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Research report

Effects of clinically relevant doses of methyphenidate on spatial memory, behavioral sensitization and open field habituation: A time related study

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HIGHLIGHTS

- Clinically relevant doses (0.25–1.0 mg/kg) of MPD enhance learning and memory.
- Memory acquisition, retention, consolidation is enhanced and extinction impaired.
- Novelty-induced exploratory behavior in an open field is also impaired.
- High (1 mg/kg) but not low doses produce behavioral sensitization.

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ABSTRACT

The psychostimulant methylphenidate (MPD) is a first-line drug for the treatment of attention deficit hyperactivity disorder (ADHD). Despite acceptable therapeutic efficacy, there is limited data regarding the long-term consequences of MPD exposure over extended periods. The present study concerns effects of clinically relevant doses of MPD, administered orally to rats for an extended period, on spatial memory, behavioral sensitization and habituation to an open field. Water maze test was used to monitor memory acquisition (2 h after training), retention (day next to training), extinction (1 week after training) and reconsolidation (weekly for 4 weeks). Administration of MPD at doses of 0.25–1.0 mg/kg improved memory acquisition, retention, reconsolidation and impaired memory extinction. Treatment with 0.25 and 0.5 mg/kg MPD for 6 weeks produced a sustained increase in motor activity but higher dose (1.0 mg/kg) elicited behavioral sensitization. High as well as low doses MPD impaired open field habituation. We conclude that clinically relevant doses of MPD enhance memory even if used for extended period. It is suggested that higher (1.0 mg/kg) clinically relevant doses of MPD, if used for extended period, may exacerbate hyperactivity and impulsivity associated with the disease.

1. Introduction

Methylphenidate (MPD) is a psychomotor stimulant most commonly prescribed for the treatment of attention-deficit hyperactivity disorder (ADHD) [1–3]. The drug is misused as a cognitive enhancer and recreationally [4]. ADHD, a neurodevelopmental disorder characterized by inattention, impulsiveness and hyperactivity [5], can begin in early childhood [6] and, in many cases, continues to adulthood [7]. Clinical evidence supports pharmacotherapy as the most effective treatment for ADHD throughout the duration of the disorder [8] and [9]. Despite widely accepted therapeutic efficacy of MPD, there is limited preclinical data on therapeutic and side effects of MPD exposure for extended period. A large increase in the use of MPD in patients to whom MPD is prescribed as well as in the general population, who believe that MPD could enhance their cognitive functions [10,11] suggests that the drug has abuse potential. Considering that MPD is prescribed at

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increasingly younger ages [6] and that many adverse effects of MPD may go unnoticed in clinical population, it is essential to monitor long term effects of MPD on cognition, abuse potential and other behaviors in preclinical research.

The translational utility of pre-clinical studies of MPD treatment is limited by a number of methodological factors, namely drug dosage and route of drug administration [12–14]. Acute doses of MPD have been seen to enhance attention, improve learning and memory, and reduce impulsivity in a variety of tasks [15]. Behavioral and cognitive deficits of neurogranin knockout mice were also improved by high doses of MPD [16]. Following repeated high-dose injections the drug produces behavioral sensitization, a process that has been implicated in drug abuse liability and addiction [17–20].

In most animal studies MPD treatment is achieved through a subcutaneous (s.c.) or intraperitoneal (i.p.) injection across a wide range of doses (0.5–80.0 mg/kg) that exceed the relatively low therapeutically recommended oral doses (0.3–1.0 mg/kg) in humans. The route of drug administration also appears to be an important determining factor; the same MPD dose applied through different routes produces widely different behavioral and neurochemical effects [13], [14], [21] and [22]. Additionally, a substantial body of pre-clinical work established associations between the effects of repeated exposure to stimulants on the brain's reward circuitry and an increased risk for substance abuse in adulthood [18,19].

The aim of the present study was to administer clinically relevant doses (0.25–1.0 mg/kg orally) of MPD to rats for an extended period and examine its effect on cognition and emotions. We used water Maze test to monitor MPD effects on memory acquisition, retention, extinction and reconsolidation in an extended, 4 weeks, treatment paradigm. In a separate experiment the effects of these doses of MPD are determined on behavioral sensitization and open field exploration.

2. Methods

2.1. Animals

Locally bred male albino Wistar rats, weighing 180–200 g, purchased from HEJ Research Institute of Chemistry (Karachi, Pakistan), a week before the start of the experiment, were housed under a 12-h light and dark cycle (lights on at 06:00 h) and controlled room temperature ($24 \pm 2\,^{\circ}C$), with free access to tap water and cubes of standard rodent diet, for familiarization with the environment. The animals were housed individually 3 days before the start of the experiment. Behavioral testing were conducted in the light phase. Before starting the experiment, rats were accustomed to various handling procedures. All animal experiments, approved by the Institutional Ethics and Animal Care Committee, were conducted in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All treatments and behavioral monitoring were performed in a balanced design to avoid order and time effects.

2.2. Drug

Commercially available methylphenidate (Ritalin, Novartis Pharma) tablets, purchased locally were used in the present study. The tablets were pulverized and dissolved in saline. Freshly prepared drug solution was administered orally using gavages. The drug was administered at doses of 0.25, 0.5 and 1.0 mg/kg, two times a day, from 10:00 to 11:00 h and from 17:00 to 18:00 h, for 4 weeks (water maze test) or 6 weeks (motor behavior).

2.3. Dose related effects of MPD on motor and exploratory activity

Dose related effects of MPD on motor behavior and novelty-induced exploratory activity were determined respectively in an activity cage and in an open field as described before [23].

2.3.1. Activity cage

Transparent, Perspex activity cages ($26 \times 26 \times 26$ cm) with sawdust-covered floor were used to monitor activity in a habituated environment [23]. Rats were placed individually in these cages before drug administration; the small area of the activity cage enabled quick habituation. After drug administration, animals were returned to the activity cages and the numbers of cage crossings were counted 30 min after placement in the activity box. A video monitoring device was used for determining the activity score.

2.3.2. Open field

Unlike activity cage, an open field provides a large exploratory area, in which activity-reducing effects of central nervous system depressants are clearly demonstrable [24]. Moreover, activity in an open field is monitored for a short time starting immediately after an animal is exposed to the novel arena of the apparatus, which does not allow habituation. The open field used in the present investigation was a square area (76×76 cm) with walls 42 cm high. The floor of apparatus was divided by lines into 25 squares of equal size. To monitor activity, an animal was placed in the central square of the open field and numbers of squares crossed with all four paws were counted for 5 min starting immediately after exposure to the open field.

2.3.3. Procedure

Thirty two animals were used in the experiment designed to monitor dose related effects of MPD on motor activity in the habituated arena of an activity cage and novel arena of an open field. The animals randomly divided to 0, 0.25, 0.5 and 1.0 mg/kg MPD treatment groups were placed individually in activity cages, 15 min before the drug administration. The drug was administered in between 10:00 and 11:00 h and animals placed back to activity cages. Activity score, monitored as the number of cage crossings/10 min, were counted from 30 to 40 min after the drug administration and animals returned to their home cages. Exploratory activity in an open field was monitored 60 min after the drug administration. It was scored as the number of squares crossed/5 min, immediately after an animal was exposed to the open field.

2.4. Dose related effects of MPD on water maze performance

2.4.1. Water maze

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

2.4.2. Procedure

The procedure of the water maze test [25] was essentially same as described before [26] and [27]. Thirty-two animals, randomly