Local kisspeptin excitation of rat oxytocin neurones in late pregnancy

Mehwish Abbasi, Michael R Perkinson, Alexander Seymour, Richard Piet, Rebecca Campbell, Karl J Iremonger, and Colin Brown

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Ryoichi Teruyama (Referee #1)

Review Timeline:

| Event                          | Date       |
|-------------------------------|------------|
| Submission Date               | 21-Oct-2021|
| Editorial Decision            | 15-Nov-2021|
| Revision Received             | 29-Nov-2021|
| Editorial Decision            | 21-Dec-2021|
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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)
Dear Professor Brown,

Re: JP-RP-2021-282531 "Local kisspeptin excitation of rat oxytocin neurones in late pregnancy" by Mehwish Abbasi, Michael R Perkinson, Alexander Seymour, Richard Piet, Rebecca Campbell, Karl J Iremonger, and Colin Brown

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

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I look forward to receiving your revised submission.

If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

Professor Laura Bennet
REQUIRED ITEMS:

- Author photo and profile. First (or joint first) authors are asked to provide a short biography (no more than 100 words for one author or 150 words in total for joint first authors) and a portrait photograph. These should be uploaded and clearly labelled with the revised version of the manuscript. See Information for Authors for further details.

- Research Governance contact: please provide an institutional email address for Richard Blaikie.

- You must start the Methods section with a paragraph headed Ethical Approval. A detailed explanation of journal policy and regulations on animal experimentation is given in Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818. A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: https://physoc.onlinelibrary.wiley.com/hub/animal-experiments. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015). The Methods section must contain details of the anaesthetic regime: anaesthetic used, dose and route of administration and method of killing the experimental animals.

- Please upload separate high-quality figure files via the submission form.

- A Statistical Summary Document, summarising the statistics presented in the manuscript, is required upon revision. It must be on the Journal's template, which can be downloaded from the link in the Statistical Summary Document section here: https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics

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In summary:

- If n (less than or equal to) 30, all data points must be plotted in the figure in a way that reveals their range and distribution. A bar graph with data points overlaid, a box and whisker plot or a violin plot (preferably with data points included) are acceptable formats.

- If n > 30, then the entire raw dataset must be made available either as supporting information, or hosted on a not-for-profit repository e.g. FigShare, with access details provided in the manuscript.

- 'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

- All relevant 'n' values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

- The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

- Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.
Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the research and should summarise the main conclusions. If possible, the image should be easily ‘readable’ from left to right or top to bottom. It should show the physiological relevance of the manuscript so readers can assess the importance and content of its findings. Abstract Figures should not merely recapitulate other figures in the manuscript. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion(s). Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type ‘Abstract Figure’. Please ensure that you include the figure legend in the main article file. All Abstract Figures should be created using BioRender. Authors should use The Journal’s premium BioRender account to export high-resolution images. Details on how to use and access the premium account are included as part of this email.

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EDITOR COMMENTS

The article is thorough and looks interesting. Both of the reviewers are supportive of the manuscript, but request major revisions.

In particular, the reviewers have some experimental concerns over (i) whether the findings from in vivo vs ex vivo kisspeptin data can truly be compared, given the length of kisspeptin treatments was markedly different between the in vivo study (60 min) vs. the in vitro study (5 min); (ii) the estrus cycle of the non-pregnant rats and the range of gestational ages used; (iii) the ratemeter data and presentation for figures 1, 4, 5; and (iv) the statistical analyses for Figures 10 and 11, which need to be addressed.

There are also a number of other more general comments that need addressing, including the overall interpretation of some of the findings.

Other comments:

The authors state: "Non-pregnant or late-pregnant rats were decapitated..". Even though the fetuses were not used for the experiments, the authors should state how the fetuses were euthanised.

Please add the ethics approval number.

Please state ~age ranges of the female rats used in the study

Were the rats in the first pregnancy?

Please provide data as mean +/- SD (not SEM) as per journal requirements.

Please also note that for a given conclusion to be assessed, the exact p values must be stated to three significant figures even when ‘no statistical significance’ is being reported. These should be stated in the main text, figures and their legends and tables. The only exception to this is if p is less than 0.0001, in which case ‘<’ is permitted.

Please provide the statistical summary document.

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REFEREE COMMENTS

Referee #1:

Previous studies from this group suggested that central kisspeptin increase the activity of oxytocin neurons specifically at the
end of pregnancy. The present study was conducted to elucidate the mechanisms underlying this excitation in oxytocin neurons. Oxytocin is an important hormone regulating the contraction of the uterus and mammary glands during parturition and lactation, respectively. Understanding the mechanism underlying regulation of oxytocin neurons' excitability at the critical period, late-pregnancy, will have a significant impact in the field.

This manuscript describes the differences in the effect of kisspeptin on the firing activity of oxytocin neurons from the late pregnancy and non-lactating rats. Using in vivo single-unit extracellular recording, changes in firing activity of oxytocin and vasopressin neurons in response to kisspeptin that was administered directly into the supraoptic nucleus by micro-dialysis. The intra-supraoptic administration of kisspeptin caused increase in firing frequency in oxytocin neurons only from late-pregnant, but not from non-pregnant animals. Hazard analysis of firing suggested that kisspeptin transiently increases excitability after each action potential. The group also conducted in vitro patch-clamp electrophysiology; however, a bath application of kisspeptin did not affect the action current frequency, EPSC, or IPSCs in supraoptic neurons either from late-pregnant or non-pregnant animals. From these results, authors concluded that central kisspeptin increases excitability in oxytocin neurons at the end of pregnancy via modulation of channels that regulate post-spike excitability. The experiments were carefully conducted with appropriate statistical power. The results were well described and seem to be solid. However, there are some major concerns that are needed to be discussed. Specifically, the methodology employed in the brain slice electrophysiology may not be compatible with the in vivo study (more detail below). Therefore, the interpretation of the results of this study, kisspeptin directly activates oxytocin neurones in late pregnancy, may not be appropriate.

Major concerns:

The duration of intra-supraoptic administration of kisspeptin in in vivo study was 60 min. The effect of kisspeptin on the firing rate was not observed until after ~20 min and peaked around 40-60 min of the application. The experiment and its results are fine themselves; however, when the results were compared with results from in vitro patch-clamp experiment in which the duration of a bath application of kisspeptin was only 5 min and change in the firing rate was not observed. Therefore, there is a possibility that the bath application of kisspeptin did not affect the action current frequency, EPSC, or IPSCs in supraoptic neurons in slices simply because the duration of kisspeptin application was too short.

In the group's previous reports, ICV infusion of kisspeptin increased firing rate of oxytocin neurons within ~5 min of application. Why the effect of intra-supraoptic application on the firing rate requires a longer duration must be discussed. Is the effect mediated by the same underlying mechanism?

The statement "Kisspeptin directly activates oxytocin neurones in late pregnancy, at least in part, via an action on channels that regulate post-spike excitability" is slightly far-fetched, since this study did not investigate any ion channel properties that regulate the post-spike excitability.

Minor comments:

Fig. 3 and 7 are not necessary (or even confusing) when vasopressin neurons were classified into continuous and phasic vasopressin neurons.

Hazard analysis is great to detect post-spike excitability; however, afterhyperpolarization, afterdepolarization, and rebound depolarization mentioned in discussion are activity dependent properties, and therefore the analysis may not be appropriate to detect these intrinsic membrane properties. Correlation between the change in firing rate and peak hazard may provide some suggestion, but are there any other ways to analyze firing rate/pattern?
Referee #2:

Manuscript JP-RP-2021-282531 reports the kisspeptin-induced potential firing rate oxytocin neurons in late pregnant rat. This action of kisspeptin can be detected after intra-supraoptic nucleus administration in vivo, but non-detectable in isolated hypothalamic slices in vitro. Difference has been proved between the non-pregnant and the late pregnant rats.

Authors have concluded that the kisspeptin may contribute to the initiation of delivery of offspring via the stimulation of oxytocin neurons in hypothalamus. As a translational significance they mentioned that the hypothalamic kisspeptin system could be a novel target to reduce the risk of premature labor.

The manuscript is quite well-written, although the study plan, presentation and interpretation of the results are not quite appropriate in some places.

Questions and concerns:

1. Row 82: The registration number of the approval of the animal experiment must be given.

2. Row 84: The age of the rats must be given.

3. Row 93: did you checked the estrus cycle of the non-pregnant rats? Do you have evidence that the estrus cycle phases do not modify the hypothalamic response and sensitivity? The late pregnant rats were in the gestational days18-21. In uterine contractility there is significant difference e.g., between 18-day and 21-day pregnant uterus. Do you have evidence that these 3-4 days differences in the gestational days have no impact on hypothalamic function? The S.E.M. values are quite big in most of the pregnant measurements as compared to the non-pregnant. It could be the consequence of different gestational days. What was the reason that you have applied rats from quite wide range of pregnancy days instead of choosing one day (e.g., day 21)?

4. Figure 1, 4, and 5: The ratemeters on parts A and B not always reflect the part C. The firing rates that are not always correlating to the part C (e.g., Fig 1. between 41-50 min no obvious change in Part A, but there is a decrease in part C; obvious increase between 11-20 min on part B, but no change on part C, Fig. 5 obvious increase in 51-70 min on part B, but even a decrease on part C...). What is the reason of these discrepancies? Fig. 5: What is the significance of the 2 min extra insets?

5. Figure 10 and 11: The statistical analysis is doubtful. Fig 10, part C shows an almost 4-fold elevation in mean frequency in late pregnant rats, but it was found no significant, while Fig 11 part F shows a very slight modification of amplitudes with significancy. These statistical analyses must be clarified.

6. Row 348: Authors state that kisspeptin might reduce the risk of preterm birth by restraining oxytocin neuron activity. This statement is speculative and does not consider the peripheral role of kisspeptin receptors in pregnant rats (Eur J Pharmacol. 2021; 896:173924. doi: 10.1016/j.ejphar.2021.17392)

7. Row 356: Mentioning the sex differences is not proper because the current manuscript has not investigated any male response.

8. Row 374: this statement suggests that the superfusion technique is even not appropriate for this kind of investigation because of the severed afferents. What would be the significance of the in vitro results in the light of this statement?
9. Row 377. The link between the noradrenergic system and oxytocin via kisspeptin is highly speculative without any evidence.

10. Row 401. The fact that the kisspeptin receptor mRNA of the oxytocin neurons was not modified over pregnancy does not mean that the receptor sensitivity remains the same. The modification of the activity of the receptor second messenger pathway can modify the kisspeptin response without the modification of receptor mRNA.

END OF COMMENTS
Response to Reviews for JP-RP-2021-282531

We thank the editors and referees for their constructive criticism of the original version of our manuscript. We have addressed all of the issues raised below to describe how each revision has been incorporated into the manuscript, or explain why a change has not been made.

In addition to the points raised by the editors and referees, we have:

- created an abstract figure using BioRender
- completed the Statistical Summary Document
- uploaded each figure as a separate high-quality TIFF file, following the Journal’s policy on data representation
- uploaded a copy of the manuscript with the changes highlighted
- uploaded an author photo and profile for the first author
- provided an institutional email address for Richard Blaikie for research governance: dvc.research@otago.ac.nz
- started the methods section with a paragraph headed “Ethical Approval”

Reviewing Editor:

...the reviewers have some experimental concerns over (i) whether the findings from in vivo vs ex vivo kisspeptin data can truly be compared, given the length of kisspeptin treatments was markedly different between the in vivo study (60 min) vs. the in vitro study (5 min); (ii) the estrus cycle of the non-pregnant rats and the range of gestational ages used; (iii) the ratemeter data and presentation for figures 1, 4, 5; and (iv) the statistical analyses for Figures 10 and 11, which need to be addressed.

There are also a number of other more general comments that need addressing, including the overall interpretation of some of the findings.

Each of the points above have been addressed in detail where they are raised by the referees below.

The authors state: "Non-pregnant or late-pregnant rats were decapitated.". Even though the fetuses were not used for the experiments, the authors should state how the fetuses were euthanised.

The text has been revised to read “Non-pregnant rats or late-pregnant rats were decapitated by guillotine. Pup death in utero was confirmed after decapitation of the dam.” In addition, we have revised the in vivo electrophysiology methods to include “Pup death in utero was confirmed after the dam was euthanised.”

Please add the ethics approval number.

The approval number has been added to the “Ethical Approval” section of the methods.

Please state ~age ranges of the female rats used in the study.

The age range has been added to “Animals” section of the methods.

Were the rats in the first pregnancy?

The rats were in their first pregnancy. The “Animals” section of the methods has been revised to read “Primiparous pregnant rats were housed individually from gestation day 14 (G14) and used on G18 – 21 (G21 being the expected day of parturition).”
Please provide data as mean +/- SD (not SEM) as per journal requirements.

SEM has been changed to SD throughout the manuscript and in the figures (not highlighted).

...exact p values must be stated to three significant figures even when 'no statistical significance' is being reported. These should be stated in the main text, figures and their legends and tables. The only exception to this is if p is less than 0.0001, in which case '<' is permitted.

All p-values have been revised to three significant figures except for oxytocin firing rate results where the statistics programme reported p < 0.001 (not highlighted) and the text of the “Statistical analysis” section has been revised to reflect this change. We have also added “Pearson product moment correlations were used for determining correlations” to this section. Finally, we have also stated the number of cells and number of animals throughout (not highlighted).

Referee #1:

Major concerns:

The duration of intra-supraoptic administration of kisspeptin in in vivo study was 60 min. The effect of kisspeptin on the firing rate was not observed until after ~20 min and peaked around 40–60 min of the application. The experiment and its results are fine themselves; however, when the results were compared with results from in vitro patch-clamp experiment in which the duration of a bath application of kisspeptin was only 5 min and change in the firing rate was not observed. Therefore, there is a possibility that the bath application of kisspeptin did not affect the action current frequency, EPSC, or IPSCs in supraoptic neurons in slices simply because the duration of kisspeptin application was too short.

We apologise for our failure to explain the difference between the methodologies. Superfusion of 10–100 nM kisspeptin depolarises GnRH neurons in brain slices in 1–3 min (e.g. Han et al J Neurosci. 2005; 25: 11349) so the failure of 1 μM over 5 min to affect oxytocin neurons is very unlikely to reflect a failure to deliver sufficient kisspeptin for long enough. The in vivo drug administration is by microdialysis and the latency to response is a result of the time required for drugs to reach an effective concentration (Ludwig & Leng G. Eur J Neurosci. 1997; 9: 2532), which typically takes tens of minutes for peptides. The recovery of the probes for peptides of the size of kisspeptin is ~1%, so the concentration in the dialysate (100 μM) likely delivered a lower concentration to the tissue in vivo than superfusion did in vitro. We have inserted the following text into the discussion “…is unlikely to reflect a failure to deliver sufficient kisspeptin to have an effect because 10–100 nM excites gonadotrophin releasing hormone neurones in 1–3 min (Han et al, 2005). Rather, it likely reflects a failure of kisspeptin to affect…”

In the group's previous reports, ICV infusion of kisspeptin increased firing rate of oxytocin neurons within ~5 min of application. Why the effect of intra-supraoptic application on the firing rate requires a longer duration must be discussed. Is the effect mediated by the same underlying mechanism?

Again, we apologise for this omission. The longer time-course in the current experiments is because microdialysis administration takes longer to achieve an effective concentration than acute injection of a bolus of kisspeptin. We inserted the following text into the discussion “Therefore, we tested whether local microdialysis administration of kisspeptin directly into the supraoptic nucleus excites oxytocin neurons; the resulting kisspeptin-induced excitation was robust and consistent, suggesting that the excitation is mediated locally. The longer time-course of the excitation by dialysed kisspeptin compared to that elicited by ICV kisspeptin likely reflects the time required for dialysed
drugs to reach sufficient concentration within the tissue to have an effect (insert Ludwig & Leng, 1997).

The statement "Kisspeptin directly activates oxytocin neurones in late pregnancy, at least in part, via an action on channels that regulate post-spike excitability" is slightly far-fetched, since this study did not investigate any ion channel properties that regulate the post-spike excitability.

We accept that we over-stated the interpretation and have modified “via an action on channels that regulate post-spike excitability” to read “via increased post-spike excitability”.

Minor comments:

Fig. 3 and 7 are not necessary (or even confusing) when vasopressin neurons were classified into continuous and phasic vasopressin neurons.

We agree that the inclusion of the overall vasopressin data can be confusing but initially included it for completeness. These data and figures have been deleted, as has reference to the data in the discussion. The figure numbering has been updated accordingly (not highlighted).

Hazard analysis is great to detect post-spike excitability; however, afterhyperpolarization, afterdepolarization, and rebound depolarization mentioned in discussion are activity dependent properties, and therefore the analysis may not be appropriate to detect these intrinsic membrane properties. Correlation between the change in firing rate and peak hazard may provide some suggestion, but are there any other ways to analyze firing rate/pattern?

We are not aware of other ways to analyse the data to directly address post-spike excitability. We believe that we comprehensively addressed this issue in the original discussion. We have not revised the manuscript in light of this comment.

Referee #2:

1. Row 82: The registration number of the approval of the animal experiment must be given.
   The approval number has been added to the “Ethical Approval” section of the methods.

2. Row 84: The age of the rats must be given.
   The age range has been added to “Animals” section of the methods.

3. Row 93: did you checked the estrus cycle of the non-pregnant rats? Do you have evidence that the estrus cycle phases do not modify the hypothalamic response and sensitivity?
   We checked the oestrus cycle of all rats. Non-pregnant rats were rats that were not used for mating. We did not select any particular day of the cycle because we have hundreds of recordings from different stages of the oestrous cycle and see no effect of the cycle on basal activity. We have revised the text in the “Animals” section of the methods to read “To prevent any potential confounding effects of the oestrous cycle, non-pregnant rats were freely-cycling virgin rats…”

   The late pregnant rats were in the gestational days 18-21. In uterine contractility there is significant difference e.g., between 18-day and 21-day pregnant uterus. Do you have evidence that these 3-4 days differences in the gestational days have no impact on hypothalamic function? The S.E.M. values are quite big in most of the pregnant measurements as compared to the non-pregnant. It could be the consequence of different gestational days. What was the reason that you have applied rats from quite wide range of pregnancy days instead of choosing one day (e.g., day 21)?
We did not choose one day because the experiments were completed on one animal at a time and the logistics of planning for all experiments to be on G21 would require frequent weekend work for months on end and we believe that work-life balance and wellbeing matter. We have run a correlation between the response to kisspeptin and the day of gestation, which shows no correlation between the oxytocin neuron responses to kisspeptin and day of gestation. We have inserted the text “There was no correlation between the response to kisspeptin and the day of gestation (r = -0.546, p = 0.204)” to the “Intra-supraoptic nucleus kisspeptin increases the firing rate of oxytocin neurones in late-pregnant rats” section of the results.

4. Figure 1, 4, and 5: The ratemeters on parts A and B not always reflect the part C. The firing rates that are not always correlating to the part C (e.g., Fig 1. between 41-50 min no obvious change in Part A, but there is a decrease in part C; obvious increase between 11-20 min on part B, but no change on part C, Fig. 5 obvious increase in 51-70 min on part B, but even a decrease on part C...). What is the reason of these discrepancies? Fig. 5: What is the significance of the 2 min extra insets?

The responses were variable and none exactly matched the mean data. We accept that this is aesthetically dissatisfying but the examples and mean data are accurate. We state “The insets show two-minute segments of recording (in 1 s bins) from each graph before (Pre-KP) and during (KP) kisspeptin administration to illustrate phasic bursts”. We have not revised the manuscript in light of this comment.

5. Figure 10 and 11: The statistical analysis is doubtful. Fig 10, part C shows an almost 4-fold elevation in mean frequency in late pregnant rats, but it was found no significant, while Fig 11 part F shows a very slight modification of amplitudes with significancy. These statistical analyses must be clarified.

We have checked that the statistics are completed and reported correctly. We have not revised the manuscript in light of this comment.

6. Row 348: Authors state that kisspeptin might reduce the risk of preterm birth by restraining oxytocin neuron activity. This statement is speculative and does not consider the peripheral role of kisspeptin receptors in pregnant rats (Eur J Pharmacol. 2021; 896:173924. doi:10.1016/j.ejphar.2021.17392)

We apologise for the confusion. This paragraph is about differences between non-pregnant and late-pregnant, not effects of kisspeptin in non-pregnant and late-pregnant. We have revised the final sentence to read “The lower excitatory synaptic drive and higher inhibitory synaptic drive on oxytocin neurones in late pregnancy appears counterintuitive but might help reduce the risk of preterm delivery by restraining oxytocin neurone activity prior to the onset of parturition.”

7. Row 356: Mentioning the sex differences is not proper because the current manuscript has not investigated any male response.

We apologise for the confusion. This comparison is between previously published data on vasopressin neurons (that shows kisspeptin excitation) with our data (that shows no effect on unidentified supraoptic nucleus neurons). We believe that we have treated the difference fairly, and must acknowledge the possibility that there are sex differences. We have not revised the manuscript in light of this comment.

8. Row 374: this statement suggests that the superfusion technique is even not appropriate for this kind of investigation because of the severed afferents. What would be the significance of the in vitro results in the light of this statement?
These experiments were necessary to determine whether the excitation was via local glutamatergic/GABAergic inputs, or directly on oxytocin neurons. While we expected to see an excitation in these experiments, the lack of response in vitro allowed us to advance knowledge by eliminating a possible mechanism. We believe that our logic is sound. We have not revised the manuscript in light of this comment.

9. Row 377. The link between the noradrenergic system and oxytocin via kisspeptin is highly speculative without any evidence.

We agree that the statement is speculative and it was written to ensure that the reader would understand it to be speculative. We believe that suggesting a way forward is an integral element of a rigorous discussion. We have not revised the manuscript in light of this comment but will delete the speculation if the reviewer insists.

10. Row 401. The fact that the kisspeptin receptor mRNA of the oxytocin neurons was not modified over pregnancy does not mean that the receptor sensitivity remains the same. The modification of the activity of the receptor second messenger pathway can modify the kisspeptin response without the modification of receptor mRNA.

We agree that we should have include this possibility and have revised the text to read “...might reflect a change in surface expression, sensitivity and/or intracellular coupling of Kiss1R...”
Dear Professor Brown,

Re: JP-RP-2021-282531R1 "Local kisspeptin excitation of rat oxytocin neurones in late pregnancy" by Mehwish Abbasi, Michael R Perkinson, Alexander Seymour, Richard Piet, Rebecca Campbell, Karl J Iremonger, and Colin Brown

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

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Authors are asked to use The Journal’s premium BioRender (https://biorender.com/) account to create/redraw their Abstract Figures. Information on how to access The Journal’s premium BioRender account is here: https://physoc.onlinelibrary.wiley.com/journal/14697793/biorender-access and authors are expected to use this service. This will enable Authors to download high-resolution versions of their figures.

I hope you will find the comments helpful and have no difficulty returning your revisions within 4 weeks.

Your revised manuscript should be submitted online using the links in Author Tasks Link Not Available.

Any image files uploaded with the previous version are retained on the system. Please ensure you replace or remove all files that have been revised.

REVISION CHECKLIST:
- Article file, including any tables and figure legends, must be in an editable format (eg Word)
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- Statistical Summary Document
- Upload each figure as a separate high quality file
- Upload a full Response to Referees, including a response to any Senior and Reviewing Editor Comments;
- Upload a copy of the manuscript with the changes highlighted.

You may also upload:
- A potential 'Cover Art' file for consideration as the Issue's cover image;
- Appropriate Supporting Information (Video, audio or data set https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#supp).

To create your 'Response to Referees' copy all the reports, including any comments from the Senior and Reviewing Editors, into a Word, or similar, file and respond to each point in colour or CAPITALS and upload this when you submit your revision.

I look forward to receiving your revised submission.

If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

Professor Laura Bennet
REQUIRED ITEMS:

- Papers must comply with the Statistics Policy https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics

In summary:

- If n \leq 30, all data points must be plotted in the figure in a way that reveals their range and distribution. A bar graph with data points overlaid, a box and whisker plot or a violin plot (preferably with data points included) are acceptable formats.

- If n > 30, then the entire raw dataset must be made available either as supporting information, or hosted on a not-for-profit repository e.g. FigShare, with access details provided in the manuscript.

- ‘n’ clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

- All relevant ‘n’ values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

- The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

- Exact p values must be stated. Authors must not use ‘greater than’ or ‘less than’. Exact p values must be stated to three significant figures even when ‘no statistical significance’ is claimed.

- Statistics Summary Document completed appropriately upon revision

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EDITOR COMMENTS

Reviewing Editor:

Please see comments of Reviewer 2. Please address or rebut accordingly.

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REFEREE COMMENTS

Referee #1:

The authors have adequately addressed the comments raised by the reviewers in the revised version of the manuscript or their letter. I have no further comments.

Referee #2:

The authors mostly has been responded the raised questions, however their response to Question 4 (Ref#2) is not really
fine. The discrepancy between the representative ratemeter record and the calculated means is not the question of aesthetic or the satisfaction of the Reviewer, but the question of scientific reliability. If the authors really got such means values after the evaluation of individual ratemeter records, they must have some of them, that reflect what they claim. Please select a ratemeter record fitting to your result. This is not an extraordinary request, but rather a basic requirement...

END OF COMMENTS

1st Confidential Review
Response to Reviews for JP-RP-2021-282531-R1

Reviewing Editor:

Please see comments of Reviewer 2. Please address or rebut accordingly.

This is addressed below and no other revisions have been made to the text or figures.

Referee #1:

No response required.

Referee #2:

The authors mostly has been responded the raised questions, however their response to Question 4 (Ref#2) is not really fine. The discrepancy between the representative ratemeter record and the calculated means is not the question of aesthetic or the satisfaction of the Reviewer, but the question of scientific reliability. If the authors really got such means values after the evaluation of individual ratemeter records, they must have some of them, that reflect what they claim. Please select a ratemeter record fitting to your result. This is not an extraordinary request, but rather a basic requirement...

We apologise that none of the individual recordings exactly match the averaged group data. We have replaced the example recording in Figure 1B. Our original selection was the one that most closely matched the group mean basal firing rate that had a clear response to kisspeptin. The replacement has a response to kisspeptin that more closely matches the group mean response but has a lower basal firing rate than the group mean. We hope that the stronger focus on the kisspeptin response will be satisfactory.
Dear Dr Brown,

Re: JP-RP-2022-282531R2 "Local kisspeptin excitation of rat oxytocin neurones in late pregnancy" by Mehwish Abbasi, Michael R Perkinson, Alexander Seymour, Richard Piet, Rebecca Campbell, Karl J Iremonger, and Colin Brown

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

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Authors should note that it is too late at this point to offer corrections prior to proofing. The accepted version will be published online, ahead of the copy edited and typeset version being made available. Major corrections at proof stage, such as changes to figures, will be referred to the Reviewing Editor for approval before they can be incorporated. Only minor changes, such as to style and consistency, should be made a proof stage. Changes that need to be made after proof stage will usually require a formal correction notice.

All queries at proof stage should be sent to TJP@wiley.com

Yours sincerely,

Professor Laura Bennet
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

The additional responses to reviewer 2 are suitable.

2nd Confidential Review

10-Jan-2022