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Original Article

DOES METHYLPHENIDATE ENHANCE COGNITION IN NORMAL RATS AND DOES IT AFFECT NEURONAL POPULATION?

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ABSTRACT

Objective: Methylphenidate [MPH] is one of the drugs of choice for children with Attention Deficit Hyperactivity Disorder [ADHD] since many decades with good effect. Consumption of this drug by normal children and adolescents to boost their cognition skills is of concern. MPH induced cognitive enhancement involves brain dopamine and norepinephrine levels in areas concerned with cognition especially hippocampus. Altered expression of these neurotransmitters can affect neuronal population of hippocampus which may have the significant effect in later part of the life. Hence we evaluate the effect of MPH on cognition and histopathological changes in the hippocampus and dentate gyrus.

Methods: Two month old male *wistar* rats were given either 2 or 5 mg/kg dose of MPH for 10 successive days and another set of rats served as control. The rats were tested for learning and memory activities followed by histopathological studies in hippocampus and dentate gyrus using Nissl staining.

Results: MPH at both the doses has enhanced learning abilities as well as retention of memory. The histopathological studies did not show any significant effect on dentate gyrus as well as hippocampus.

Conclusion: Though MPH is known to provide sound results in ADHD, from the present study it is clear that MPH treatment in normal rats also temporarily enhance the cognitive skills especially declarative memory. However, its effect on long term memory is to be investigated. MPH treatment has not affected the neuronal population hence possible cytotoxic effects on neurons can be ruled out from the present study.

Keywords: Attention deficit hyper activity disorder, Cognitive function, Learning, Memory, Methylphenidate, Hippocampus.

INTRODUCTION

MPH is generally used to treat ADHD in children. MPH is proved to be effective in reducing hyperactivity and improving attentiveness [1] in children with ADHD. Of late there is a tendency to use MPH by people who are seeking to boost their cognition [2]. In United States of America 7% of the students are using nootrophics and this percentage rises to 25% at some establishments [3]. MPH is known to be an inhibitor of dopamine and nor-epinephrine transporter [4]. Inadequate research was performed in normal children in which improvement in attention was observed [5], recall [6], and spatial working memory [7]. However, the exact effect of MPH depends on many factors. While research in children with ADHD is limited, research in healthy animals would be more useful.

Numbers of studies using oral administration of MPH in rats at the doses relevant to humans are targeted. Studies have focused mainly on nucleus accumbens (NAc), prefrontal cortex (PFC), and hippocampal regions of the rats, which are mainly concerned with cognition. These studies have revealed an increased dopamine [DA] in the NAc and in the PFC [8] and increased nor-epinephrine [NE] levels in the hippocampus [9]. Both NE and DA have a critical influence on PFC and hippocampus dependent cognitive functioning [10]. Though these studies throw light on MPH-induced cognitive enhancement involves the prefrontal cortex NE and DA system, whether these changes involve morphological changes in the regions of the brain involved in cognition is not known. Manipulation of neurotransmitters like NE and DA can cause adverse effect on neuronal population of the hippocampus [11]. Dentate gyrus is an area of active neuronal population which in turn required for hippocampal structural and functional plasticity [12]. Temporary enhancement of cognition though sounds good, it is necessary to evaluate MPH administration effect on neuronal population. Adverse effect if any on neuronal population has to be investigated. Hence in the present study we evaluate the MPH treatment effect in young rats on cognition and neuronal morphology of the hippocampus and dentate gyrus.

MATERIALS AND METHODS

Animals

In-house bred two months old male albino *Wistar* rats were used in the present study. Rats were fed with water and food *ad libitum*. The rats were maintained under controlled conditions of light-dark cycle (12:12), temperature (22±3 °C), humidity (approximately 50±10%) and pathogen free environment. Polypropylene cage with paddy husk as bedding material was used for housing the rats. Institution animal ethics committee approval was obtained before commencing the experiment [letter dated 28/02/2014].

Animal groups

The experiment consisted of following animal groups (n=6).

Group 1: Control rats

Group 2: Rats received 2 mg/kg MPH twice daily for 10 consecutive days

Group 3: Rats received 5 mg/kg MPH twice daily for 10 consecutive days

MPH was administered intraperitoneally. The rats were tested for learning and memory activities as well as histomorphological studies immediately after cessation of MPH treatment.

Condition avoidance test (Active avoidance/Shuttle box test)

This test evaluates the learning and memory abilities of the rats. The shuttle box consisted of a closed wooden box with shutters in front. The floor area was made up of grids, which were separated into two parts by a median grid. Each part was connected to separate electric circuits and a buzzer was fixed inside the box. Individual rats were allowed to explore the test box for 5 min. After 10 seconds, a discriminative stimulus was given through a buzzer. During that period, the rat could avoid the shock by crossing to the other compartment. If the rat failed to respond during the discriminative

stimulus period, it received a shock of 2.5mA for a maximum period of 10 seconds, during which, it could escape by crossing to the other side. The contingency for avoiding the shock was a single crossing over the median grid from one side of the shuttle box to the other. The test consisted of 30 trials daily for 5 consecutive days. The number of shock avoidances increases from day 1 to 5 of the test under normal circumstances. The average shock avoidance numbers on all 5 d were termed as the mean score during 5 d of testing. Any decrease in this score was an indication of learning impairment. Each rat was retested one week after the last trial to assess retention of memory. A comparison of rat's performance with its previous performance gives the assessment of memory and is presented as the retention score (RTS), which is calculated as in [11].

$$RTS = \frac{\text{Mean of retest score X Mean scoring during 5 d of testing}}{\text{Mean score during day 5 of the testing}}$$

Any decrease in the retest score and retention score was an indication of memory impairment. Each rat was trained for 4 d before starting the test.

Neuronal assay of the hippocampus& dentate gyrus (cresyl violet staining)

Perfusion: Rats were deeply anaesthetized with ether and secured on a dissection board, and its chest cavity was opened to expose the heart. About 15 ml of 0.9% saline was perfused through the left ventricle at the rate of 1 ml/min. This was followed by perfusion of 10% formalin at the same flow rate. The animal was decapitated and the brain was removed and kept in 10% formalin for 48h (post fixation). Paraffin blocks were made in an embedding bath. Coronal sections of 5µm thickness were cut in the dorsal hippocampus using a rotary microtome (Jung Biocutt 2035, Leica, Germany). Ten sections from each animal were mounted serially on air dried gelatinized slides.

Staining

The sections were stained with cresyl violet stain. One hundred milligrams of cresyl violets was dissolved in 100 ml of distilled water. To this 0.5 ml of 10% acetic acid was added to give a pH of 3.5-3.8. The stain was filtered before use [11].

Scoring

The slides are screened using a Nikon trinocular microscope (H600L) under 40X magnification. In each hippocampal section, cornua amonis (CA2, CA3, CA4; 250µm length) of hippocampus and dentate gyrus (200µm^2 length) were selected using imaging software NIS Elements Br version 4.30and the numbers of viable neurons were counted. Slides from different groups of rats were decoded to avoid manual bias while counting the cells. The cell counts were expressed as the number of cells per unit length of the cell field (cells/250µm length).

Statistical analysis

The data were expressed as mean±SD. The significance of differences among the groups was assessed using one-way analysis of Variance (ANOVA) test followed by Bonferroni's multiple comparison post hoc test. *P* values<0.05 were considered as significant.

RESULTS

Condition avoidance test

MPH at both doses has significantly (p<0.001) increased mean of 5 d score compared to control rats. This is an indication of increased learning ability. There was also a moderately significant (p<0.05) difference between the two doses of MPH treatment, where 5 mg/kg dose found to be more affective. The retest score was also increased significantly (p<0.001) in both the doses of MPH treatment compared to control. The memory retention score was significantly increased in both the doses of MPH treatment compared to control. At 2 mg/kg dose the significant was p<0.01 and at 5 mg/kg dose was p<0.001. There was no statistically significant difference between two doses of MPH treatment as far as memory retention score was concerned (fig. 1).

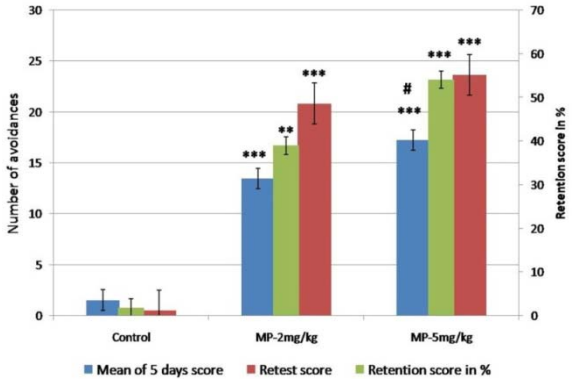

Fig. 1: Observation of condition avoidance test by control and MPH treated rats. Values are expressed as mean. Error bar indicates±SD. Comparison between control Vs MPH treatment, ** =P<0.01, *** =p<0.001, Comparison between MPH2 mg/Kg Vs MPH 5 mg/kg, # = p<0.05

Neuronal assay of dentate gyrus and hippocampus:

The quantitative estimation of neurons in dentate gyrus region of the rat did not show any statistically significant (p>0.05) difference between control and MPH treated groups. However there was a significant (p<0.001) difference in number of neurons between 2 and 5 mg/kg dose of MPH treated rats. The quantification of neurons in CA4, CA3 and CA2 regions of the rat hippocampus did not show any statistically significant (p>0.05) difference between control and MPH treated groups (fig. 2, 3 and 4).

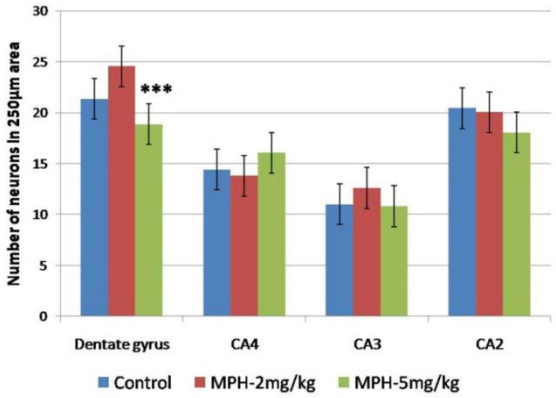

Fig. 2: Observation of neuronal assay in hippocampus and dentate gyrus. In hippocampus the numbers of neurons were counted in 250 µm length area and in dentate gyrus 200 µm^2 area. Values are expressed as mean. Error bar indicates±SD. Comparison between MPH2 mg/Kg Vs MPH 5 mg/kg, *** =p<0.001

DISCUSSION

The results of the present study clearly demonstrate that MPH treatment in normal rats improves their learning ability and also retention of learnt things in the form of memory with an immediate effect. Though MPH is prescribed in clinically confirmed conditions of ADHD in children as well as in adults, of late it has been misused as stimulant to enhance the performance in examinations by students [13]. Earlier studies have proved that MPH treatment in children as well as adults with ADHD, to improve the working memory [14-16]. In the present study the condition avoidance task not only tests the learning ability of the rats but also memory retention. Rats were tested whether they can escape from the foot shocks from their past memory, which can be considered as declarative memory. Declarative memory is the ability to recall the facts, ideas or plans from past memory. As far as MPH effect on such memory function is less reported. These kinds of declarative memory are very much required in daily life and in social interaction [17].

There are also different reports with respect to declarative memory. A study reports [6] considerable memory enhancement in healthy volunteers who received MPH, but another study [18] did not find any significant effect of MPH on memory function in healthy volunteers. MPH treatment in patients with ADHD [19] has benefited in improving the memory. In the present study normal rats showed a significant improvement in learning and memory abilities and our results are in consistent with Riordan *et al.* Since MPH can enhance the cognitive skills in normal condition, its misuse among the student community is of serious concern. Its long-term effect like substance abuse in later life is also another concern.

There is also an apprehension that usage of MPH in normal children can boost short term memory at the cost of long term memory [20]. Hence further study is warranted to evaluate whether MPH would adversely affect long term memory.

Fig. 3: Photomicrographs of dentate gyrus region of the rat brain under 40X magnification, stained with cresyl violet. Square box indicates 50πm² area

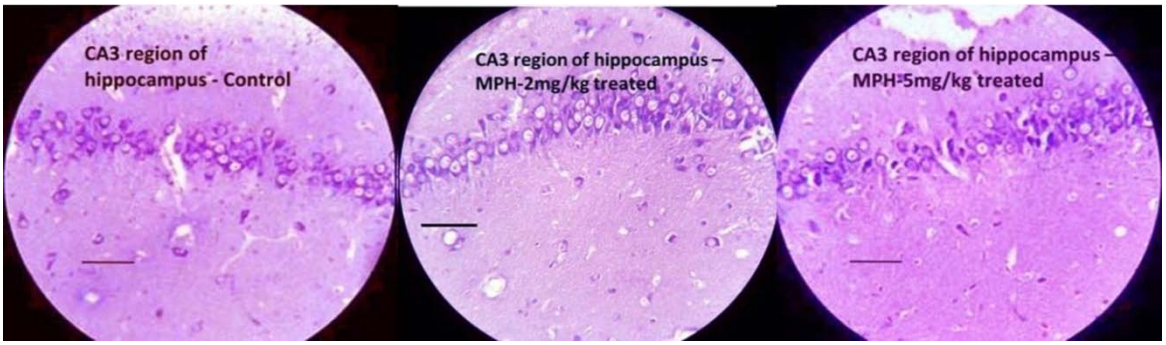

Fig. 4: Photomicrographs of CA3 region of the rat hippocampus under 40X magnification, stained with cresyl violet. Scale bar indicates 50πm length area

The mechanism of ADHD is due to under performance of dopamine and norepinephrine especially in the hippocampus and prefrontal cortex of the brain. These areas are concerned with higher functions like motivation, inhibition, memory, planning, reasoning and foresight [21]. MPH is primarily acts by inhibiting transport of dopamine and also norepinephrine, which in turn leads to elevation of its concentration in synaptic cleft. It would be interesting to evaluate these effects on neurons of the hippocampus after MPH treatment. In the present study, the neuronal assay of dentate gyrus and hippocampus showed that MPH treatment has not affected the number of normal neurons. This implies that, despite the efficiency of MPH in inducing changes in the dopaminergic system and thereby enhancing cognition, it has not caused either loss of neurons or promoting neurogenesis especially in dentate gyrus where neurogenesis continues even during adulthood.

The rationale of evaluating the neuronal number was because there was also a report claiming loss of dopaminergic neurons after MPH treatment in mice [22]. Though our study did not evaluate the dopaminergic neurons in specific, but considering the fact that hippocampus present a high number of dopaminergic neuronal population, actual loss if any would fairly provide an indication. It would be interesting to see whether it promotes the survival of healthy neurons, but this would require immunohistochemistry studies. In the present study there was a significant decline in neuronal number in MPH-5 mg/kg treated rats compared to 2 mg/kg dose, but their number did not differ from the base line. Few of the *in vitro* studies have claimed that MPH enhances the neuronal stem cell proliferation [23].

MPH is prescribed in ADHD conditions, but in the present study we tested its effect on normal rats, since there is an increased tendency to use MPH among normal children, adolescents and even adults as a performance enhancer. The present study did not focus on hyperactivity, which is a common feature of ADHD, because we aimed to see its effect in normal rats.

CONCLUSION

MPH treatment certainly enhances learning abilities and memory retention in normal rats. MPH induced cognitive enhancement does not affect the neuronal population of dentate gyrus and hippocampus. MPH is used mainly in children with ADHD, which is known to reverse the symptoms of ADHD and promote the learning skills in children and in adolescents.

However, from the present study it is clear that MPH treatment in normal rats also temporarily enhance the cognitive skills especially declarative memory. However, its effect on long term memory is to be investigated. MPH treatment has not affected the neuronal population in the present study. Use of MPH to enhance the academic performances by students and its long term effects needs to be discussed further.

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CONFLICT OF INTERESTS

Declared None

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