International Journal of High Risk Behaviors and Addiction
Volume 12 · Issue 2 · e131285

OPEN PEER REVIEW

# The Effects of Deep Brain Stimulation in Medial Prefrontal Cortex on Morphine Dependency and Electrical Recording of the Nucleus Accumbens

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| SUBMITTED | REVISED | ACCEPTED | DOI |
| --- | --- | --- | --- |
| 29 Aug 2022 | 28 Feb 2023 | 2 Mar 2023 | 10.5812/ijhrba-131285 |

## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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## 1 Original submission

3 comments · 29 Sep 2022 – 24 Nov 2022

### Referee 1 Reviewer

MAJOR REVISION 29 Sep 2022

Abstract

The methods section needs to be revised to describe the procedures properly, including the order of the experiments performed on the groups: first, implanting the electrodes in the brain, then CPP, and then recording from the electrodes, ….

It is not clear whether stimulation was applied to the PFC and recordings were obtained from the NAc, because the conclusion mentions that stimulation of the NAc increased locomotor activity.

Line 16, " … average of spikes in the cortical neurons of the nucleus accumbens". What are cortical neurons of the NAc?

Please rewrite the abstract, considering the order of the experiments and results, and then base the conclusion on them.

Introduction

There is no need to write the word substance with a capital S (line 28, 32).

Line 33 and 34, what is meant by "unique" in "…because of its unique 34 human and geographical characteristics"?

The English language needs to be checked and corrected. Line 33, " …control headquarters indicates the …": headquarters is plural, and the verb indicate must follow without "s". Similar mistakes involving subject–verb agreement or incorrect tenses occur throughout the text.

Line 58, "…, DBS research has been developed in various areas of the brain to treat 59 neuropsychiatric conditions such as Alzheimer's disease, Tourette's syndrome, obsessive compulsive disorder, and advanced depression ". A reference is needed.

Line 65, what do the authors mean by "orbital effects"?

Line 67, 2 afferents? " …specific afferents and afferents …"

Line 76, "…But we consider 76 that this intervention in LH orexin neurons may also provide promising results for addiction 77 treatment…". What do the authors mean by this sentence? Did they observe this in their own studies, or is it from other studies? A reference and further explanation are needed.

Line 78, "..To the best of our knowledge, there is little information about the effect of DBS on morphine dependency…". Contrary to this notion expressed by the authors, there is a substantial body of literature discussing the specific effect of DBS on related regions of the brain in "morphine" dependency and addiction, particularly by Iranian researchers!

Methods

Line 91, "…results of the CPP (…..…..…) test": what does (…) mean?

For sham groups, the standard procedure is to open the animal or drill holes in the skull, but not to insert the cannula. Do you have any references indicating that a cannula is also placed in sham animals?

Also, what was the role of the sham group in your experiments? The experimental design, especially with CPP involved, does not require a sham group, because apparently the authors did not inject anything into the sham rats. Therefore, it seems more logical to remove the sham group and perform the analysis using two-way ANOVA, with rats receiving either morphine or saline, with and without DBS. That makes more sense.

Again, English language correction is needed. Line 97, "…The electrical response of AC neurons to DBS is determined …". "Is" must be replaced with was. In line 101, body temperature … was maintained is correct.

Line 105, the coordinates must be presented as follows: AP: -4, ML: 1.6 and DL: -8.4 mm.
Line 117, do light and dark, as used to describe the chambers, refer to light intensity or color? If they refer to color, this should be mentioned, although it is customary to distinguish the interiors of the chambers by differences in design rather than color, such as vertical lines for one and horizontal lines for another.
It is not clear how stimulation (DBS) was administered to the rats: during the 3 days of conditioning, in both the morning and afternoon or only one of these, OR after conditioning? Also, in the discussion, the authors mentioned 3–6 days of stimulation. When did that happen? Also, which period of DBS does the graph of DBS spikes relate to?
A diagram illustrating the timing of each event would be useful.

The histological assessment needs to be rewritten to present the steps in the proper order: first, dissecting the brain; then immersing it in 10% paraformaldehyde for 24 hours, followed by 4% paraformaldehyde for preservation and fixation; then sectioning; and finally, staining and microscopic examination.
Also, please provide an image of the electrode perforation sites in the brain.
As explained above, using two-way ANOVA is more suitable for this study.

Results
Each figure must have its own descriptions of the symbols (@, &, …).
In the text, the authors have not mentioned anything about baseline recordings of spikes in the cortex and NAc. Please add this to the text.
The results of the statistical analysis must include the degrees of freedom, as in F(dn, df) = ??.??, P<…).
Why is there no description of a comparison with the saline group? Morphine and saline are the main groups, not sham.
In the first part of the results, the first paragraph includes some repeated reports. Please correct this. "… morphine (5mg/kg) has significantly enhanced conditioned place 167 preference [487.5 ± 59.91] in comparison with the sham group [18.33 ± 1.56. Based on Fig.2, administration of morphine [487.5 ± 59.91] without electrical stimulation 169 increased conditioned place preferences compared with the sham group [18.33 ± 1.56; P"
Discussion
The first paragraph repeats the introduction. Please avoid redundancy.
Lines 201, 202, .." Further, applying electrical stimulation for 3–6 days ??? reduced the preference score in the CPP test compared with the sham DBS group."
This section does not discuss the relationship between the mPFC and NAc, how this relationship affects morphine dependency, or how this connection is disrupted by DBS. Most sentences in the discussion section are not relevant to the main subject of your manuscript: a new connection between the mPFC and NAc in morphine dependency. Please rewrite the discussion to make it relevant to the subject and results of your study.

**Associate Editor** MAJOR REVISION 24 Nov 2022

major revision

**Editor-in-Chief** MAJOR REVISION 24 Nov 2022

major revision

2

# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**1/2/2023**

**Response to comments of reviewers**

**Journal of High Risk and Behavior**

Dear Editor

The authors are grateful to respected editor and reviewers for their attention, constructive comments and valuable suggestions on previous version of the manuscript. We tried to consider the Biomedicine and Pharmacotherapy journal guidelines as strictly as possible. We have revised the manuscript according to reviewer's comments and suggestions as point by point in detail with highlighted and red color texts.

Best wishes,

Dr. Mohammad Ali Mirshekar

**Reviewer comment:** Reviewer #1:

Abstract

The method section is needed to be reconsidered by writing it properly including the order of the experiments applied to the groups,… first; implanting the electrodes in the brain, then cpp , then recording from the electrodes,….

Response to reviewer: The order of the experiments applied to the groups, was reviewed and corrected. The corrected sentence was added as follows: "Then, the rats were treated with saline or morphine, subjected to stereotaxic surgery to insert the stimulator electrode in mPFC and the recorder electrode in AC. The electrical response of AC neurons to DBS is

determined by the single unit recording method.”

It is not clear was the stimulation to pfc and recording from NAc? Because at the conclusion is mentioned that stimulation of NAc produced increase locomotor activity??

Response to reviewer: The answer to this question was added in the text as below: The nucleus accumbens sends efferents to areas such as the frontal cortex, limbic areas, amygdala, hypothalamus and midbrain, which play an important role in the processes involved in reward. Studies have shown that addiction can decrease the activity of areas such as the cingulate cortex and the dorsolateral prefrontal cortex (DLPFC), which decrease the activity of these areas can decrease a person's control over his own behavior and ultimately lead to addiction [10]. Nevertheless, the nucleus accumbens receives important inputs from the prefrontal cortex, which affects the activity of the accumbens region and finally the addictive behaviors of the individual [11]. One of the target areas studied in the discussion of DBS and addiction is the prefrontal cortex and especially the medial prefrontal cortex (mPFC).

Line 16, “ … average of spikes in the cortical neurons of the nucleus accumbens”. What are cortical neurons of NAc??

Response to reviewer: The Nucleus of Accumbens has two cortical and central parts, and the stimulation of each part has unique effects.

Please re-write the abstract considering the order of experiments and results and then build up the conclusion upon it.

Response to reviewer: the abstract re-writed considering the order of experiments and results and then conclusion. The order of experiment edited as follows: During conditioning in the conditioned place preference (CPP) box, rats received DBS with a frequency of 130 Hz, amplitude of 0.2 to 0.5 mA, and repeated periods of 15 minutes with an interval of 45 minutes for 3 hours. Then, the rats were treated with saline or morphine, subjected to stereotaxic surgery for insertion the stimulator electrode in mPFC and the recorder electrode in AC. The electrical response of AC neurons to DBS is determined by the single unit recording method. Then, motor activities of different groups were assessed to evaluate the effects of DBS on animal movement activities.

Introduction

There is no need to write the substance with capital S (line 28, 32).

Response to reviewer: Corrected.

Line 33 and 34, what it is meant by “unique” in “…because of its unique 34 human and geographical characteristics” ?

Response to reviewer: The sentence was changed as follows: Because of its geographical location, which borders with drug producing countries.

English language has to be checked and corrected. Line 33 “ …control headquarters indicates the …” headquarters is plural and the verb indicate must follow without “s”.

Response to reviewer: Corrected.

Similar mistakes so that verb and nouns are not compatible or the tenses (times) are not correct, are all in the text.

Response to reviewer: As much as possible, the errors in the manuscript were corrected

Line 58, "…, DBS research has been developed in various areas of the brain to treat 59 neuropsychiatric conditions such as Alzheimer's disease, Tourette's syndrome, obsessive compulsive disorder, and advanced depression ". Needs reference.

Response to reviewer: For each of the mentioned diseases, the relevant reference was added in the text

Line 65, what do authors mean by "orbital effects"??

Response to reviewer: The sentence was changed as follows: Instead, DBS has been shown to stimulate axonal terminals, leading to main effects on neural circuits.

Line 67, 2 afferents? " …specific afferents and afferents …"

Response to reviewer: "its specific afferents and efferents…"

Line 76, "…But we consider 76 that this intervention in LH orexin neurons may also provide promising results for addiction 77 treatment…". What do authors mean by this sentence, did they had this experience in their studies themselves or is from other studies? Reference and more explanation is needed.

Response to reviewer: It seems that the above sentence was irrelevant to the text of the manuscript. So it was deleted.

Line 78, “..To the best of our knowledge, there is little information about the effect of DBS on morphine dependency…”. In contrary to this notion by authors, there are a good number of literature discussing the exact effect of DBS on related regions of the brain in “morphine” dependency and addiction, specifically by Iranian researchers!

Response to reviewer: The opinion of the respected reviewer is absolutely correct. The correct sentences were replaced as follows: there is a little information about the effect of DBS in mPFC on morphine dependency and motor activity.

Methods

Line 91, “…results of the CPP (……..) test” what does (…) mean?

Response to reviewer: The term “conditional place preference”, which is abbreviated as CPP. At first, "conditional place preference" was mentioned in full and then its abbreviation was written.

For sham groups, the custom procedure is that the animal is opened or drill is pulled into the skull and holes are made, but the cannula is not inserted. Do you have any reference indicating that in sham animals the cannula is also been placed?

Response to reviewer: In the sham group, the cannula inserted into the brain tissue in the location, and then removed. By doing this, only the effect of surgical stress is the same in all groups. However, the insertion of the electrode into the cerebral tissue and the resulting stress can have an effect on the electrical response of the cerebral nuclei, including the accumbens.

Also, what has been the role of the sham group in your experiments? The design of experiment specially with CPP involving, does not need sham. Because apparently authors did not inject anything to the sham rat. Therefore, it seems more logic to take off the sham group and do the analysis with 2 way ANOVA, with rats received either morphine or saline with and without DBS. That makes more sense.

Response to reviewer: In the sham group, the electrode was inserted into the target area in the cerebral tissue. To eliminate the stress caused by surgery in groups, the presence of this group seems logical. However, if the respected reviewer insists on removing this group, the authors consider themselves obliged to remove the desired group.

The effects of morphine on the response of cells in different brain regions, including the prefrontal cortex and the nucleus accumbens, have been shown in previous studies and have shown promising results in several areas of the reward system. In this study, since only the effect of deep brain stimulation on different groups was examined, one-way ANOVA test was used.

Again English language correction is needed, line 97, "…The electrical response of AC neurons to DBS is determined …". "Is" must be was. Line 101, body temp … was maintained is right.

Response to reviewer: corrected.

Line 105, coordination must be addressed like this : AP: -4, ML: 1.6 and DL: -8.4mm.

Response to reviewer: The coordinates of the desired area were entered like above.

Line 117, are the light and dark in describing the chambers, related to the light intensity or color? If color, it should be mentioned, although it is customary to distinguish the inside of the chambers by difference in design not color, like vertical lines for one and horizontal lines for another.

Response to reviewer: The sentence was modified as follows: In this experiment, a wooden device with dimensions of 30[L]x16[W]x45[H] consists of two chambers, one with vertical lines and other with horizontal lines was used.

It is not clear how the stimulations (DBS) have been given to the rats, during 3 days of conditioining, both morning and afternoon or either of them, OR post conditioning? Also, in the discussion authors mentioned 3-6 days of stimulation, when did that happen?? Also, the graph about DBS spikes, is related to which period of DBS??

Using a diagram illustrating the timing of each event would be useful.

Response to reviewer: During conditioning in the CPP box, rats received DBS with a frequency of 130 Hz, amplitude of 0.2 to 0.5 mA, and repeated periods of 15 minutes with an interval of 45 minutes for 3 hours after completing the afternoon course. Experimental design illustrating the timing of each event was added.

The histological assessment needs to be rewritten with proper ordering the evidence, like;

first dissecting the brain, immersing in the paraformaldehyde 10% for 24 hours then in 4% for preservation and fixation and then cutting and last staining and microscope view.

Response to reviewer: According to the point of view of the esteemed reviewer regarding the attachment of a photograph of the electrode entering the cerebral tissue, since the study has ended and it is not possible to take photographs, the histology section was removed and the motor activity measurement section was added.

Also, please provide an image of the electrodes perforation locations on brain.

As explained above, using 2 way ANOVA is more suitable for this study.

Response to reviewer: It is explained in the previous comment.

Results

Each figure must have its own descriptions of marks (@, &, …)

Response to reviewer: The description of each graph was added below.

In the text authors have not mention anything about basal recording of the spikes in cortex and NAc, please add it to the text.

Response to reviewer: the corrected sentence was added as follows: When a responsive neuron was identified, 5 min of baseline activity were recorded.

The result of the statistical analysis must include the degrees of freedom, like F(dn, df)=??.?? , P<…).

Response to reviewer: The degrees of freedoms were added in statistical analysis.

Why there is not any description of comparison with saline group? morphine and saline are the main groups not sham.

Response to reviewer: The saline group is not the same as the control group. Because the results of the saline group were not significantly different from the sham group, all groups were compared with the sham group. As it is mentioned in the text, normal saline was injected in the saline group. But in sham group, electrode was inserted and then removed. Therefore, it seems logical to compare all the groups with the sham group

In the first part of the result the first paragraph includes some repeating reports, please correct that. "…morphine (5mg/kg) has significantly enhanced conditioned place 167 preference [487.5 ± 59.91] in comparison with the sham group [18.33 ± 1.56. Based on Fig.2, administration of morphine [487.5 ± 59.91] without electrical stimulation 169 increased conditioned place preferences compared with the sham group [18.33 ± 1.56; P"

Response to reviewer: Duplicate parts were removed.

Discussion

The first paragraph is repeating the introduction, please avoid redundancy.

Response to reviewer: Duplicate parts were removed.

Lines 201, 202, .." Further, applying electrical stimulation for 3–6 days ???

Response to reviewer: Applying electrical stimulation for 3 days reduced the preference score in the morphine+ DBS group in the CPP test compared with the morphine group.”

Response to reviewer: According to Table 2, electrical stimulation decreased the duration of conditioned place preference in the morphine-treated group.

In this part there is no discussion about the relation between mPFC and NAc and how their relation is affecting the morphine dependency and as a result of DBS how this connection is disrupted. Most sentences in the discussion section are not relevant to the main subject of your manuscript: a new connection between mPFC and NAc in morphine dependency. Please rewrite the discussion relevant to the subject and results ***of your study.***

Response to reviewer: According to your pertinent remark, the discussion rewrited according to the subject and results of our study.

The END

## 2 Revision 1

0 comments

*No public comments in this round.*

3

# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**2/10/2023**

**Response to comments of reviewers**

**Journal of High Risk and Behavior**

Dear Editor

The authors are grateful to respected editor and reviewers for their attention, constructive comments and valuable suggestions on previous version of the manuscript. We tried to consider the Journal of High Risk and Behavior guidelines as strictly as possible. We have revised the manuscript according to reviewer's comments and suggestions as point by point in detail. Red purple highlighted texts are corrected and blue highlighted texts are added

Best wishes,

**Editor comment:**

Rewrite discussion based on your results and write limitation and suggestion in this part.

Response to reviewer: The discussion part of the manuscript was revised and updated according to the results part.

check English language of manuscript (grammer, structure).

Response to reviewer: The writing and grammar of the manuscript was edited and reviewed.

On the other hand, because the "title" is clearly indicative of the design of this research, the title was changed as follows:

**the effects of deep brain stimulation in medial prefrontal cortex on morphine dependency and electrical recording of the nucleus accumbens**

## 3 Revision 2

0 comments

*No public comments in this round.*

4

# Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**2/25/2023**

**Response to comments of reviewers**

**Journal of High Risk and Behavior**

Dear Editor

The authors are grateful to respected editor and reviewers for their attention, constructive comments and valuable suggestions on previous version of the manuscript. We tried to consider the Journal of High Risk and Behavior guidelines as strictly as possible. We have revised the manuscript according to reviewer's comments and suggestions as point by point in detail. Green highlighted texts are corrected and added.

Best wishes,

**Editor comment:**

Discussion

1) in the end of line 292, after reference (34) write your limitations and suggestions.

Response to Editor: Our limitations and suggestions were added in the end of line 292 as follows: We also recommend that in future studies, the investigation of the possible effect of DBS on ion-channel currents, especially potassium ion currents, should be considered using patch clamp recording. Also obtaining appropriate stimulation patterns for DBS was one of the limitations of this study.

2) In the beginning of line 293, delete the word in conclusion, and write Conclusions in Heading.

Response to Editor: Edited.

## 4 Revision 3

0 comments

*No public comments in this round.*