useful for ADHD patients. Higher doses, on the other hand, are expected to produce adverse effects on memory

and should be avoided. Future research on animal models with memory deficit may help to understand further mechanisms involved in MPD-induced impairment of memory.

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