The spontaneously hypertensive rat/Izm (SHR/Izm) shows attention deficit/hyperactivity disorder-like behaviors but without impulsive behavior: Therapeutic implications of low-dose methylphenidate

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Abstract

The spontaneously hypertensive rat (SHR) has been used as a genetic animal model of attention deficit/hyperactivity disorder (ADHD). SHR/Izm is derived from stroke-resistant SHR as SHR/NIH and SHR/NCrl but from 22nd-23rd generation descendants of the SHR/NIH ancestor and therefore may show different behavioral phenotypes compared to other SHR sub-strains. In this study, ADHD-like behaviors in SHR/Izm were evaluated compared to Wistar rats. SHR/Izm showed high locomotor activity in the habituation phase in a novel environment, although locomotor activity in the initial exploratory phase was low. In a behavioral test for attention, spontaneous alternation behavior in the Y-maze test was impaired in SHR/Izm. However, impulsive behavior in the elevated-plus maze test, which is designed to detect anxiety-related behavior but also reflects impulsivity for novelty seeking, was comparable to Wistar rats. Hyperactivity and inattention, detected as ADHD-like behaviors in SHR/Izm, were ameliorated with methylphenidate at a low dose (0.05 mg/kg, i.p.). Therefore, SHR/Izm represents a unique animal model of ADHD without anxiety-related impulsive behavior.

Keywords

Spontaneously hypertensive rat (SHR), attention deficit/hyperactivity disorder (ADHD), methylphenidate (MPH), hyperactivity, Y-maze test, elevated plus maze test

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder of childhood onset that is characterized by behavioral symptoms, including inattention, motor hyperactivity and impulsivity [1, 2]. ADHD is a heterogeneous and heritable disorder resulting from complex gene-gene and gene-environmental interactions [3]. To simplify and understand the complex features of ADHD, animal models of ADHD can provide insights into the etiology, pathophysiology and drug therapy of ADHD [3, 4].

The spontaneously hypertensive rat (SHR) has frequently been used as a genetic animal model of ADHD, since SHR is reported to fulfill validation criteria for assessing an ADHD model [4, 5]. However, there are also reports that debate the validity of SHR because of lack of some aspects of ADHD validation criteria: hyperactivity [6], inattention [7] or impulsivitiy [8]. One reason for the inconsistency across behavioral assessments of SHR is due to inappropriate use of reference strains with different basal response rates [9]. Another reason is the use of various behavioral tasks with different sensitivity and/or specificity for each of behavioral deficits, which are heterogeneous among ADHD subtypes [10]. Thus, SHR is a useful animal model of ADHD, but still insufficient for the inconsistency of behavioral phenotypes.

Among various sub-strains of SHR, SHR/NCrl (the SHR sub-strain established at Charles River, Germany) is reported as the best validated animal model for the ADHD combined subtype (ADHD-C) with behavioral symptoms of inattention and hyperactivity-impulsiveness [11]. SHR/NCrl is derived from SHR/NIH, which was separated from stroke-resistant SHR (SHRSR) in the F_{13} generation of inbreeding in Japan. SHR/Izm is also derived from SHRSR (B1 sub-line) at the $F_{35\text{-}36}$ generations of inbreeding in Japan, which are descendants of the 22nd-23rd generations from the SHR/NIH ancestor. Because SHR/Izm is genetically more homogeneous than SHR/NCrl, SHR/Izm may show less variable and/or limited ADHD-like behavioral phenotypes. However, the behavioral profiles of SHR/Izm have not been characterized.

Methylphenidate (MPH) is one of the most commonly prescribed psychostimulants for the treatment of ADHD. MPH is known to block the reuptake of dopamine (DA) and noradrenaline (NA), but the precise mechanisms of MPH action on the ADHD symptoms are not understood. The effects of MPH over a wide range of doses (0.1 - 20 mg/kg, i.p.) have been evaluated in SHR [12, 13] **.** The doses used in SHR are relatively or extremely high compared with optimal doses in ADHD children. For example, Berridge et al. [14] reported that in Sprague-Dawley (SD) rats, MPH administration at 0.25 mg/kg (i.p.) resulted in plasma MPH levels of 16 ng/ml 5 min after administration, which is comparable to the peak plasma levels of a high oral dose of MPH (0.6-0.8 mg/kg) in ADHD children [15]. In our previous study using *in vivo* microdialysis, a low dose of MPH at 0.05 mg/kg (i.p.), but not higher doses of MPH (0.1, 0.25 and 1.0 mg/kg, i.p.), decreased the NA content and normalized the ratio of the NA and DA contents (NA/DA) in the prefrontal cortex (PFC) of SHR/Izm, which was high due to the low DA content under basal conditions [16]. The findings suggest that adjusting the imbalance of NA and DA systems may play a role in the therapeutic action of MPH in ADHD.

In this study, ADHD-like behaviors in SHR/Izm were characterized. To assess three core symptoms of ADHD in SHR/Izm, behavioral tests for 1) hyperactivity, 2) spontaneous alternation behavior (as an index of attention using a Y-maze test) [17], and 3) anxiety-related behavior (in an elevated-plus maze test as a measure of impulsive behavior) [18, 19] were performed compared to Wistar rats. The elevated-plus maze test is originally developed to detect the anxiety-related behavior, but used to detect impulsivity in this study, because the exploratory behavior in open arms may reflect impulsivity for novelty-seeking [20]. Furthermore, the effect of a low-dose of MPH on ADHD-like behaviors in SHR/Izm was evaluated.

2. Materials and methods

2.1. Animals

Five-week old male SHR/Izm [118.88±1.12 g (n=106); SLC, Shizuoka, Japan] and male Wistar rats [143.40±1.34 g (n=106); Kyudo, Tosu, Japan] were maintained at 23 ± 2 °C under a 12-h light–dark cycle with free access to food and water. Rats were divided into three experimental groups. Rats in each experimental group were used for only one of the following three behavioral tests; 1) locomotor activity, 2) the Y-maze test or 3) the elevated plus maze test. All of the rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and the specific protocols were approved by the Committee for Animal Experimentation at Kurume University School of Medicine. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

[(±)-methylphenidate hydrochloride (MPH)] (Sigma-Aldrich, St. Louis, MO, USA) at a low (0.05 mg/kg) or high (10 mg/kg) dose, calculated as the salt form, was dissolved in 0.2 ml of saline and injected intraperitoneally. In the Y-maze test and the elevated plus-maze test, MPH was administered 90 min prior to the tests.

2.3. Locomotor activity

Rats were placed in a cage (40 cm × 25 cm × 20 cm) in a novel environment immediately after injection of MPH (0.05 mg/kg or 10 mg/kg in 0.2 ml saline) or saline (0.2 ml). The number of horizontal and vertical (or rearing) movements was determined as activity counts using an area sensor (NS-AS01; Neuroscience, Tokyo, Japan) for 120 min, and data were stored and analyzed with a computerized system (DAS system; Neuroscience).

2.4. The Y-maze test

Spontaneous alternation behavior determined by the Y-maze test requires attention [21, 22] and working memory [17]. The methods of the Y-maze test were similar to those described previously [17] and used to identify inattention behavior. Impaired attention results in low percentage of spontaneous alteration, because rats need to explore a new arm rather than returning to arms that were visited in the last two entries. The Y-maze consisted of three arms made of black plastic (50 cm long, 20 cm high, 10 cm wide) extending from a central platform at an angle of 120°. After 90 min of saline or MPH administration (i.p.) (0.05 mg/kg or 10 mg/kg in 0.2 ml saline) or saline (0.2 ml), each rat was placed at the end of one arm and was allowed to move freely in three arms of the maze during the test session for 5 min [23]. 'Arm entry' was defined as the entry of half of the body trunk into one arm. Testing was performed between 1 p.m. and 5 p.m. The sequence of arm entries was recorded visually. Alternation was defined as multiple entries into the three different arms in overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of the actual-to-possible alternations (defined as the total number of arm entries − 2) multiplied by 100. Actual alternations are the number of three consecutive entries into three different arms (A, B, C) such as ABC, ACB, BAC, BCA, CAB, or CBA during the test session.

2.5. The elevated plus-maze test

The elevated plus-maze consisted of two open arms (10 × 45 cm), two enclosed arms (10 × 45 cm) with protective walls (20 cm high) and a central arena (10 × 10 cm). The maze was elevated 72 cm off the ground. The apparatus was situated in a separate small shielded square room within the laboratory so that rats were not disturbed by environmental stimuli. The light intensity was 100 lux in the central arena.

At the beginning of the experiment, the rat was placed in the central arena of the apparatus with the head pointing mid-way between a closed and an open arm. The 5-minute test session was started after 90 min of saline (0.2 ml) or MPH administration (i.p.) (0.05 mg/kg or 10 mg/kg in 0.2 ml saline). Movements were videotaped during the session, and

the following parameters were recorded by the computer program VideoMot (TSE, Bad Homburg, Germany): the number of entries into the open arms/total number of entries (%), time spent in the open arms/time spent in all arms (%) and total distance travelled (cm) [18, 19, 24].

2.6. Statistical analysis

All data were expressed as the mean ± S.E.M. for 10-14 rats in each group of behavioral tests. Exact number of rats used in each behavioral test was described in figure legends. The locomotor activity data were analyzed with a repeated measures two-way analysis of variance (ANOVA) followed by the Bonferroni test and t-test. The Y-maze and the elevated plus-maze data were evaluated by a t-test and a one-way ANOVA followed by the Dunnett test (GraphPad Prism software Inc., San Diego, CA, USA). The level of significance was set at $p < 0.05$.

3. Results

3.1. Locomotor activity

Locomotor activity was recorded after the administration of saline or MPH (0.05 and 10 mg/kg) (i.p.) (Fig. 1 A-C). Locomotor counts in SHR/Izm were significantly different from those in Wistar rats in all groups (Fig. 1A: saline group, $F_{(1,312)}$=32.68, p<0.0001; Fig. 1B: low-dose MPH group, $F_{(1,216)}$=9.819, p<0.01; Fig. 1C: high-dose MPH group, $F_{(1,216)}$=36.18, p<0.0001). In the saline-treated groups, SHR/Izm showed significantly lower locomotor counts than Wistar rats during the initial exploratory phase of 0-30 min when they were placed in a novel cage environment. However, the locomotor counts in SHR/Izm did not decrease as they did in Wistar rats during the habituation phases of 30-60 and 60-90 min, and the counts became higher than those in Wistar rats (Fig. 1D). MPH at a low dose (0.05 mg/kg, i.p.) decreased the locomotor counts only in SHR/Izm during the habituation phases of 30-60 and 60-90 min (Fig. 1B). The locomotor counts accordingly became comparable between SHR/Izm and Wistar rats after 30 min of MPH administration, although they were still low in SHR/Izm during the initial exploratory phase (Fig. 1E). MPH at a high dose (10 mg/kg, i.p.) increased the locomotor counts in both SHR/Izm and Wistar rats, but the increased locomotor counts were higher in SHR/Izm than in Wistar rats (Fig. 1C), especially during the phase from 60-90 min (Fig. 1F). The total locomotor counts at 120 min in SHR/Izm were significantly higher than those in Wistar rats in the saline groups (Fig. 1G), but MPH at a low dose suppressed the high locomotor counts (Fig. 1H). In contrast, the responses of locomotor counts to MPH at a high dose were larger in SHR/Izm than in Wistar rats (Fig. 1I).

3.2. The Y-maze test

In the Y-maze test, SHR/Izm pre-treated with saline (90 min before) showed a significantly lower percentage of spontaneous alternation behavior than Wistar rats (Fig. 2A), suggesting that SHR/Izm exhibits the impairment of attention. Pretreatment with MPH at a low dose (0.05 mg/kg, i.p.), but not at a high dose (10 mg/kg, i.p.), improved the alternation behavior in

SHR/Izm (Fig. 2C), whereas MPH did not affect the alternation behavior at low or high doses in Wistar rats (Fig. 2B). The total arm entries of SHR/Izm pre-treated with saline were similar to those of Wistar rats (Fig. 2D). MPH at a high dose increased the total arm entries in Wistar rats (Fig. 2E) but had no effect in SHR/Izm (Fig. 2F). MPH at a low dose did not affect the total arm entries either in Wistar rats or SHR/Izm.

3.3. The elevated plus maze test

The percentage of entries into open arms or time in open arms in SHR/Izm was not significantly different from that in Wistar rats in the saline-pretreated group (Fig. 3A and D). The total distance travelled during 5 min of the test session in SHR/Izm was significantly lower than for Wistar rats (Fig. 3G). The results were consistent with the decreased locomotor activity in SHR/Izm when placed in a novel environment (Fig. 1A and D). MPH at a low dose (0.05 mg/kg, i.p.) did not affect any parameters (the percentage of entries into open arms or time in open arms or total distance in Wistar rats or SHR/Izm) (Fig. 3B, C, E, F, H and I). MPH at a high dose (10 mg/kg, i.p.) induced increases in the percentage of time in open arms only in Wistar rats (Fig. 3E) and in the total distance in both Wistar rats and SHR/Izm (Fig. 3H and I). These results suggest that SHR/Izm does not exhibit behavioral abnormalities that can be detected with the elevated plus maze test, namely anxiety or impulsivity.

4. Discussion

We demonstrated that SHR/Izm displayed hyperactive and inattentive behaviors but no anxiety-related impulsive behavior when compared to Wistar rats. Our previous *in vivo* microdialysis study suggested that MPH at a low dose (0.05 mg/kg, i.p.) adjusts the imbalance of NA and DA systems in the PFC of SHR/Izm [16]. In this study, MPH at a low dose was shown to ameliorate hyperactivity and inattention in SHR/Izm. Therefore, SHR/Izm has different behavioral profiles from SHR/NCrl and is a unique animal model of ADHD without anxiety-related impulsive behavior.

4.1. Hyperactive and inattentive behaviors in SHR/Izm

In children with ADHD [25, 26], hyperactivity is absent in a novel situation but develops gradually over time after becoming familiar to a surrounding situation. Similar to children with ADHD, SHR/Izm used in the present study showed increased locomotor activity in the habituation phase following high exploratory activity. The findings in SHR/Izm are in agreement with other SHR sub-strains such as SHR/NIH [27], SHR/NCrl [4, 28], SHR/NHsd [29] and stroke-prone SHR (SHRSP)/Ezo [19]. It was previously reported that, when SHR/NCrl was exposed to the same testing box for two consecutive days, the initial increase in exploratory activity in response to a novel environment was reduced on day 2 similarly to Wistar rats, but activity in the habituation phase was still high on day 2 and day 3 [28]. Although similar experiments need to be performed in SHR/Izm, SHR including SHR/Izm may show hyperactivity even after becoming familiar to the surrounding situation as observed in children with ADHD.

The locomotor activity in the exploratory phase was reduced in SHR/Izm in comparison with Wistar rats. SHR/NCrl is reported to exhibit similar exploratory activity to Wistar rats at the initial 10 min in the open field test [28]. In children with and without ADHD, similar activity levels at the initiation phase of fixed-interval/extinction tasks or variable-interval/extinction tasks have been reported [30]. It is possible that SHR/Izm is a substrain of

SHR with low sensitivity to a novel environment. Since the object-recognition deficit has been reported in SHR [31], the cognitive performance in SHR/Izm needs to be investigated in comparison with other SHR substrains and Wistar rats.

Inattention in SHR/Izm was detected with spontaneous alternation behavior in the Y-maze test compared with Wistar rats. The Y-maze test has been used to demonstrate inattention in other SHR sub-strains, including SHR/NCrl [23] and SHRSP/Ezo [19], although the Wistar-Kyoto rat (WKY) was used as a control. ADHD children suffer from deficits in sustained attention when stimuli are widely spaced in time [4, 5]. Deficient sustained attention was found in SHR/NCrl using a visual discrimination task [32] and a five-choice serial reaction time task [33] and in SHRSP/Ezo using the five-choice serial reaction time task [34]. The Y-maze test detected inattention in subpopulations of SHR with deficient sustained attention (SHR/NCrl and SHRSP/Ezo), suggesting that the Y-maze test might be a sensitive method to evaluate inattention in the animal model of ADHD.

4.2. Anxiety-related impulsive behavior in SHR/Izm

Impulsivity has a multi-faceted nature, and different aspects of impulsivity, influenced by a variety of biological mechanisms, may represent different subtypes of ADHD [35; 36]. Various behavioral paradigms have been developed to evaluate different aspects of impulsivity categorized into impulsive action and impulsive choice [35]. The elevated plus-maze test has been extensively used to measure anxiety-related behavior, in which novelty produces both approach/exploration (open arm) and avoidance (closed arm) interpreted as curiosity and anxiety, respectively [37]. A study in Roman high and low avoidance rats proposed that the increases in exploratory behavior in open arms might not simply be less anxious, but also be more impulsive or novelty-seeking due to a complex interaction between divergent anxiety/emotionality characteristics and novelty/reward seeking [20]. There are also evidences showing that prenatal malnutrition, which results in increased exploration of open arms in the elevated plus-maze test [38, 39], induces impairment of

inhibiting operant responses for food/water reward [40]. Anxiety-related impulsivity is connected to impulsivity detected with other behavioral tests for impulsive action control, e.g. stop-signal reaction time, go/no-go task and five-choice serial reaction time task [35, 41], although controversial results are reported [42, 43].

In this study, anxiety-related impulsivity in SHR/Izm was evaluated with the elevated plus-maze test. As described above, the increases in entry into open arms and the time in open arms might reflect impulsivity [19, 24]. However, SHR/Izm did not exhibit anxiety-related impulsive behavior in the elevated plus maze test compared with Wistar rats. The results are consistent with a previous report demonstrating that SHR/NCrl and Wistar rats show less anxiety-related behavior than WKY/NCrl in the elevated plus maze test but no difference between SHR/NCrl and Wistar rats [24]. However, in another study using the elevated plus maze test, the reduced anxiety-related behavior in SHR/NCrl was detected compared with SD rats and WKY/NCrl [44]. Increased impulsivity in SHR/NCrl compared with SD rats was also reported in the water radial arm maze [45]. Interestingly, Adriani [46] proposed that SHR/NCrl is subdivided into impulsive and non-impulsive subpopulations using the intolerance-to-delay task. SHR/Izm may be established by selecting the non-impulsive SHR line because SHR/Izm is derived from the B1 sub-line of SHRSR in the $F_{35\text{-}36}$ generations in which breeding has been repeated more than 20 times from the ancestor of SHR/NIH and SHR/NCrl at the F_{13} generations. Identification of candidate genes associated with impulsivity in SHR as well as ADHD patients will help to understand trait impulsivity of SHR in molecular levels [47, 48] .

ADHD children do not exhibit impulsiveness in novel situations, but impulsiveness develops gradually over time [4, 5]. Such unique features of impulsiveness have been demonstrated in SHR sub-strains including SHR/NCrl using the multiple fixed-interval extinction schedule of reinforcement when compared with WKY [4, 5]. In our study, the possibility that novel situations during the test period (5 min) in the elevated plus maze test mask anxiety-related impulsive behavior in SHR/Izm needs to be ruled out. However, when

compared with Wistar or SD rats, impulsivity in SHR/NCrl or SHR/NHsd, regardless of the gradual development during tasks, was not detected in the visual discrimination task [32] or the five-choice serial reaction time task [29, 33, 49]. In fact, the presence of impulsivity in SHR, which is not detected as well in other studies examining the tolerance to delay of reinforcement [8, 50] , has been debated. Behavioral tests evaluating anxiety-unrelated impulsivity are required to characterize various aspects of impulsivity in SHR/Izm in a future study.

4.3. The reference strain for SHR

WKY, derived from the same ancestral outbred Wistar colony at Kyoto University as SHR, was frequently used as the control strain [11]. However, WKY sub-strains display the substantial genetic, neurobiological and behavioral divergence [32, 51]. Among WKY sub-strains, WKY/NCrl, which has 76.6% genotypic concordance with SHR/NCrl, has been proposed as an animal model of ADHD predominantly inattentive subtype (ADHD-PI), whereas WKY/NHsd, which has less concordance (66.5%) with SHR/NCrl, has been proposed as the control strain for SHR/NCrl [32]. Furthermore, researchers have criticized using WKY as an adequate control for SHR [9]. Especially for evaluating impulsivity with anxiety-related behavioral tests, WKY should not be used as a control for SHR because WKY sub-strains, including WKY/NCrl [52], WKY/NHsd [53, 54] and WKY/Izm [55], have been proposed as animal models of depression and display hypoactivity and depression- and anxiety-like behaviors. Therefore, in this study, SHR/Izm was compared with Wistar rats, the outbreed strain representing the normal heterogeneous population [32, 56].

4.4. Effects of methylphenidate (MPH) on the behavioral alternations in SHR/Izm

MPH at a low dose (0.05 mg/kg, i.p.) ameliorated the increased locomotor activity in the habituation phase and the reduced spontaneous alternation behavior in SHR/Izm. When MPH at a low dose is administered in SHR/Izm, the maximum plasma level of MPH is

estimated as ~4 ng/ml 5 min after administration, according to reference data [14, 57]. MPH at 0.05 mg/kg (i.p.) in SHR/Izm might be enough to increase the plasma MPH concentration up to the low therapeutic range in ADHD children, which is obtained with oral administration of MPH at 0.2 mg/kg (p.o.) [15]. However, the behavioral effects of MPH at a low dose in SHR/Izm were observed after 90 min of MPH administration, when the estimated plasma levels of MPH declined and became much lower (~1.5 ng/ml) [14, 57]. It is possible that MPH levels in the brain stay high compared to plasma levels because of longer half-life [15], and that there is a delay of more than 90 min to adjust the imbalance of NA and DA systems in the PFC by MPH in SHR/Izm, which was demonstrated in our previous microdialysis study [16]. In support of our study, the lowest dose of MPH at 0.01 mg/kg (i.p.) was shown to ameliorate ADHD-like behaviors, especially hyperactivity and spontaneous alternation behavior, but not anxiety-related behavior, in SHRSP/Ezo [19].

In clinical studies, time-action and dose response for motor and cognitive effects of psychostimulants are known to be divergent [58], and the relationship of MPH dose and its therapeutic effects on various behavioral abnormalities is somewhat different in SHR and ADHD children. In ADHD children, MPH is associated with improvement of hyperactivity and impulsivity more than inattention [59, 60]. Solanto [61] reported that MPH at a sub-clinical dose (0.1 mg/kg, p.o.) induced the reduction of hyperactivity but did not improve attention in ADHD children. Sprague and Sleator [62] showed a dose-dependent dissociation between MPH effects on cognitive performance and social behavior in ADHD children. Cognitive performance improved with a low dose of MPH and was hindered with a higher dose, suggesting an inverted U dose response curve of MPH for cognition. However, social behavior only improved with higher doses of MPH. The effect of MPH on cognitive function may be dependent on the order of cognitive functions; higher orders of cognitive functions, such as learning, require higher doses of MPH than simpler functions, such as target detection [58]. These clinical studies support the interpretation of data in SHR/Izm that the low dose of MPH ameliorates hyperactivity and inattention, which is mediated through the

improvement of simple cognitive functions. Therapeutic values of MPH at a low dose need to be evaluated in other animal models of ADHD.

Conclusions

SHR/Izm represents a unique animal model of ADHD and displays behavioral profiles of hyperactivity and inattention but not anxiety-related impulsivity when compared with Wistar rats. The fact that a low dose of MPH ameliorates ADHD-like symptoms in SHR/Izm suggests the importance of evaluating the therapeutic effect of MPH at a low dose in animal models of ADHD, including SHR sub-strains.

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Conflict of interest statement

The authors declare no conflicts of interest.

Figure Legends

Figure 1. Locomotor activities in a novel cage environment. SHR/Izm and Wistar rats received saline (0.2 ml, i.p.) (A, D, G), methylphenidate (MPH) at a low dose (0.05 mg/kg, i.p.) (B, E, H), or MPH at a high dose (10 mg/kg, i.p.) (C, F, I) and were placed in a novel cage environment. (A, B, C) Locomotor counts were recorded every 5 min. *p<0.05, **p<0.01 vs. the counts at 0-5 min, $^{\#}$ p<0.05, $^{\#\#}$ p<0.01 vs. Wistar rats. (D, E, F) Sum of locomotor counts for every 30 min. *p<0.05, **p<0.01, ***p<0.001 vs. Wistar rats. (G, H, I) Sum of locomotor counts for 2 hours. *p<0.05 vs. Wistar rats. Scales of Y-axes for (C, F, I) are different from corresponding figures because of larger responses to MPH at the high dose. Data are expressed as the mean ± S.E.M. (n=14 rats in the saline group; n=10 rats in each MPH group).

Figure 2. Spontaneous alternation behavior and total arm entries in the Y-maze test. Comparison of spontaneous alternation behavior (A) and total arm entries (D) between Wistar rats and SHR/Izm after 90 min of saline administration (control conditions). Effects of MPH administration at low (0.05 mg/kg, i.p.) and high (10 mg/kg, i.p.) doses on spontaneous alternation behavior (B, C) and total arm entries (E, F) in Wistar rats and SHR/Izm. Data are expressed as the mean ± S.E.M. (n=12 rats in each group) *p<0.05 vs. Wistar rats/saline; $^{\#\#}$$p$<0.01 vs. SHR/Izm/saline; $^{\#\#\#}$$p$<0.001 vs. Wistar rats/MPH or SHR/Izm/MPH at the high dose.

Figure 3. Anxiety-related behavior in the elevated plus maze test. Comparison of entries into open arms (A), time in open arms (D) and total distance (G) between Wistar rats and SHR/Izm after 90 min of saline administration (control conditions). Effects of MPH administration at low (0.05 mg/kg, i.p.) and high (10 mg/kg, i.p.) doses on entries into open arms (B, C), time in open arms (E, F) and total distance (H, I) in Wistar rats and SHR/Izm.

Data are expressed as the mean ± S.E.M. (n=12 rats in each group) *p<0.05 vs. Wistar rats; $^{\#}p$<0.05, $^{\#\#\#}p$<0.001 vs. saline.

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Figure 1

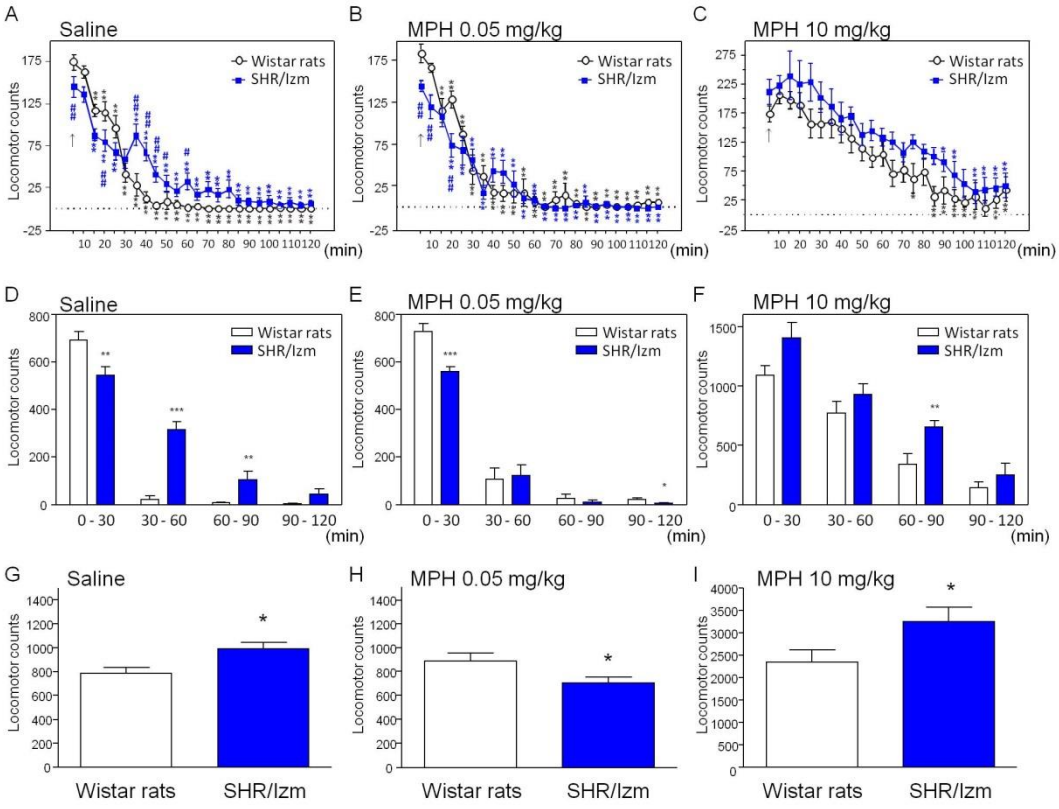

A
Saline
B
MPH 0.05 mg/kg
C
MPH 10 mg/kg
Wistar rats
SHR/Izm
Locomotor counts
(min)
D
Saline
E
MPH 0.05 mg/kg
F
MPH 10 mg/kg
0 - 30
30 - 60
60 - 90
90 - 120
G
Saline
H
MPH 0.05 mg/kg
I
MPH 10 mg/kg

Figure 2

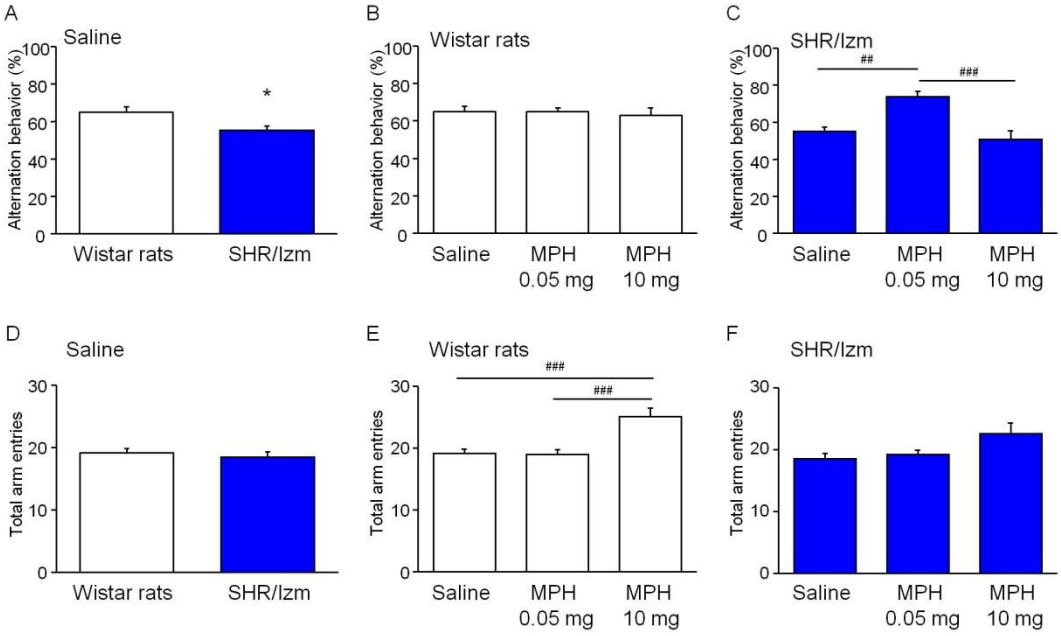
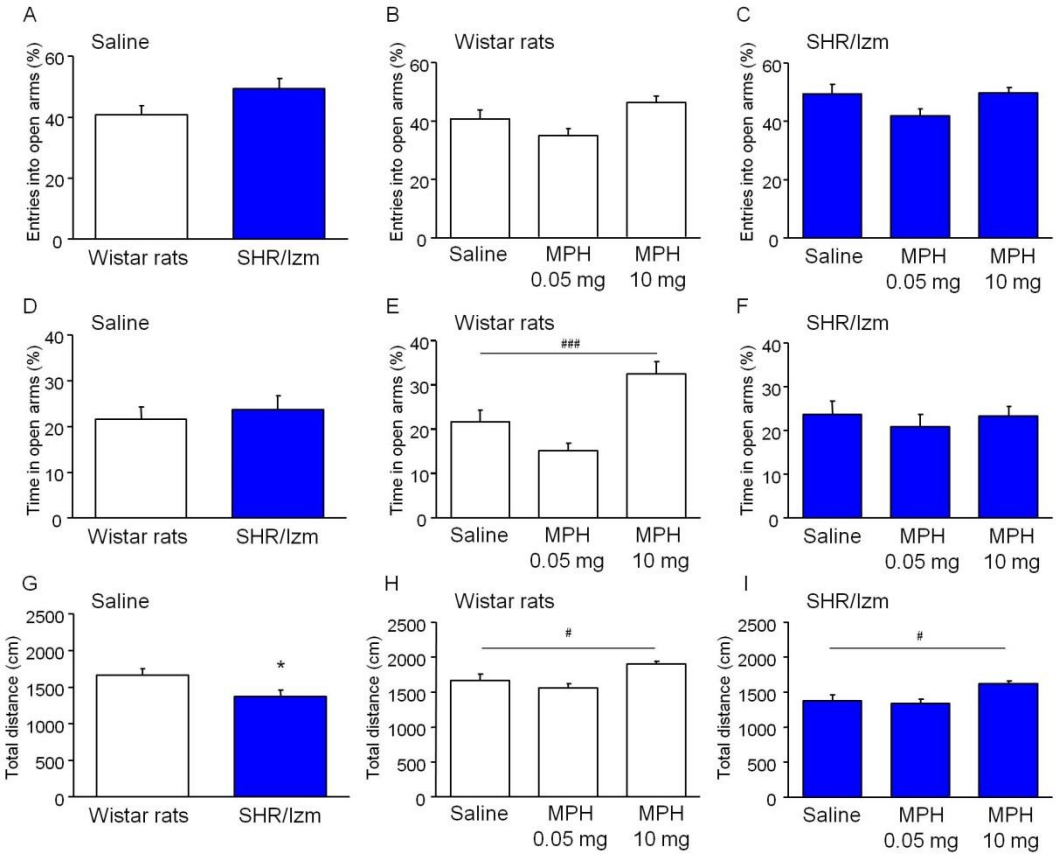

A
Saline
Alternation behavior (%)
100
80
60
40
20
0
*
Wistar rats
SHR/Izm
B
Wistar rats
Alternation behavior (%)
Saline
MPH 0.05 mg
MPH 10 mg
C
SHR/Izm
##
###
Alternation behavior (%)
Saline
MPH 0.05 mg
MPH 10 mg
D
Saline
Total arm entries
30
20
10
0
Wistar rats
SHR/Izm
E
Wistar rats
###
###
Total arm entries
Saline
MPH 0.05 mg
MPH 10 mg
F
SHR/Izm
Total arm entries
Saline
MPH 0.05 mg
MPH 10 mg

Figure 3


A
Saline
Entries into open arms (%)
Wistar rats
SHR/Izm
B
Wistar rats
Saline
MPH 0.05 mg
MPH 10 mg
C
SHR/Izm
D
Saline
Time in open arms (%)
E
Wistar rats
###
F
SHR/Izm
G
Saline
Total distance (cm)
*
H
Wistar rats
#
I
SHR/Izm
#