

Full Length Research Paper

Methylphenidate effects on search strategy of an animal model of attention-deficit/ hyperactivity disorder

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This study is aimed at investigating the effects of methylphenidate about search strategy use on the spontaneously hypertensive rat, an animal model of attention-deficit/hyperactivity disorder in the Morris water maze. The methylphenidate group rats were given intraperitoneal injection of methylphenidate (10 mg/kg) dissolved in saline 30 min before daily training for 6 consecutive days. In the same way, the model group and the control group rats were only administered equalized volume of saline, respectively. From the second day of trials, the frequency of using tendency-straight strategy to search the hidden platform was significantly increased in all of the training rats. Additionally, the methylphenidate group rats used straight strategy more frequently than the model group rats did on days 1, 3 and 6, respectively. The data indicated that methylphenidate might play a positive role in improving spatial learning ability of the spontaneously hypertensive rat by adding its straight search strategy use in the Morris water maze.

Key words: Methylphenidate, spontaneously hypertensive rat, Wistar Kyoto rat, behaviour, search strategy.

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a clinically heterogeneous neuropsychiatric syndrome. Its symptoms such as inattention, hyperactivity, impulsivity and learning deficit cause impairment to academic performances and intellectual function of children (Faraone et al., 2003), and cognitive impulsiveness often makes them to show forgetfulness (Sagvolden, 2000).

With stimulant treatment for children starting in the 1960s, ADHD have been treated with medications such as methylphenidate and so on for over half a century, which serve to alleviate symptoms and to improve ADHD children performances in school (Solanto, 1998). Today, methylphenidate is still widely used in developed and developing countries (Scheffler et al., 2007) despite the emergence of new non-stimulant drugs; however, the effects of methylphenidate on behaviour and learning are not yet fully understood. Search strategies have been used to analyze the performance of rats in the Morris

water maze (MWM), which is a useful tool to assess learning and memory ability in rodent models (Vorhees and Williams, 2006). Based on rats' trace images, strategies are often grouped into categories including spatial strategies, repetitive looping strategies and non-spatial strategies, and spatial strategies are considered to be the most efficient method of finding the hidden platform in the MWM (Brody and Holtzman, 2006; Janus, 2004). Additionally, another search strategy classification includes straight, tendency, marginal and random strategies (Jiang et al., 2004).

Although some water maze research has been done on the spontaneously hypertensive (SH) rat (Prediger et al., 2005; Clements and Wainwright, 2006; Pamplona et al., 2009), a genetic animal model for ADHD, because of the similar behavioural problems it shows and deficits in learning and memory as well (Davids et al., 2003; Meneses et al., 2011; Sagvolden, 2000); little is known about the

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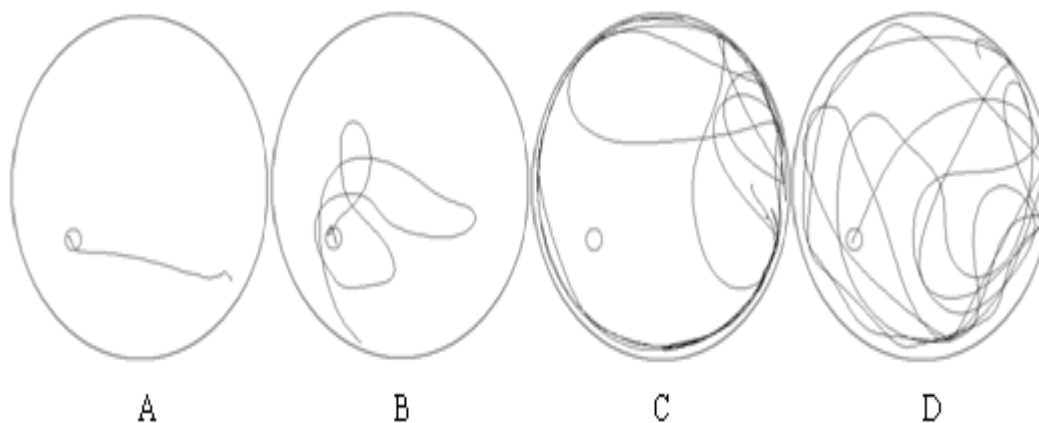


Figure 1. Search strategies used by rats in locating the hidden platform in the Morris water maze. Representative traces of male spontaneously hypertensive rats and Wistar Kyoto rats are shown for (A) Straight strategy, (B) Tendency strategy, (C) Marginal strategy, and (D) Random strategy in the Morris water maze training.

about the effect of methylphenidate on strategy. Therefore, the present study is designed to focus on the effect of methylphenidate on the SH rat's search strategy in place navigation test of MWM.

MATERIALS AND METHODS

Animals

Male SH rats ($n=16$) and their controls: Wistar Kyoto (WKY) rats ($n=8$) (Okamoto and Aoki, 1963) of 7-week-old (Slac Laboratory Animal Co. Ltd., Shanghai, China), were used for this experiment. All the rats were kept four per cage (measuring $480 \times 355 \times 195$ mm) for 6 days acclimation in an experimental room with a constant relative humidity ($50 \pm 5\%$) and ambient temperature ($23 \pm 1^\circ\text{C}$). They were maintained on a 12:12 h light: dark cycle (7:00 a.m. to 7:00 p.m.), and had free access to food and water.

Apparatus

The maze consisted of a cylindrical, black painted pool, 130 cm in diameter and 60 cm deep. The apparatus was full of water to a depth of 25 cm, with a temperature of $23 \pm 1^\circ\text{C}$. A black circular escape platform, 10 cm in diameter, was located 1 cm below the water surface, placed in one of four equal-size quadrants, 40 cm away from the wall of the pool. The position of the pool and the extra maze cues were maintained unchanged throughout the study. The position of the rat in the pool was automatically recorded by a video tracking/computer digitizing system (Sunny Instruments Co. Ltd., Beijing, China).

Study design

The SH rats were randomly divided into two groups (each $n=8$): a model group (MG) and a methylphenidate hydrochloride group (MPDG). The MPDG rats were given intraperitoneal injection of methylphenidate (10 mg/kg, First Suzhou Pharmaceutical Co. Ltd., Suzhou, China) dissolved in saline (0.9% NaCl) 30 min before daily training for 6 consecutive days. The WKY rats were used as a

control group (CG). In the same way, the MG and CG rats were only administered equalized volume saline, respectively. All procedures were performed in accordance with Xi'an Jiaotong University guidelines for animal research.

All the rats were allowed to swim 120 s in the pool containing no platform for habituation one day before the experiment. In the following 6 days, the rats were trained to find the hidden platform, which was the only escape from the water. They were given four trials on each day with 15 min intertrial intervals. In each trial, the rat was placed gently in the water facing the pool wall at one of the four starting points (in a random order). If the rat failed to locate the escape platform within 120 s, the experimenter would guide it to the platform where they were allowed to stay for 10 s.

According to swimming traces of rats' locating the hidden platform, the following four search strategies were recorded, including straight (Figure 1A), tendency (Figure 1B), marginal (Figure 1C) and random (Figure 1D). Draw an imaginary straight line from the point the rat was placed into the water to the midpoint of the platform and treat it as the maze's axis. If the distance of each point of a rat's trace from the axis does not exceed 25% of the diameter of the maze, it indicates that the rat formed reference cognition according to the outside fixed clues and the straight strategy which the rat used is considered to be the most efficient method of finding the platform. Essentially different from the random strategy and the marginal strategy, the tendency strategy is similar to the straight strategy; however, the scope of the trace is within 55% of the diameter of the maze. With using the tendency strategy, a rat will not be able to accurately determine the location of the target, but it knows the direction of it. The marginal strategy reflects instinctive behaviour and the tracks of rats usually appear in the edge of the pool; and the random strategy, which is thought to be a blind movement, is used by rats that presented themselves in the most areas of the maze (Jiang et al., 2004).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 13.0 statistical software (Spss Inc., Chicago, IL, USA). The differences of the search strategies used by the rats among the groups were determined by Mann-Whitney non-parametric test. Significance was assumed at $P < 0.05$, 0.01 or 0.001.

RESULTS

Search strategies findings

With the experiment carried out, each group using the straight or the tendency strategy was gradually increasing in percentage. From the beginning to the end of the training, the MPDG rats decreased their use of the random strategy from 56.3 to 0%, increased their use of the tendency strategy from 28.1 to 50% and the straight strategy from 15.6 to 50% (Figure 2B). During the training of the MG, the random strategy use was decreased from 62.5 to 6.2%, meanwhile, the tendency strategy use was increased from 31.2 to 68.8%, and the straight strategy use was also increased from 3.1 to 25% (Figure 2A). As for the CG rats, they decreased their use of the random strategy from 56.2 to 6.2%, increased their use of the tendency strategy from 25 to 71.9% and the straight strategy from 3.1 to 21.9%. The marginal search strategy was mainly seen in the 1st day training of the WKY rats (Figure 2C).

Straight strategy findings

Overall, we could see that the MG rats used the straight strategy with a higher percent as compared to the CG ($P=0.006<0.01$); meanwhile, the MPDG rats used the same strategy with a higher percent as compared to the MG rats ($P<0.001$) during the whole training. Specifically, the MPDG rats used the straight strategy more frequently than the MG rats did on day 1 ($P=0.002<0.01$), day 3 ($P=0.002<0.01$) and day 6 ($P<0.001$), respectively, however, daily comparisons were not significant between the MG and the CG.

Effective strategy findings

Since the straight strategy and the tendency strategy are similar but apparently different from the rest of the two strategies, they were regarded as the effective strategy as compared to the non-effective strategy which included the marginal strategy and the random strategy for further analysis in this study. The results showed that the tendency-straight strategy used from days 2 to 6 was significantly increased as compared to that used on day 1 in the MG ($P_{\text{day}2}=0.047<0.05$; $P_{\text{day}3,4,5,6}<0.001$, respectively), the MPDG ($P_{\text{day}2}=0.001<0.01$; $P_{\text{day}3}=0.001<0.01$; $P_{\text{day}4,5,6}<0.001$, respectively) and the CG ($P_{\text{day}2}=0.024<0.05$; $P_{\text{day}3}=0.001<0.01$; $P_{\text{day}4,5,6}<0.001$, respectively), respectively (Figure 3).

DISCUSSION

As a first-line drug for ADHD, methylphenidate has been

clinically effective in treating the symptoms of the disorder for decades, which are believed to enhance neurotransmission of dopamine and norepinephrine (Spencer, 2004). However, the effects of methylphenidate on behaviour and learning are far from clear. In the MWM hidden platform training, animals can not directly see the platform, so they must perform a search for it until they find the platform to escape from the maze. Accordingly, the SH rat, a validated rat model of ADHD, was used to make investigations on the search strategy in the MWM in the present study.

In view of previous behavioural studies of learning and memory including testing in the water maze, SH rats exhibited a better, poorer or similar performance relative to WKY rats (Ferguson and Cada, 2004; Gattu et al., 1997; King et al., 2000; Wyss et al., 2000). In this study, the data showed that the MG rats and the CG rats had a significant difference in percentage on straight strategy use. Overall, the SH rats seemed better than the WKY rats on accurate target location, but their daily comparisons were not of statistical significance. Although, some WKY rats tends to choose the marginal strategy in the beginning of the training, using tendency-straight strategy was also gradually increasing in frequency over 6 days of trials as well as the SH rats. The two male rat strains showed their improved search ability respectively with the experiment carried out, suggesting a more efficient approach to find the target.

It is well known that the MWM is mainly used to evaluate the hippocampal cognitive function in rodents. A recent study investigated the effects of methylphenidate on cell proliferation and neuronal differentiation in the hippocampus. Among 2.5, 5 and 10 mg/kg, they observed that the numbers of neurons were significantly increased only in the 10 mg/kg methylphenidate group as compared to the vehicle group (Lee et al., 2012). In this study, it was also found out that the SH rats treated with 10 mg/kg methylphenidate chose less random strategy and more straight strategy to locate the hidden platform in the experiment, which suggests that the search strategy used by the MPDG rats might be considered more efficient than that used by the MG rats. However, another study showed that testing in a water maze task revealed no significant effects of any dose including 10 mg/kg of methylphenidate on learning (McFayden et al., 2002). The aforementioned different results may be due to the differences in animal species, medicine administration, etc.

In this study, methylphenidate might improve hippocampal functions including learning and memory. Sometimes the rats were far from accurate target location, but they had less blind movement more quickly, their looking for the hidden platform ability was significantly improved. Thus, we conclude that methylphenidate might play a positive role in improving spatial learning ability of the SH rats by adding its straight search strategy use in MWM test.

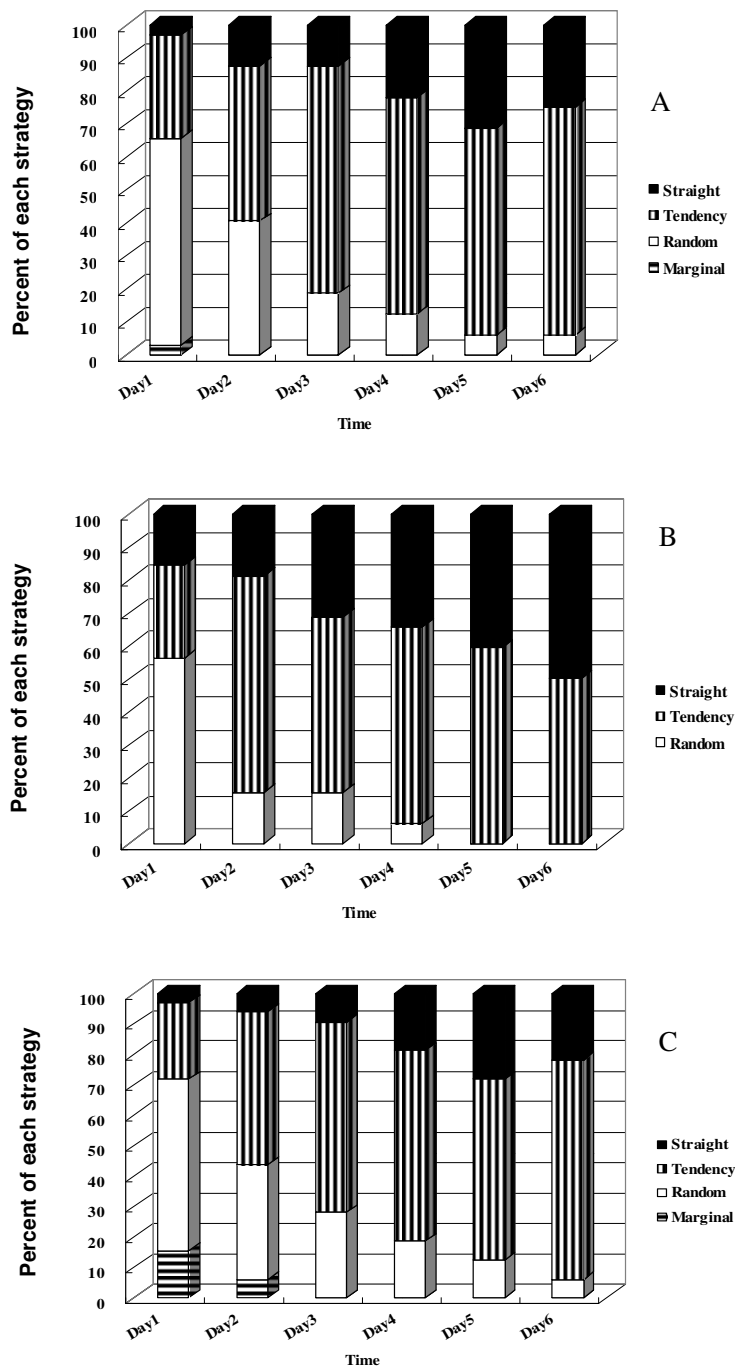


Figure 2. Percentage of each search strategy used by each group over 6 days of trials. Representative traces were shown for straight strategy (black), tendency strategy (vertical stripe), random strategy (white), and marginal strategy (horizontal stripe) in the (A) Model group, (B) Methylphenidate group, and (C) Control group, respectively. During the training in the Morris water maze, all the rats were more and more in favour of using the straight strategy and the tendency strategy as well to search the hidden platform. The MG used the straight strategy with a higher percent compared to the CG ($P < 0.01$), meanwhile, the MPDG used the same strategy with a higher percent as compared to the MG ($P < 0.001$) during the whole training. Specifically, the MPDG rats used the straight strategy more frequently than the MG rats did on day 1 ($P < 0.01$), day 3 ($P < 0.01$) and day 6 ($P < 0.001$), respectively, however, daily comparisons were not significant between the MG and the CG.

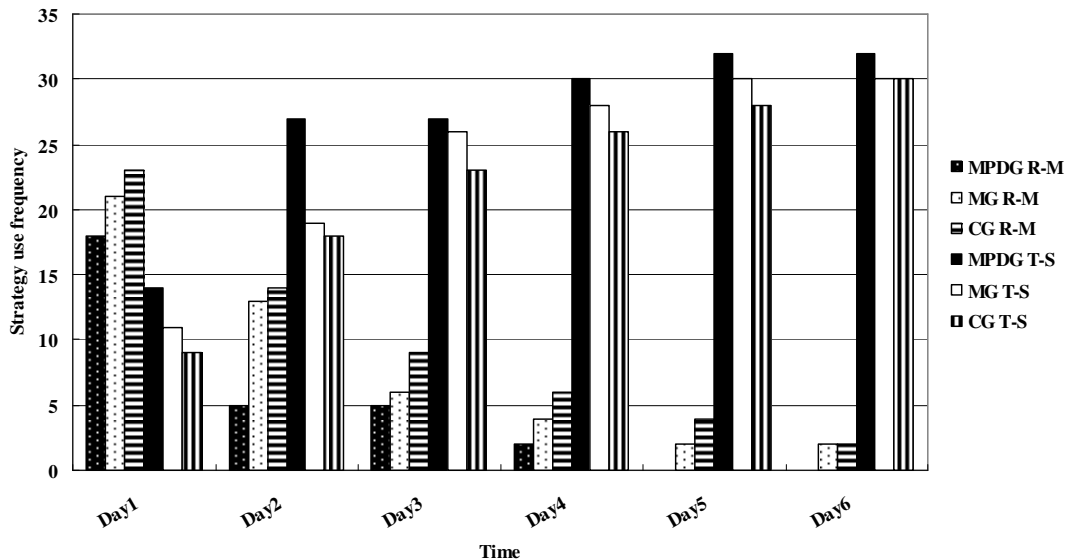


Figure 3. Frequency of the random-marginal strategy and the tendency-straight strategy used by each group over 6 days of trials.

Traces were shown for the R-M strategy (black with white dots) and the T-S strategy (black) in the MPDG; the R-M strategy (white with black dots) and the T-S strategy (white) in the MG; the R-M strategy (horizontal stripe) and the T-S strategy (vertical stripe) in the CG, respectively. From the 2nd day of training, the frequency of the T-S strategy was significantly increased in the three groups, respectively ($P < 0.05$). R-M: Random-marginal; T-S: tendency-straight; MPDG: methylphenidate hydrochloride group; MG: model group; CG: control group.

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ABBREVIATIONS

ADHD, Attention-deficit/hyperactivity disorder; **SH**, spontaneously hypertensive; **WKY**, Wistar Kyoto; **MWM**, Morris water maze; **MG**, model group; **MPDG**, methylphenidate hydrochloride group; **CG**, control group; **T-S**, tendency-straight; **R-M**, random-marginal.

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