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Research Article

The enriched cross-maze test as a model of impulsivity and attention deficit in spontaneously hypertensive rats

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Abstract

Introduction: Attention deficit hyperactivity disorder (ADHD) is one of the problems of modern medicine, which requires development of appropriate treatments using animal models. One of the valid genetic models of ADHD in animals is the spontaneous hypertensive (SH) outbred rats line. Results of the present paper demonstrate heterogeneity among the SH rats line regarding measures of impulsivity and attention deficit obtained by the use of the enriched cross-maze test and assesses the effects of clinically effective anti-ADHD drug atomoxetine.

Materials and Method: 106 adult male SH outbred rats were used. Their exploratory behavior was assessed in the enriched cross-maze test, which provides correlates of impulsivity and attention to enriching objects regardless of overall motor activity. The device consists of 4-arm radial maze, two arms of which contain objects (enriched compartments). Normal non-impulsive and attentive animals exploring the maze usually spend more time in the central compartment before entering the enriched arms and then spend more time in these arms as compared to when in the empty arms**.** Impulsive and inattentive individuals have the opposite tendency. Atomoxetine (3 mg/kg) was administered orally, once daily for 4 days.

Results and Discussion: Frequency distribution of the impulsivity index had clear bimodal form, which statistically significantly differed from the normal distribution. The outcome indicates existence of subpopulations of individuals with high and low impulsivity. Subpopulation with high impulsivity and low attention to enriching objects (34% of individuals from experiment 1), which are parallel to patients with ADHD, was collected for experiment 2 in which they were treated with atomoxetine or placebo. Subchronic atomoxetine selectively improved impulsivity and attention measures in the second enriched cross-maze test.

Conclusion: The enriched cross-maze test may be useful in neurobiology studies of impulsivity and attention deficit and for screening new drug candidates.

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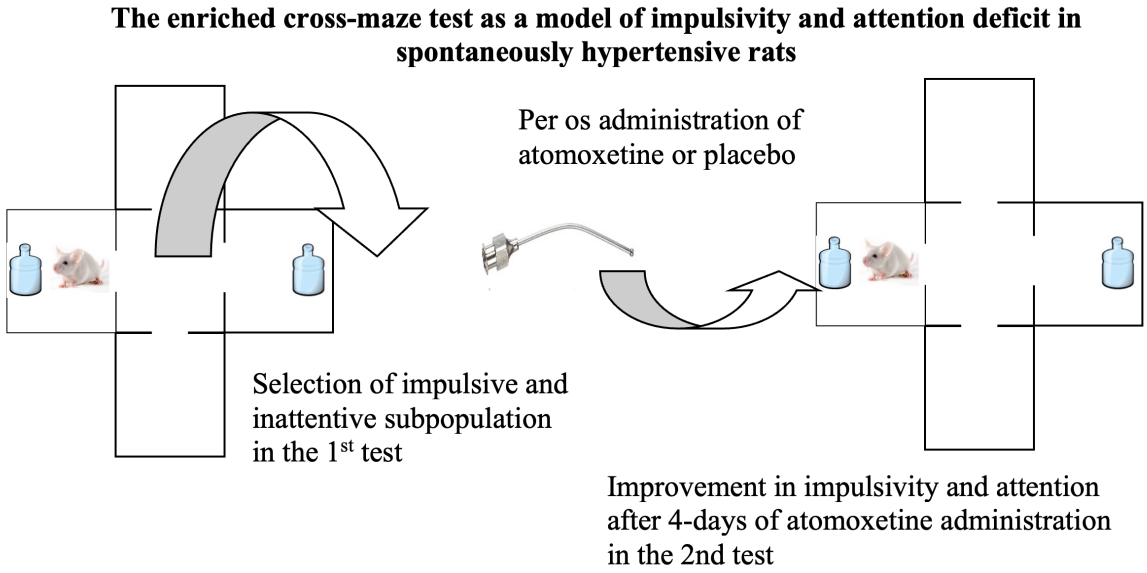
Graphical abstract



Keywords

impulsivity; attention deficit; enriched cross-maze; rats with spontaneous hypertension; Atomoxetine

Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the problems of modern medicine, which requires development of appropriate treatments. The ADHD syndrome involves inattention, which can be combined with impulsivity/hyperactivity and in general is not accompanied by decline in overall intelligence (American Academy of Pediatrics 2000; Paloyelis et al 2010) or deficiency in motor skills deficits (Al-Menabbawy et al. 2006). Left untreated, up to 70% of children with ADHD continue to experience symptoms of the disease as adults (Mannuzza et al. 1993). ADHD symptoms may be corrected by psychostimulants or some antidepressants, in particular, by novel non-stimulating drug atomoxetine (American Academy of Pediatrics 2000; Kates 2005; Asherson et al. 2007). However, these drugs are helpful in less than 60% of cases (Kates 2005), suggesting the necessity of development of novel drugs for treatment of ADHD (Naderi et al. 2010) using animal models of ADHD.

One of the valid genetic animal models of ADHD is considered the outbred line of rats with spontaneous hypertension (SH) (Davids et al. 2003; Sagvolden et al. 2005; Bizot et al. 2011). The SH rats show reduced sustained attention to environmental cue as compared to their original normotensive line (Meyer et al. 2010; Calzavara et al. 2011; Langen and Dost 2011; Salimov and Kovalev 2012, 2013), greater variability in rewarded operant responses, and slower elimination of ineffective responses (Johansen et al. 2007). This ADHD-like pattern in SH rats is not related with their blood pressure levels (Wickens et al. 2004; Salimov and Kovalev 2012). Interestingly, the SH rat line is not homogeneous regarding these variables and includes subpopulations of individuals diverging in attention to the elements of the enriched environment (Salimov and Kovalev 2012), as well as in impulsivity in instrumental paradigms with delayed reward (Adriani et al. 2003; Johansen et al. 2007).

The most recent studies of rat behavior have demonstrated that there is a correlation between general measures of impulsivity in the operant paradigm and time spent in the center of plus-maze before entering its open or closed arms (Rico et al. 2016). This suggests that it may be possible to use the latency before entering the different maze arms during exploratory behavior as one of potential correlates of impulsivity in rodents.

The present study employs previously published paradigm of exploratory behavior that does not require from animals any training and provides independent measures of time in the center and arms of the cross-maze before and after entering different maze arms. Previously, using this method, it was shown the presence of subpopulations of ED-low and ED-high individuals diverging in time in arms containing objects (as compared with empty arms) in the SH rats (Salimov and Kovalev 2012) and outbred mice of CD-1 strain (Salimov and Kovalev 2013), which was considered as differences in the level of attention to the environmental objects. Improved attention was found also in the ED-low rodent subpopulation after repeated administration of atomoxetine (Salimov and Kovalev 2012, 2013), suggesting face and predictive validity of this test.

The present study is **aimed to** further evaluate using this method the homogeneity of the SH rat population in terms of impulsivity (latency before entering the maze arms). In case of population inhomogeneity, to evaluate the effect of the clinically effective drug atomoxetine in the subpopulation of rats with high impulsivity and low attention to enriched arms.

Materials and Methods

Animals

One hundred and six male rats of the SH strain (body weight 205-270 g) were obtained from the Pushchino animal facility (Moscow Region, Russian Federation) and kept under standard vivarium conditions in a 12:12-hour dark-light cycle with free access to standard dry feed granules and sterile drinking water. The care and use of the animals and procedures reported in this study were in accord with Directive 2010/63/EU of the European Parliament and of the Animal Welfare Council used for scientific purposes.

Drugs

Atomoxetine (Strattera, Eli Lilly, USA) was dissolved in sterile water containing 0.5% Tween-80 (P1754, Sigma-Aldrich, USA) and administered orally via stainless steel gavage at a dose of 3 mg/kg once daily. The volume of administration was 2.5 ml/kg. The placebo contained 0.5% Tween-80 in sterile water.

Apparatus

The maze (TS0605-1, OpenScience Ltd., Russian Federation) was made of black plastic and consisted of 4 closed arms (numbered clockwise 1, 2, 3 and 4) connected to each other via central compartment through rectangular doorways. The dimensions of the arms were 20 × 20 × 20 cm with doorways of 10 × 10 cm in each arm. Two (opposite) of the four arms contained enriching objects – a closed cylindrical glass bottle (7.5 cm in diameter, 13 cm in height) placed vertically at the far end of the arm. The maze was closed with a transparent plastic cover equipped with small ventilation holes.

General procedure

On Day 1, Experiment 1 was conducted to evaluate the behavior of all 106 animals in the first cross-maze test. The frequency distribution of the Im-ratio index (see section Enriched cross-maze test) was analyzed to assess its difference from the normal distribution shape. Due to heterogeneity of the rat population regarding this measure showing existence of two subpopulations of individuals (Im+ and Im -), for experiment 2 with atomoxetine, only rats with high impulsivity and low attention (Im-ratio and ED-ratio indices less than 100) were used (n=36).

Thirty-six rats with these Im-ratio and ED-ratio indices were randomly divided into four subgroups assigned to either single or multiple administration of placebo or atomoxetine. The multiple administration regimen was performed on Days 4-7 of the study. The single dose regimen was performed on study Day 7. On study day 7, the animal behavior was assessed in the second enriched cross-maze test one hour after placebo or atomoxetine administration. The dosing regimen was selected according to a previously performed study with atomoxetine administration to SH rats (Salimov and Kovalev 2012).

Enriched cross-maze test

The test was described in detail earlier (Salimov and Kovalev 2012).

The animal was placed in the central compartment and allowed to explore the maze. The sequence of the arm visits and the time spent in the compartments were recorded. Each trial ended when 12 arm visits occurred within 15 minutes. The position of the objects in the pair of opposite arms (# 1 and # 3 or # 2 and # 4) was changed in a quasi-random order. The main variables for subsequent analysis were:

1) Total time spent in the center of the maze before entering empty or enriched arms. The impulsivity index Im-ratio was calculated by the formula 1:

$$Im-ratio = \frac{100 \times C\ time\ before\ arm\ enriched}{C\ time\ before\ empty} \quad (1)$$

where C time before arm enriched is the total time spent in the central compartment before entering arms containing enriching objects, C time before arm empty – total time spent in the central compartment before entering empty arms.

If there is no difference in the time spent in the maze center before entering the enriched and empty arms, then the ratio is 100. This index was considered as an indicator of impulsivity based on the results by Rico et al. (2016). Impulsive animals (Im+) have an index of Im-ratio of 100 or less; the rest of population (Im-) have an index of Im-ratio of more than 100.

2) Total time spent in empty or enriched arms. The index was used to estimate the ED ratio attention index (Salimov and Kovalev 2012, 2013), which was calculated by the formula 2:

$$ED-ratio = \frac{100 \times T\ arm\ enriched}{T\ arm\ empty} \quad (2)$$

where T arm enriched is the total time spent in arms containing enriching objects, T arm empty is the total time spent in empty arms.

If there is no difference in the time spent in the enriched and empty arms, then the ratio is 100. Animals exploring the objects stay longer in the enriched part of the maze than in empty arms and have higher enrichment recognition rates. Inattentive animals have an index of 100 or less.

Statistical analysis

The results were analyzed using Statistica 6.0 software. In experiment 1, a frequency distribution of the Im-ratio variable was compared with the normal distribution using the Chi-square test. Comparison of the mean values of the two groups was performed according to Student's t-test for independent groups. Pearson correlation was used to evaluate the relationship between the Im-ratio and ED-ratio indices. In experiment 2, the effect of atomoxetine on the behavior of rats with the Im+/CD-low phenotype (with the indices less than 100) was evaluated using 2-way ANOVA, where the independent factors were the substance administered (placebo or atomoxetine) and duration of the therapy (administration once or for 4 days). The difference between pairs of means was further assessed using the ANOVA contrast analysis.

Results

Experiment 1

In the first test, the frequency distribution of the Im-ratio variable had a clear bimodal shape (Fig. 1, data represented by bars) which was statistically significantly different from the curve expected under the normal distribution hypothesis (represented by line) (Chi-Square = 59.21, df = 4, $p<0.001$). The result indicates existence of two subpopulations (phenotypes) of Im+ and Im – individuals (Fig. 1) diverging in the impulsivity measure in this test.

The high impulsivity Im+ phenotype (having low Im-ratio index) is represented by the left side of the distribution and has an Im-ratio index within 0-129. The distribution shows that this rat phenotype has latency before entering the enriched maze arms almost

no more than that before entering the empty arms. This phenotype was represented by 59.4% of individuals of the rat population. The right side of the distribution represents the phenotype of rats with low impulsivity measure (high Im-ratio), in the range of 130-300 or more. The distribution indicates that majority of SH rats are inclined to the impulsive-like behavior.

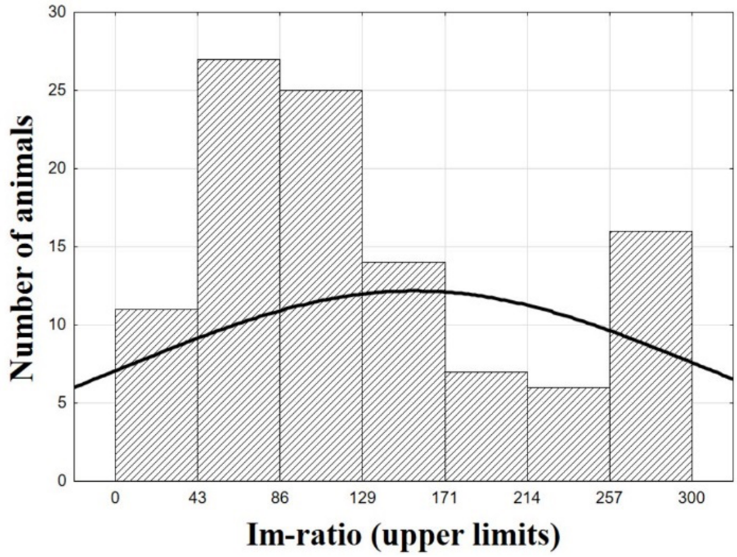


Figure 1. The frequency distribution of the Im-ratio of SH rats estimated during Experiment 1 (represented by bars) is bimodal and significantly different from the theoretical normal curve (represented by line) (Chi-square = 59.21, df = 4, p <0.001).

There was a statistically significant Pearson correlation between the Im-ratio impulsivity variable and the ED-ratio index of attention to enriched maze arms (r = 0.358, n = 106, p <0.001). There is also a statistically significant difference in the Im-ratio impulsivity index between the subgroups of rats with low and high ED-ratio attention indices (Table 1). Due to that, 36 rats of the Im+/ED-low phenotype (indices less than 100) were selected for Experiment 2.

Table 1. Im-ratio scores of SH rats diverging in attention index in the first enriched cross-maze test (mean±SEM)

Variable	Rats with low attention index, ED-low, n = 71	Rats with high attention index, ED-high, n = 35	Statistical significance of difference by Student's t-test
Im-ratio	121±17	225±25	t=3.55, df=104, p<0.001

Experiment 2

Animals from the placebo groups showed no significant differences in either the Im-ratio or ED-ratio variables obtained in the second test of Experiment 2 as compared with those obtained in the first test of Experiment 1. The fact demonstrates that they represent a relatively stable phenotypic subpopulation.

The two-way ANOVA of Im-ratio and ED-ratio indices of the Im+/ED-low rat from the second test having been treated with single or multiple atomoxetine or placebo revealed statistically significant interaction between the type of drug administered and the treatment duration (Tables 2 and 3). The Im-ratio and ED-ratio indices increased after multiple atomoxetine administration as compared with repeated placebo administration, namely by 125% and 191%, respectively.

Table 2. Results of 2-way ANOVA of behavioral measures from the second enriched cross-maze test performed with SH rats of Im+/ED-low phenotype after single or multiple (once daily, for 4 days) placebo or atomoxetine administration per os (mean ± SEM)

Factors	Im-ratio		ED-ratio	
	F-value	p	F-value	p
Factor 1 "Drug duration" (df=1)	1.905	0.177	8.05	0.008
Factor 2 "Drug type" (df=1)	3.632	0.066	4.017	0.054
Interaction of Factors 1 & 2 (df=1)	4.369	0.045	5.14	0.03

Table 3. Behavioral measures from the second enriched cross-maze test performed with SH rats of Im+/ED-low phenotype after single or multiple (once daily, for 4 days) placebo or atomoxetine administration per os (mean ± SEM)

Variable	Placebo		Atomoxetine (3 mg/kg)	
	Single	Multiple	Single	Multiple
Number of animals	8	9	9	10
Im-ratio	105.2 ± 37.5	98.7 ± 35.4	80 ± 35.5	221.9 ± 33.6 #
ED-ratio	91.3 ± 32.9	83.1 ± 31	109 ± 31.1	241.8 ± 29.4 ##

Note: # – statistically significant difference from the placebo group (F (1.32) = 6.38, p = 0.017, ANOVA contrast analysis); ## – statistically significant difference from the placebo group (F (1.32) = 13.8, p = 0.0008, ANOVA contrast analysis).

Discussion

The first main result of the present study is that the frequency distribution of the Im-ratio index of rats from an unselected SH rat population has clear bimodal shape that is statistically significantly different from the expected Gaussian curve. The result shows the existence of phenotypes among rats of the SH line which differ in impulsivity-like behavior in this test. The Im-ratio impulsivity index was correlated with the ED-ratio attention index. The outcome demonstrates the presence of Im+/ED-low phenotype that is similar to patients with ADHD syndrome. The membership of rats to the Im+/ED-low phenotype seems to be a relatively stable group type since these characteristics did not significantly change in the second test performed a week later with rats having received placebo.

The second main result of the present study is the fact that repeated administration of clinically effective anti-ADHD drug atomoxetine administered over 4 days improves impulsivity and attention measures. Interestingly, no improvement was observed after a single dose of atomoxetine. This result in terms of attention index replicates the previously obtained data (Salimov and Kovalev 2012). Because different drugs were used to treat ADHD, future studies would be necessary to evaluate the effects of different drugs and their combinations in the present test with individuals of Im+/ED-low phenotype.

Overall, the data from the present study suggest face and predictive validity of this test as an animal model of impulsivity and attention deficit, which may be useful in comparative studies proposed in rodents to investigate the neurobiology of ADHD and, especially, to screen new candidates for ADHD drugs.

Additional information

Conflict of interest

The authors declare no conflict of interests.

Acknowledgements

The authors have no support to report.

Data availability

All of the data that support the findings of this study are available in the main text.

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