



(RESEARCH ARTICLE)



Clinically relevant doses of methylphenidate elicit behavioral sensitization and impair cognition on drug withdrawal in normal adult rats

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Abstract

Methylphenidate (MPD), a psychostimulant, is the first line drug for improving cognitive performance in attention deficit hyperactivity disorder (ADHD). A non-prescription use of this drug for improving performance is also becoming increasingly known. A growing rise in its medical and nonmedical use suggests that the drug is addictive. The present study was designed to ascertain the reinforcing and withdrawal effects of clinically relevant doses of methylphenidate on cognitive behavior of normal adult rats. Potential addictive effects and withdrawal effects on cognition were also determined. Effects of MPD in improving cognition were monitored after drug administration as well as withdrawal using Morris Water Maze test. Taking behavioral sensitization as an important contributing factor of drug addiction; addictive effects of MPD were also determined. Data analysis was done on SPSS version 13 by one-way and two-way ANOVA (repeated measure design) where applicable; post hoc comparisons were done by Tukey's test. Repeated oral administration of MPD (0.5 and 1mg/kg) for six days produced behavioral sensitization and reduced daily food intake. After six days of treatment rats were repeatedly administered/withdrawal from repeated administration of MPD to investigate effects of MPD on cognitive behaviors. Results showed an improvement in cognition in rats repeatedly administered with MPD (0.5 and 1 mg/kg). Whereas, withdrawal from repeated administration of MPD impaired short term memory, long term memory and memory retention. Doses of MPD which improve learning and memory are potentially addictive and elicit behavioral sensitization. Use of drug in healthy subjects can impair performance below basal levels particularly in drug withdrawal conditions.

Keywords: Methylphenidate; Sensitization; Drug addiction; Drug withdrawal; learning and memory

1. Introduction

Psychostimulants were initially prescribed for treatment of attention deficit hyperactivity disorder (ADHD) [1-3]. ADHD is a disorder of brain development usually characterized by severe and persistent impulsiveness, lack of attention and hyperactivity [4]. The psychostimulant methylphenidate (MPD; ritalin) has now become the most prescribed medication for ADHD [5,6]. The anorectic effect of methylphenidate has also been reported previously. Chronic use of MPD reduces daily intake of food and body weight gain so it can be used for treatment of hyperphagia and obesity [7]. Acute and chronic methylphenidate enhances cognitive performance [8,9] as well as motor activity [8, 10, 11]. Behavioral

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experiments in animals demonstrated that repeated exposure to Psychostimulants elicit behavioral sensitization in term of the progressive augmentation of the initial, behavioral responses to a psychostimulant [12]. Behavioral sensitization in response to repeated exposure to a psychostimulant or a drug of abuse is taken as an early manifestation of neuronal plasticity that resulted in drug craving, relapse and development of addictive behavior [1]. Repeated administration of MPD has been reported to produce behavioral sensitization in terms of progressive increase in locomotor activity [13] mostly in adult animals [1].

Memory deficits in ADHD patients are well documented and improved by the treatment with methylphenidate [14-17]. Methylphenidate as a “cognitive inducer” is not only abused by the patients with depression like symptoms but also by healthy individuals [18].MPD has been reported to enhance cognitive performance and produce rewarding effect by increasing the brain levels of dopamine and noradrenalin through inhibition of their uptake [19].This neurochemical and cellular mechanism of brain reward circuitry is a key element in development of addiction [20]. Drug addiction is also considered as a cognitive disorder [21].Addiction induced neurocognitive deficits have been well documented previously [22-27] and they effect on variety of brain functions, such as attention, working memory, memory, planning, impulse control, and decision-making [28].

Use of methylphenidate by depressed or normal individuals has been increased all over the world. There were different studies have been done yet to evaluate MPD induced improvement in cognition. Present study was designed to investigate the clinically relevant doses of methylphenidate (0.5mg/kg and 1mg/kg) which improve motor activity and cognition in normal adult rats. Potential addictive effects and withdrawal effects on cognition were also determined.

2. Material and methods

2.1. Animals

42 male adult Albino Wistar rats (weighing 180-220g) purchased from Dow University of Health Sciences, Karachi and housed individually under a 12-hour light and dark cycle (lights on at 06:00 hours) with free access to cubes of standard rodent diet and tap water at least 3 days before experimentation. The experiment was performed according to a protocol approved by local Animal Care Committee.

2.2. Drug and doses

Methylphenidate (Ritalin) tablets purchased from Medicine Department of the Agha Khan University Hospital, Karachi were pulverized and dissolved in distilled water. Drug was administered orally through gavage in volumes of 0.5 mg/ml/kg and 1 mg/ml/kg of body weights.

2.3. Skinner's Box Activity

Specifically designed transparent Perspex cages of area (26 x 26 x 26 cm) with sawdust covered floor i.e., Skinner's box were used to monitor motor activity in a familiar environment. Animals were placed in the center of the Skinner's box and allow moving freely. Number of cage crossings was monitored for a cut-off time of 10 minutes.

2.4. Morris Water Maze Test

Morris Water Maze (MWM) test is a well-known, conventional cognitive test, performed to examine the effects on spatial memory. 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 end 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 × 10cm) was placed at 2cm beneath the surface of water [8, 29].

2.5. Experimental protocol

42 male Albino Wistar rats were initially divided into three groups. Group 1: water treated controls (18 animals), Group 2: 0.5 mg/kg of methylphenidate (12 animals) and Group 3: 1 mg/kg of methylphenidate (12 animals). Food intake of all rats was monitored daily in the morning. Freshly prepared dose of 0.5 mg/kg and 1 mg/kg methylphenidate were administered orally to the respective group of animals, whereas at the same time water treated controls were administered with tap water daily from 9:00 to 9:30h and from 17:00 to 17:30h for 6 days. Dose related effects of

methylphenidate on motor behavior were determined in a familiar environment of Skinner's box 30 min post administration daily for six days for a cut-off time of 10 min.

On 7th day, three groups of rats were further divided into total seven groups of 6 animals each:

- Water treated controls
- Water treated 0.5 mg/kg
- Water treated 1 mg/kg
- 0.5 mg/kg withdrawal
- Repeated 0.5 mg/kg
- 1 mg/kg withdrawal
- Repeated 1 mg/kg

To study the effects of repeated administration/withdrawal from clinically relevant doses of MPD on cognition; Morris water maze test was performed. Acquisition and retention of memory was assessed as the latency time to locate the hidden platform. Procedure was consisted of two phases: the training phase and the test phase. In the training phase, platform was placed in north quadrant of the tank and animals were placed in the center of the water maze. 2 minutes were given for each animal to find and mount onto the hidden platform. If the rat succeeded, it was allowed to stay on it for 10 sec and if failed, was guided towards the platform. In the test phase we assessed short term memory, long term memory and daily changes in memory same as training session for a cut off time of 2 minutes. Less latency time to reach at hidden platform indicates enhancement or improvement of memory.

On 7th day of experiment animals were trained in Morris water maze tank at 9:00h to 10:00h. Immediately after training session water or drug was administered to respective group of animals. Short term memory was assessed 3-h post drug administration; long term memory was assessed 24-h post drug administration. Daily changes in memory were monitored as 45 min post administration of drug for next 5 days.

2.6. Statistical Analysis

Results are represented as means \pm SD. Data were analyzed by one-way and two-way ANOVA (repeated measure design) where applicable. Following ANOVA, post hoc comparisons were done by Tukey's test. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Daily food intake of rats for six days

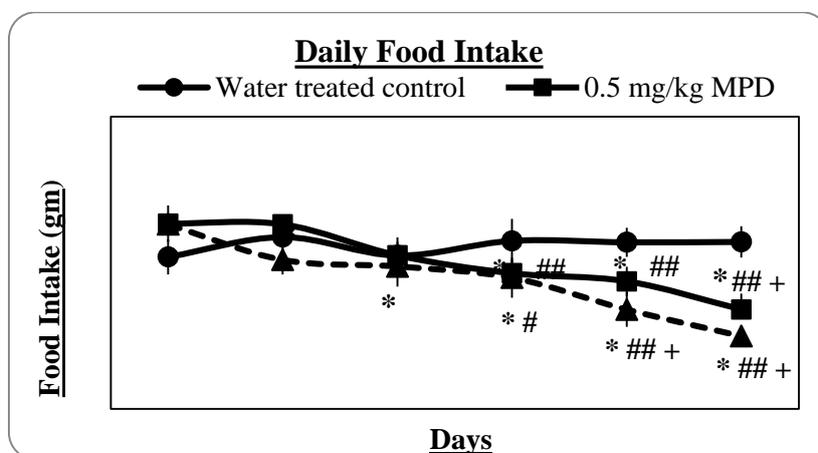


Figure 1 Dose dependent effects of Alpha Methylphenidate administration for six days on daily food intake of rats. Values are means \pm SD ($n = 18$ for controls, $n = 12$ for 0.5 mg/kg and 1mg/kg animals). Significant differences by Tukey's test: * $p < 0.01$ from 1st day value of similarly treated group: # $p < 0.05$, ## $p < 0.01$ from respective water treated controls: + $p < 0.01$ from respective 0.5mg/kg animals following two-way ANOVA (repeated measure design)

Fig 1 shows the dose dependent effects of repeated administration of methylphenidate for six days on daily food intake of rats. Analysis of data on daily food intake by two-way ANOVA (repeated measure design) showed that effects of methylphenidate ($F=12.5$ $df=1,39$ $p<0.01$), repeated monitoring ($F=22.1$ $df=5,39$ $p<0.01$) and interaction between methylphenidate and repeated monitoring ($F=12.9$ $df=10,39$ $p<0.01$) were all significant. Post hoc analysis by Tukey's test showed that food intake was decreased at a dose of 0.5mg/kg from day 3 to day 6 and at a dose of 1mg/kg from day 2 to day 6 as compared to food intake on day 1. Methylphenidate 0.5 and 1 mg/kg decreased food intake from day 4 to day 6 than respective day values of water treated controls. Decreased food intake was more effective at 1 mg/kg than 0.5 mg/kg on day 5 and 6.

3.2. Motor activity of rats in familiar environment (Skinner's box)

Fig 2 shows the dose dependent effects of repeated administration of alpha methylphenidate for six days on motor activity of rats monitored in familiar environment of Skinner's box. Analysis of data on number of crossings by two-way ANOVA (repeated measure design) showed that effects of alpha methylphenidate ($F=658.0$ $df=1,39$ $p<0.01$) and repeated monitoring ($F=44.2$ $df=5,39$ $p<0.01$) and interaction between alpha methylphenidate and repeated monitoring ($F=68.9$ $df=10,39$ $p<0.01$) were all significant. Post hoc analysis by Tukey's test showed that motor activity was increased at a dose of 0.5mg/kg from day 4 to day 6 and at a dose of 1 mg/kg from day 3 to day 6 than day 1. Motor activity was increased at 0.5 and 1 mg/kg from day 1 to day 6 than water treated control. Methylphenidate induced increased motor activity was greater at 1 mg/kg than 0.5 mg/kg from day 2 to day 6.

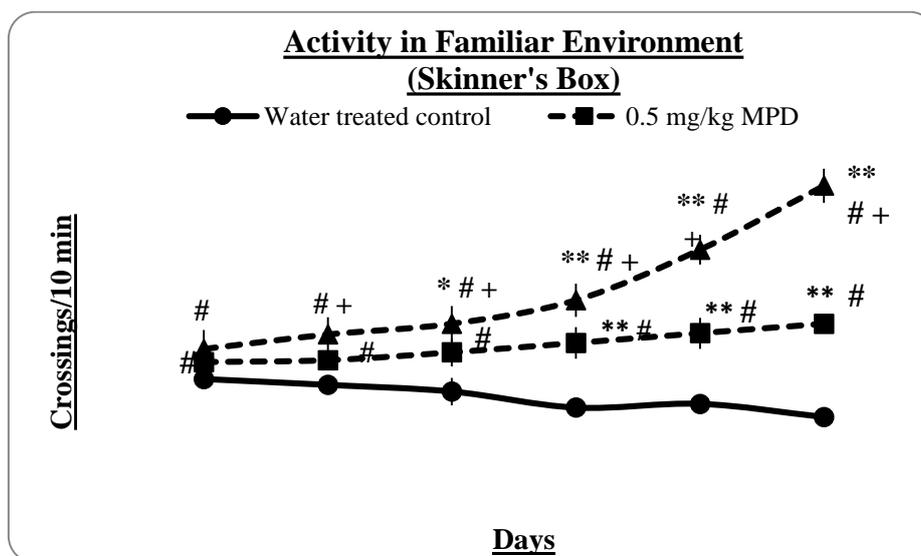


Figure 2 Dose dependent effects of Alpha Methylphenidate administration for six days on motor activity of rats in familiar environment. Values are means \pm SD ($n= 18$ for controls, $n= 12$ for 0.5 mg/kg and 1mg/kg). Significant differences by Tukey's test: * $p<0.05$ and ** $p<0.01$ from 1st day value of similarly treated group: # $p<0.01$ from respective water treated controls: + $p<0.01$ from respective 0.5mg/kg following two-way ANOVA (repeated measure design)

3.3. Dose dependent effects of alpha methylphenidate induced sensitization and its withdrawal on daily food intake of rats

Fig 3 shows the dose dependent effects of alpha methylphenidate induced sensitization and its withdrawal on daily food intake of rats. Analysis of data on daily food intake by two-way ANOVA (repeated measure design) showed that effects of alpha methylphenidate ($F=1160.5$ $df= 6,35$ $p<0.01$), repeated monitoring ($F=3.1$ $df=5,35$ $p<0.05$) and interaction between alpha methylphenidate and repeated monitoring ($F=38.01$ $df=30,35$ $p<0.01$) were all significant. Post hoc analysis by Tukey's test showed that water treated controls exhibited greater food intake from day 4 to day 6 than day 1. Water treated 0.5 mg/kg animals exhibited smaller food intake from day 3 to day 6 than day 1 and then respective day value of water treated controls. Water treated 1 mg/kg animals exhibited smaller food intake from day 2 to day 6 than day 1 and water treated controls, on day 5 and 6 than respective day value of water treated 0.5 mg/kg animals. 0.5 mg/kg withdrawal animals showed greater intake of food from day 4 to day 6 than day 1, smaller food intake from day 1 to day 6 than respective day value of water treated controls, and greater food intake on day 6 than respective day value of water treated 0.5 mg/kg animals. Repeated 0.5 mg/kg sensitized animals showed smaller food intake from day 4 to day 6 than day 1 and respective day value of 0.5 mg/kg withdrawal animals and from day 1 to day 6 than respective day value of water treated controls and water treated 0.5 mg/kg animals. 1 mg/kg withdrawal animals showed greater

food intake from day 4 to day 6 than day 1, smaller food intake from day 1 to day 6 than water treated control, smaller food intake on day 1,2 and 3 and greater food intake on day 4,5 and 6 than water treated 1 mg/kg and smaller food intake from day 1 to day 4 than respective day value of 0.5mg/kg withdrawal animals. Repeated 1 mg/kg sensitized animals showed smaller food intake from day 4 to day 6 than day 1, from day 1 to day 6 than respective day value of water treated controls and water treated 1 mg/kg animals, smaller food intake on day 1,2,4,5 and 6 than respective day value of repeated 0.5 mg/kg sensitized animals and from day 4 to day 6 than respective day value of 1 mg/kg withdrawal animals.

Daily Food Intake X-Axis: Days

Y-Axis: Food intake (gm)

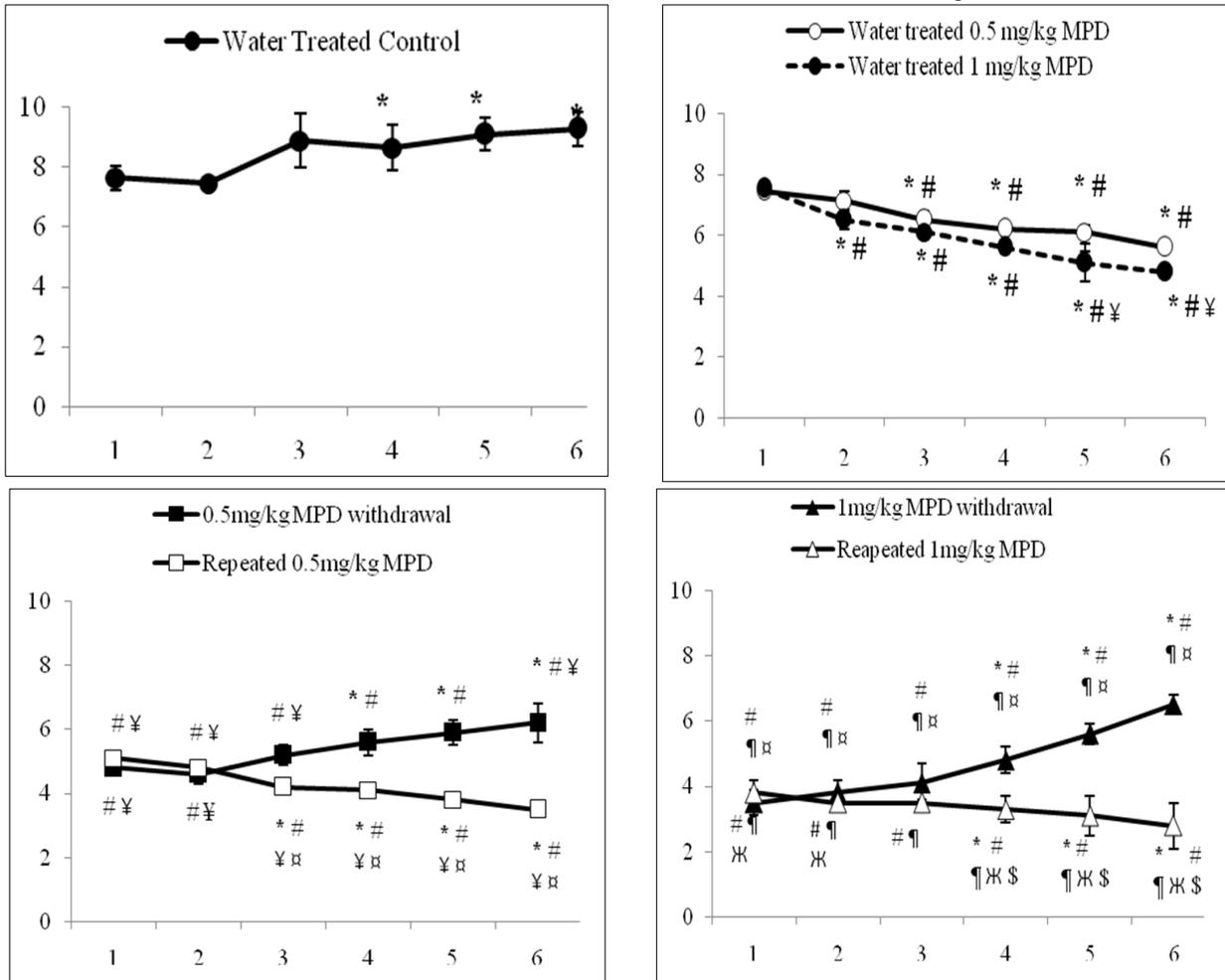


Figure 3 Dose dependent effects of alpha methylphenidate induced sensitization and its withdrawal on daily food intake of rats. Values are means \pm SD (n= 6). Significant differences by Tukey’s test: *p<0.01 from 1st day value of similarly treated group: # p<0.01 from respective day value of water treated controls: ¥ p<0.01 from water treated 0.5 mg/kg: ¶ p<0.01 from water treated 1 mg/kg: ⌘ p<0.01 from 0.5 mg/kg withdrawal: ⌚ p<0.01 from repeated 0.5 mg/kg: \$ p<0.01 from 1 mg/kg withdrawal following two-way ANOVA (repeated measure design)

3.4. Short term and long-term memory (Morris water maze)

Fig 4 shows the dose dependent effects of alpha methylphenidate induced sensitization and its withdrawal on short term (a) and long term memory (b) of rats monitored in Morris water maze tank. Analysis of data on short term memory by one-way ANOVA showed that effect of alpha methylphenidate (F=258.2 df= 6, 41p<0.01) was significant. Post hoc analysis by Tukey’s test showed that single administration of methylphenidate in water treated 0.5 mg/kg animals increased short term memory as compared to water treated controls. Single administration of methylphenidate in water treated 1 mg/kg animals and withdrawal from 0.5 mg/kg of methylphenidate impaired short term memory than water treated controls and water treated 0.5 mg/kg animals. Repeated administration of 0.5 mg/kg methylphenidate

(repeated measure design) showed that effects of alpha methylphenidate ($F=276.5$ $df= 6,35$ $p<0.01$), repeated monitoring ($F=638.6$ $df=4,35$ $p<0.01$) and interaction between alpha methylphenidate and repeated monitoring ($F=178.9$ $df=24,35$ $p<0.01$) were all significant.

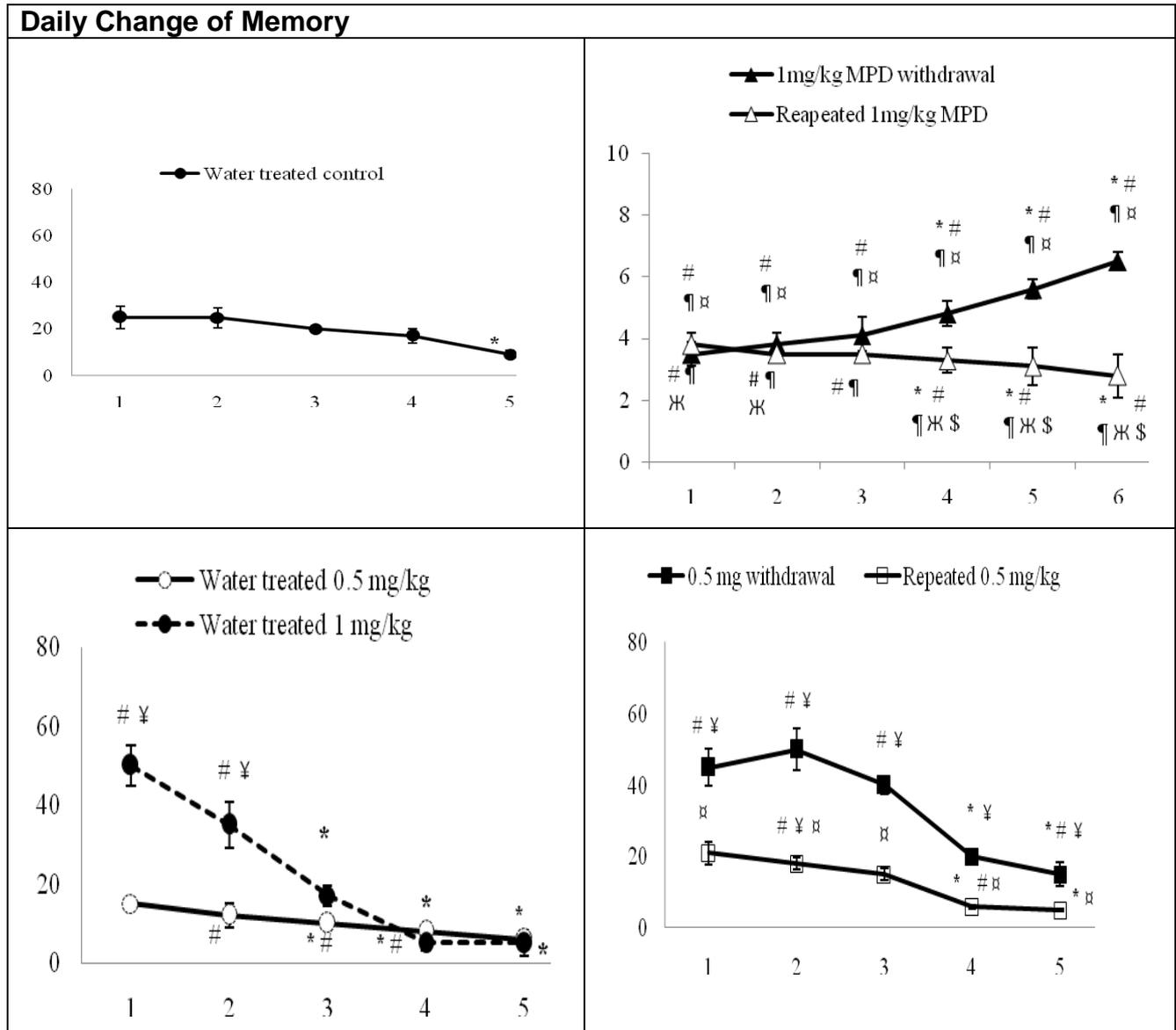


Figure 5 Dose dependent effects of alpha methylphenidate induced sensitization and its withdrawal on daily changes of memory of rats in Morris water maze tank. Values are means \pm SD ($n= 6$). Significant differences by Tukey's test: * $p<0.01$ from 1st day value of similarly treated group; # $p<0.01$ from respective day value of water treated controls; ¥ $p<0.01$ from water treated 0.5 mg/kg; ¶ $p<0.01$ from water treated 1 mg/kg; □ $p<0.01$ from 0.5 mg/kg withdrawal; Ж $p<0.01$ from repeated 0.5 mg/kg; \$ $p<0.01$ from 1 mg/kg withdrawal following two way ANOVA (repeated measure design)

Post hoc analysis by Tukey's test showed that water treated controls exhibited enhanced memory on day 5 than day 1. Water treated 0.5 mg/kg animals exhibited enhanced memory from day 3 to day 5 than day 1 and from day 2 to day 5 than respective day value of water treated controls. Water treated 1mg/kg animals showed enhanced memory from day 3 to day 5 than day 1 and impaired memory on day 1 and day 2 than respective day value of water treated controls and water treated 0.5 mg/kg animals. Withdrawal from 0.5 mg/kg of methylphenidate enhanced memory on day 4 and 5 than day 1 and impaired memory from day 1 to day 5 than respective day value of water treated controls and water treated 0.5 mg/kg animals. Animals that were sensitized with repeated administration of 0.5 mg/kg methylphenidate showed enhanced memory on day 4 and 5 than day 1 and from day 1 to day 5 than respective day value of 0.5 mg/kg

withdrawal animals. 1mg/kg withdrawal animals showed enhanced memory on day 5 than day 1 but impaired memory from day 1 to day 5 than respective day value of water treated controls and water treated 1 mg/kg animals and on day 4 and 5 than respective day value of 0.5 mg/kg withdrawal animals. Repeated 1 mg/kg sensitized animals showed memory enhancing effects of methylphenidate from day 2 to day 5 than day 1, from day 1 to day 4 than respective day value of water treated controls, water treated 1 mg/kg and 1 mg/kg withdrawal, on day 2 and 3 than respective day value of repeated 0.5 mg/kg sensitized animals.

4. Discussion

Present study shows that oral administration of clinically relevant doses (0.5 and 1mg/kg) of methylphenidate enhanced motor activity and cognition. Different preclinical studies have been carried out to study the reinforcing as well as cognition enhancing effects of methylphenidate. In the present study we investigated the effects of methylphenidate (0.5 and 1mg/kg orally) on food intake, motor activity and cognition. Our study comprised of two phases; in the first phase we monitored the anorectic and reinforcing effects of 0.5 and 1mg/kg methylphenidate and in the second phase we monitored cognition enhancing effects in rats acute administered with, repeatedly administered with or withdrawal from 0.5 and 1 mg/kg methylphenidate.

Dose dependent decrease of food intake by methylphenidate has been reported previously [30]. Results of the present study showed that methylphenidate at a dose of 0.5 mg/kg and 1 mg/kg produced hypophagia (Fig 1). However, these effects were produced from third day of 1 mg/kg of methylphenidate and from fourth day of 0.5 mg/kg of methylphenidate administration. Thus, 1 mg/kg methylphenidate is more effective to produce hypophagia and to reduce body weight gain so it can be used for the treatment of hyperphagia and obesity.

Methylphenidate upon repeated administration has been reported to produce sensitization in a dose dependent manner [8]. Our results showed a dose dependent progressive increase in motor activity of rats monitored in familiar environment of Skinners box (Fig 2). Methylphenidate increases the availability of dopamine and norepinephrine in extra cellular area by blocking their reuptake [31, 9]. In the present study methylphenidate 0.5 and 1 mg/kg increased motor activity of animals but behavioral sensitization was markedly observed from the 5th day of the treatment with 1 mg/kg of methylphenidate (Fig 2). It is suggested that repeated administration of 1mg/kg methylphenidate produced reinforcing effects by promoting hypophagia and behavioral sensitization in normal adult rats.

From the first phase of the experiment we suggested that methylphenidate caused reduction in daily food intake and progressive increase in motor activity of rats i.e. sensitization. Taking behavioral sensitization as a marker of drug addiction we further extended our study to investigate the effects of MPD induced addiction and its withdrawal on daily food intake and cognitive profile of rats.

Different studies state a relation of drug addiction and food intake. Drug addiction is often associated with hypophagia [32] and withdrawal from drugs of abuse increases food consumption and body weight [33]. Therefore, we monitored daily food intake of animals to investigate the effects of methylphenidate induced sensitization and its withdrawal on food intake. Results showed that repeated administration of methylphenidate 0.5 and 1 mg/kg reduced food intake. An increased food intake was observed from 3rd day of the withdrawal from methylphenidate but this increase is still less than the food intake of water treated control rats (Fig 3).

Different preclinical studies used methylphenidate doses significantly higher than those used clinically in humans [34, 35]. Dose dependent effects of MPD on learning and memory monitored in Morris water-maze have been reported previously. Oral administration of clinically recommended doses of MPD (0.25-1 mg/kg) enhanced short term and long term memory of rats in Morris Water Maze test [8]. Methylphenidate at a dose of 0.2 and 0.4 mg/kg enhanced short term and long term memory but methylphenidate 0.8 mg/kg enhanced only long term memory of rats [8]. Results showed that single as well as repeated administration of methylphenidate 0.5 mg/kg increased short term and long term memory of rats. However, 1mg/kg of methylphenidate enhanced short term and long term memory only upon repeated administration. Results suggested that clinically relevant lower dose (0.5mg/kg) of methylphenidate has cognition enhancing effects even after single administration (Fig 4 a & b).

Conflicting results with chronic administration of methylphenidate and its effects on cognitive performance have been reported. For example adult rats treated with methylphenidate 5 and 10 mg/kg showed impairment on recognition memory but this effect was evident only 14 days later after daily drug administration [36]. Another study demonstrated that i.p administration of methylphenidate 5 mg/kg for 15 days did not effect on memory (inhibitory avoidance and object recognition task) [34]. In the present study methylphenidate improved retention of memory in Morris Water

Maze up to 4 days at both doses. On 5th day, learning and memory of water treated control rats also become maximum and they also behaved like drug treated rats because of familiarization to water maze tank (fig 3.5). In the brain, the prefrontal cortex and hippocampus are involved in encoding and retrieval [37, 38]. Dopamine (DA) regulates activity of these regions of brain, as well as the communication between them [39]. Methylphenidate enhances extra cellular concentration of dopamine [40] by increasing DA signaling in the brain because it blocks DA transporters [41-43]. It is suggested that methylphenidate enhances learning and memory functions by increasing DA levels in the brain.

Withdrawal from abused drugs either a depressant or a stimulant caused dysfunction of memory [44]. Withdrawal from chronic consumption of alcohol impaired memory of rats monitored in Morris Water Maze [45], T-maze, foot shock avoidance, shuttle box active avoidance and step-down passive avoidance (tests for assessing spatial learning and memory) [46-49]. Besides this, memory deficits from the withdrawal of psychostimulants like amphetamine and cocaine has also been reported [50-52]. Results of the present study support above statements; withdrawal from repeated administration of 0.5 and 1 mg/kg methylphenidate impaired memory acquisition and memory retention of rats (fig 4 & 5). Different studies have reported a direct association of drug abuse with neuronal plasticity. Chronic administration of addictive drugs is associated with neurochemical and morphological changes, neuronal plasticity and alteration in the levels of neurotransmitters in specific regions of the brain which are involved in cognition and memory processes like neocortex, basal forebrain and hippocampus [53] and withdrawal from these drugs may potentiates these alterations that exert adverse effects upon cognitive performance and results in decline of memory functions [44]. Although the whole mechanism of cognitive decline after withdrawal from drug abused is not completely understood, multiple mechanisms are said to be involved. Contributing factors for cognitive impairment after drug withdrawal include neurotransmitters and neuropeptides such as dopamine, glutamate, glucocorticoids and cannabinoids [44].

Dysfunctioning of dopaminergic system leads to the development of several neuropsychiatric disorders that involve abnormal cognitive and affective functions [54]. Chronic intake of ethanol increases the secretion of glutamate (an excitatory amino acid) and inhibition of N-Methyl D-Aspartate (NMDA) receptors [55-56] and withdrawal from ethanol caused glutamate excitotoxicity and thus damage on the frontal lobe (one of the critical regions for memory function) [57, 58]. Some other studies also reported a role of glucocorticoids in drug withdrawal induced memory decline. Chronic consumption of ethanol increased concentration of glucocorticoids in memory associated regions of brain including hippocampus and prefrontal cortex [59, 44]. Morphine-withdrawal resulted in activation of Hypothalamic-Pituitary-Adrenal axis (HPA) pathway [60]. So, in the view of all these statements it is suggested that withdrawal from methylphenidate impaired cognitive functions because of the abnormal functions and interconnection of all these systems of neurotransmitters and neuropeptides. It is suggested that methylphenidate may be useful in improving performance in ADHD patients but in normal adults with normal performance the drug can impair performance on withdrawal.

5. Conclusion

Present study shows anorexigenic, reinforcing and cognition enhancing effects of clinically relevant doses of methylphenidate (0.5 and 1 mg/kg) in normal adult rats. Withdrawal from methylphenidate impaired spatial memory. Methylphenidate 1 mg/kg produces behavioral sensitization and 0.5 mg/kg enhances learning and memory functions. Methylphenidate 0.5mg/kg is clinically relevant for enhancing motor activity and cognitive functions. Further investigations may help to understand neurochemical alterations, cellular, and molecular mechanisms in brain after withdrawal from methylphenidate.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Abbreviations

MPD (methylphenidate), ADHD (attention deficit hyperactivity disorder), DA (dopamine), NA (noradrenalin), NMDA (N-Methyl D-Aspartate), HPA (Hypothalamus-Pituitary-Adrenal axis).

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