Behavioural Brain Research 218 (2011) 206–217

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Age and genetic strain differences in response to chronic methylphenidate administration

Pamela B. Yang[c], Derly O. Cuellar III[a], Alan C. Swann[b], Nachum Dafny[a,b,*]

[a] Department of Neurobiology and Anatomy, The University of Texas-Medical School at Houston, P.O. Box 20708, Houston, TX 77225, USA
[b] Department of Psychiatry and Behavioral Sciences, The University of Texas-Medical School at Houston, P.O. Box 20708, Houston, TX 77225, USA
[c] Department of Anatomy and Neurobiology, University of California, Irvine, School of Medicine, Irvine, CA 92697, USA

ARTICLE INFO

*Article history:*
Received 14 September 2010
Received in revised form
11 November 2010
Accepted 16 November 2010

*Keywords:*
Locomotor
Behavior
Ritalin
Adolescent
Adult
Genetic
Strain

ABSTRACT

Methylphenidate hydrochloride (MPD) is a psychostimulant used in the treatment of attention deficit hyperactive disorder (ADHD) in adolescents and adults alike. Adolescence involves a period of neural development that is highly susceptible to environmental and pharmacological influence. Exposure to a psychostimulant like MPD during this crucial time period may cause permanent changes in neuronal function and formation. Another factor that may influence changes in neuronal function and formation is genetic variability. It has been reported that genetic variability affects both the initial behavioral response to drugs in general and psychostimulants in particular, and subsequently whether tolerance or sensitization is induced. The objective of the present study is to investigate the dose-response effects of repeated MPD administration (0.6, 2.5, or 10.0 mg/kg, i.p.) using an open field assay to investigate if there are differences between adolescent and adult Wistar-Kyoto (WKY), Spontaneously Hyperactive rat (SHR), and Sprague-Dawley (SD) rats, respectively, and if the genetic variability between the strains influences the degree of change in locomotion. The acute and chronic administration of MPD resulted in unique differences in the level of increasing intensity in locomotor activity in each rat strain, with adult rats for the most part having a more intense increase in locomotor activity when compared to their adolescent counterparts. In conclusion, significant response differences among rat strains and age to acute and chronic MPD administration were observed only following the 2.5 and 10.0 mg/kg i.p. doses and not following the lower MPD dose (0.6 mg//kg i.p.). In addition the variability in activity among the rat strain and age suggests that MPD may affect the same neuronal circuit differently in each strain and age. The unique differences among the individual locomotor indices suggest also that each locomotor index is regulated by different neuronal circuits, and each affected differently by MPD.



## 1. Introduction

Attention deficit hyperactive disorder (ADHD), a behavioral disorder, is one of the most commonly diagnosed psychiatric disorders affecting 3–5% of school-aged children in the United States, possibly 17% if subclinical cases are included. ADHD affects 3–5% of adults in the United States, suggesting the disorder persists into adulthood [1,2]. Methylphenidate hydrochloride (MPD), commonly known as Ritalin is one of the most prescribed treatments for ADHD in adolescents and adults alike [3–5]. Its efficacy and safety have been well documented in many studies [6], but there is still a lack of information regarding the influence of MPD on the developing brain and its long-term effects on neuronal function.

In humans, normal neurodevelopment consists of an overproduction of synaptic connections with a subsequent elimination of these synapses by competitive inhibition. Synaptic pruning, usually occurs between 5 and 15 years of age, when the synaptic density of the prefrontal cortex decreases by approximately 40% [7,8]. It is thought that this synaptic reorganization may be a predisposing factor for many behavioral/psychiatric disorders including ADHD [7,9,10]. The treatment of ADHD in children using MPD parallels the timeframe of synaptic pruning, during which environmental and pharmacological influences exert a strong influence on neuronal formation and function [10,11]. Furthermore, rats exposed to MPD at an age approximating human childhood experienced behavioral changes that endured into adulthood, which suggests that MPD has a long-term effect on normal neurodevelopment [10,12]. The response to psychostimulants has been reported to vary with age

* Corresponding author at: Department of Neurobiology and Anatomy, The University of Texas-Medical School at Houston, P.O. Box 20708, Houston, TX 77225, USA. Tel.: +1 713 500 5616; fax: +1 713 500 0621.
*E-mail address:* Nachum.Dafny@uth.tmc.edu (N. Dafny).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.11.034

[12,13], including the ability to reduce impulsivity when administered to adolescents, while having no effect in adults [14]. Therefore it is essential to investigate whether there are age differences in response to acute and/or chronic MPD administration.

Pharmacogenetic research using genetically defined rodent strains has provided insight into the possibility that genetic factors influence drug-related behaviors [15]. Humans as well as experimental animals exhibit considerable individual variability, both in the initial behavioral response to psychostimulants and in the development of tolerance and/or sensitization [15–17]. It has also been reported that different rat strains, comprised of distinct gene pools, show different susceptibility to psychostimulants and a varied chronic response, i.e., tolerance and/or sensitization [14,18–21]. Although the etiology of ADHD is not yet fully understood, there seems to be a strong underlying genetic component, which has been supported by familial, adoptive, and twin studies [22,23]. Furthermore, strain differences in the acute and chronic response to cocaine in the development of behavioral sensitization in mice have been reported [24–26]. However, rat strain comparisons of the acute and chronic effects of MPD are limited. Several animal ADHD models are currently used. The rat strain most commonly used as a genetic model for ADHD is the Spontaneous Hypertensive/Hyperactive rat (SHR), since it mimics several key characteristics found in ADHD patients including: motor hyperactivity, decreased sustained attention, motor and cognitive impulsiveness, increased behavioral variability, and decreased dopamine transmission [27,28]. The SHR strain has been bred from its progenitor the Wistar-Kyoto (WKY) rat [29], which is used as a control to the SHR strain [30]. It is also of importance to compare the effects of MPD with another rat stain that is frequently used in studying the effects of psychostimulants, the Sprague-Dawley rat (SD). This strain has been reported to develop behavioral sensitization after repeated exposure to MPD [12,21,31–36]. In order to better understand the role genetics plays in the treatment of ADHD with MPD, a study using animal models of different strains is required. The objective of the present study is to investigate whether age and genetic variability influences the locomotor response to repeated MPD administration using the open field assay.

## 2. Materials and methods

### 2.1. Animals

A total of 216 male rats were used for this study as follows: SHR ($N = 71$), WKY ($N = 73$), and SD ($N = 72$). The rats were purchased with a postnatal age of 30 days (P-30) for adolescents and 52 days (P-52) for adults. The rats in groups of four per Plexiglas cage were placed in the experimental room for 5–7 days for acclimation. The ambient temperature of the room was maintained at $21 \pm 2\,^{\circ}C$ and a relative humidity of 37–42%. The rats were maintained on a 12:12 h light/dark cycle(light on starting at 05:30 h) with food and water given ad libitum. After 5–7 days of habituation to the experimental room, rats were randomly divided into groups as summarized in Table 1. Then rats were placed individually into automated animal activity monitoring chambers (test cage) for an additional 24–36 h for habituation. This test cage then became their home cage during the subsequent 11 consecutive experimental days, i.e., during the locomotor activity recording (Table 1). At the beginning of the recording the animals were at P-40 (adolescent) and P-62 (young adult). All efforts were made to minimize the number of animal used and their suffering. Housing conditions and experimental procedures were approved by our animal welfare committee. This study was done over three years. Only one age, sex and strain were studied at a time to eliminate additional factors that could affect the recording.

### 2.2. Drugs

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrodt Inc. (Hazelwood, MO), and was dissolved in 0.9% isotonic saline solution at 0.6, 2.5, and 10.0 mg/kg. Syringes containing different dosages were equalized to 0.8 ml by adding saline, to ensure that total volume of the injection did not vary from animal to animal. The injections were administered intra-peritoneally (i.p.) around 07:00 h. The dosages used and times of injection were selected based on previous MPD dose dependent response studies which showed that this time is optimal for eliciting dose dependent tolerance and/or behavioral and electrophysiological sensitization [20,21,31,35,37]. In general, the treatment schedule included an initial saline administration (day 1), followed by 6 consecutive days of MPD administration, then 3 days without any treatment (washout) followed by a rechallenge of MPD on the final 11th day (Table 1).

**Table 1**
Treatment protocol for adolescent and adult rats during the 11 experimental days.

| | | Experimental day | | | | |
|---|---|---|---|---|---|---|
| | | −5 to 0 | 1 | 2 to 7 | 8 to 10 | 11 |
| WKY: Group A | | | | | | |
| Adult | | | | | | |
| $N = 8$ | A 1-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/kg |
| $N = 8$ | A 1-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 8$ | A 1-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | A 1-4 | Habituate | Saline | Saline | Washout | Saline |
| Adolescent | | | | | | |
| $N = 8$ | A 2-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/k |
| $N = 13$ | A 2-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 12$ | A 2-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | A 2-4 | Habituate | Saline | Saline | Washout | Saline |
| SHR: Group B | | | | | | |
| Adult | | | | | | |
| $N = 8$ | B 1-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/kg |
| $N = 8$ | B 1-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 11$ | B 1-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | B 1-4 | Habituate | Saline | Saline | Washout | Saline |
| Adolescent | | | | | | |
| $N = 12$ | B 2-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/kg |
| $N = 8$ | B 2-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 8$ | B 2-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | B 2-4 | Habituate | Saline | Saline | Washout | Saline |
| SD: Group C | | | | | | |
| Adult | | | | | | |
| $N = 8$ | C 1-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/kg |
| $N = 13$ | C 1-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 8$ | C 1-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | C 1-4 | Habituate | Saline | Saline | Washout | Saline |
| Adolescent | | | | | | |
| $N = 8$ | C 2-1 | Habituate | Saline | 0.6 mg/kg | Washout | 0.6 mg/kg |
| $N = 11$ | C 2-2 | Habituate | Saline | 2.5 mg/kg | Washout | 2.5 mg/kg |
| $N = 8$ | C 2-3 | Habituate | Saline | 10.0 mg/kg | Washout | 10.0 mg/kg |
| $N = 8$ | C 2-4 | Habituate | Saline | Saline | Washout | Saline |

The experimental protocol from the date the animals was purchased, i.e., experimental day −5 to 0, through the recording and administration schedule. On experimental day 1 all animals were injected with saline, on experimental day 2–7 they were injected with 0.6 mg/kg methylphenidate (MPD) (subgroup 1), 2.5 mg/kg MPD (subgroup 2), 10.0 mg/kg MPD (subgroup 3), or saline (subgroup 4), all injections were administered intra-peritoneally around 07:00 h. Three animal strains were used: Wistar-Kyoto rat (WKY), Spontaneously Hyperactive rat (SHR), and Sprague-Dawley rat (SD).

### 2.3. Apparatus

An open field monitoring system was used to record locomotor activity during the duration of the experiment. Testing chambers consisted of a clear acrylic box (40.5 cm × 40.5 cm × 31 .5 cm) fitted with two sets of 16 infrared motion sensors located 6 and 12 cm above the floor (AccuScan Instruments, Inc., Columbus, OH). Briefly, the system records activity by counting the number of interruptions in the beams at a frequency of 100 Hz. The interruption of any beam was transformed it into an activity score. Cumulative counts of scores were compiled and downloaded every 10 min into the OASIS data collection software and organized into various locomotor indices. Total distance traveled (TD), measured the total amount of forward movement in centimeters during a given period; horizontal activity (HA), recorded the total number of beams interrupted at the horizontal sensor (lowest tier) level during a given period a measure of the overall activity; vertical activity (VA) measured the total number of beams interrupted at the vertical (highest tier) level during a given period, which mainly represented rearing; number of stereotypic movements (NOS), measured the number of repetitive purposeless episodes with at least a 1 s interval before the initiation of another episode, which could represent general stereotyped behaviors, such as sniffing and grooming [20,21,31,37–39]. This system has been previously described in detail [21,31,40–42].