Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury

Marta Zaforas, Juliana M Rosa, Elena Alonso-Calviño, Elena Fernández-López, Claudia Miguel-Quesada, Antonio Oliviero, and Juan Aguilar

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Arko Ghosh (Referee #1); Ken D O’Halloran (Referee #3)

Review Timeline:

| Event                  | Date         |
|------------------------|--------------|
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| Editorial Decision     | 10-Jun-2021  |
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Senior Editor: Harold Schultz

Reviewing Editor: Richard Carson

Transaction Report:

(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 Dr Aguilar,

Re: JP-RP-2021-281901 “Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury” by Marta Zaforas, Juliana M Rosa, Elena Alonso-Calviño, Elena Fernández-López, Claudia Miguel-Quesada, Antonio Oliviero, and Juan Aguilar

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 3 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.

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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,

Harold D Schultz
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REQUIRED ITEMS:

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- Please ensure that any tables are in Word format and are, wherever possible, embedded in the article file itself.

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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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-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.

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-'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

-A Data Availability Statement is required for all papers reporting original data. This must be in the Additional Information section of the manuscript itself. It must have the paragraph heading "Data Availability Statement". All data supporting the results in the paper must be either: in the paper itself; uploaded as Supporting Information for Online Publication; or archived in an appropriate public repository. The statement needs to describe the availability or the absence of shared data. Authors must include in their Statement: a link to the repository they have used, or a statement that it is available as Supporting Information; reference the data in the appropriate sections(s) of their manuscript; and cite the data they have shared in the References section.
Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. If sharing data compromises ethical standards or legal requirements then authors are not expected to share it, but must note this in their Statement. For more information, see our [Statistics Policy](#).

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EDITOR COMMENTS

Reviewing Editor:

It is evident that the study described in this submission is technically sophisticated, and has been conducted with great care. There is every reason to suppose that the data are of high quality. The expert reviewers agree that the results are robust, unique, and have the potential to advance significantly our knowledge of (layer-specific) cortical adaptation arising from sensory deprivation - due to spinal cord injury. Indeed, Referee 1 is of the opinion that the significance of the outcomes could be further accentuated. In this regard, it is recommended that the Introduction be amended in order to frame the study to best effect. Corresponding suggestions are offered in relation to the Discussion. Indeed, both referees make valuable recommendations concerning ways in which the presentation of the work might be enhanced. There are also a number of technical points, and issues of grammar and referencing, that should be addressed in the course of a careful revision.

Senior Editor:

Summary data must be shown with standard deviation, not standard error. Please correct figure 1, 4, 5, 7.

Actual p values must be stated throughout, including tables and in figures. Do not use asterisks to denote p values. Table and figures legends must state statistical test(s) used.

Table 1-3 supplement must be incorporated into the manuscript.

Please include the required Statistical Summary Document using the form and requirements in Instructions to Authors,

Urethane as a sole agent for euthanasia is not documented as a standard method to euthanize rodents. While overdose of an anesthetic agent used in terminal experiments is acceptable (Schedule 1 ASPA), please provide documentation of efficacy of the dose used and methods used to ensure the animal was killed.

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REFEREE COMMENTS

Referee #1:

This is a carefully conducted study on the immediate electrophysiological changes that occur after SCI. The study uses a beautiful combination of methods, and provides the field with what could be a landmark dataset that spans population to multi-unit activity across different cortical
layers. I congratulate the authors on their efforts, but I believe the report needs some consideration in terms of interpretation, making the data more accessible to the reader, and reasoning the selective forms of analysis. I also do hope that this data would become available to the scientific community to maximize the impact of this outstanding effort.

Major considerations:

1. The framework of deprivation. I found the framework of deprivation a bit confusing. Now, a thoracic spinal cord injury does lead to sensory deprivation but I believe the experimental design focuses on the aspect the cortex is traumatically ‘disconnected’ from the body as opposed to deprivation. Conventional studies on deprivation typically are concerned with the reduced sensory flow/inputs and their consequences. Essentially, the ‘experience-dependent’ processes gain prominence under that framework. However, in this study, the focus is on the recently altered input statistics compounded with the traumatic consequences (such as retrograde messaging of the injury from the distal axons). I think this zone of change is poorly understood and using the appropriate semantics will help place this work in the right context.

2. The complex source of reorganization. This group has previously performed some excellent research on injury-induced cortical alterations. Essentially, the reorganization immediately after the injury may be attributed to (a) retrograde messaging say due to cut axons (b) altered spontaneous activity directly related and un-related to ‘a’ (c) altered input statistics partly due to ‘b’ in conjunction with the inability of the original input pathways to operate. I think a clearer synthesis of these ideas to frame their current work is needed. For instance, what is particularly exciting is that they are recording from multiple cortical layers simultaneously after the injury. As the authors indicate, previous studies have focused on the deeper layer V where ‘a’ ‘b’ and ‘c’ are all compounded. It would be reasonable to expect that the impact of ‘a’ is less in the ‘not layer V’ neurons. An introduction into why a multi-layered recording (either based on the circuit properties and/or the ability to study neurons under different conditions due to their distinct relations to the injury) is needed beyond what is currently mentioned (current focus is on the aspect that it has not been well studied and thus needs to be studied now). A more sophisticated framing would elevate the importance of this work to its correct stature. Indeed, circuit-level arguments are present in the results and discussion section but these need to be introduced better.

3. Impact of anesthesia. The experimental design does not consider anesthesia in itself to induce cortical alterations. Could the authors - perhaps using existing data or even data in the literature - clarify that when the parameters as evaluated here are gathered over the same period do not spontaneously alter due to anesthesia? In other words, a control group would be really useful and perhaps this can be harnessed from existing data.

4. Choice of the analysis used. The data collected here is fundamentally rich and I am excited by the prospect of more discoveries stemming from this very data. However, this can be explicitly facilitated. For instance, will the authors clarify: (a) how will the data be shared, (b) the authors
choose some rather basic analytical tools to address their initial questions, what other analytical tools would they suggest as a next step? For instance, the same data could be used to develop functional information flow models - by using forms of source localization and coherence analysis - across different layers and then study how the SCI impacts that flow. A discussion paragraph could be added along such exciting outlook.

Minor:

Introduction>

‘This sensory deprivation initiates the so-called process of cortical reorganization. Please consider my above major consideration (1), and perhaps note here that both retrograde messaging of the injury and altered input flow may be key players.

The final paragraphs of the introduction are missing references. I assume it's a bibliographic error. For instance, the citation is missing in the sentence (among others). ‘Changes in the cortical activity after SCI have been mostly studied by using electrophysiological recordings from layer 5 neurons.’.

Methods>

A 14% loss of animals is striking. Is this number compared to what is found in previous reports? I also appreciate their transparent reporting of this number and many studies fail to report such numbers.

I appreciate how the authors performed histology on the recording site. Did the authors also perform histology at the spinal injury site? Now, I appreciate the electrophysiological data showing loss of signals from below the injury stimulations but it remains possible some fibers were spared. Can the authors provide some qualitative or quantitative overview of how the 'complete' transection was verified? (this may explain the different groups found in the section 'SCI induces layer-dependent functional changes in the sensory deprived cortex.')
Layer-specific anatomical changes after spinal cord injury have been suggested by 10.1093/cercor/bhr191 (Ghosh et al., 2012) to occur on the layer V dendrites. How does this anatomy relate to physiology?

I think the pre-clinical relevance paragraph is a bit of a stretch. The authors do indeed identify variation in how the cortex responds between animals but this could be due to a variety of reasons and cannot be used to explain the clinical observations like the development of pain. I think the basic science and data are interesting in themselves and perhaps this tangent dilutes the apparent rigor of the study.

Linking to the human data, in general, seems to be a useful exercise at first but such measurements cannot be conducted in humans so the comparative claims are not well-founded. It is impossible to reasonably conjecture if the very rapid alterations observed here have anything to do with what is observed in human studies on cortical plasticity. I strongly recommend focusing on the rodent findings and the corresponding circuitry. I believe focusing on the core results derived from rodents here will inspire human spinal cord injury researchers to be inspired and may lead to the development of next-generation reports that explicitly attempt to find parallels in human observations. This critical work is important but beyond the scope of the current research article.

Given the number of measures could the authors consider a summary figure or scheme to bring all of the findings under one view?

Referee #2:

Zaforas and co-authors present data describing details of the immediate affects of SCI on sensory cortex. This work follows-up on previous work by this group and provides important information regarding the immediate basis for long-term reorganization of sensory cortex after SCI. The work is original, well done and the results are clear. The study design is appropriate and the data are robust. The authors find that acute SCI induces layer-dependent changes in local circuits through alterations of both corticocortical and thalamocortical connections. The insight into the role of changes in the LFP slope to suggest unequal reduction of local inhibition across layers that allows infragranular cells to better integrate evoked sensory inputs is intriguing and provides important new context to our understanding of the effect of SCI on sensory cortical processing. Finally, comparing the latency of responses to suggests that the onset of evoked-LFP responses in infragranular layers is more conditioned by changes in thalamic inputs, is important for our understanding of the mechanisms. The conclusions are appropriate and lend context to the impact of the results.
Overall, there are several grammatical errors so a good proofing of the article is in order. There are a few issues that should be addressed

1. Introduction is missing a few important references
   a. Reference to electrophysiological experiments are missing e.g. Ganzer et al., 2013
   b. References missing for the statement about ephys recordings from layer 5 (perhaps want to change to infragranular) e.g. Manohar et al., 2017

2. Methods - more detail on how rMUA was calculated is necessary

3. Discussion -
   a. along with the discussion around slow-wave oscillations, it is important to acknowledge that these oscillations are developed in response to anesthesia. Therefore, their discussion needs to make clear how these anesthesia induced changes in oscillations are related to their discussion about the relevance of these change in up-states resulting in reorganization.
   b. For the discussion of infragranular a missing reference related to size of receptive fields in infragranular cortex - Tutuncular et al. 2006

Referee #3 (ethics review):

Thank you for submitting your work to The Journal of Physiology. There are some matters relating to animal ethics and welfare that must be clarified.

It would be better to introduce the experimental procedures first and then clarify the method of killing at the end of the relevant sections, so that the timeline of the animals' fate is clearer. Please state animals were killed (or euthanised), not sacrificed.

I commend the authors for the transparent reporting of all animals used in the study, including those who died under anaesthesia before data collection.

The dose of anaesthetic is clearly stated and the measures taken to ensure adequacy of depth of anaesthesia are included. I suggest moving the text which clarifies the approach taken following spinal transection (ie assessment of forelimb reflexes, spontaneous whisker movement and corneal reflexes) to an earlier portion of the text when spinal transection is first introduced.
This immediately addresses the issue of adequacy of depth of anaesthesia, which must be monitored throughout procedures.

It appears that an additional dose of urethane (1.5g/kg) was used to kill the animals by overdose. Please indicate how death was confirmed. Later, under the sub-heading "Histology", it is stated that rats were transcardially perfused with saline and 4% PFA. Please clarify if this was performed post-mortem following urethane overdose. Or, if the heart was still beating, please state that this was the method of euthanasia, in deeply anaesthetised rats.

Under the sub-heading "Thalamocortical recordings...".

Studies in 7 rats are briefly described. Notwithstanding the apparent redundancy of repeating text used earlier in the manuscript, it is better to clearly state the dose of anaesthetic for these animals and to provide a statement (again) in respect of steps taken to ensure adequacy of depth of anaesthesia before and after spinal transection. The fate of all animals must be clearly reported in manuscripts. Although one can infer the dose and approach used from the text as currently written, it is best to re-state this for absolute clarity. There are no word limits in The Journal.

The authors highlight that the novel data presented in this sub-section were collected in a separate study which is published, reporting on a different data set collected contemporaneously. I commend the authors on the transparent reporting of this detail. Please include in this sub-section the ethics approval details relating to that historical study, or re-state the ethics approval details if the same as the current study. Please state the method of killing of the animals in this sub-section.

END OF COMMENTS
1st July 2021

Subject: JP-RP-2021-281901 “Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury”

Dear Editor and Senior Editor

First of all, we would like to thank you for all comments and arguments provided about our previous submitted version.

We are now submitting a revised version of our manuscript that fully address all concerns raised by both Editors and Reviewers as well as complying with the Journal of Physiology’s declaration of transparency, statistical requirements and scientific rigour.

We do believe that our study was substantially improved by following all the givens considerations (i.e. changes in semantics, better original focused, and addition of important physiological arguments) and We hope that the manuscript in its present form will be acceptable for publication in the Journal of Physiology.

Please, see below the point-by-point answers to Answers to Reviewing Editor, Senior Editor and Referees.

Yours sincerely

Juan Aguilar
EDITOR COMMENTS

Reviewing Editor:

It is evident that the study described in this submission is technically sophisticated, and has been conducted with great care. There is every reason to suppose that the data are of high quality. The expert reviewers agree that the results are robust, unique, and have the potential to advance significantly our knowledge of (layer-specific) cortical adaptation arising from sensory deprivation - due to spinal cord injury. Indeed, Referee 1 is of the opinion that the significance of the outcomes could be further accentuated. In this regard, it is recommended that the Introduction be amended in order to frame the study to best effect. Corresponding suggestions are offered in relation to the Discussion. Indeed, both referees make valuable recommendations concerning ways in which the presentation of the work might be enhanced. There are also a number of technical points, and issues of grammar and referencing, that should be addressed in the course of a careful revision.

We thank the valuable insights given from Editors and Reviewers that we believe have significantly improved our study and the way we present our data in this new version. Following these suggestions, we have carried out changes in the text (Introduction and Discussion) to better fit our results with the new semantics pointed, specially, by Reviewer 1. We have also included new arguments pointed by Reviewer 2 that we considered important such as the inclusion of the role of slow wave activity in the cortical reorganization as well as added few missing citations.
A new figure has also been provided in order to summarize the data in a schematic way as asked by Reviewer 1 and changes in the Methods regarding Ethics and better description of few methodological points were made following Reviewer’s 3 arguments. A more detailed answer to all points is provided for each Reviewer below.

Senior Editor:

Summary data must be shown with standard deviation, not standard error. Please correct figure 1, 4, 5, 7.
All figures have been corrected and standard deviation is shown throughout.

Actual p values must be stated throughout, including tables and in figures. Do not use asterisks to denote p values. Table and figures legends must state statistical test(s) used.
Asterisks have been changed to the exact p value in all figures and tables.

Table 1-3 supplement must be incorporated into the manuscript.
It has been incorporated.

Please include the required Statistical Summary Document using the form and requirements in Instructions to Authors,
It has been completed and submitted in this new version.
Urethane as a sole agent for euthanasia is not documented as a standard method to euthanize rodents. While overdose of an anesthetic agent used in terminal experiments is acceptable (Schedule 1 ASPA), please provide documentation of efficacy of the dose used and methods used to ensure the animal was killed.

We would like to clarify that in our previous version, we wrongfully wrote that animals were euthanized by urethane. This was not the case, as we used an overdosis of urethane at the end of the experiments to induce a deep state of anesthesia in order to proceed with transcardial perfusion for histological experiments. A fully answer to this question is provided to Reviewers 1 and 3 and can be found below.

We have also modified the text in the Methods section to clearly describe the procedures (page 9, new version.)

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REFEREE COMMENTS

Referee #1:

This is a carefully conducted study on the immediate electrophysiological changes that occur after SCI. The study uses a beautiful combination of methods, and provides the field with what could be a landmark dataset that spans population to multi-unit activity across different cortical layers. I congratulate the authors on their efforts, but I believe the report needs some consideration in terms of interpretation, making the data more accessible to the reader, and reasoning the selective forms of analysis. I also do hope that this data would become available to the scientific community to maximize the impact of this outstanding effort.

We would like to thank the reviewer for all the positive comments regarding our study as well as for all the constructive criticism applied to it. After discussion within the lab and thought about all the comments concerning the interpretation of the main idea and the overall concept in which we built our study, we completely agreed with the reviewer that a much better focused could be applied to our results. For that, we have included other ideas suggested by the reviewer and that are explained below point by point.

We have also made explicit our data availability statement and uploaded some of our populational analysis in an open repository. This point is clearly explained below in the Major considerations #4 from this Answer to Reviewers’ doc.

Major considerations:

1. The framework of deprivation. I found the framework of deprivation a bit confusing. Now, a thoracic spinal cord injury does lead to sensory deprivation but I believe the experimental design focuses on the aspect the cortex is traumatically 'disconnected' from the body as opposed to deprivation. Conventional studies on deprivation typically are concerned with the reduced sensory flow/inputs and their consequences. Essentially, the 'experience-dependent' processes gain prominence under that framework. However, in this study, the focus is on the recently
altered input statistics compounded with the traumatic consequences (such as retrograde messaging of the injury from the distal axons). I think this zone of change is poorly understood and using the appropriate semantics will help place this work in the right context.

We thank the reviewer for such insightful inputs that have helped us to better focused the original hypothesis of our study. Regarding the concept of sensory deprivation, we have now modified the text to focus on cortical deafferentation, as we also agree that it better fits our experimental approach (for example in page 4, paragraph 1).

Moreover, further explanation about the others concerns in this Reviewer`s consideration is better explained in the next point as we considered they are related (see Major consideration #2).

2. The complex source of reorganization. This group has previously performed some excellent research on injury-induced cortical alterations. Essentially, the reorganization immediately after the injury may be attributed to (a) retrograde messaging say due to cut axons (b) altered spontaneous activity directly related and un-related to ‘a’ (c) altered input statistics partly due to ‘b’ in conjunction with the inability of the original input pathways to operate. I think a clearer synthesis of these ideas to frame their current work is needed. For instance, what is particularly exciting is that they are recording from multiple cortical layers simultaneously after the injury. As the authors indicate, previous studies have focused on the deeper layer V where ‘a’ ‘b’ and ‘c’ are all compounded. It would be reasonable to expect that the impact of ‘a’ is less in the ‘not layer V’ neurons. An introduction into why a multi-layered recording (either based on the circuit properties and/or the ability to study neurons under different conditions due to their distinct relations to the injury) is needed beyond what is currently mentioned (current focus is on the aspect that it has not been well studied and thus needs to be studied now). A more sophisticated framing would elevate the importance of this work to its correct stature. Indeed, circuit-level arguments are present in the results and discussion section but these need to be introduced better.

As pointed by the Reviewer, the complex process of cortical reorganization after SCI that takes into account multiple anatomical and circuitries levels was not considered properly in our previous version. We have now rewritten most of the Introduction to include the prospects of altered spontaneous excitability, altered retrograde messaging due to the axotomy and changes in thalamic and cortical connectivity that together may promote a differential layering-cortical reorganization. We have also included a better explanation about the scientific value of using a vertical multielectrode array spanning all cortical layers that allows to determine how SCI affects the neuronal activity within individual layers, the vertical relationship between layers as well as horizontal connections from adjacent intact cortical columns.

Examples of these changes are the following:

(Page 4, paragraph 1): “Spinal cord injury (SCI) produces a physical disconnection between the brain and spinal cord regions below injury level. Such deafferentation interrupts the ascending sensory information towards the somatosensory cortex, which promotes extensive cortical reorganization of sensorimotor areas (CoRe).”

(Page 5, paragraph 1): “… In this regard, SCI has been described to: 1) alters the neuronal excitability at thalamic level and cortical layer V activity (Liang and Mendell 2013; Alonso-
Calviño et al., 2016; Jain et al., 2008); 2) induces spine loss in the proximal segments of the apical dendrite of axotomized and non-axotomized corticospinal layer 5 neurons in the deafferented cortex (Ghosh et al., 2012) and 3) increases the strength of corticocortical connections (Endo et al., 2007; Humanes-Valera et al. 2017; Ganzer 2013; Manohar 2017)."

(Page 5, paragraph 1): “…Therefore, the effects that a SCI will produce at cortical level might not be restricted as it might span across different cortical layers as a result of an altered input statistics that take into account changes in the spontaneous excitability, altered retrograde messaging due to the axotomy and changes in thalamic and cortical connectivity.”

3. Impact of anesthesia. The experimental design does not consider anesthesia in itself to induce cortical alterations. Could the authors - perhaps using existing data or even data in the literature - clarify that when the parameters as evaluated here are gathered over the same period do not spontaneously alter due to anesthesia? In other words, a control group would be really useful and perhaps this can be harnessed from existing data.

First of all, the scientific reason for using urethane is that this anesthetic has been largely used in rodents for study of the somatosensory system (mainly barrel cortex). Most of authors provide information about stability during long periods of recordings and preservation of basic neuronal properties that allows the study of sensory responses at cortical level. However, the reviewer’s question has been made previously by other reviewers along our trajectory in which we have used acute model of spinal cord injury to study early effects at cortical level. For this reason, in previous articles we have demonstrated that our results are due to the direct effect of SCI and not to a possible confounding factor of anesthesia. More in detail, in Aguilar et al., 2010 we described that SCI immediately changes the state of the brain. In this work we showed that this change was not due to a possible cumulative effect of anesthesia. In addition, we used a pharmacological block of spinal cord conduction (with lidocaine/TTX, please see Figure 4 in Aguilar et al 2010) to show that massive sensory deprivation was sufficient to produce cortical effects. In study Foffani et al., 2011, we quantified the anesthetic requirements of animals with and without SCI during several hours. Our results demonstrated that animals with SCI required lower levels of anesthesia than control animals in order to maintain the same level of cortical activity (please see Figure 2, Foffani et al., 2011 Spinal Cord). This means that control animals didn’t show changes in cortical activity even after several additional doses of anesthesia, while animals with SCI showed immediate changes without increased doses of anesthesia, therefore the changes were due to SCI. In a posterior article Humanes-Varela et al., 2013, we show how to control the level of anesthesia to induce a slow-wave activity before SCI avoid a possible conounding factor due to anesthetic. Finally, in Fernández-López et al., 2019 we studied spontaneous activity after in layer V immediately after SCI and in the long-term. In this article, we used a group of animals in which a protocol of double duration time in order to discard that a longer duration under anesthesia doesn’t modify/affect cortical activity previous SCI. Therefore, based on all the previous experiments already published by our group, we assume that under our experimental conditions, the anesthesia itself is not inducing changes in cortical activity.

4. Choice of the analysis used. The data collected here is fundamentally rich and I am excited by the prospect of more discoveries stemming from this very data. However, this can be explicitly facilitated. For instance, will the authors clarify: (a) how will the data be shared, (b) the authors choose some rather basic analytical tools to address their initial questions, what other analytical tools would they suggest as a next step? For instance, the same data could be used to develop functional information flow models - by using forms of source localization and
coherence analysis - across different layers and then study how the SCI impacts that flow. A discussion paragraph could be added along such exciting outlook.

We thank the reviewer for such constructive criticism. Using our electrophysiological data to perform an in-depth neuronal modelling would be a very interesting approach and we would be very happy to share our data to establish new collaborations to work on these aspects. To explicitly show our interest, we have now made available the population data used throughout the manuscript in the lab’s Github database. We have also added the section “Data Availability Statement” (page 32) stating our sharing policies and making clear that anyone interested in the original raw data used in this study (as well as other raw data from previous publications) to carry out further analysis can directly contact us, so we can provide them with the data, explain our experimental protocols and help to setup some background ideas for analysis.

Regarding the suggestions made by the reviewer about further analysis, we do think that algorithms based on information flow optimisation and wiring cost minimisation (i.e. MST or ECO methods) could be used in order to reveal how SCI (or any other pathological conditions that interrupts flow, i.e. stroke, TBI) affects the information transfer within and between cortical areas. To state this idea in the text and to follow the reviewer's advice, we have now included a new subsection in the Discussion (page 27-28) and detailed below.

“SCI alters the spread of sensory-evoked activity across layers

....

Changes in the neuronal ability to integrate synaptic inputs may lead to an altered cortical information flow transfer as previously shown by altering the development of the thalamorecipient layer 4 (McLaughlin et al., 2005 Cer Cortex). Under physiological conditions, cortical layers are considered local circuitries functionally connected exhibiting synchronised fluctuations of neuronal activity that is transferred within (vertical flow) and between (horizontal flow) cortical columns. The strength and the ability to integrate and transmit the information are jointly determined by the confluence of neuronal inputs from distinct brain areas (i.e. brainstem, thalamus and cortical regions), but also by the intrinsic properties of the neurons in the network hub. In this line, SCI abruptly interrupts the input statistics in the affected cortex leading to a severe disruption of information-flow highways as observed by the altered transfer of both spontaneous and evoked cortical activity (Figure 5 and 7). In this scenario different possibilities could be taking place. In one hand, the altered transfer information could result from a simple change in the weight of horizontal over vertical input statistics in the affected cortex, that is, a synaptic reduction and a disruption in the coherence between layers (vertical flow) that would favour the horizontal input statistic. On the other hand, a more extreme condition would be that SCI leads to a disassembling of the layering connectivity triggering neurons to work as individual nodes without statistical relationship between edges, that is, local neural circuitries that are no longer functionally connected and start working as isolated, independent and non-regulated cortical hubs. In any case, SCI represents deviations from the physiological network that could be accurately inferred and modelled in future to study functional connectivity alterations and to determine if changes in the information flow affect the global functional network and dictate the strength of the cortical reorganisation at long-term.”

We have also included a better description about the results, and statistics in the graph, of our data regarding the propagation and initiation of up-states during spontaneous activity in the Results section related to Figure 7C-F (page 21, paragraph 1).
Minor:
Introduction
This sensory deprivation initiates the so-called process of cortical reorganization. Please consider my above major consideration (1), and perhaps note here that both retrograde messaging of the injury and altered input flow may be key players.

We have now included in the Introduction (page 4-5) the physiological aspects that may be taking place in the somatosensory cortex as pointed by the reviewer in Major considerations #1.

The final paragraphs of the introduction are missing references. I assume it's a bibliographic error. For instance, the citation is missing in the sentence (among others). 'Changes in the cortical activity after SCI have been mostly studied by using electrophysiological recordings from layer 5 neurons.'.

We have now included the references for the sentence as well as the acknowledged that there are also anatomical changes in the pyramidal layer 5 neurons after a SCI. The sentence now reads (page 4, paragraph 2):

“Interestingly, changes in the cortical activity after SCI have been mostly studied by using electrophysiological recordings from layer 5 neurons (Humanes-Valera et al., 2017, Ganzer et al., 2013 Humanes-Valera et al., 2013, Ghosh et al., 2012, Aguilar et al., 2010; Manohar et al., 2017).”

Methods
A 14% loss of animals is striking. Is this number compared to what is found in previous reports? I also appreciate their transparent reporting of this number and many studies fail to report such numbers.

Urethane anesthesia has been described to cause approximately a 10% loss of animals. This percentage rate is similar to what we have when working with animals without SCI, that is, in control conditions with no other procedures than only anesthetic application. To our knowledge, this effect is assumed in the field as natural reaction in a part of the population of animals to a particular anesthesia. This assumption could be the reason why many studies do not declare it, as it could be perceived as normal mortality using this compound, and this is the main reason why we did not declare it in previous works. In the present work and considering the current worldwide frame for animal research in which ethical regulation is a priority, and following the rules of the Journal of Physiology, we have decided to be transparent and included those numbers in the manuscript.

Indeed, in this particular topic, we have found an article (see Field et al., 1993 in Laboratory Animals 27:258-269) in which a comparison between three anesthetics was made in rats: chloral hydrate, pentobarbitone and urethane. Interestingly, authors declare that mortality of 25% was found only when urethane was used. These data show higher mortality rate than we have observed during the last two decades in our lab, but interestingly confirm that urethane produces higher animal mortality than other anesthetics.
There are some other comparisons between the use of urethane and different anesthetics, but most of them were focused on the effect that each anesthetic produce on sensory evoked responses or spontaneous activity. Finally, at the same time that urethane has been extensively used in rats to study somatosensory system (since 1970 as for example in Angel A & Unwin J, 1970 “The effect of urethane on transmission along dorsal column sensory pathway in the rat”. J Physiol 208(1):23p-33p), it has been validated as good anaesthetic to replicate brain states during natural sleep and transitions between REM and no-REM brain activity (Pagliardini et al., 2012, “State-dependent modulation of breathing in urethane anesthetized rats” J Neurosci. 32(33):11259-270; Clement et al., 2008 “Cyclic and sleep-like spontaneous alternation of brain state under urethane anesthesia” Plos One 3(4): e2004). This particular aspect related to brain rhythms has been useful to a proper interpretation of results in the present and previous works from our lab.

In summary, urethane is a widely used anaesthetic in rodent model for study of sensory systems and brain rhythms, with the downside to produce a higher rate of spontaneous mortality (10-12%) that do not alter results of our scientific works. Due to this singularity of the urethane, our group has been working lately with the inhalatory anaesthetic isoflurane, with it is a much better fit for a chronic longitudinal study in mice.

To better clarify this issue, we have added the above reference Field et al. 1993 in the Ethical Approval section of the Methods (page 7, paragraph 1).

I appreciate how the authors performed histology on the recording site. Did the authors also perform histology at the spinal injury site? Now, I appreciate the electrophysiological data showing loss of signals from below the injury stimulations but it remains possible some fibers were spared. Can the authors provide some qualitative or quantitative overview of how the 'complete' transection was verified? (this may explain the different groups found in the section 'SCI induces layer-dependent functional changes in the sensory deprived cortex')

We thank the reviewer’s comment regarding the electrophysiological data showing immediate loss of evoked responses when stimuli were applied below the lesion level (in both Figure 2 and 3). Actually, the data in Figure 2 showing the neuronal cortical response during the precise moment of the spinal cord transection is unique in the field, as far as we know, and of great scientific value. Therefore, we wanted to include in the study to demonstrate the quality and stability of recordings in order to make proper comparisons in pre-lesion and post-lesion conditions.

Regarding the histological data of the lesion site, this is something we have largely tried without success in our acute model. The anatomical verification of the injury is a routine procedure when using a SCI experimental model that requires assessment after days-to-months to determine either the severity (lesion extension) or the presence of spared fibers. In these cases, a simple Nissl staining of coronal or sagittal slices is sufficient to determine the anatomical features of the injury because of two main reasons: 1) the spine tissue has undergone necrosis, which is easily detect by the absence of Nissl bodies or other anatomical markers and, 2) despite of the injury, the spine tissue represents a continuous due to the extracellular matrix that maintain a proper tissue structure that enables a properly cutting and staining and a clear post hoc visualization of the fibers. However, in our case, the histological assessment using an acute model of SCI does not provide an accurate visualization of the injury because there is no physical continuity neither in coronal nor sagittal plane.
To clarify this point for the Reviewer in order to answer the question, allow us to briefly describe our procedure and provide some original pictures taken from our own experiments (Figure to Reviewer 1). First, the dorsal musculature is softly removed to expose the vertebral bone. After that, we carefully remove the vertebral bone between T8-T10 to expose the intact spinal cord. Note that in this step, the dura mater is still intact as well (Figure to Reviewer 1, panel B). The spinal cord is then protected by covering the opened vertebra with a small piece of absorbable hemostatic gelatin sponge (©Spongostan) with saline. This procedure is made before starting the electrophysiological recordings in control conditions. Once we lowered the electrode, confirm that we are in the hindlimb somatosensory cortex by stimulating the contralateral hindlimb and obtaining MUA activity across cortical layers. Once the experimental protocol pre-lesion is obtained, the spinal cord is transected using a special scissors and a surgical needle that is passed through the lateral and basal vertebral bone to remove any possible spared fibers close to the bone, for that, it is helpful to remove the basal dura mater as confirmation that no nervous tissue still connected between stumps. Next, we visually confirm that both spinal stumps are physically separated by using a forceps and a small piece of spongostan that is inserted between the two parts of the spinal cord in order to detect the “empty” space as shown in Figure for Reviewer 1, panel C. After that and before starting the post-lesion protocol, few stimuli (5-10) are applied at maximal intensity (10mA) in order to obtain a physiological prove of the total transection and to discard any possible connection from lower limbs to the brain. The experiment is then continued only if no short latency (evoked potentials) or long-latency (up-states) or any signal of cortical activation is observed in response to stimulation. Note that, we have experienced few times in which after the transection we observed some kind of cortical activation related to the stimulation, and we had to go over again with the surgical needle to remove spared fibers, proving that our experimental design to transect and verify the transection is reliable. (Note 1: We want to remark that, although this procedure seems invasive, the level of
anesthesia (note it is fixed to slow-wave activity) and the short time of depolarization and fast return to slow-wave activity observed during spinal transection guarantee that in this level of anesthesia and based on cortical activity no pain process or animal suffering was produced.)

Regarding the question whether possible spared fibers could be the origin of animals in which no changes were detected, we consider this an unlikely possibility. The reason is that to produce and maintain a physiological activity it is required a minimum number or percentage of preserved fibers which provide sufficient neuronal drive to produce an action or effect (both sensory and motor actions). Taking into account the type of spinal lesion we perform, which is a direct cut in coronal plane, a complete damage of dorsal columns axons (sensory ascending pathway) and corticospinal tract (motor descending pathway) located in the middle upper region of spinal cord is directly performed without doubts (See panel C, separated stumps in all dorsal region). Moreover, as described previously, once the complete transection with scissors was performed, a needle was used to ensure that spared fibers are eliminated, mainly for ventral and lateral funiculus containing the spinothalamic tract. It could be possible that in occasional cases few spared fibers could still be intact, if so, we cannot be sure if it happens with equal probability in animals with or without cortical changes. Therefore, although we perform both physical and physiological assessment of the complete transection, we cannot exclude the possibility that differences between groups are related to the presence of some spare fibers as we have no tools to confirm it in our experimental approaching. To make this possibility transparent in our study, we have included the following sentence in the Discussion section:

Page 25, paragraph 1:
“The presence of some spared fibers close to the lateral and/or ventral bone that were not fully transected after our spinal cord injury, could also be a source of variability leading to the group separation. However, considering the physical and physiological assessment performed after SCI to determine the total disconnection from spinal cord to brain, we see this possibility unlikely.”

Discussion>
Layer-specific anatomical changes after spinal cord injury have been suggested by 10.1093/cercor/bhr191 (Ghosh et al., 2012) to occur on the layer V dendrites. How does this anatomy relate to physiology?

This is a very interesting point that we have discussed several times in the lab. In this paper, Ghosh et al used an elegant approach to retrogradely label corticospinal neurons to determine the anatomical changes in the deafferented hindlimb cortex after a SCI. The fact that they found that SCI induces a reduction in the spine density of proximal dendrites, but a much less in the distal segments, leads to the assumption that the amount of spine might be directly related to the level of cortical activity. In this way, supragranular layers are still able to maintain the sufficient activity to stabilise spines and could be key in the process of cortical reorganization. On the other hand, a recent work using an in vitro model to study axon injury of pyramidal neurons (Nagendran et al., 2017, Nat. Comm.) greatly improves the knowledge of the rapid effects (24-48 hr) of axotomy in the synaptic remodelling. The main results from this study show that axotomized neurons undergo retrograde dendritic spine loss followed by hyper-presynaptic excitability, via elimination of inhibitory inputs onto the affected neurons. The increased neuronal excitability supports our results regarding the increased LFP and MUA responses observed in infragranular layers, especially layer V, after a SCI. Therefore, the immediate alteration in the neuronal excitability observed by our study and that could lead to the anatomical elimination of
inhibitory inputs, may set the physiological basis for the short- and long-term changes in spine density observed by Ghosh. All of these would directly affect the strength of the cortical reorganization.

To clearly state this line of thought, we have now included in the discussion the following argument (page 25-26):

“In fact, a synaptic reorganization in terms of a retrograde dendritic spine loss of both axotomized and non-axotomized layer 5 neurons in the hindlimb somatosensory cortex has been observed few days following SCI (Ghosh et al., 2012). Interestingly, the reduction in spines depends on the layer in which the same apical dendrite is passing through, that is, proximal segments in layer 5a are much more vulnerable to spine loss than distal segments in layer 2/3 indicating that corticocortical connections in supragranular layers may maintain a sufficient cortical activity that favours the synaptic maintenance. Under our experimental conditions, it is unlikely that such drastic spine remodelling could be triggering the observed functional changes in the first few minutes after the injury, indicating the possibility of a mechanism not directly related to anatomical aspects such as changes in connectivity. However, a recent study using an in vitro model to study axon injury of pyramidal neurons (Nagendran et al., 2017, Nat. Comm.) greatly improves the knowledge of the rapid effects (24-48 hr) of axotomy in the synaptic remodelling. It has been described that axotomized neurons undergo retrograde dendritic spine loss followed by hyper-presynaptic excitability, via elimination of inhibitory inputs onto the affected neurons. The increased neuronal excitability supports our results regarding the increased LFP and MUA responses observed in infragranular layers, especially layer V, after a SCI. In addition, the immediate alteration in the neuronal excitability observed by our study and that could lead to the anatomical elimination of inhibitory inputs, may set the physiological basis for the short- and long-term changes in spine density observed in Ghosh et al, 2012.”

I think the pre-clinical relevance paragraph is a bit of a stretch. The authors do indeed identify variation in how the cortex responds between animals but this could be due to a variety of reasons and cannot be used to explain the clinical observations like the development of pain. I think the basic science and data are interesting in themselves and perhaps this tangent dilutes the apparent rigour of the study.

Linking to the human data, in general, seems to be a useful exercise at first but such measurements cannot be conducted in humans so the comparative claims are not well-founded. It is impossible to reasonably conjecture if the very rapid alterations observed here have anything to do with what is observed in human studies on cortical plasticity. I strongly recommend focusing on the rodent findings and the corresponding circuitry. I believe focusing on the core results derived from rodents here will inspire human spinal cord injury researchers to be inspired and may lead to the development of next-generation reports that explicitly attempt to find parallels in human observations. This critical work is important but beyond the scope of the current research article.

We appreciate the reviewer’s suggestion about considering the arguments used in the manuscript to try to correlate the animal findings to the human pathophysiology. We have now removed such considerations from the Discussion as we agree that they are merely speculative considering the data we are presenting and moved up (to page 24-25) the written part about the
group division. We have also removed the written text in the Results Section associating the group division with human data.

Given the number of measures could the authors consider a summary figure or scheme to bring all of the findings under one view?

We have now included a final figure showing the schematic of the findings.

Referee #2:

Zafiras and co-authors present data describing details of the immediate affects of SCI on sensory cortex. This work follows-up on previous work by this group and provides important information regarding the immediate basis for long-term reorganization of sensory cortex after SCI. The work is original, well done and the results are clear. The study design is appropriate and the data are robust. The authors find that acute SCI induces layer-dependent changes in local circuits through alterations of both corticocortical and thalamocortical connections. The insight into the role of changes in the LFP slope to suggest unequal reduction of local inhibition across layers that allows infragranular cells to better integrate evoked sensory inputs is intriguing and provides important new context to our understanding of the effect of SCI on sensory cortical processing. Finally, comparing the latency of responses to suggests that the onset of evoked-LFP responses in infragranular layers is more conditioned by changes in thalamic inputs, is important for our understanding of the mechanisms. The conclusions are appropriate and lend context to the impact of the results.

Overall, there are several grammatical errors so a good proofing of the article is in order. There are a few issues that should be addressed Thank. All the team has been through the paper and carefully check and correct grammatical errors and typos.

1. Introduction is missing a few important references
a. Reference to electrophysiological experiments are missing e.g. Ganzer et al., 2013
b. References missing for the statement about ephys recordings from layer 5 (perhaps want to change to infragranular) e.g. Manohar et al., 2017
We thank the reviewer for highlighting few important papers that were not cited in the previous version. We have now included them in our Introduction and Discussion sections.

2. Methods - more detail on how rMUA was calculated is necessary

We have now modified the text in Methods section in order to better clarify the methods used to obtain and measure rMUA.

(Page 11, paragraph 3, Data Analysis section): “…In order to quantify MUA, local field potentials were bandpass filtered (FIR bandpass 0.3-3 kHz, gap 0.12 kHz) to obtain multi-unit activity. MUA was then rectified (rMUA), downsampled to 2 kHz and averaged across 100 stimuli to measure the total voltage resulting from the averaged area of responses (µV). The background voltage corresponding to basal activity was subtracted to response voltage (equal time than analysis window but preceding stimulus). For layer analysis, electrode sites were grouped according to layer thickness following these depths: layer 2/3 (150-650 µm), layer 4 (700-1000 µm), layer 5 (1050-1450 µm) and layer 6 (1500-2000 µm; Fiáth et al., 2016). For LFP data analysis, electrode measures were averaged across layers while neuronal signals obtained from individual channels (rMUA) were summed up within a layer to allow robust detection of the neuronal activity.”

We have also added more information about the signal processing method used to obtain rMUA from the original traces at the Figure Legend 3C.

(Page 35, Figure 3C): “…(C) Signal processing approach used to extract the multi-unit activity: low frequency components corresponding to local field potentials in the original traces (top) were removed with a bandpass filter (FIR bandpass filter, 0.3-3 kHz, gap 0.12 kHz) to obtain multi-unit activity (MUA, middle). Filtered MUA was then rectified and downsampled to 2 kHz for faster processing (rMUA, bottom). The area of the rectified MUA was then calculated to plot the populational response.”

3. Discussion -

a. along with the discussion around slow-wave oscillations, it is important to acknowledge that these oscillations are developed in response to anesthesia. Therefore, their discussion needs to make clear how these anesthesia induced changes in oscillations are related to their discussion about the relevance of these change in up-states resulting in reorganization.

Thank you for the insight about this matter, which we believe it is an important point that was not discussed in our previous version. We have now included in Discussion section a full paragraph discussing the importance of the possibility of changes in slow wave activity to initiate cortical reorganization following SCI (page 28, paragraph 2).

We have also included in Results: Page 16, paragraph 1: “Before SCI, all layers exhibited slow-wave oscillations composed of up (periods of synchronized activity) and down (periods of neuronal silence) that emerge as a consequence of anaesthesia or sleep conditions.”
b. For the discussion of infragranular a missing reference related to size of receptive fields in infragranular cortex - Tutuncular et al. 2006

We have now included this citation in the Discussion text where we discuss the properties of layer 5 neurons including the receptive fields (page 25).

Referee #3 (ethics review):

Thank you for submitting your work to The Journal of Physiology. There are some matters relating to animal ethics and welfare that must be clarified.

It would be better to introduce the experimental procedures first and then clarify the method of killing at the end of the relevant sections, so that the timeline of the animals’ fate is clearer. Please state animals were killed (or euthanised), not sacrificed.

First of all, we would like to thank the reviewer for such constructive criticism regarding the Ethics.

We have rewritten parts of the Methods in order to clarify how animals in the two experimental sets used in this study were treated at the end of experiments. Briefly, at the end of electrophysiological recordings, animals were prepared for transcardial perfusion to preserve the brain tissue, which were used for histological procedures. Before the transcardial perfusion, an overdose of the same anesthetic (urethane, 1.5g/kg, i.p.) was applied while electrocorticogram recordings indicate a deep state anesthesia (slower activity than during experimental protocol, almost flat EEG), which guarantee total unconsciousness and all reflex were avoided, while heart beating and respiration still preserved. These changes can be seen in page 9.

The same was also applied to the Thalamic recording section, in which now explains the animal’s fate (pages 10-11).
I commend the authors for the transparent reporting of all animals used in the study, including those who died under anaesthesia before data collection.

The dose of anaesthetic is clearly stated and the measures taken to ensure adequacy of depth of anaesthesia are included. I suggest moving the text which clarifies the approach taken following spinal transection (ie assessment of forelimb reflexes, spontaneous whisker movement and corneal reflexes) to an earlier portion of the text when spinal transection is first introduced. This immediately addresses the issue of adequacy of depth of anaesthesia, which must be monitored throughout procedures.

Following the reviewer’s recommendation, we have moved the text to a more correct location related to spinal transection, regarding to clarify our methods and ethics in animal handling. It now reads:

(page 8): “The optimal level of anaesthesia was settled at stage III-4 of cortical activity described by Friedberg et al. (1999). The absence of reflexes to forelimb stimuli, spontaneous whisker movements and corneal reflex was also used to verify the level of anesthesia. Constant verification of EEG activity and reflexes were monitored throughout the experiment to guarantee the optimal level of anesthesia.”

At the same time, we wanted to remark that, in our experimental approaching, the depth of anaesthesia is constantly monitored by the EEG signal, which is a more precise way to ensure constant level of unconsciousness (slow-wave activity) and absence of any reflex, as it has been described by Friedberg et al. (1999) in a comparison between halothane and urethane. This work describes that halothane and urethane show the same effects when increasing levels of anaesthesia were applied to animals, which is mainly observed in changes of predominant neuronal activity. We wanted to note that, as in our previous works, our results were obtained at state III-4 described by Friedberg et al., then to prepare animals for transcardial perfusion an overdose of urethane was applied intended to set the animals at stage 4 (which is a flat cortical activity). All these information is now incorporated in Methods.

It appears that an additional dose of urethane (1.5g/kg) was used to kill the animals by overdose. Please indicate how death was confirmed. Later, under the sub-heading “Histology”, it is stated that rats were transcardially perfused with saline and 4% PFA. Please clarify if this was performed post-mortem following urethane overdose. Or, if the heart was still beating, please state that this was the method of euthanasia, in deeply anaesthetised rats.

We thank the reviewer for this clarifying point. Indeed, what we described in the manuscript regarding the explanation about the end life of animals can be confusing. As the reviewer indicate, because all our animals were submitted to transcardially perfusion, what we have done in all animals is to apply an overdose of urethane to guarantee a deeper level of anesthesia to the complete unconsciousness (level III-4 to level IV described by Fiedberg et al., 1999, for details see above response) while heart beating and respiration were preserved to make a successful transcardial perfusion. To avoid confusion, this point has been clarified in the revised manuscript that now includes a brief explanation about how animals were prepared for transcardial perfusion by application of an overdose that develops a signal of flat EEG while respiration and heart beating were kept constant (page 9 to 11).
Under the sub-heading “Thalamocortical recordings...”.

Studies in 7 rats are briefly described. Notwithstanding the apparent redundancy of repeating text used earlier in the manuscript, it is better to clearly state the dose of anaesthetic for these animals and to provide a statement (again) in respect of steps taken to ensure adequacy of depth of anaesthesia before and after spinal transection. The fate of all animals must be clearly reported in manuscripts. Although one can infer the dose and approach used from the text as currently written, it is best to re-state this for absolute clarity. There are no word limits in The Journal.

The authors highlight that the novel data presented in this sub-section were collected in a separate study which is published, reporting on a different data set collected contemporaneously. I commend the authors on the transparent reporting of this detail. Please include in this sub-section the ethics approval details relating to that historical study, or re-state the ethics approval details if the same as the current study. Please state the method of killing of the animals in this sub-section.

We appreciate the reviewer’s comments on the transparency of our data report. We have now included a detailed statement about the procedures, experiments and ethics from the section describing both the Ethical Approval (page 7) and the thalamocortical recordings (pages 10, section Thalamocortical recordings, paragraph 1).

We have also added information about the anaesthetic requirements for the experiments (section Thalamocortical recordings, paragraph 2):

“In order to obtain comparable data, animals used for this analysis presented a deep state of anaesthesia (III-4; Friedberg et al., 1999; Erchova et al., 2002) corresponding to a slow-wave cortical activity, and evoked cortical responses to peripheral forelimb stimulation were used at 5mA (0.5Hz).”

END OF COMMENTS
Dear Dr Aguilar,

Re: JP-RP-2021-281901R1 “Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury” by Marta Zaforas, Juliana M Rosa, Elena Alonso-Calviño, Elena Fernández-López, Claudia Miguel-Quesada, Antonio Oliviero, and Juan Aguilar

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 1 expert Referee 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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Yours sincerely,

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REQUIRED ITEMS:

NEW REQUIREMENT: 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.
EDITOR COMMENTS

Reviewing Editor:

The authors are thanked for a careful and comprehensive revision. There remains however a minor concern that the link that is made (in the Discussion - page 24) to findings derived through human neuroimaging is not supported sufficiently, particularly given the limited spatial and temporal resolution of fMRI. It is recommended that this be removed, and that any more directly analogous work in other species be given greater prominence.

REFEREE COMMENTS

Referee #1:

The authors have attempted to address all of my concerns. I am glad the report now is so much stronger due to the careful revisions. I have one minor remaining concern:

One of the suggestions in the first round of feedback was to focus on animal studies and root out the human claims made in the study. The authors have largely succeeded in implementing this suggestion. However, the authors still link to human studies in the paragraph on page 27 - Taking our results in the perspective of long-term physiological changes -. I think the discussion would be much stronger if the studies discussed here were closer to the reported measurements (electrophysiology) as opposed to human fMRI where both the time-scale of observations reported here and the underlying neural foundations do not match this study in order to provide a comprehensive physiological perspective. In contrast to the human fMRI studies, this study is much closer to the work of Jon Kaas, Neeraj Jain, et al., in primates and their own work in rodents in terms of their methodological and conceptual frameworks. I hope the authors will be able to link to the more appropriate literature in the revised final form.

END OF COMMENTS

1st Confidential Review 01-Jul-2021
18th August 2021

Subject: JP-RP-2021-281901R1 “Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury”

Dear Editor and Senior Editor

First of all, we would like to thank you for all comments and arguments provided about our previous submitted version.

We are now submitting a revised version of our manuscript that fully address all concerns raised by both Editor and Reviewer in this revision round regarding the link made in the Discussion about our results and neuroimaging and human results.

We do believe that our study was substantially improved by following all the prior considerations in the first round and we hope that the manuscript in its present form will be acceptable for publication in the Journal of Physiology.

Please, see below the point-by-point answers to Answers to Reviewing Editor, Senior Editor and Referees.

Yours sincerely

Juan Aguilar
EDITOR COMMENTS

Reviewing Editor:

The authors are thanked for a careful and comprehensive revision. There remains however a minor concern that the link that is made (in the Discussion - page 24) to findings derived through human neuroimaging is not supported sufficiently, particularly given the limited spatial and temporal resolution of fMRI. It is recommended that this be removed, and that any more directly analogous work in other species be given greater prominence.

We thank the editor's comment, which undoubtedly is intended to refine all minimum details. After carefully checking of references and context in Discussion page 24 (as indicated) we haven't found references to human neuroimaging (fMRI) most probably due to changes in page formats. However, it is possible that the expression “brain scanning” in the sentence: "Under our experimental conditions, increased magnitude of evoked-LFP in response to stimulation of the contralateral forelimb was observed across layers of the deprived cortex (Fig 2B), which is very consistent with results obtained using brain scanning approaches (Endo et al., 2007; Ghosh et al., 2010)” could be interpreted as a research performed in human. However, we want to clarify that both references included in the text were made in animal models for study cortical effects of SCI. Here, we used the expression “brain scanning” because the work of Endo et al., 2007 used fMRI as experimental tool to explore rats with a similar thoracic SCI to show cortical reorganization. On the other hand, the work of Ghosh et al., 2010 shows cortical changes in mice with SCI by using voltage sensitive dye (VSD). Our intention including these two works using brain imaging in animals was to show the consistency of our electrophysiological results with other results obtained in similar animal models using different experimental tools (fMRI and VSD), as the basis to explain in posterior steps how our present results provide higher quality of information about the physiology of the phenomenon. As we cannot find other references in the text (page 24) to fMRI in human data, we hope the Editor could admits this explanation about the expression “brain scanning”. And so, allow us to keep both references of animal models in the context they appear. However, if we still wrong, please could the Editor indicate us a bit more precisely the non-appropriate reference?

Moreover, we did find references to human data on page 30 as pointed by Referee #1. In the current version, all references to human data have been excluded from the Discussion. A more extensive response to this question is given on the answer to Referee #1 below.

REFEREE COMMENTS

Referee #1:
The authors have attempted to address all of my concerns. I am glad the report now is so much stronger due to the careful revisions. I have one minor remaining concern:

One of the suggestions in the first round of feedback was to focus on animal studies and root out the human claims made in the study. The authors have largely succeeded in implementing this suggestion. However, the authors still link to human studies in the paragraph on page 27 - Taking our results in the perspective of long-term physiological changes -. I think the discussion
would be much stronger if the studies discussed here were closer to the reported measurements (electrophysiology) as opposed to human fMRI where both the time-scale of observations reported here and the underlying neural foundations do not match this study in order to provide a comprehensive physiological perspective. In contrast to the human fMRI studies, this study is much closer to the work of Jon Kaas, Neeraj Jain, et al., in primates and their own work in rodents in terms of their methodological and conceptual frameworks. I hope the authors will be able to link to the more appropriate literature in the revised final form.

We appreciate the words of reviewer about our previous improved version and we apologise for the missing link with human data that was not eliminated in the first round. We have revised all the Discussion section and have removed any reference and rephrased sentences making references to human data. For example in page 29, we have now eliminated Ortmann et al 2010 and have modified the entire paragraph related to human data in page 30 (the one pointed by the reviewers: Taking our results in the perspective of long-term…..). In the current version the content of this paragraph appears in framework which only takes into account experimental data obtained in animal models of SCI to study cortical reorganization. Here we would like to thank the reviewer the idea to provide a proper context to our results obtained in animal models.

The paragraph appears now as follow:

*Based on previous reports, there are two main factors that have been directly related to the development of CoRe: 1) structural changes of neuronal networks and connections linked to anatomical rewiring of axons and dendrites (Ghosh et al., 2012; Nagendran et al., 2017; Zhang et al, 2015), and 2) functional changes linked to activity-dependent plasticity and/or homeostatic plasticity (Jain et al., 1998, 2008; Fernández-López., et al 2019). Since our results were obtained in a narrow time window (from minutes to few hours after deprivation), the possibility of structural and/or anatomical changes is limited as previously shown (Jain et al., 1995; Chand and Jain, 2005). On the contrary, we consider that the observed neuronal network alterations in a specific layer will depend on the importance of lacking the preferred connectivity (thalamocortical or corticocortical), the inhibitory neuronal composition and how the network integrates non-preferential and weak connections (Endo et al., 2007; Humanes-Valera et al. 2017; Ganzer et al., 2013; Manohar 2017). Then, immediate functional changes described in our results point to an initiation of homeostatic processes intended to compensate input deprivation by rebalancing excitation:inhibition as it has been described in other sensory systems (Keck et al., 2013; Teichert et al., 2017), which can be followed in the long-term by a process of activity-dependent plasticity (…)*
Dear Dr Aguilar,

Re: JP-RP-2021-281901R2 “Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury” by Marta Zaforas, Juliana M Rosa, Elena Alonso-Calviño, Elena Fernández-López, Claudia Miguel-Quesada, Antonio Oliviero, and Juan Aguilar

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

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

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

The authors are thanked for the comprehensive manner in which they have addressed the remaining minor issues of concern.

2nd Confidential Review 18-Aug-2021