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SHORT- AND LONG-TERM EFFECTS OF METHYLPHENIDATE ON COST-BENEFIT DECISION MAKING IN ADULT RATS

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Decision making is one of the most complicated and controversial topics in neuroscience. Today, there are important classes of chemicals that increase cognitive performance; in particular these are psychostimulants (e.g., methylphenidate, MPH). However, long-term effects of MPH on a cost-benefit decision making in healthy animals remain unknown. Therefore, we aimed to compare the short- and long-term effects of MPH in adult healthy male rats on the decision in two distinct T-maze tasks, the ability of animals to adjust the height of an obstacle in a T-maze or to process information on the reward amount. We found that sort-term effects of MPH (2 weeks) played a significant role in making the correct decision in T-maze tasks, while the respective effects of long-term administration (12 weeks) were much weaker. These data suggest that chronic application of MPH has short- but not long-term effects on cost-benefit decision making in healthy adult animals.

Keywords: methylphenidate (MPH), T-maze, cost-benefit decision making, cognitive performance.

INTRODUCTION

Understanding of the mechanism due to which we prefer a particular choice among a set of options, and also of how different factors can influence that decision, is one of the important topics of cognitive neuroscience. An appropriate choice may increase the likelihood of survival and improve the quality of life. The worth or the quality of a number of benefits must be evaluated before decision making, including the likelihood of an event occurring, the time taken for it to occur, and the effort required to obtain success [1]. Animal models of such physical effort-based decision making may help to interpret central neural mechanisms involved in recruiting a physical attempt. It is well accepted that methylphenidate (MPH, Ritalin) improves cognitive abilities (e.g., attention, decision making, and working memory) in the attention deficit/hyperactivity disorder (ADHD); it also produces comparable positive effects in animals and healthy humans [2-4]. Some studies reported that MPH increased dopamine and noradrenaline concentrations, primarily in the prefrontal cortex [5, 6]. Additionally, some evidence was accepted that the prefrontal cortex plays an important role in decision making [7-9]. However, long-term effects of MPH on the adult brain are far from being clear. To our knowledge, there are no or few direct evidence to identify the short-term and long-term effects of chronic administration of MPH on cost-benefit decision making in healthy adult rats.

The aim of our study was to elucidate whether MPH treatment can modulate the sensitivity of adult intact rats to differences in the effort requirements when making decisions. For such purpose, we assessed the short-term and long-term effects of MPH application on the ability of animals to adjust the height of an obstacle in a T-maze or to process information on the reward amount.

METHODS

Animals. Twenty-seven male Wistar rats (250-300 g) purchased from the Pasteur Institute (Tehran, Iran) were used in our experiments. The rats were grouped in three per cage with free access to food and water, except the times that the food amounts were changed

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according to the phase of our experiment. Lightening in the animal colony was maintained on a 12/12 h light/dark schedule with light on at 7:00.

T-Maze Apparatus. The rats were tested in an evaluated T-maze based upon the original apparatus described by Walton et al. [10]. The apparatus consisted of a start arm and two goal arms (60 cm length, 10 cm width, and 30 cm height). A food well was placed at the end of each goal arm. In forced trials, a barrier was used to prevent the animal from entering one goal arm. The barriers that the rat had to climb were constructed of wire mesh with a right-angled triangle. The rats had to climb the vertical face of the triangle and to go down at a 45 deg angle to attain the reward. The height of the barriers was increased during training from 15 to 40 cm.

Habituation. Rats were habituated to the T-maze during four days. On these days, the animals were placed in the start arm and were allowed to explore the maze for 20 min. Plentiful food (50 mg food pellets) was left in both feeding wells in the goal arms.

Discrimination Training. Discrimination training consisted of three phases. The first phase involved putting eight pellets in the feeding well of the high-reward arm (HR) and two pellets in the low-reward arm (LR). For half of the rats, the HR arm was to the left, while for the other half it was to the right. This side destination was maintained throughout the remaining training and test trials. Each rat in this phase received five trials per day for two days. The trial ended when the rat had eaten from both food cups, or 150 sec elapsed. Each trial began with placing the rat at the beginning of the start arm, and the animal was allowed to sample both food arms on each trial. In phase 2 of discrimination training, each rat received 10 trials per day for two days, and also the access to one of the goal arms was prevented by placing a wooden block at the entrance (forced trials), thus forcing the rat to sample a particular arm on each trial. Rats were forced into the HR or LR arm five times. They were not forced into the same arm more than two times in a line. The experiment finished after the rats ate from the food cup, or 150 sec over and done. In the third phase, each rat received 10 trials per day for three days. On trials 5 and 10, admission to the previously selected arm was blocked with the box in order to avoid rats from adopting a side bias. The experiment lasted immediately after the rat ate the food from the cup, or 150 sec over. Rats were investigated in this phase for three days (Table 1). Within the final day of this phase, all animals selected the HR arm on more than 90% of the occasions during the training session.

Barrier Training. During barrier training, the first barrier (15 cm) was placed in the HR arm. The height of the barrier was increased by 5 cm every three days up to 30 cm. Animals received five trials per day. On the first trial of the first day, the trial lasted only after the rat had climbed the barrier and eaten the pellets, or 300 sec over. On the last four trials of the first day and all remaining trials, the experiment ended immediately after the rat selected one of the arms and consumed the pellets, or 150 sec elapsed.

Drug Treatment. MPH was obtained from Novartis (Great Britain). Our study was performed in four groups (9 animals in each group); control, sham, MPH + + 2-week latency (2WL), and MPH + 12-week latency (12WL). MPH was gavaged (10 mg/kg) twice a day over 11 consecutive days and then ceased. The dose of drug administration was based on prior studies [3]. A behavioral study started 2 weeks after cessation of MPH in the 2WL group, to study the short-term effects of chronic intake of MPH on the cost–benefit task in the T-maze, and also 12 weeks after cessation of that in the 12WL group for evaluation of the long-term effects of MPH. There was no manipulation in the control group. Sham animals received only normal 0.9% saline (0.5 ml) instead of MPH twice a day for 11 days.

Experimental Design. Six experiments were designed to evaluate the sensitivity of animals to differences in the height of the barriers and also to the reward amount. In experiment 1, a 30-cm-high barrier was placed in the HR arm for evaluation of effort-based decision making of intact rats to obtain a high reward with such a barrier. Each animal then ran 10

Table 1. Time Lines of Behavioral Manipulation

Таблиця. Часові межі маніпуляцій із поведінковими факторами

Habituation	Discrimination training	Barrier training			MPH treatment	Experiments
4 days	7 days	3 days	3 days	3 days	11 days and cease of treatment on day 12	Group 2W: 2 weeks after cease of MPH; group 12W: 12 weeks after cease of MPH

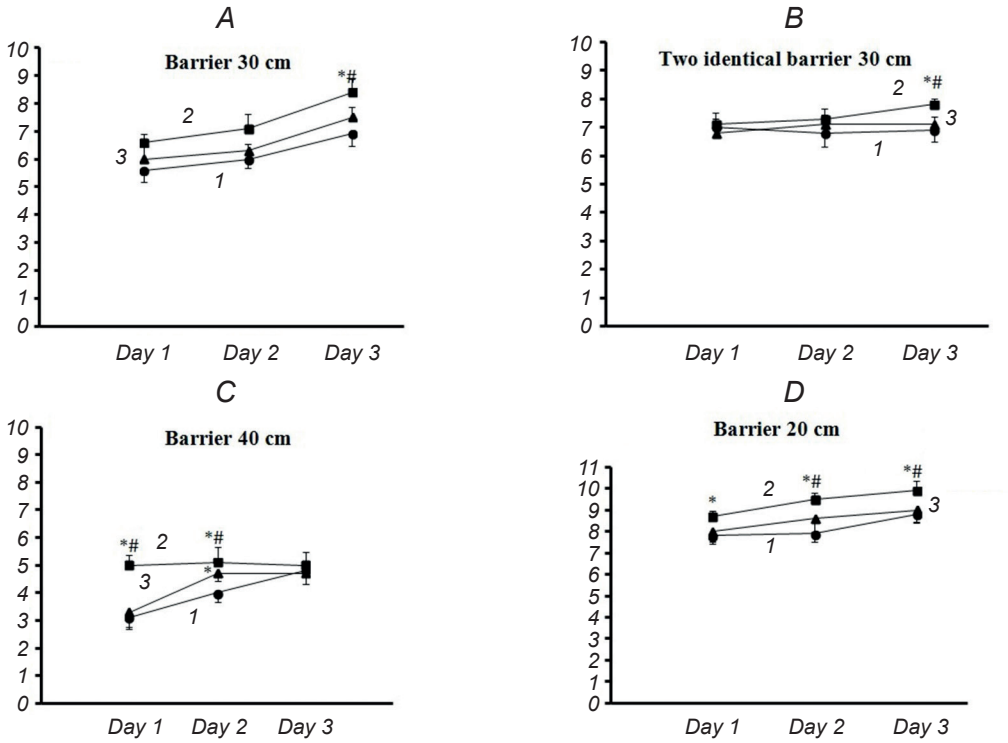
choice trials each day for three days. In experiment 2, two identical barriers (30 cm) were placed in both HR and LR arms. This test was performed to evaluate whether the animal can distinguish the HR arm from the LR, or habituation for the 30-cm barrier can be induced. In the experiment 3, the 30-cm barrier was replaced by a 40 cm one to evaluate whether increasing of the height of the barrier would increase the effort of the animal to gain a high reward. In the experiment 4, the 40-cm barrier was replaced by the 20-cm one, to evaluate whether decreasing of the barrier height would decrease the effort of animal to gain the above reward. Experiment 5 was designed to evaluate whether decreasing of the award would have some effect or not. Therefore, the reward ratio was then changed by four pellets in the HR arm and two in the LR one, and also the 30-cm barrier was placed in the HR arm. In experiment 6, four pellets in the HR arm and two in the LR one were placed, and also the 30-cm barrier was placed in the HR arm. Each experiment was conducted each day over a period of three days.

Statistics. All data were analyzed by SPSS software using one-way analysis of variance (ANOVA) and the Tukey's test as the post test. Results were expressed as means ± s.e.m., and intergroup differences were considered significant at $P < 0.05$.

RESULTS

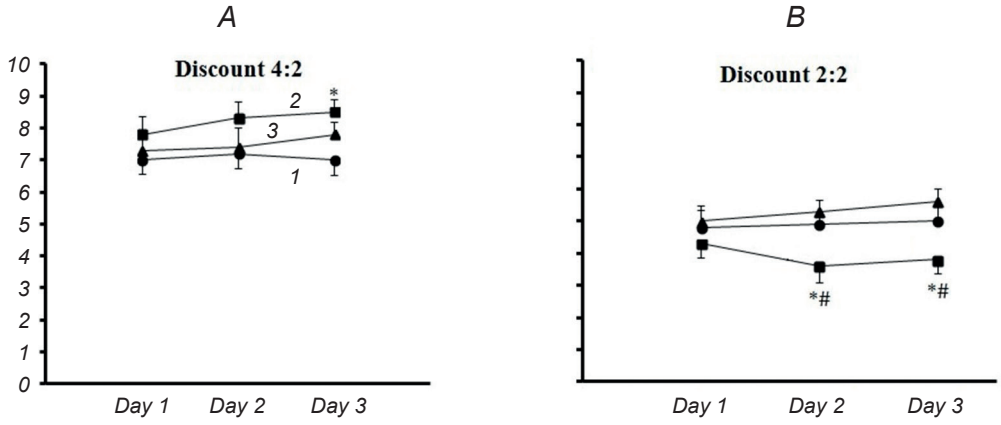
Experiment 1. As is shown in Fig. 1A, chronic administration of MPH significantly increased the number of HR selection only two weeks after cessation of that on day 3 of the experiment with the 30 cm barrier ($P < 0.05$), as compared with the control and MPH + 12WL groups. This increase of HR selection returned to the control level after 12 weeks after cessation of MPH treatment.

Experiment 2. As is shown in Fig. 1B, two identical barriers (30 cm) placed in both HR and LR arms significantly shifted the animals' choices for the HR selection only on day 3 of the experiment in the



F i g. 1. Effects of MPH on the number of high-reward (HR) arm selection with different sizes of the barriers in experiments 1 to 4 (A-D, respectively). 1, 2, and 3) Animal groups, control, MPH 2W, and MPH 12W (see the text), respectively. Each point represents the mean ± ± s.e.m. (9 rats per group). * indicates a significant difference ($P < 0.05$) from control animals. # indicates a significant difference ($P < 0.05$) between the treatment groups.

Р и с. 1. Впливи метилфенідату на кількість виборів «вірного» розгалуження лабіринту (з високою харчовою винагородою) при різних висотах бар'єрів у експериментах 1–4 (*A–D* відповідно).



F i g. 2. Effects of MPH on the number of high-reward (HR) arm selection with different ratios of the rewards in experiments 5 and 6 (A and B, respectively). Other indications are the same as in Fig. 1.

Р и с. 2. Впливи метилфенідату на кількість вибору «вірного» розгалуження лабіринту з різними кількостями винагороди в розгалуженнях в експериментах 5 та 6 (*A* та *B* відповідно).

group MPH + 2WL ($P < 0.05$), as compared with the control and MPH + 12WL groups. Therefore, chronic administration of MPH was unable to induce any significant change in the number of HR selection (in the group MPH + 12WL).

Experiment 3. The replacement of the 30-cm barrier by a 40-cm one in the HR arm produced the significant effort in the animals two weeks after cessation of MPH treatment ($P < 0.05$) to gain a high reward on days 1 and 2 of the experiment compared with the control and MPH + 12WL groups (Fig. 1C). Additionally, the replacement of the barrier evoked the significant effort in animals of group MPH + 12WL ($P < 0.05$) to gain the high reward on day 2 of the experiment compared with the control group (Fig. 1C).

Experiment 4. The replacement of the 40 cm barrier with a lower (20-cm) one in the HR arm decreased the effort of animals of the MPH + 2WL group ($P < 0.05$) to gain the high reward on all three days of the experiment, as compared with the control and MPH + + 12WL groups (Fig. 1D). However, the replacement of the barrier could not produce any significant changes in the number of HR selection in the MPH + + 12WL group, as compared to the control group, in all days of the experiment (Fig. 1D).

Experiment 5. When the ratio of food pellets were changed from 8:2 to 4:2, this caused the significant increase ($P < 0.05$) in the number of HR selection in the MPH + 2WL group only on day 3 of the experiment, as compared with the control group (Fig. 2A). Additionally, there was no difference in the performance between the 12WL and control groups.

Experiment 6. When the ratio of food pellets was changed from 4:2 to 2:2, this resulted in the significant decrease ($P < 0.05$) in the number of HR selection in the MPH + 2WL group on days 2 and 3 of the experiment, as compared with the control and MPH + + 12WL groups (Fig. 2B). Additionally, there was no difference in the performance between the 12WL and control groups.

There were also no significant differences between the control and sham groups in all experiments.

DISCUSSION

The main finding of our study was that exposure to MPH exerts only relatively short-term effects on the cost-benefit decision making in adult male intact rats. MPH is a psychostimulant; it is currently abused by adolescents and students when they have exams or need to stay awake for a long time [11]. The effect of MPH on decision making has been studied before [12], but short- and long-term consequences of chronic MPH intake on cost-benefit decision making, especially in the mature brain, still remain unclear.

It is highly likely that the prefrontal cortex provides an important contribution in the performance of behavioral tasks and also in decision making [7, 8]. Developing of the prefrontal cortex in humans continues at least until young adulthood [13]. Although, several studies evaluated the effects of

MPH on attention in ADHD humans and rats [4, 14, 15]. Our study differed from the above ones by special attention to the short- and long-term effects of MPH on effort-based decision making in healthy adult animals. Here, we administered relatively high doses of MPH (10 mg/kg) for such rats over 11 days and then stoped the treatment. Then we evaluated the MPH effect on the cost-benefit decision making on two and twelve weeks after cessation of MPH application. We found that, after two weeks following cessation of chronic MPH treatment, adult rats significantly adjusted their attempts with the height of the barrier and chose the HR arm more frequently than control animals. However, MPH did not endure changes in the cost-benefit decision making about the barrier height within a remote period after cessation of chronic treatment with this drug (12 weeks), as compared with the control group.

Moreover, our behavioral study demonstrated that MPH exerted an increasing effect on the number of HR selection when the ratio of food pellets was changed from 8:2 to 4:2. This increasing effect of MPH was observed 2 weeks after cessation of chronic treatment and nearly disappeared after 12 weeks (Fig. 2A). However, when the ratio of food pellets changed from 4:2 to 2:2, MPH began to exert a depressing effect 2 weeks after treatment cessation as compared with the control rats, but returned to the control level after 12 weeks.

Some studies emphasized the enhancing effects of MPH on the healthy brain. For example, Berridge et al. [5] showed that introduction of high MPH doses (5–10 mg/kg, i.p.) increased locomotor activity and impaired attention and performance of the prefrontal cortex-dependent cognitive skills in rats. At the same time, administration of low doses of MPH (0.25–1.0 mg/kg, i.p.) in normal adult rats enhanced the performance of attention-related tasks. Controversially, our behavioral study revealed that oral application of high doses of MPH (10 mg/kg) in normal adult rats facilitated the performance and increased attention to the barrier height in the HR arm and/or amount of reward. Moreover, this increasing effect of MPH on attention to the height of the barriers in the HR arm was manifested only for a relatively short time (2 weeks) after treatment cessation.

It seems that there is certain age-dependent difference in MPH actions on attention, in particular between healthy juveniles and adult individuals. For example, Urban et al. [16] showed that a single low MPH dose (1 mg/kg, i.p.) significantly decreased the neuronal excitability in the prefrontal cortex of juvenile rats. Chronic treatment with that for three weeks led to further decrease, while the same low dosage exerted excitatory effects in adult rats. Additionally, chronic treatment with 3 and 9 mg/kg resulted in depression of prefrontal neurons lasting for 10 weeks. Our behavioral experiments, however, revealed that chronic treatment with MPH (10 mg/kg) increased attention in healthy adult rats. It is likely that other regions of the brain (in addition to the prefrontal cortex) have also contribution to the attention increase. Canese et al. [13] examined responses of adolescent and adult rats to MPH using an MRI technique. They revealed age-related differences in neuronal activation patterns following acute introduction of MPH in both age groups of the rats. They reported that MPH (4 mg/kg, i.p.) increased the blood oxygenation level-dependent signals in the *nucl. accumbens* and prefrontal cortex of adult rats. In contrast, the same MPH dose reduced the above signals in both above-mentioned cerebral structures of adolescent rats, suggesting that neurological effects of pharmacological manipulations differ between adolescent and adult animals.

Thus, our study demonstrated that facilitatory effects of chronic MPH treatment on cost-benefit decision making in healthy adult rats remain during relatively short terms after cessation of such treatment. However, exposure of adult rats to MPH exerts no enduring effects on the attention level.

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All procedures were performed in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee (Shahid Beheshti University of Medical Sciences, Tehran, Iran; 194/-90/3/18; 2011).

The authors of this study, S. Daniali, H. Manaheji, V. Nazemian and M. Taheri, confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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КОРОТКО- ТА ДОВГОТРИВАЛІ ВПЛИВИ ВВЕДЕННЯ МЕТИЛФЕНІДАТУ НА ПРИЙНЯТТЯ РІШЕНЬ У СИТУАЦІЇ ОЦІНКИ «ВИГОДА/ВИТРАТИ» У ЩУРІВ

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Р е з ю м е

Механізми прийняття рішення є однією з найбільш складних проблем у нейронауках. На сьогодні існують значні класи фармакологічних агентів, котрі посилюють когнітивну діяльність; зокрема, це психостимулятори (наприклад, метилфенідат – МРН). Довготривалі впливи введення МРН на прийняття рішень у ситуації оцінки «вигода/витрати» в модельних експериментах на здорових тваринах поки що залишаються невивченими. Отже, ми порівнювали короткота довготривалі впливи курсового введення МРН здоровим дорослим самцям щурів на два аспекти прийняття такими тваринами рішень у різних тест-завданнях у Т-подібному лабіринті. Розглядали здатність тварин оцінювати висоту перешкоди в лабіринті та процес обробки інформації щодо кількості харчової винагороди. Було виявлено, що короткотривалі ефекти введення МРН (через два тижні) відігравали істотну роль у прийнятті правильного рішення при тестуванні в Т-подібному лабіринті, але такі ефекти ставали неістотними при довготривалому введенні агента (12 тижнів). Таким чином, слід вважати, що курсове введення МРН забезпечує лише короткотривалі (але не довготривалі) впливи на прийняття рішень у ситуації оцінки «вигода/витрати» у здорових дорослих тварин.

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