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Effect of fluoxetine on seizures in rats with high susceptibility to audiogenic stress

Khadija Ismayilova, Ulduz Hashimova, Mohammad Reza Majidi, Farhad Rustamov

Corresponding author
Farhad Rustamov
Academician Abdulla Garayev Institute of Physiology, Azerbaijan National Academy of Sciences (ANAS), Baku, Azerbaijan

Handling editor:
Michal Heger
Department of Pharmaceutics, Utrecht University, the Netherlands
Department of Pharmaceutics, Jiaxing University Medical College, Zhejiang, China

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1st Editorial decision
25-Dec-2020

Ref.: Ms. No. JCTRes-D-20-00123
Effect of fluoxetine on seizures in rats with high susceptibility to audiogenic stress caused by the imbalance of neurotransmitters
Journal of Clinical and Translational Research

Dear Mr Rustamov,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below and attached to this email.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jan 24, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission

record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The paper contains data on the changes of audiogenic seizure intensity after fluoxetine treatment in Wistar audiogenic-sensitive rats. This change occurred in parallel with shifts in noradrenaline, 5-HT and dopamine levels in the hypothalamus and prefrontal neocortical area.These data are relatively new and deserve publication, although the paper requires rather profound changes concerning the general data from the literature of audiogenic epilepsy data/. It contains several mistakes as well.
1. It is strange that the authors did not notice the paper on (chronic) fluoxetine treatment in rats of several genotypes, in which the comorbidity of comorbidity in audiogenic seizure and depressive behavior had been analyzed in control rats and in rats after fluoxetine treatment. These data should be mentioned in the respective places of the paper (Sarkisova et al. Epilepsy Behav. 2017 Mar;68:95-102). i/ The existence of this paper should change the text of introduction as well as the discussion of the paper under review.
2. The audiogenic epilepsy is the complicated physiological trait and glutamate and GABA systems are now considered of great importance in the expression of this pathological reaction to loud sound. Thus this moment should be mentioned in the paper as well. The latter notion also means that 5-HT and changes of its level due to fluoxetine treatment are not the critical issue for the the sound-induce seizure development. The production of this pathology involves several links, and the interference in each of them could induce changes in the seizure pattern, the 5-HT system being one of them. Concerning 5-HT system role in these processes the phenomenon of SUDEP (extensively analyzed by C. Faingold et al.) should also be mentioned in the paper.
3. It stays enigmatic why the monoamine content had been determined in hypothalamus and prefrontal cortex, while the structures, crucial for audiogenic seizyre development are in the brain stem (colliculi inferior, PAG etc)/ The respective comments in the paper are needed.
4. The wild-run stage of audiogenic fit bears the similarity of panic attack, but their identity should be proved in special experiments, So the claim that the initial stage of audiogenic fit is the panic reaction is not correct.
5. Page 4. Line 12. The seizures induced by sound are not the stress reaction as the development of stress (elevation of plasma corticosterone etc) requires several minutes for expression. - It should be corrected.
6. Page 5, line 44. The origin of Wistar rats should be indicated, as the differences in population scores of audiogenic epilepsy exist.
7. Page 5 line 48 -the DBA is the MOUSE strain
8. Page 5, line 52 - this statement probably is correct for GEPR strains, but not, for example, for the KM strain (mentioned in the citation list by number 4), in which about 100% of animals display tonic seizures in response to loud sound.
9. The table (page 8-9) repeats the information ,included in the text- it should be corrected.
10. The scaling of seizure severity used by authors is the one which had been introduced by Krushinsky while the selection of audiogenic prone strain. It means that the citation by number 13 is erroneous, as these authors worked with GEPR s and thus used another scaling

(0-9).
If authors are ready to submit their manuscript to sevberal rather deep changes, the paper could be published in the Journal.

Reviewer #2:
This study is novel and interesting. However, there are some concerns that needs to be addressed by the authors.

1. The biggest concern is that authors have not performed any behavioral study, which I feel is very important for this kind of study.

2. A sentence should never start with a number. e.g., "1 h prior to the..."The author should check the whole manuscript for this.

Reviewer #3: Abstract
1) Abstract background is too long, Please shorten the background part of the abstract part very long.

Introduction
1) Page 2 line 35, please correct the 'iSnhibitors' spelling error 'inhibitors'
2) Page 4 line 27, 28, 29, 30 'There are several strains of rodents including natural and synthetic mutants (Wag/Rij, WAR, tottering etc.), which exhibit spontaneous seizures of different types (vs chemically or electrically induced seizures).
The sentence should be written more clearly, natural(……..) and synthetic mutants (……).
And also add refeneces.
3) Page 17/4 line 50 references
Material and method
1) Material and method part 7/17 is not clear.
'From the total number (111) of the rats, 29 ST and 27 SS rats were selected'
By drawing a diagram, the material method should be written in more detail.
2) The rats were housed in standard cages (6-7 rats per cage); what were the dimensions of the cage. 6-7 rats in a cag? İt is too much, isnt it.
3) 8/17 Statistical analysis should be rewritten, for which data the nonparametric Mann - Whitney U test and Parametric Student's t-test were used. Which test was used to determine whether it conformed to normal distribution?
4) Why was ECoG not recorded by attaching electrodes to rats? The seizure could also be evaluated with the EcoG recording. It was only evaluated behaviorally. Was video recorded while behaviorally evaluating the seizure?
5) How was the dose of 25 mg / kg fluoxetine determined? Give references
6) You could get the seizure patterns included in Figure 2 from your own video recording. Images are not good.
7) It is seen in Figure 1 that there is a much decrease in tonic-clonic seizures compared to the control group. Was there no statistically significant difference? It should be written clearly.
8) Also, a column for A - control animals, B - experimental animals; 1 - running around in circles + jumping; a separate column chart would be more understandable for 2 - tonic-clonic seizures.

9) In ELISA findings, when ST (control), ST (fluoxetine) compared to SS (Control) and SS (fluoxetine) rats are compared to rats, that is, when the quadruple group is compared with anova, is there a difference in 5-HT, NA and DA levels in the hypothalamus and frontal cortex? What I am particularly curious about is the change in 5-HT, NA and DA levels in the hypothalamus and frontal cortex compared to control rats (ST control) when an odyegenic seizure occurs? what is fluoxetine's reaction to this.
It should also be discussed in the discussion section in detail.
Statnick, M. A., Maring-Smith, M. L., Clough, R. W., Wang, C., Dailey, J. W., Jobe, P. C., & Browning, R. A. (1996). Effect of 5, 7-dihydroxytryptamine on audiogenic seizures in genetically epilepsy-prone rats. Life sciences, 59(21), 1763-1771.

10) Discussion

In clinical and experimental studies, proconvulsant and anticonvulsant effects of fluoxetine have been demonstrated. For example, in one study, in the penicillin-induced experimental epilepsy model, fluoxetine at a dose of 5, 10 mg / kg decreased seizures while at a dose of 20 mg / kg it increased seizures. Fluoxetine (10 and 20mg/kg) shortened the latency to first seizure and increased mortality rate and proconvulsant effect in a dose-related manner in pilocarpine-induced status epilepticus rats .
Ferrero AJ, Cereseto M, Reinés A, Bonavita CD, Sifonios LL, Rubio MC, et al. Chronic treatment with fluoxetine decreases seizure threshold in naive but not in rats exposed to the learned helplessness paradigm: correlation with the hippocampal glutamate release. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(5):678-86.
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Aygun, H. (2019). The effect of fluoxetine on penicillin-induced epileptiform activity. Epilepsy & Behavior, 95, 79-86.
It is seen that you used a single dose of 25 mg / kg in your study. Why wasn't the dose study done? Because the effect on seizures could be different at low doses. In the abovementioned articles, generally 20 mg / kg has an enhancing effect on seizures. The dose used in the presented study is a high dose of 25 mg / kg. It should be discussed with the above references why it might have reduced seizures in the audiogenic seizures model. It is useful to discuss it together.
Also, many experimental studies demonstrated that fluoxetine was effective against audiogenic seizures in mice and rats.

There are two important studies on audiogenic seizures, which are not discussed:
Sparks DL, Buckholtz NS. Combined inhibition of serotonin uptake and oxidative deamination attenuates audiogenic seizures in DBA/2J mice. Pharmacol Biochem Behav 1985;23:753-7.
Dailey JW, Yan QS, Adams-Curtis LE, Ryu JR, Ko KH, Mishra PK, et al. Neurochemical correlates of antiepileptic drugs in the genetically epilepsy-prone rat (GEPR). Life Sci 1996;58(4):259-66

There is much missing in the discussion. It should be revised very carefully.

There is additional documentation related to this decision letter. To access the file(s), please

click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Authors' response

Dear Editor,

Coauthors and I very much appreciated the encouraging, critical and constructive comments on this manuscript by the reviewers. The comments have been very thorough and useful in improving the manuscript. We strongly believe that the comments and suggestions have increased the scientific value of revised manuscript by many folds. We have taken them fully into account in revision. We are submitting the corrected manuscript with the suggestion incorporated the manuscript. The manuscript has been revised as per the comments given by the reviewer, and our responses to all the comments are as follows:

Reviewer #1: The paper contains data on the changes of audiogenic seizure intensity after fluoxetine treatment in Wistar audiogenic-sensitive rats. This change occurred in parallel with shifts in noradrenaline, 5-HT and dopamine levels in the hypothalamus and prefrontal neocortical area.These data are relatively new and deserve publication, although the paper requires rather profound changes concerning the general data from the literature of audiogenic epilepsy data/. It contains several mistakes as well.

1. It is strange that the authors did not notice the paper on (chronic) fluoxetine treatment in rats of several genotypes, in which the comorbidity of comorbidity in audiogenic seizure and depressive behavior had been analyzed in control rats and in rats after fluoxetine treatment. These data should be mentioned in the respective places of the paper (Sarkisova et al. Epilepsy Behav. 2017 Mar;68:95-102). i/ The existence of this paper should change the text of introduction as well as the discussion of the paper under review.

The corresponding changes have been made to the text of the manuscript.

2. The audiogenic epilepsy is the complicated physiological trait and glutamate and GABA systems are now considered of great importance in the expression of this pathological reaction to loud sound. Thus this moment should be mentioned in the paper as well. The latter notion also means that 5-HT and changes of its level due to fluoxetine treatment are not the critical issue for the the sound-induce seizure development. The production of this pathology involves several links, and the interference in each of them could induce changes in the seizure pattern, the 5-HT system being one of them. Concerning 5-HT system role in these processes the phenomenon of SUDEP (extensively analyzed by C. Faingold et al.) should also be mentioned in the paper.

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3. It stays enigmatic why the monoamine content had been determined in hypothalamus and prefrontal cortex, while the structures, crucial for audiogenic seizyre development are in the brain stem (colliculi inferior, PAG etc)/ The respective comments in the paper are needed.

The corresponding changes have been made to the text of the manuscript.

4. The wild-run stage of audiogenic fit bears the similarity of panic attack, but their identity should be proved in special experiments, So the claim that the initial stage of audiogenic fit is the panic reaction is not correct.

We agree with the comment, and the corresponding changes have been made to the text of the manuscript.

5. Page 4. Line 12. The seizures induced by sound are not the stress reaction as the development of stress (elevation of plasma corticosterone etc) requires several minutes for expression. - It should be corrected.

We agree with the comment, and the word "stress" has been replaced by "stimulus".

6. Page 5, line 44. The origin of Wistar rats should be indicated, as the differences in population scores of audiogenic epilepsy exist.

The corresponding changes have been made to the text of the manuscript.

7. Page 5 line 48 -the DBA is the MOUSE strain

We have deleted DBA from the sentence.

8. Page 5, line 52 - this statement probably is correct for GEPR strains, but not, for example, for the KM strain (mentioned in the citation list by number 4), in which about 100% of animals display tonic seizures in response to loud sound.

We agree with the comment, and the corresponding changes have been made to the text of the manuscript.

9. The table (page 8-9) repeats the information ,included in the text- it should be corrected.

The table has been removed from the text of the manuscript.

10. The scaling of seizure severity used by authors is the one which had been introduced by Krushinsky while the selection of audiogenic prone strain. It means that the citation by number 13 is erroneous, as these authors worked with GEPR s and thus used another scaling (0-9).

We agree with the comment, and the corresponding changes have been made to the text of the manuscript.

If authors are ready to submit their manuscript to sevberal rather deep changes, the paper could be published in the Journal.

Reviewer #2:

This study is novel and interesting. However, there are some concerns that needs to be addressed by the authors.

1. The biggest concern is that authors have not performed any behavioral study, which I feel is very important for this kind of study.

The experiments were performed on two groups of male Wistar rats: seizure-tolerant (ST) and seizure-susceptible (SS), the division of which is explained by their response to the acoustic stimulus.

In our studies, the behavior of the rats of both groups in the open-field changed dramatically after the acute oral administration of fluoxetine at a dose of 25 mg/kg. Under the drug, the ST rats exhibited reduced exploratory behavior and increased frequency and duration of grooming episodes, which indicates the rise in the level of anxiety thereby confirming the anxiogenic property of fluoxetine. Our findings are consistent with literature data (Sarkisova, Folomkina, 2010) showing that fluoxetine has anxiogenic-like effect in "normal" Wistar rats (decrease in the total number of visits to the center of open-field and increase in the number of grooming episodes). Similarly, the anxiogenic-like effect of the acute administration of fluoxetine on behavior in the tests for anxiety was shown in the overview of Borsini et al (Borsini et al., 2002). In addition, the overview demonstrates that the serotonergic system of the brain plays a key role in the mechanisms underlying anxiety. It is suggested that the possible mechanism of anxiogenic-like effect of the selective serotonin reuptake inhibitors in the ST rats is the increase in the level of 5-HT in the synaptic cleft and activation of 5-HT2c serotonin receptors (Salchner, Singewald, 2006). There are also data on the inhibitory effect of 5-HT in the mechanism of motor activity (Gershteyn et al., 2000).

As regards the SS rats, the acute administration of fluoxetine led to decrease in motor activity, frequency, and duration of grooming episodes in the open-field test, which indicates the reduction in anxiety and fear. These findings corroborate the views of the researcher (Sarkisova, Folomkina, 2010) on the fact that the administration of fluoxetine may have anxiolytic-like effect.

It should be noted that there is correlation between the multidirectional effects of psychotropic drugs and genotype of animals, test conditions (Griebel et al., 2000), as well as the initial psycho-emotional state of individuals (Ben-Porath, Taylor, 2002).

It is known that epilepsy, as the system disease of the Central Nervous System, is accompanied by cognitive and behavioral impairment. Therefore, the clinical and experimental studies of epilepsy are mainly focused on comorbidity (Sarkisova et al., 2017). It was shown that the individual (and, apparently, genetic) variability plays a key role in comorbidity of anxiety and depressive disorders, which is confirmed by the clinical (Bozak et al., 2015) and experimental (Swau et al., 2009) studies.

In our studies, the anxiety-related behavior of the ST and the SS male Wistar rats in the elevated plus maze (EPM) test, as well as their responses to a sound stimulus were assessed prior to and 1h after the acute oral administration of fluoxetine at a dose of 25 mg/kg. It is suggested that anxiety combines different types of behavior: active avoidance response to the stimulus and passive avoidance response to the danger – freezing (Avgustinovich et al., 1998). Thus, the ST rats exhibited the passive avoidance response (decrease in motor activity and number of rearing episodes, as well as increase in the number of boluses) in open arms of the maze in comparison with the control group. As regards the SS rats, the administration of fluoxetine led to increase in anxiety indicative by the active avoidance response to open spaces of the maze and by preferring closed arms, where they stayed until the end of the test (increase in the number of grooming episodes and their duration).

It is known that the values of the background parameters in the EPM test and their fluctuations prior to and after the 14-day intraperitoneal administration of fluoxetine (20 mg/kg per day) indicate the absence of the direct link (and, apparently, comorbidity) between

the symptoms of anxiety and the severity of audiogenic seizures in Wistar rats. (Sarkisova et al., 2016). Perhaps, the findings obtained by the authors (Sarkisova et al., 2017) after the chronic administration of fluoxetine are consistent with our results obtained from the research only on the seizure-tolerant Wistar rats in the EPM test after the acute administration of the drug.

2. A sentence should never start with a number. e.g., "1 h prior to the..."The author should check the whole manuscript for this.

The corresponding changes have been made to the text of the manuscript.

Reviewer #3: Abstract

1) Abstract background is too long, Please shorten the background part of the abstract part very long.

The whole abstract has been shortened according to the scientific paper that were already published.

Introduction

1) Page 2 line 35, please correct the 'iSnhibitors' spelling error 'inhibitors'

The spelling error has been corrected.

2) Page 4 line 27, 28, 29, 30 'There are several strains of rodents including natural and synthetic mutants (Wag/Rij, WAR, tottering etc.), which exhibit spontaneous seizures of different types (vs chemically or electrically induced seizures).

The sentence should be written more clearly, natural(……..) and synthetic mutants (……).

And also add refeneces.

The corresponding changes have been made to the text of the manuscript.

3) Page 17/4 line 50 references

This comment is not clear to us.

Material and method

1) Material and method part 7/17 is not clear.

'From the total number (111) of the rats, 29 ST and 27 SS rats were selected'

By drawing a diagram, the material method should be written in more detail.

The corresponding changes have been made to the text of the manuscript.

2) The rats were housed in standard cages (6-7 rats per cage); what were the dimensions of the cage. 6-7 rats in a cag? İt is too much, isnt it.

We have now submitted the precise dimensions of the housing cage in the text of the manuscript.

3) 8/17 Statistical analysis should be rewritten, for which data the nonparametric Mann - Whitney U test and Parametric Student's t-test were used. Which test was used to determine whether it conformed to normal distribution?

The corresponding changes have been made to the text of the manuscript.

4) Why was ECoG not recorded by attaching electrodes to rats? The seizure could also be evaluated with the EcoG recording. It was only evaluated behaviorally. Was video recorded while behaviorally evaluating the seizure?

Unfortunately, we did not record the ECoG, because it was not one of the objectives of the study due to the fact that the polygraphic recording during wild running and clonic seizures would be significantly complicated by artifacts arising from sudden movements of the animals. In this regard, we have submitted the video recorded while behaviorally evaluating the seizure.

5) How was the dose of 25 mg / kg fluoxetine determined? Give references

The corresponding changes have been made to the text of the manuscript.

6) You could get the seizure patterns included in Figure 2 from your own video recording. Images are not good.

Unfortunately, the resolution of the video is not high enough, therefore, the Figure 2 will be very unclear, if we include pictures of rat from the video. However, we have tweaked it a bit in order to make it more understandable.

7) It is seen in Figure 1 that there is a much decrease in tonic-clonic seizures compared to the control group. Was there no statistically significant difference? It should be written clearly.

The corresponding changes have been made to the Figure 1.

8) Also, a column for A - control animals, B - experimental animals; 1 - running around in circles + jumping; a separate column chart would be more understandable for 2 - tonic-clonic seizures.

The corresponding changes have been made to the Figure 1.

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Unfortunately, we did not compare ST (control), ST (fluoxetine) to SS (Control) and SS (fluoxetine) rats with anova, because we thought that it will distract attention from the main idea of the paper. As regards the second part of the question, such information is given at the end of the Results section of the paper.

It should also be discussed in the discussion section in detail.

Statnick, M. A., Maring-Smith, M. L., Clough, R. W., Wang, C., Dailey, J. W., Jobe, P. C., & Browning, R. A. (1996). Effect of 5, 7-dihydroxytryptamine on audiogenic seizures in genetically epilepsy-prone rats. Life sciences, 59(21), 1763-1771.

The reference mentioned above has now been discussed in the manuscript.

10) Discussion

In clinical and experimental studies, proconvulsant and anticonvulsant effects of fluoxetine have been demonstrated. For example, in one study, in the penicillin-induced experimental epilepsy model, fluoxetine at a dose of 5, 10 mg / kg decreased seizures while at a dose of 20 mg / kg it increased seizures. Fluoxetine (10 and 20mg/kg) shortened the latency to first seizure and increased mortality rate and proconvulsant effect in a dose-related manner in pilocarpine-induced status epilepticus rats .

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Also, many experimental studies demonstrated that fluoxetine was effective against audiogenic seizures in mice and rats.

There are two important studies on audiogenic seizures, which are not discussed:

Sparks DL, Buckholtz NS. Combined inhibition of serotonin uptake and oxidative deamination attenuates audiogenic seizures in DBA/2J mice. Pharmacol Biochem Behav 1985;23:753-7.

Dailey JW, Yan QS, Adams-Curtis LE, Ryu JR, Ko KH, Mishra PK, et al. Neurochemical correlates of antiepileptic drugs in the genetically epilepsy-prone rat (GEPR). Life Sci 1996;58(4):259-66

All references that you mentioned above have now been discussed in the manuscript.

There is much missing in the discussion. It should be revised very carefully.

2nd Editorial decision
18-Mar-2021

Ref.: Ms. No. JCTRes-D-20-00123R1
Effect of fluoxetine on seizures in rats with high susceptibility to audiogenic stress caused by the imbalance of neurotransmitters
Journal of Clinical and Translational Research

Dear Mr Rustamov,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Apr 17, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The points which should be discussed in this paper are in the attached file

Reviewer #2: The authors have successfully addressed all the comments and the manuscript is now suitable for publication.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Authors' decision

Dear Editor,
Dear Reviewers,

Thank you again for giving us the opportunity to submit a revised version of the manuscript for publication. We appreciate the time and effort that you and the reviewers dedicated to

providing feedback on our manuscript and are grateful for the insightful comments on and valuable improvements to our paper. Those changes are highlighted within the manuscript. Please see below for a point-by-point response to the reviewers' comments and concerns.

1) The imbalance of brain monoamines, announced in the heading of the paper was not investigated by authors, so the heading in this respect is misleading.

We agree with the comment, and the corresponding changes have been made to the title of the manuscript.

2) It looks that authors ignore or are not aware of the paper in which the chronic fluoxetine effects were analyzed in rats of 4 genotypes pairwise different by AER-proneness and genetic background. It is important to include this information into discussion and the reference list. (*Sarkisova et al., Genetic background contributes to the co-morbidity of anxiety and depression with audiogenic seizure propensity and responses to fluoxetine treatment. Epilepsy and Behavior, 2017, v. 68, p. 95-102*).

The corresponding changes have been made to the text of the manuscript.

3) Wistar rats originated from different animal farms differ by AE proneness as well, so it is important to indicate the source of animals used in the study (*Honndorf et al., Female Wistar rats obtained from different breeders vary in anxiety-like behavior and epileptogenesis. Epilepsy Res. 2011 Mar;94(1-2):26-38)*

The study was performed Wistar rats, which were bred at the Animal Research Facility of the Institute of Physiology, ANAS. This information has also been copied to the "Methods" section of the manuscript.

3rd Editorial decision
29-Mar-2021

Ref.: Ms. No. JCTRes-D-20-00123R2
Effect of fluoxetine on seizures in rats with high susceptibility to audiogenic stress
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger

Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: