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Research Article

# Comparison of CD-1 and ICR mouse strains for impulsivity in the enriched cross-maze test: Effects of atomoxetine

Ramiz M. Salimov[1], Natalia A. Sukhorukova[2], Georgy I. Kovalev[2]

[1]*Independent scientist, 31 Shkolnaya St., Krasnogorsk district, Moscow region 143442 Russia*
[2]*Laboratory of Mental Disorders Pharmacology, Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, 8 Baltiyskaya St., Moscow 125315 Russia*

*Corresponding author:* Ramiz M. Salimov (rmsalimov@yandex.ru)

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## Abstract

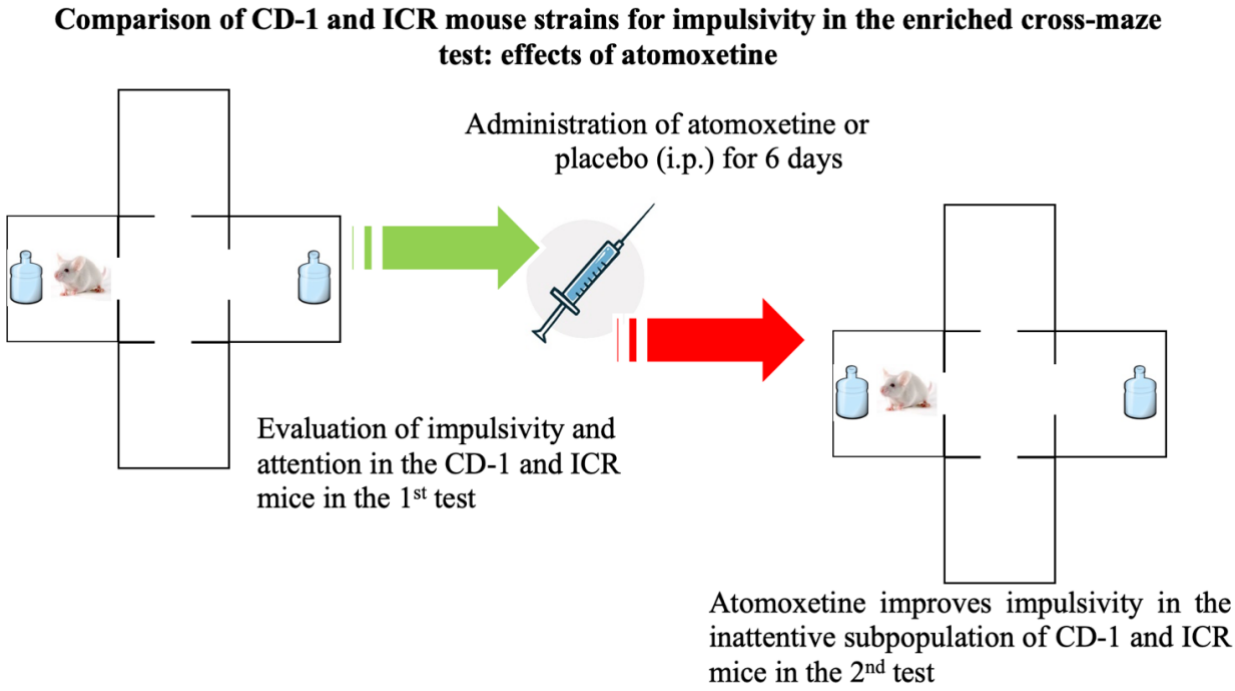
**Introduction**: Attention deficit hyperactivity disorder (ADHD) in children and adults is a neuropsychiatric condition that is characterized by difficulty sustaining attention and behavioral impulsivity. It is one of the problems of modern medicine requiring development of appropriate treatments and valid animal models. Previously, we have developed a model of the enriched cross-maze test suitable for express-evaluation of attention deficiency in rodents. Recently, we have also revealed that while employing spontaneously hypertensive rats it is possible to estimate impulsivity indices in the test. The present study is aimed at evaluation of the impulsivity indicators in the enriched cross-maze test employing mice of outbred CD-1 and ICR strains, belonging to different breeding cores.

**Materials and Method:** Adult male mice of the both outbred CD-1 (n=199) and ICR (n=148) strains were used in the study. Atomoxetine (3 mg/kg) as the drug of choice for ADHD was administered intraperitoneally once daily for 6 consecutive days.

**Results and Discussion**: Frequency distribution of the impulsivity index obtained from mice of both strains had a clear bimodal shape that statistically significantly differed from the normal distribution. The outcome indicates existence of subpopulations of individuals with high and low impulsivity. In inattentive mice, the subchronic atomoxetine administration selectively improved impulsivity indicators in the second enriched cross-maze test.

**Conclusion**: The enriched cross-maze test may be useful in neurobiology studies of ADHD and for screening new drug candidates for the ADHD treatment.

# Graphical abstract





# Keywords

Impulsivity; attention deficit, enriched cross-maze, mice, atomoxetine


# Introduction

Attention deficit hyperactivity disorder (ADHD) in children and adults is a neuropsychiatric condition that is characterized by difficulty sustaining attention, hyperactivity, and cognitive and behavioral impulsivity. Patients with ADHD have difficulties in sustaining attention that lead to problems in academic and job performance (American Academy of Pediatrics 2000; Paloyelis et al 2010). If untreated, up to 70% of the children continue to experience symptoms of the disease as adults (Mannuzza et al. 1993). It is one of the problems of modern medicine (Mannuzza et al. 1993; Volkow and Swanson 2013) requiring development of appropriate treatments and valid animal models.

Previously, we have developed a rodent model of the enriched cross-maze test that is suitable for express-evaluation of attention deficiency in the animals. Recently, we have also revealed employing spontaneously hypertensive rats that it is possible to estimate impulsivity indices in the test validated with atomoxetine as the drug of choice for ADHD treatment (Salimov and Kovalev 2025). The present study is aimed at evaluation of the impulsivity indicators in the enriched cross-maze test employing mice of CD-1 and ICR strains.

# Materials and Methods

### Animals

The animals were male mice (body weight 27-30 g) of two outbred strains – CD-1 and ICR belonging to different breeding cores. The CD-1 mice (n=199) were obtained from the Pushchino Animal Facility (Moscow Region, Russian Federation). The ICR (n=148) mice were obtained from the Stolbovaya Animal Facility (Moscow Region, Russian Federation). The animals were housed in the standard vivarium condition, exposed to 12:12-hour dark-light cycle with *ad libitum*

access to standard dry feed granules and tap water. The care and use of the animals and procedures in this study were in accord with EU Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes. Minutes of the institutional ethical committee No. 6 were approved on March 21, 2025.

**Drugs**

Atomoxetine (Strattera, Eli Lilly, USA) was dissolved in sterile water with 0.5% Tween-80 (P1754, Sigma-Aldrich, USA). The solution of the drug or placebo was administered intraperitoneally at a dose of 3 mg/kg in a volume of 2.5 ml/kg. The treatment was performed once daily for 6 consecutive days.

**Apparatus**

The maze (TS0605-1, OpenScience Ltd., Russian Federation) was made of black plastic. It included 4 closed arms (numbered clockwise 1, 2, 3 and 4) that connected to each other via central compartment through rectangular doorways. The dimensions of the arms were 12 × 12 × 12 cm with doorways of 7 × 7 cm in each arm. Two (opposite) of the four arms contained enriching objects – a closed cylindrical glass bottle (4.5 cm in diameter, 4 cm in height). The enriching objects were located at the far end of the arm. The maze had a transparent plastic cover with small holes for ventilation.

**General procedure**

On Day 1, Experiment 1 was performed to estimate behavior of all the mice in the first cross-maze test. The frequency distribution of the Im-ratio index (see next section) was evaluated to assess its difference from the normal distribution curve. For experiment 2 with atomoxetine that was aimed to assess effect of the drug on the Im-ratio index, 60 individuals of CD-1 strain and 40 individuals of ICR strain were selected. Mice of each strain were divided into cohorts belonging to phenotype of low or high attention index (ED-low or ED-high) having been recognized in Experiment 1 (as it was described in Salimov and Kovalev 2025). Each of the cohorts was randomly divided into subgroups assigned to administration of placebo or atomoxetine. The administration regimen was performed on Days 2-7 of the study. On study day 7, the animal behavior was assessed in the second enriched cross-maze test one hour after the last placebo or atomoxetine administration. The dosing regimen was selected according to the previously performed study with atomoxetine administration to spontaneously hypertensive rats (Salimov and Kovalev 2025).

**The enriched cross-maze test**

In details, the method was described earlier by Salimov and Kovalev (2025). An animal was placed in the central compartment and allowed to explore the maze. The sequence of arm visits and the time spent in the compartments were recorded. Each trial ended when the animal had made visits 12 to arms within 15 minutes. The objects' location in the pair of opposite arms (# 1 and # 3 or # 2 and # 4) was altered in a quasi-random order. The main variables for analysis were:

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

$$Im - ratio = \frac{100 \times C\_time\_before\_arm\_enriched}{C\_time\_before\_arm\_empty} \quad (1),$$

where C_time_before_arm_enriched is the total time spent in the central compartment before entering the arms containing enriching objects; C_time_before_arm_empty is the 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. According to the previously reported data (Rico et al. 2016; Salimov and Kovalev 2025), this index was considered as an indicator of impulsivity. 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 2025), 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. Inattentive animals have the index of 100 or less. Animals exploring objects stay longer in the enriched part of the maze than in empty arms and the index is more than 100.

### 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 effects of atomoxetine on the behavior of mice of both strains belonging to ED-low or ED-high phenotype was evaluated using 2-way ANOVA, where the independent factors were the phenotype (ED-low or ED-high) as well as the substance administered (placebo or atomoxetine). 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 obtained from mice of both strains had a bimodal shape that was statistically significantly different from the curve expected under the normal distribution hypothesis (Fig. 1). The result reveals existence of two subpopulations (phenotypes) of Im+ and Im – individuals among CD-1 and ICR strains 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. The distribution shows that this mouse phenotype has latency before entering the enriched maze arms similar to that before entering the empty arms. The right side of the distribution represents the phenotype of mice with low impulsivity measure (high Im-ratio). The distribution indicates that majority of the mice are inclined to the impulsive-like behavior.

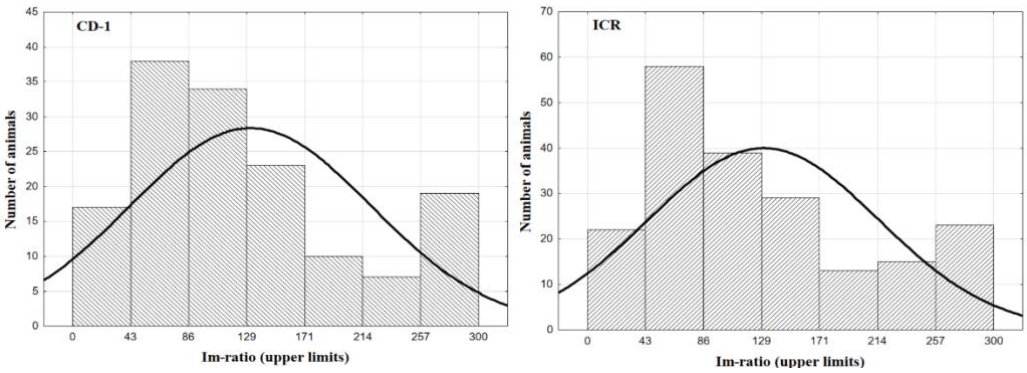



**Figure 1.** The frequency distribution (represented by bars) of the Im-ratio of CD-1 (left panel) and ICR (right panel) mice tested in Experiment 1 is bimodal and significantly different from the theoretical normal curve (represented by lines). For CD-1 mice: chi-square = 32.6, df = 4, $p < 0.001$. For ICR mice: chi-square = 49.5, df = 4, $p < 0.001$.

There was statistically significant difference in the Im-ratio impulsivity index between the phenotypes displaying low and high ED-ratio attention indices (Table 1). The proportion of ED-low individuals in the CD-1 strain (25%) was significantly lower than in the ICR strain (41.7%, chi-square = 10.5, df = 1, $p = 0.001$). There was also statistically significant Pearson correlation between the Im-ratio and the ED-ratio (for CD-1 mice: $r = 0.537$, $n = 148$, $p < 0.001$; for ICR mice: $r = 0.56$, $n = 199$, $p < 0.001$).

**Table 1.** Im-ratio scores of CD-1 and ICR mice phenotypes diverging in ED-ratio attention index obtained from the first enriched cross-maze test (mean ± S.E.M.)

| Mouse strain | Mice with low attention index, ED-low | Mice with high attention index, ED-high | Statistical significance of difference by Student's t-test |
|---|---|---|---|
| CD-1 | 77 ± 14 (n = 37) | 150 ± 8 (n = 111) | t = 4.57, df = 146, $p < 0.001$ |
| ICR | 81 ± 8 (n = 83) | 165 ± 7 (n = 116) | t = 7.85, df = 197, $p < 0.001$ |

### Experiment 2

The Im-ratio variables obtained in the second test of Experiment 2 from mice of placebo groups were similar to those obtained the first test of Experiment 1 in naïve mice of each CD-1 and ICR strains (Tables 1 and 2). The fact demonstrates that they represent relatively stable phenotypic subpopulations.

The two-way ANOVA yielded effects of phenotype (ED-low or ED-high) and substance administered (placebo or atomoxetine) on the Im-ratio. For CD-1 mice, there were insignificant general effects of phenotype [$F(1,56)=1.363$, $p=0.248$], significant general effect of substance type [$F(1,56)=9.427$, $p=0.003$] and significant interaction of the factors [$F(1,56)=4.208$, $p=0.045$]. For ICR mice, there were insignificant general effects of phenotype [$F(1,36)=3.049$, $p=0.089$], significant general effect of substance type [$F(1,36)=5.001$, $p=0.032$] and significant interaction of the factors [$F(1,36)=10.992$, $p=0.002$]. As it follows from Table 2, atomoxetine treatment resulted in normalization of Im-ratio index in the ED-low subpopulations in mice of the both strains (Table 2).

**Table 2.** Behavioral measures of Im-ratio from the second enriched cross-maze test performed with CD-1 and ICR mice after 6-day placebo or atomoxetine administration (mean ± S.E.M.)

| Strain | Variable | Placebo | | Atomoxetine (3 mg/kg) | |
|---|---|---|---|---|---|
| | | ED-low | ED-high | ED-low | ED-high |
| CD-1 | Number of animals | 14 | 16 | 13 | 17 |
| | Im-ratio | 74 ± 38* | 195 ± 40 | 266 ±36 # | 233 ± 35 |
| ICR | Number of animals | 10 | 10 | 10 | 10 |
| | Im-ratio | 53 ± 22* | 167 ± 23 | 178 ± 24 # | 143 ± 24 |

***Note:*** Statistically significant difference ($p < 0.05$, ANOVA contrast analysis): # – between placebo and atomoxetine groups; * – between ED-low and ED-high subpopulations.

# Discussion

The results of the present study demonstrate presence of impulsive individuals among the mice strains of subpopulation that demonstrate the same impulsivity indices in the second Enriched cross-maze test performed a week later. The animals of both strains selected in Experiment 1, having been treated with placebo, had demonstrated similar impulsivity indices between the strains in the second test of Experiment 2. In addition, subchronic atomoxetine administration produced selective improvement in the impulsivity indicators in the low-attentive mice subpopulations of both strains. The results of the present study are parallel to the previously reported outcomes obtained employing the same enriched cross-maze test performed with spontaneously hypertensive rats that was considered as a valid model of ADHD (Salimov and Kovalev 2025).

The present study proves that the enriched cross-maze test can serve as an animal model of impulsivity and attention deficit. The test is recommended for investigation of neurobiology of ADHD and, especially, to screen new candidates for anti-ADHD drugs. In addition, the enriched cross-maze test can be useful in the framework of examination of specific toxicity of novel medicine drugs in order to predict their potential effects on human abilities to operate mechanisms and drive vehicles. Future studies are recommended to evaluate the effects of different drugs and their combinations in the present test.

# Conclusion

The present study provides data suggesting that the enriched cross-maze test includes criteria for face validity and predictive validity of the ADHD. Thus, the proposed enriched cross-maze test can serve as a non-invasive model of ADHD in animals.

# Additional Information

### Conflict of interest

The authors declare the absence of a conflict of interests.

**Acknowledgements**

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**Data availability**

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

**Ethics Statements**

The methods used in the work were approved by the Minutes of the institutional ethical committee No. 6 were approved on March 21, 2025, Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies Moscow 125315 Russia.

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# Author Contribution

- **Ramiz M. Salimov,** Doctor of Biological Sciences, independent scientist, Shkolnaya Street, 31, Krasnogorsk district, Moscow region, Russia; e-mail: rmsalimov@yandex.ru; **ORCID ID** https://orcid.org/0000-0002-9297-9847. Personal contribution: designing the experiments, statistical analysis of results, writing and designing the text of the article.
- **Natalia A. Sukhorukova,** PhD, Scientific researcher, Laboratory of Mental Disorders Pharmacology, Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, 8 Baltiyskaya St., Moscow 125315 Russia; e-mail: sukhorukova_na@academpharm.ru; **ORCID ID** https://orcid.org/0000-0002-6412-4833. Personal contribution: designing and performing the experiments, writing and designing the text of the article.
- **Georgy I. Kovalev,** Professor, Doctor of Medical Sciences, Chief Scientific Officer, Laboratory of Mental Disorders Pharmacology, Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, 8 Baltiyskaya St., Moscow 125315 Russia, e-mail: kovalev_gi@academpharm.ru; **ORCID ID** https://orcid.org/0000-0002-8597-7018. Personal contribution: designing the experiments, writing and designing the text of the article.