Chinese Journal of Physiology 41(1): 53-58, 1998

The Effects of Methylphenidate and Maturation on Exploratory Activity in Rats

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Abstract

Treatment of attention-deficit hyperactivity disorder with stimulants such as methylphenidate reduces motor activity and improves performance of tasks requiring attention, learning, and memory. The present study reports the patterns of behavioral activity of rats of different ages, and the effect of methylphenidate on the behavioral activity. The behavioral activity of Wistar male rats was measured on the nine hole-board apparatus. In experiment I, the behavioral activity of rats from three age groups (4, 8 and 12 weeks old) were measured in term of the activity time, specific exploratory behavior, diverse exploratory behavior and defecation number. The rats were re-exposed to the hole-board again every two weeks until they 14 weeks old. The younger rats showed higher activity level compared to the older rats. The activity level decreased as the rats grew older. The younger rats also showed more diverse exploratory behavior, but less specific exploratory behavior compared with the older rats. These suggested that the younger rats may be more hyperactive in nature, and less prone to focus on the specific targets. In experiment II, the methylphenidate (4 mg/kg, i.p.) injected rats showed higher activity level than the controls across the three age groups. The exploratory behavioral patterns were not significantly different among the three age groups. This suggests that the methylphenidate injection raises the motor activity level without affecting the exploratory tendency of rats.

Key Words: hole-board task, hyperactivity, exploratory behavior, methylphenidate

Introduction

Attention-deficit hyperactivity disorder (ADHD) is characterized by a history of impulsivity, hyperkinesis, short attention span, and poor peer relations (1, 20). Treatment of ADHD with stimulants such as methylphenidate reduced motor activity, improved performance of tasks require attention, learning, and memory (1, 20). However, the stimulant itself is an excitatory substance that causes tremendous changes in the locomotion of the individual (10, 17, 27). Thus, this paradoxical effect of stimulant treatment in the alleviation of ADHD syndromes suggests that the hyperactive children have a central neurophysiological disturbance involving catecholamine neural system for which stimulant drugs specifically compensate, thereby accounting for a behavioral response to these drugs that would have been unexpected in normal children and adults (2, 25). Another theory has been proposed suggesting that hyperactive children are characterized by lower reticular formation activity and consequent decreased control of sensory function and motor outflow (23, 24).

The evidences of the importance of catecholamines in learning and memory (30) had prompted much research on the effects of stimulant drugs on conditioned behavior in animals (9, 14, 31). Reports showed that amphetamine markedly increases the rate of pleasurable self-stimulation in rats. Although the exact effect of methylphenidate is not as well investigated as amphetamine, but presumably it may modulate the learning and memory processes as amphetamine does. A recent study has high-lighted the effect of methylphenidate on reward strength of the ADHD children (34). Therefore, ADHD pathology may not be as simple as an "uninhibitory" locomotor

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activity; there must be other neural systems involved, e.g. arousal and motivational systems that involve learning processes. Assessments of the effects of drugs on exploratory behavior in laboratory rodents are recognized as an essential part of the evaluation of psychotropic compounds (3, 7, 18, 19, 32). A popular index of responsiveness to complexity is found in the "head-poke" test of exploration which measures the tendency for rodents to insert their heads into holes in the floor or "hole-board" (5, 6). Oades (1982) reported that the rats with ventral-tegmental damage showed impairment in search strategies on a hole-board (16). Moreover, study also showed that the dopaminergic neurons are responsible for the development of methamphetamine-induced anticipatory activity in rat (26).

There are two possible factors, which influence the animal "exploratory" behavior (4, 33). 1. The spontaneous activity may be another factor in the facilitation of the "exploratory" behavior. The spontaneity in the production of the motor activity may be the crucial factor, especially in the ADHD syndrome. 2. The maturation factor in the production of the motor activity. It involves developmental cognition in the production of experience-dependent behavior.

In order to understand the etiology of ADHD using animal behavior model, the precise aspect of the behavioral component at a certain situation (or under certain manipulation) has to be clarified. In fact, a simple locomotor activity may be influenced by the following factors: 1. locomotor activity (speed); 2. exploration (investigatory); 3. exploration (spontaneous); and 4. maturation (experience-dependent: developmental cognitive). In the present study, the activities of rats with wide spectrum of ages were monitored, and the effects of methylphenidate on their activities were evaluated.

Materials and Methods

Subjects

Pregnant Wistar rats were purchased from the National Breeding center for Experimental Animal. The male offspring male were used in the behavioral test. The conditions for animal quarter were maintained at the standard requirement, 12:12 hr light/dark cycle with light on at 7:00 am.

In Experiment I, the rats were divided into three groups (n = 9 each). Their behavioral patterns were measured on the hole-board apparatus. The behavioral activities of rats in group A were measured at the age of 4 weeks, and were re-measured every two weeks until age of 14 weeks. Group B rats were measured at the age of 8 weeks, and were re-measured every two weeks until age of 14 weeks. Group C rats were measured at 12 weeks old, and re-measured at 14 weeks old.

In Experiment II, rats of 4, 8, 12 weeks old were used (n = 20, each). Rats in each aged group were sub-divided into two groups (n=10, each), methylphenidate-injected and vehicle-injected groups. Behavioral activities on the hole-board apparatus were measured 30 min after the injection.

Behavioral Activity Measurement

Hole-board Apparatus: A modified wooden hole-board apparatus was used to measure the rat's behavioral activity (5, 6). The board (100×100 cm) was divided by lines into a hundred squares (10×10 cm). Nine holes (dia. 5 cm) were arranged in a square figure where the distance between two adjacent holes was 30 cm apart. The bottom of the hole was covered with a dark Plexiglas, and with a depth of 1 cm.

The behavioral measurement were carried out during the period from 18:00 hr to 21:00 hr, in the room with dim ceiling light (10W bulb). Rats were handled for three days consecutively before behavioral measurement. The rat was placed on the hole-board gently, and it was allowed to explore or to move around the board for 20 min. The behavioral activity was recorded with a video-camera for detail analyses.

Behavioral Indices: The behavioral indices were used to describe rat's prominent behavioral patterns on the hole-board: 1. Activity Time (AT): the total time minus the non-moving time. 2. Specific Exploratory Behavior (SEB): the number of hole-dipping behaviors in the total moving time. 3. Diverse Exploratory Behavior (DEB): the number of non-specific types of behaviors in the total moving time, these includes rearing and margin activity. The number of feces was also recorded as an indication for the animal tense response. In Experiment II, the 20 min behavioral measurement session was divided into four five-minute phases, the activity time in each phase was recorded. The maximum activity time minus the minimum activity time was used as an index for habituation on the hole-board.

Drug Administration: The rats in the Experiment II were injected (i.p.) 30 min prior to the behavioral test (36). The dosage used was referred to the study reported by Wultz and his colleagues (36). The rats in the experimental group were injected with 4 mg/kg methylphenidate (Sigma, U.S.A.), whereas the controls were injected with vehicle (0.9% NaCl, v/v).

Data Analysis

Data from behavioral measurements were

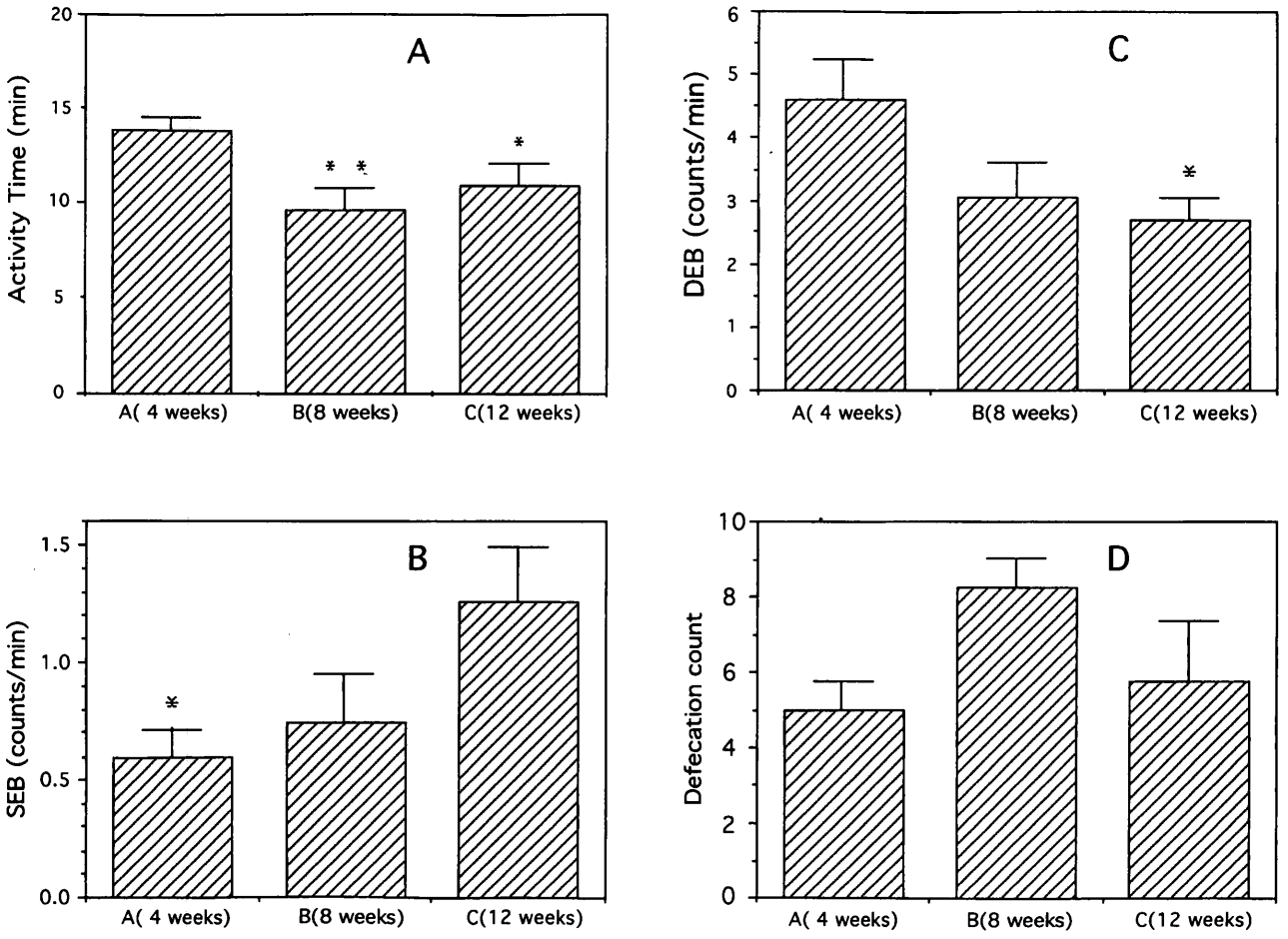

Fig. 1. The behavioral activity of rats during the first hole-board experience. A. The younger rat AT was significantly higher than those of the older rats ($F(2,23)=4.29$, $p<0.05$). The AT of the 4 weeks old rats was significantly higher than those of the 8 weeks ($t(15)=3.05$), as well as the 12 weeks old rats ($t(15)=2.24$). B. Four weeks old rats showed significantly fewer SEB compared to 12 weeks old rats ($t(15)=-2.42$). C. The 4 weeks old rats showed significantly higher number of DEB compared to 12 weeks old rats ($t(15)=2.78$). D. There were no differences in the number of defecation of rats from all groups. (*$p<0.05$; **$p<0.01$).

analyzed by analyses of variance and Student t-tests.

Results

The reliability of the nine hole-board task was evaluated by comparing first 5-min period of the first and second behavioral measurements of rats from all age groups. The data was analyzed with Pearson product-moment correlation method. In the activity time (AT) measurement, the reliability coefficient (r) is 0.7053, $p<0.01$. The reliability coefficients for specific exploratory behavior (SEB) and diverse exploratory behavior (DEB) measurements were $r=0.452$, $p<0.05$ and $r=0.4552$, $p<0.05$, respectively. These suggested that the behaviors measured on the nine hole-board were consistent.

During the first hole-board experience, the younger rat activity time (AT) was significantly higher than those of the older rats ($F(2,23)=4.29$, $p<0.05$; Fig. 1a). The AT of the 4 weeks old (group A) rats was significantly higher than those of the 8 weeks old (group B) rats ($t(15)=3.05$, $p<0.01$), as well as the 12 weeks old (group C) rats ($t(15)=2.24$, $p<0.05$). There were no significant differences in AT during the first hole-board experience between the rats from group B and C. In term of the specific exploratory behaviors (SEB), group A rats showed significantly fewer SEB compared to group C rats ($t(15)=-2.42$, $p<0.05$; Fig. 1b). Inversely, the group A rats showed significantly higher number of diverse exploratory behaviors (DEB) compared to group C rats ($t(15)=2.78$, $p<0.05$; Fig. 1c). There were no differences in the number of defecation of rats from all groups (Fig. 1d). Rats showed similar learning effect on the patterns of AT, SEB and DEB measurements (Fig. 2). At the age of 8 weeks old, the group A rats showed no significant differences in AT, SEB, DEB and number of defecation compared to the group B rats. However, at the age of 12 weeks old, the group C rats showed significant

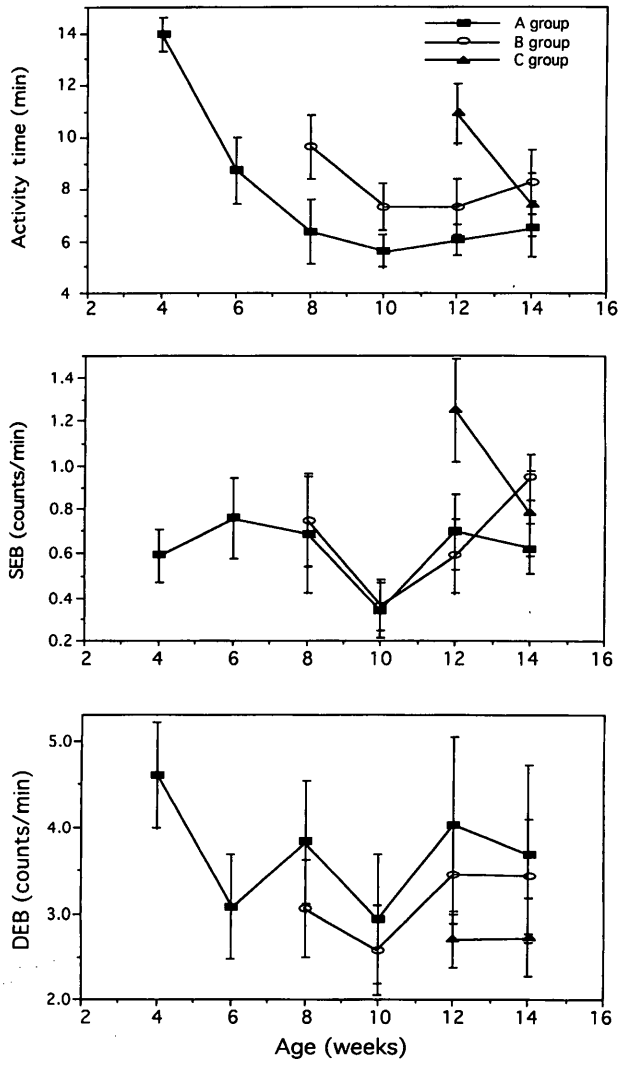

Fig. 2. The patterns of behavioral activity of rats after re-exposure to the hole-board apparatus. These include AT, SEB and DEB measurements.

differences in AT ($t(16)=-2.30$, $p<0.05$) and SEB ($t(16)=-2.30$, $p<0.05$) compared to the rats from groups A and B. Although a linear trend was apparent in the SEB measurement, yet it was not statistically significant ($F(2,23)=3.09$, $p=0.06$). In Experiment II, the methylphenidate-injected rats showed significantly higher AT compared with the controls across three age groups ($F(2,54)=5.16$, $p<0.005$; Fig. 3a). The methylphenidate-injected rats showed significantly lower HI compared with the controls ($F(1,54)=19.03$, $p<0.001$; Fig. 3b). There were no significant differences in SEB and DEB between the methylphenidate-injected and the controlled rats (Fig. 3c and 3d). In addition, the drug effect apparently reversed the lower SEB and higher DEB of 4 weeks old rats from Experiment I.

Discussion

The initial AT of the 4 weeks old rats was significantly higher than those of the 8 and 12 weeks old rats. The younger rats showed more locomotor activity compared to the older rats suggested that the maturational factor may play a crucial role in the regulation of locomotor activity in rats, especially when encountering a novel environment. The locomotor activity decreased as the rats were re-exposed to the hole-board every two weeks. The same pattern occurred for the rats from all three groups. When comparing AT at the age of 8 weeks old, group A rats showed remarkably lower AT compared to the group B rats; and at the age of 12 weeks old, group B rats showed significantly lower AT compared to those of group C rats at the age of 12 weeks old. Thus, besides the maturational factor, the repeated exposure experiences may modify the locomotor activity in rats.

During the first hole-board experience, group A rats showed significantly fewer SEB but significantly more DEB compared with the group C rats. Presumably, as the younger rats possessed more locomotor activities, they were more likely to be distracted by the non-specific and distal cues. Moreover, the older rats showed more SEB, this suggested that the younger rats were less attentive relative to the older rats. Studies have reported that the younger rats revealed more activities in the emotional stressful environment because they did not perceive the fear situation as their older counterparts (8, 11). However, there were no differences in the number of defecation of rats from all groups. Therefore, no evidence for the dissimilar emotional responses was found among the three age groups.

In the present report, the younger rats showed more rearing and marginal activity compared to the older rats. These suggested that the hyperactive tendency of rats was inhibited as they matured. In short, there are two possibilities for the differences in the SEB and DEB measurement across the age groups. First, the younger rats possess a more hyperactive tendency. Second, the younger rats are less perceptive that cause more locomotor activity apparently.

In Experiment II, the methylphenidate-injected rats showed significantly higher AT compared to the controls across the three age groups. This suggested that the drug injected rats were more hyperactive than the controls. A previous study has reported 3 - 6 mg/kg dose methylphenidate injections raised the animal activity to its maximum (36). There were no significant differences in SEB and DEB between the methylphenidate-injected and controlled rats. These findings were similar to what reported by File

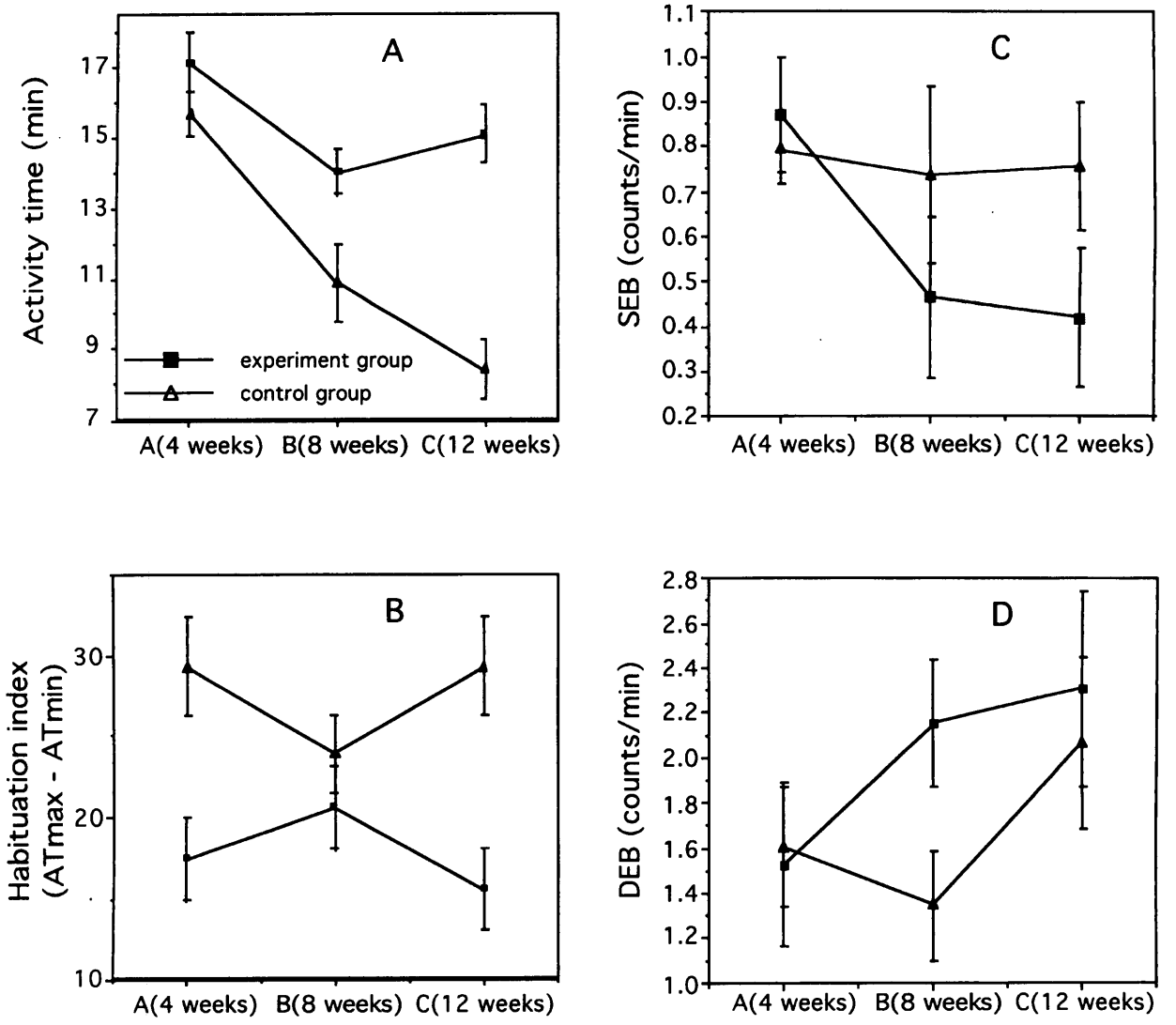

Fig. 3. Comparisons of the behavioral activity of rats after methylphenidate injection, the controlled rats were injected with saline. A. Methylphenidate-injected rats showed significantly higher AT compared to the controls across the three age groups ($F(2,54)=5.16$, $p<0.005$). B. The methylphenidate-injected rats showed significantly lower HI compared to the controls ($F(1,54)=19.03$, $p<0.001$). C and D. There were no significant differences in SEB and DEB between the methylphenidate-injected and the controlled rats.

and Wardill (6). The excitatory influences of stimulants appeared to be more distinctive on the locomotor activity than on the specific exploratory activity.

In sum, there is a high locomotor activity of rat at the early age, and it decreases with age. The exploratory behavioral patterns also alter with age; at the early age, the rat is less focusing on the specific targeted object. As the rat matured, it tends to fixate on the specific targeted object. The methylphenidate injection raises the rat locomotor activity regardless of age. However, the methylphenidate injection does not alter the exploratory behavior patterns. This suggests that methylphenidate affects the rat at the activity level only. Using the neuron lesioning method, Kostrzewa et al. (1994) had reported that the dopaminergic neuron lesioned rats showed higher behavioral activity compared to the controls (13). With the stimulant injection, the high behavioral activity of the dopaminergic neuron lesioned rats were depressed; however, the same injection increased the behavioral activity of the controlled rats. Therefore, the effect of stimulant causes the normal behavioral activity to increase, which may be acted through the dopaminergic neuronal system. Methylphenidate acts on the dopaminergic system (15, 21, 28, 29). There is a gamma amino-butyric acid neuronal projection from the nucleus accumbens to the globus pallidus (12), which is suggested to play a crucial role in the regulation of behavioral activity (22, 35). Thus, the biochemical alterations in the nucleus accumbens and globus pallidus in this

exploratory behavioral paradigm will be pursued in the future.

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