# Properties and modulation of excitatory inputs to the locus coeruleus

Christopher Ford, Kelsey Barcomb, Samantha S Olah, and Matthew Kennedy
**DOI: 10.1113/JP283605**

Corresponding author(s): Christopher Ford (christopher.ford@cuanschutz.edu)

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

## Transaction Report:

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Dear Dr Ford,

Re: JP-RP-2022-283605 "Properties and modulation of excitatory inputs to the locus coeruleus" by Christopher Ford, Kelsey Barcomb, Samantha Schwartz, and Matthew Kennedy

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

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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,

David Wyllie
Senior Editor
The Journal of Physiology


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-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.


-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.


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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.

-Statistics Summary Document completed appropriately upon revision

-Please provide (in the article file) a legend to accompany your 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.

-Please include a full title page as part of your article (Word) file (containing title, authors, affiliations, corresponding author name and contact details, keywords, and running title).

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EDITOR COMMENTS

Reviewing Editor:

Barcomb et al., used a viral-mediated optogenetic approach in CaMKII-Cre mice to compare glutamate afferents from the prefrontal cortex (PFC), the lateral hypothalamus (LH) and the periaqueductal grey (PAG). The authors found that the lateral hypothalamus and periaqueductal grey also drove robust excitatory events in locus coeruleus norepinephrine neurons. They further characterised the modulation of inputs by peptides, showing that the different excitatory inputs were modulated by dynorphin and corticotrophin releasing factor in different ways.

This is a well written manuscript that will likely make an important contribution to the literature. The manuscript advances knowledge with respect to the functional neuroanatomy and electrophysiology of inputs to the locus coeruleus. Two expert reviewers have assessed the manuscript and made comments that should be addressed when revising the manuscript. Furthermore, please:

- include the ethics approval number for IACUC University of Colorado ethics.

- report exact p-values for all statistical tests (e.g. $p = 0.004$), with the exception of $p < 0.0001$, and supplement reporting of p-values with effect size calculations (e.g. Cohen's d for t-tests, partial eta-squared for RMANOVA).

- report results in text and figures using mean and standard deviation, as per the Journal's statistics policy. Alternatively, for figures, instead of standard deviation error bars, violin plots (easy to produce in GraphPad prism) are an excellent wat to show the data distribution as well as the variability.

- describe the post operative care following stereotaxic surgery.

Senior Editor:

Your manuscript has been positively assessed by two expert Referees and a Reviewing Editor. Each agree that your manuscript reports data that will be of interest to the field. They make several suggestions for clarification. I would also add that it would be preferable to pool data obtained from the same animal and then take the mean of these values and report N - you do document n/N and indeed your N values are large. You can still report the cells etc but for robustness please use N for the basis of statistical comparisons.

Please report precise p values and SD (not SEM).

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REFEREE COMMENTS

Referee #1:


This manuscript by Barcomb and colleagues studies the properties of excitatory inputs from the prefrontal cortex (PFC), lateral hypothalamus (LH) and periaqueductal grey (PAG) to the locus coeruleus (LC) in mice. Using a variety of virally delivered tools to facility neuroanatomical tracing and photostimulation of afferent neurons, in combination with electrophysiological recordings from brain slices containing the LC, the authors thoroughly characterize and compare afferent excitatory projections from the PFC, LH, and PAG to the LC. Furthermore, they study the excitatory effects of these projections in the presence of key neuromodulators.


Overall, this study is well done and adds to our understanding of the LC-norepinephrine system in the brain. The experiments seem to be performed and analyzed with care and appropriate statistical tests. The manuscript is well-written, and the visualization of data is excellent (I particularly appreciate the use of a consistent color scheme to depict PFC, LH, and PAG data throughout the manuscript).


I have a small number of concerns to address for a resubmission of this manuscript:


(1) The authors claim that afferent input from the PAG does not seem to have anteroposterior projection pattern. However, to my eye, projections from the PAG to the LC seem to be exclusively and strongly located in the posterior LC (Figure 1D). To a lesser degree, projections from the PFC seem to be exclusively located in the anterior LC. Are these figures in Figure 1D truly representative, or perhaps not good enough depictions of what the authors report in the text?

(2) Perhaps consistent with the comment above, it is difficult to discern overlap between mCherry signal and TH immunostaining in the histological images in Figure 1D, perhaps because it is hard to visualize overlap between the blue and red colors. Perhaps the authors could adopt a different color scheme that better depicts overlap between these two colors. Alternatively (or additionally), the authors could add subfigures showing higher magnification of the LC to complement these images.

(3) In the Abstract, stating that the PFC is "a well-known input" while projections from the LH and PAG "have not been explored to date" seems like an exaggeration (or perhaps not precise). The anatomical projections from PFC, LH, and PAG have all been characterized previously in a way that does not detract from the novelty of this study. I suggest rephrasing.

(4) In the Introduction (first sentence of second paragraph), I am not sure what the authors mean by stating that "the afferent landscape of the LC is not fully mapped." Previous studies, such as those by Schwarz (in the lab of Liqun Luo), used cell type specific retrograde tracing to monosynaptically label sources of afferent input to the LC in a non-biased anatomical survey of the entire brain. Why do the authors say that sources of LC input are not fully mapped? Maybe the authors could rephrase this sentence or be more specific.

(5) In the Methods, please provide a citation for the original study that produced the CamKIIa Cre mice.

(6) In the Methods, please provide the specific manufacturer and product numbers for the pharmacological agents used (e.g., picrotoxin, various neuropeptides, bestatin, thiorphan, etc.)

(7) Figure 1H does not seem to be cited in the text.


Referee #2:


Barcomb et al present an important and timely manuscript on the role of glutamatergic afferent regulation of the locus coeruleus. Briefly, the authors use a CaMKII-Cre mouse to target distinct excitatory afferents from the prefrontal cortex, lateral hypothalamus, and periaqueductal gray. Through a combination of viral tracing, histological, and in-depth electrophysiological and pharmacological studies the authors demonstrate that these glutamatergics inputs to the LC have a

variety of distinct properties. Importantly, this study greatly enhances are understanding of glutamatergic input to the LC generally, and more specifically gives great insight into projections from the LH and PAG that were almost unstudied to date. The manuscript is very well-written and the results are clearly conveyed. Overall I am very positive about this important manuscript, but have a few suggested points of further data analysis hat should already be on hand or easily attained by the authors, and the remaining limitations of the current study should also be well documented in the final manuscript. More detailed comments can be found below:

More detail is needed regarding the cell bodies that send these projections:

a) If animals were included if the center of expression was outside of the target, what was the tolerance for the edges of expression outside of the target site? This is mentioned in the discussion, but were there instances of substantial expression outside of the target whether it was centered or not?

b) Again this was alluded to in the discussion, but the manuscript would benefit from representative quantification of the number of cells at each afferent site. How many cells are projecting from each location? This could influence EPSC amplitude among other considerations. Ideally this would include the standard anterograde viral injection quantified with a retrograde label from the LC. However, a correlation between Figure 1E and just an antergrade cell count could be sufficient.

c) Differential viral expression could also impact the oEPSC results - the authors are clearly aware of this possibility so I just commend them for including these considerations throughout the results and discussion.

It is not clear from the figure legend what the n corresponds to in E-G & K. Is it mouse or slice? Same is true for I, but I am assuming that is slice and mouse. This data should likely be nested per animal.

Figure 1K is very interesting, though I am not sure the data supports the claim that the "PFC more heavily target distal dendritic areas". It seems to point towards a more conservative conclusion would focus on the distinct distribution pattern rather than the lack of statistical significance at the further point. This particular point in both the results and the discussion might benefit from the inclusion of more raw data (i.e. images) showing distinct distribution patterns of the PFC compared to other targets.

The firing rate in the cell-attached recordings is relatively low compared to other published data (though not outside of what is observed). Is there a reason (outside of somewhat lower bath temperature) I am missing as to why this would be the case in the current preparation?

In the discussion regarding pericoerulear LC neurons, the authors should consider discussing and citing Kuo et al, JP, 2020. That manuscript points to an important role of pericoerular GABA neurons in regulating phasic bursts. Likewise, it might be worth considering that phasic bursts typically do not occur in horizontal slices as performed here, but appear to be present in coronal slices.

Similarly, the authors should consider citing the Luskin et al, 2022 preprint on which they are also authors.

Finally, I would encourage more precise language regarding PFC nomenclature throughout the manuscript as suggested by Laubach et al, eNeuro, 2018.

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END OF COMMENTS

**Confidential Review**                                                                                    **17-Jul-2022**

Dear Editors and Reviewers:

Below is a point by point response to the reviewer's comments. Changes to the text in the manuscript have been made in red.
Sincerely,
Kelsey Barcomb & Chris Ford


**In summary comments:**
-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.

All data points for figures are now individually plotted


-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.

All data points for figures are now individually plotted. As the cumulative distribution plot already represents the raw data, Fig 2 H-J remain unchanged.


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

Noted


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

Done


-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.

Data is presented in figures as mean+SEM but has been described in the results section as well as in the Statistical Summary Document as mean+SD

-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.

Exact p-values have been used.


-Statistics Summary Document completed appropriately upon revision

The required Statistics Summary Document has been completed.

-Please provide (in the article file) a legend to accompany your Abstract Figure.

A revised Abstract Figure, created in BioRender, has been included along with a Figure Legend

-Please include a full title page as part of your article (Word) file (containing title, authors, affiliations, corresponding author name and contact details, keywords, and running title).

Done.


**Reviewing Editor**
- include the ethics approval number for IACUC University of Colorado ethics.

IACUC number has been included.

- report exact p-values for all statistical tests (e.g. $p = 0.004$), with the exception of $p < 0.0001$, and supplement reporting of p-values with effect size calculations (e.g. Cohen's d for t-tests, partial eta-squared for RMANOVA).

Exact p-values have been used.

- report results in text and figures using mean and standard deviation, as per the Journal's statistics policy. Alternatively, for figures, instead of standard deviation error bars, violin plots (easy to produce in GraphPad prism) are an excellent wat to show the data distribution as well as the variability.

Data is presented in figures as mean+SEM but has been described in the results section as well as in the Statistical Summary Document as mean+SD

- describe the post operative care following stereotaxic surgery.

This has been included in the Stereotaxic Surgery section of the Methods.


**Senior Editor**
- I would also add that it would be preferable to pool data obtained from the same animal and then take the mean of these values and report N - you do document n/N and indeed your N values are large. You can still report the cells etc but for robustness please use N for the basis of statistical comparisons.

While it would be preferable ideally to pool cells (n) from animals (N), we have chosen not to do this since it would artificially overrepresent some cells. The study was not originally designed to have equivalent number of cells from each animals. As such some animals may only have 1 cell recorded.  We believe that in this case it is better to present the data by cells (n) rather than animals (N).  The only exception to this is Figure 1E, where injection sites (hemispheres) were pooled.

Please report precise p values and SD (not SEM).

Exact p-values and SD have been used throughout the text and Statistics Summary Document.

**Reviewer #1:**

(1) The authors claim that afferent input from the PAG does not seem to have anteroposterior projection pattern. However, to my eye, projections from the PAG to the LC seem to be exclusively and strongly located in the posterior LC (Figure 1D). To a lesser degree, projections from the PFC seem to be exclusively located in the anterior LC. Are these figures in Figure 1D truly representative, or perhaps not good enough depictions of what the authors report in the text?

- Figure 1D (PAG) has been replaced with a more representative image. Figure 1D (PFC) remains unchanged since it is a representative image of the overall data set of the PFC projections to the LC.


(2) Perhaps consistent with the comment above, it is difficult to discern overlap between mCherry signal and TH immunostaining in the histological images in Figure 1D, perhaps because it is hard to visualize overlap between the blue and red colors. Perhaps the authors could adopt a different color scheme that better depicts overlap between these two colors. Alternatively (or additionally), the authors could add subfigures showing higher magnification of the LC to complement these images.

- We respectfully find that the current color scheme for Figure 1D fairly easily shows the mCherry signal and TH overlap.  We prefer to keep the existing colors since they are separate from other colors used in the manuscript, which avoids any potential confusion for readers.


(3) In the Abstract, stating that the PFC is "a well-known input" while projections from the LH and PAG "have not been explored to date" seems like an exaggeration (or perhaps not precise). The anatomical projections from PFC, LH, and PAG have all been characterized previously in a way that does not detract from the novelty of this study. I suggest rephrasing.

- The abstract has been revised and now simply states:
"The current study used an optogenetic approach to isolate three glutamatergic afferents: the prefrontal cortex (PFC), the lateral hypothalamus (LH) and periaqueductal grey (PAG)."


(4) In the Introduction (first sentence of second paragraph), I am not sure what the authors mean by stating that "the afferent landscape of the LC is not fully mapped." Previous studies, such as those by Schwarz (in the lab of Liqun Luo), used cell type specific retrograde tracing to monosynaptically label sources of afferent input to the LC in a non-biased anatomical survey of the entire brain. Why do the authors say that sources of LC input are not fully mapped? Maybe the authors could rephrase this sentence or be more specific.

- The introduction has been revised and now states:
"Through mapping of the afferent inputs to the LC, studies suggest that LC-NE neurons receive input from many regions (Aston-Jones et al., 1991; Cedarbaum and Aghajanian, 1978; Luppi et al., 1995; Schwarz et al., 2015)."


(5) In the Methods, please provide a citation for the original study that produced the CamKIIa Cre mice.

- The reference has been included (Tsien et al., 1996).

(6) In the Methods, please provide the specific manufacturer and product numbers for the pharmacological agents used (e.g., picrotoxin, various neuropeptides, bestatin, thiorphan, etc.)

- Details are now included in the Methods section.

(7) Figure 1H does not seem to be cited in the text.

- A reference to Figure 1H has now been included in the results.

**Reviewer #2:**
More detail is needed regarding the cell bodies that send these projections:
a) If animals were included if the center of expression was outside of the target, what was the tolerance for the edges of expression outside of the target site? This is mentioned in the discussion, but were there instances of substantial expression outside of the target whether it was centered or not?

- We found that out of roughly 50 animals that were PAG injected throughout the course of the study that less than 5 had the center of the injection site located outside of the region of interest, which were excluded from the results. All PFC and LH injections accurately targeted the region of interest. With respect to the spread outside of the target region, we did not quantify the degree of fluorescence in the periphery though the images shown in Figure 1 of the injection sites are representative and show minimal spread into adjacent brain regions. We have now included a better description of this in the methods section that reads:
"After visual inspection of the injection sites, animals were excluded if the center of the injection sat outside of the target area."


b) Again this was alluded to in the discussion, but the manuscript would benefit from representative quantification of the number of cells at each afferent site. How many cells are projecting from each location? This could influence EPSC amplitude among other considerations. Ideally this would include the standard anterograde viral injection quantified with a retrograde label from the LC. However, a correlation between Figure 1E and just an antergrade cell count could be sufficient.

- While this is an interesting point, we unfortunately do not have sufficient tissue saved or imaged to allow us to do a serial reconstruction to determine the total number of cells from each region. As redoing such a serial histological analysis would be beyond the scope of the current manuscript, we feel that this level of anatomical analysis may be better addressed in future work. While this is an anatomically interesting questions, it is important to note that the results of the current study do not rely on an equal number of afferent cells.

c) Differential viral expression could also impact the oEPSC results - the authors are clearly aware of this possibility so I just commend them for including these considerations throughout the results and discussion.

- No change required.

It is not clear from the figure legend what the n corresponds to in E-G & K. Is it mouse or slice? Same is true for I, but I am assuming that is slice and mouse. This data should likely be nested per animal.

- The figure legend for Figure 1 has been updated to more clearly describe the n's/N's for Fig 1E-G, and K.

Figure 1K is very interesting, though I am not sure the data supports the claim that the "PFC more heavily target<s> distal dendritic areas". It seems to point towards a more conservative conclusion would focus on the distinct distribution pattern rather than the lack of statistical significance at the further point. This particular point in both the results and the discussion might benefit from the inclusion of more raw data (i.e. images) showing distinct distribution patterns of the PFC compared to other targets.

- We agree that a more conservative conclusion is warranted. As such we have now altered the text in the results to read "Thus the PFC, LH and PAG all innervate LC-NE neurons, with the LH

and PAG more heavily targeting proximal somatodendritic regions with the PFC displaying a broader distribution."

In regards to including more images (raw data), we feel that this would make for a very large figure as we would need to present multiple images from LC cells showing innervation from the three different regions. Instead, we rather prefer to show only the quantified data of these images that is currently presented in Figure 1K.

The firing rate in the cell-attached recordings is relatively low compared to other published data (though not outside of what is observed). Is there a reason (outside of somewhat lower bath temperature) I am missing as to why this would be the case in the current preparation?

- The firing rate of LC cells in cell-attached recordings is withing the range of other published data. It is true that it is slightly lower than some other studies. Multiple factors likely account for this including the orientation of the slices (horizontal, recording conditions and temperature).

In the discussion regarding pericoerulear LC neurons, the authors should consider discussing and citing Kuo et al, JP, 2020. That manuscript points to an important role of pericoerular GABA neurons in regulating phasic bursts. Likewise, it might be worth considering that phasic bursts typically do not occur in horizontal slices as performed here, but appear to be present in coronal slices.

- The Kuo et al., 2020 reference has now been included.

Similarly, the authors should consider citing the Luskin et al, 2022 preprint on which they are also authors.

- The Luskin et al., 2022 reference has now been included.

Finally, I would encourage more precise language regarding PFC nomenclature throughout the manuscript as suggested by Laubach et al, eNeuro, 2018.

-Better nomenclature has now been included as follows: "As per nomenclature suggestions outlined in Laubach et al., 2018, injections to the medial prefrontal cortex specifically targeted the prelimbic and infralimbic cortices, with the target plane corresponding to 1.78 mm anterior from bregma in the mouse brain atlas (Franklin & Paxinos 3rd Edition, 2007). For simplicity, this afferent is referred to in the text as "PFC"."

Dear Dr Ford,

Re: JP-RP-2022-283605R1 "Properties and modulation of excitatory inputs to the locus coeruleus" by Christopher Ford, Kelsey Barcomb, Samantha S Olah, and Matthew Kennedy

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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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)

- Abstract figure file (see above)

- 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:

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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,

David Wyllie
Senior Editor
The Journal of Physiology


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REQUIRED ITEMS:

-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.



-The Journal of Physiology funds authors of provisionally accepted papers to use the premium BioRender site to create high resolution schematic figures. Follow this link and enter your details and the manuscript number to create and download figures. Upload these as the figure files for your revised submission. If you choose not to take up this offer we require figures to be of similar quality and resolution. If you are opting out of this service to authors, state this in the Comments section on the Detailed Information page of the submission form. The link provided should only be used for the purposes of this submission. Authors will be charged for figures created on this premium BioRender account if they are not related to this manuscript submission.




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

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.

-Statistics Summary Document completed appropriately upon revision

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EDITOR COMMENTS

Reviewing Editor:

Well done on the thorough job addressing the comments of the reviewers. This manuscript makes an important contribution to understanding the afferent projections to the locus coeruleus.

Senior Editor:

Thank you for revising your manuscript. Outstanding issues remain - in some figure legends you have not provided precise p values. These are required as Journal policy unless $p < 0.0001$. Moreover, error bars should represent SD (and not SEM). I appreciate you have provided SD values but the error bars should also indicate this - again Journal policy. Please make the required changes - your revised manuscript will not go for further review, but rather I will check its compliance.

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REFEREE COMMENTS

Referee #1:


The authors have addressed all of my previous comments and this manuscript is greatly improved from the original submission.


My only comment for this resubmission is that I continue to believe that the presentation of histological data in Figure 1D is not optimal and could be substantially improved. The authors have chosen to keep the previously submitted color scheme (red and blue), which is their choice. However, to my eye these colors don't allow the reader to discern overlap in a meaningful way. More importantly, it is difficult to discern mCherry projections in the greyscale images on the left-hand side of these figures. I also continue to believe that the images of projections from the PFC to the LC do not represent the data quantified in Figure 1F--to my eye there is absolutely no projections in the posterior LC even though Figure 1F shows a higher density of projections in the posterior region compared to the anterior region. Figure 1D is an important figure in this study, and it is unfortunate that there are not more high quality, higher magnification images provided (such as the quality depicted in Figure 2A).


This comment aside, I believe this study is overall excellent and a great contribution to the field.


Referee #2:


The authors have addressed my most pressing concerns. They should be commended for their excellent addition to the literature.

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END OF COMMENTS

**1st Confidential Review**                                                                                      **13-Sep-2022**

Dear Editors,

As requested, we have revised the manuscript. All figure legends now report exact p-values. We have replaced the error bars from being SEM to now being SD.

Sincerely,
Kelsey Barcomb and Chris Ford

-We have revised the figures and figure legends. Now all figure legends report exact p-values, unless p<0.0001. Error bars in the summary figures now have been changed from SEM to SD.

The only exception is for the time course data that is presented in the upper panels of Figure 4, which has been left as SEM since converting these panels to SD would make it difficult to see the underlying electrophysiological traces. To ensure clarity, we have noted this in both the figure legend ("*Time course data are mean ± SEM. Summary data in bar graphs are mean ± SD*") as well as the in the methods (*"Data shown are all mean +/- SD in figures and mean +/- SD in text, with the exception of the time course data showing drug application that is presented in the upper panels of Figure 4 which illustrate mean +/- SEM."*). The summary bar graph data at the bottom of Figure 4, which summarizes the data presented in these upper panels is shown as mean +/- SD as requested. We hope this is acceptable, however please let us know if it is required to convert these time course panels to SD.

also continue to believe that the images of projections from the PFC to the LC do not represent the data quantified in Figure 1F--to my eye there is absolutely no projections in the posterior LC even though Figure 1F shows a higher density of projections in the posterior region compared to the anterior region. Figure 1D is an important figure in this study, and it is unfortunate that there are not more high quality, higher magnification images provided (such as the quality depicted in Figure 2A).

This comment aside, I believe this study is overall excellent and a great contribution to the field.

- As we have also included gray scale images of the mCherry projections we have attempted in the presentation of this figure to best illustrate the projections from each region to the LC. Unfortunately, we did not image the slides of the data presented in Figure 1D at a higher resolution. Repeating these experiments and substantially revising the figure would be a significant change to the manuscript. We believe that this anatomical characterization would best be done in a subsequent manuscript which would be entirely focused on anatomy.

Referee #2:

The authors have addressed my most pressing concerns. They should be commended for their excellent addition to the literature.

- No changes required.

Dear Dr Ford,

Re: JP-RP-2022-283605R2 "Properties and modulation of excitatory inputs to the locus coeruleus" by Christopher Ford, Kelsey Barcomb, Samantha S Olah, and Matthew Kennedy

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.

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Yours sincerely,

David Wyllie
Senior Editor
The Journal of Physiology


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EDITOR COMMENTS

Thank you for revising your manuscript and for submitting this work to The Journal of Physiology - with the changes made, I am happy to accept this for publication.

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**2nd Confidential Review**                                                                                    **19-Sep-2022**