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Alpha oscillations govern interhemispheric spike timing coordination in the honeybee brain

Tzvetan Popov and Paul Szyszka

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Original submission:	2 August 2019
1st revised submission:	18 January 2020
2nd revised submission:	1 February 2020
Final acceptance:	3 February 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-1817.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this manuscript, authors have investigated the mechanistic role of alpha oscillations in shaping neuronal activity using honey bee brain as a model system. The significance of alpha oscillations in neuronal processing has been investigated by several studies in humans, but less so in animal models. Hence, the mechanistic role of alpha oscillations in shaping neuronal processing has remain unclear. This manuscript adds on to this relevant topic by showing that the phase of alpha oscillations modulates neuronal spiking after odor inducement. The text is well written, and the methods are sounds. I have only minor comments to the manuscript.

• The effects in the current study induced by odor stimulation are found in the ~18 Hz. This is termed alpha-band because of its similarity to human observations. Yet, classically, however, 18Hz is defined as a low-beta band. To avoid confusion in the field, the authors should stick to the classical nomenclature. Several prior studies using animal models (for instance by Fries., Pesaran, Womelsdorf) show that low-beta oscillations carry out top-down attentional functions similarly to that found in alpha band for human studies. Hence, the human alpha and animal model low-beta band oscillations and synchronization reflect very likely reflect the same phenomena, which could be further discussed.

• Please define the frequency range for high-frequency gamma activity in the text.

• For the cross-frequency coupling as a mechanism for neuronal communication in cortical circuits refer only to Canolty & Knight 2010. I would suggest adding references to various other reviews on this topic.

• I did not find any description on how cross-frequency coupling between frequencies was estimated. There are several possible approaches. Please add a description to the Method section.

• Indicate surrogate phase distribution in Figure 2D.

• Fig. 2E shows the spike rate per bee during spontaneous activity and odor evoked activity as well as for high and low alpha power. Points and error bars show the mean and SED of trials. I would appreciate if these data could be shown as bar or violin plots which would show the actual distribution of the spikes rates across conditions.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
No

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
Popov and Szyszka present an interesting paper documenting a novel oscillation observed in the mushroom body of the honey bee. Their principal finding is that this novel oscillation exhibits characteristics that are strikingly similar to alpha oscillations observed in primates. This has broad implications as it promotes the honey bee as a model system for understanding oscillatory brain activity, connecting a substantial body of work using surface recordings in humans (EEG, MEG) to more mechanistic insights obtainable in animals. As such this work has broad interest.

The study is also methodologically well-conducted and includes all major analyses that I would expect to substantiate the core claim that the observed phenomenon is 'alpha-like'. I have one major comment on the conclusions and a few minor comments.

Major comment:

1. Alpha oscillations in primates are predominant around 10Hz, whereas this oscillation is

observed at 18Hz. Primate alpha oscillations are associated with the inhibition of spikes - spike rate tends to be higher at the trough than the peak - whereas this oscillation shows the opposite effect. Is it truly sensible to call this an 'alpha' oscillation given these differences which are not trivial? At a minimum this should be clarified in the discussion; it should be as clear as possible to what degree this phenomenon is comparable with primate alpha and to what degree it is different.

Minor comments:

Methods section/Animals: Please report number of animals and number of recording sessions per animal. Were recording sessions concatenated averaged for stats purposes or was each session treated as a separate observation (fixed effects analysis)?

Fig 1B: 'raw power' should have a unit.

Data availability: Authors indicate data are available at www.fieldtriptoolbox.org, however this seems to be the home page of a data analysis package. Authors should provide a link to a specific page so the data can be found.

Decision letter (RSPB-2019-1817.R0)

15-Oct-2019

Dear Dr Szyszka:

I am writing to inform you that your manuscript RSPB-2019-1817 entitled "Alpha oscillations govern interhemispheric spike timing coordination in the honey bee brain" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.

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Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

In your revision process, please take a second look at how open your science is; our policy is that all data involved with the study should be made openly accessible-- see:
https://royalsociety.org/journals/ethics-policies/data-sharing-mining/
Insufficient sharing of data can delay or even cause rejection of a paper.

Sincerely,
Professor John Hutchinson, Editor
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
We have now heard from two experts in the field. Although both are positive about your manuscript, both also have some concerns -- particularly with respect to whether or not you are actually measuring alpha oscillations. On the basis of these concerns, I am recommending rejection. Nevertheless, I would encourage you to submit a revised version of the manuscript that tackles their concerns.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
In this manuscript, authors have investigated the mechanistic role of alpha oscillations in shaping neuronal activity using honey bee brain as a model system. The significance of alpha oscillations in neuronal processing has been investigated by several studies in humans, but less so in animal models. Hence, the mechanistic role of alpha oscillations in shaping neuronal processing has remain unclear. This manuscript adds on to this relevant topic by showing that the phase of alpha oscillations modulates neuronal spiking after odor inducement. The text is well written, and the methods are sounds. I have only minor comments to the manuscript.

• The effects in the current study induced by odor stimulation are found in the ~18 Hz. This is termed alpha-band because of its similarity to human observations. Yet, classically, however, 18Hz is defined as a low-beta band. To avoid confusion in the field, the authors should stick to the classical nomenclature. Several prior studies using animal models (for instance by Fries., Pesaran, Womelsdorf) show that low-beta oscillations carry out top-down attentional functions similarly to that found in alpha band for human studies. Hence, the human alpha and animal model low-beta band oscillations and synchronization reflect very likely reflect the same phenomena, which could be further discussed.

• Please define the frequency range for high-frequency gamma activity in the text.

• For the cross-frequency coupling as a mechanism for neuronal communication in cortical circuits refer only to Canolty & Knight 2010. I would suggest adding references to various other reviews on this topic.

• I did not find any description on how cross-frequency coupling between frequencies was estimated. There are several possible approaches. Please add a description to the Method section.

• Indicate surrogate phase distribution in Figure 2D.

• Fig. 2E shows the spike rate per bee during spontaneous activity and odor evoked activity as

well as for high and low alpha power. Points and error bars show the mean and SED of trials. I would appreciate if these data could be shown as bar or violin plots which would show the actual distribution of the spikes rates across conditions.

Referee: 2

Comments to the Author(s)
Popov and Szyszka present an interesting paper documenting a novel oscillation observed in the mushroom body of the honey bee. Their principal finding is that this novel oscillation exhibits characteristics that are strikingly similar to alpha oscillations observed in primates. This has broad implications as it promotes the honey bee as a model system for understanding oscillatory brain activity, connecting a substantial body of work using surface recordings in humans (EEG, MEG) to more mechanistic insights obtainable in animals. As such this work has broad interest.

The study is also methodologically well-conducted and includes all major analyses that I would expect to substantiate the core claim that the observed phenomenon is 'alpha-like'. I have one major comment on the conclusions and a few minor comments.

Major comment:
1. Alpha oscillations in primates are predominant around 10Hz, whereas this oscillation is observed at 18Hz. Primate alpha oscillations are associated with the inhibition of spikes - spike rate tends to be higher at the trough than the peak - whereas this oscillation shows the opposite effect. Is it truly sensible to call this an 'alpha' oscillation given these differences which are not trivial? At a minimum this should be clarified in the discussion; it should be as clear as possible to what degree this phenomenon is comparable with primate alpha and to what degree it is different.

Minor comments:
Methods section/Animals: Please report number of animals and number of recording sessions per animal. Were recording sessions concatenated averaged for stats purposes or was each session treated as a separate observation (fixed effects analysis)?

Fig 1B: 'raw power' should have a unit.

Data availability: Authors indicate data are available at www.fieldtriptoolbox.org, however this seems to be the home page of a data analysis package. Authors should provide a link to a specific page so the data can be found.

Author's Response to Decision Letter for (RSPB-2019-1817.R0)

See Appendix A.

RSPB-2020-0115.R0

Review form: Reviewer 2 (Tom Marshall)

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have addressed all my concerns.

Decision letter (RSPB-2020-0115.R0)

28-Jan-2020

Dear Dr Szyszka

I am pleased to inform you that your Review manuscript RSPB-2020-0115 entitled "Alpha oscillations govern interhemispheric spike timing coordination in the honey bee brain" has been accepted for publication in Proceedings B. Congratulations!!

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of

your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

Before uploading your revised files please make sure that you have:

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2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2020-0115 which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor John Hutchinson, Editor
mailto:proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
I am pleased to recommend acceptance of your manuscript "Alpha oscillations govern interhemispheric spike timing coordination in the honey bee brain". Congratulations on an excellent paper.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).
The authors have addressed all my concerns.

Decision letter (RSPB-2020-0115.R1)

03-Feb-2020

Dear Dr Szyszka

I am pleased to inform you that your manuscript entitled "Alpha oscillations govern interhemispheric spike timing coordination in the honey bee brain" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 8 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online

figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear Professor John Hutchinson, January 19, 2020

Thank you for sending us the reviews for our manuscript "Alpha oscillations govern interhemispheric spike timing coordination in the honey bee brain", which helped us much in improving the manuscript.

Please find our responses to the reviewers below (we copied them into the reviewers comment).

We marked all changes in the resubmitted manuscript (Manuscript ID RSPB-2019-1817) with "Track Changes".

Yours sincerely,

Tzvetan Popov and Paul Szyszka

Referee: 1

In this manuscript, authors have investigated the mechanistic role of alpha oscillations in shaping neuronal activity using honey bee brain as a model system. The significance of alpha oscillations in neuronal processing has been investigated by several studies in humans, but less so in animal models. Hence, the mechanistic role of alpha oscillations in shaping neuronal processing has remain unclear. This manuscript adds on to this relevant topic by showing that the phase of alpha oscillations modulates neuronal spiking after odor inducement. The text is well written, and the methods are sounds. I have only minor comments to the manuscript.

- *The effects in the current study induced by odor stimulation are found in the ~18 Hz. This is termed alpha-band because of its similarity to human observations. Yet, classically, however, 18Hz is defined as a low-beta band. To avoid confusion in the field, the authors should stick to the classical nomenclature. Several prior studies using animal models (for instance by Fries., Pesaran, Womelsdorf) show that low-beta oscillations carry out top-down attentional functions similarly to that found in alpha band for human studies. Hence, the human alpha and animal model low-beta band oscillations and synchronization reflect very likely reflect the same phenomena, which could be further discussed.*

Response 1:

Thank you for raising this point. We appreciate your careful evaluation of the present findings and potential translational aspect of alpha/beta activity. We have revised the manuscript to reflect your suggestion and avoid confusion. We have now clarified (Line 233 in the "clean" version of the revised manuscript, we also provide a version with "Track Changes"):

"We termed these 18 Hz oscillations "alpha" based on their similarity with alpha oscillations in humans (around 10 Hz) and alpha/beta oscillations in non-human primates (ranging between 10 - 20 Hz) (Buffalo et al., 2011; Haegens et al., 2011; van Kerkoerle et al., 2014; Bastos et al., 2015). Similar as primate's alpha/beta oscillations, honey bees' 18 Hz oscillations were spontaneously generated, reduced in amplitude during sensory stimulation, and biased spike timing and higher frequency neuronal activity. We believe that it is adequate to term honey bees' 18 Hz oscillations "alpha" rather than "beta", because the distinction between human "alpha" and monkey "beta"

oscillations is based on frequency bands rather than on function. However, human alpha and monkey alpha/beta oscillations carry out similar functions. In monkeys for example, 10 - 20 Hz oscillations regulate communication between cortical modules in the visual system (e.g. V4 to V1) (van Kerkoerle et al., 2014; Bastos et al., 2015). This mechanism of top-down control of alpha/beta oscillations within an anatomically defined cortical hierarchy also occurs in the human visual cortex (Michalareas et al., 2016), strengthening the notion that alpha/beta oscillations serve similar functions across phyla. The present finding of asymmetric, alpha oscillation-mediated functional connectivity between the brain hemispheres (Figure 2) is in line with this view. We therefore believe that it is reasonable to subsume honey bees' 18 Hz, monkeys' 10 - 20 Hz and humans 10 Hz oscillation under the term "alpha"."

- *Please define the frequency range for high-frequency gamma activity in the text.*

Response 2:

We define the frequency range for high-frequency gamma as 40 to 450 Hz (Line 26, 195).

- *For the cross-frequency coupling as a mechanism for neuronal communication in cortical circuits refer only to Canolty & Knight 2010. I would suggest adding references to various other reviews on this topic.*

Response 3:

Thank you. We have reviewed the literature and updated the manuscript accordingly (Line 177):

"The cross-frequency coupling of high gamma activity to the phase of the spontaneous alpha oscillation is considered a mechanism of neural communication in cortical circuits (Jensen and Colgin, 2007; Tort et al., 2008; Axmacher et al., 2010; Canolty and Knight, 2010; Tort et al., 2010; Foster and Parvizi, 2012; Lopez-Azcarate et al., 2013; Hyafil et al., 2015; Jiang et al., 2015; Samiee and Baillet, 2017)."

- *I did not find any description on how cross-frequency coupling between frequencies was estimated. There are several possible approaches. Please add a description to the Method section.*

Response 4:

Thank you for pointing out the lack of this important detail. We applogize for the omission and revised the manuscript accordingly. We have now clarified (Line 152):

" Cross-frequency coupling was computed according to the procedures of Tort and colleagues (Tort et al. 2010). Modulating frequencies from 2 to 40 Hz in steps of 2 Hz and modulated frequencies from 10 to 450 Hz in steps of 10 Hz were analyzed. A time-domain, zero phase lag, finite impulse response filter served to bandpass the data in the frequencies of interest. The filter order was frequency-dependent: number of cycles × sampling frequency / frequency of interest.

Phase and amplitude estimates were derived from a Hilbert transform of the filtered data corresponding to angle and magnitude, respectively. Three cycles were used for estimation of the modulating and the modulated frequency. The modulation index was calculated for each electrode, each low-frequency phase, and each high-frequency amplitude estimate. This measure quantifies cross-frequency coupling as the divergence of the observed amplitude distribution for each phase bin from a uniform distribution."

Please note that while checking the analyses we realized that we erroneously assigned one recording to two different honey bees. We have corrected this now and re-done the analyses. The results did not change qualitatively.

- *Indicate surrogate phase distribution in Figure 2D.*

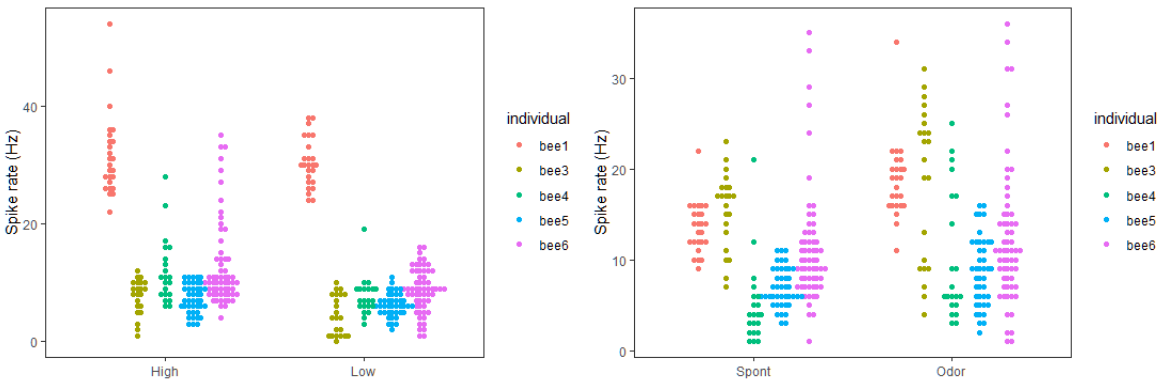
Response 5:

We realized that the analysis shown in the original Figure 2D is redundant to the analysis shown in Figure 2A and B which show the phase coupling between spikes and LFP oscillation. We therefore deleted 2D, as it does not add any information.

- *Fig. 2E shows the spike rate per bee during spontaneous activity and odor evoked activity as well as for high and low alpha power. Points and error bars show the mean and SED of trials. I would appreciate if these data could be shown as bar or violin plots which would show the actual distribution of the spikes rates across conditions.*

Response 6:

We followed the reviewer's suggestion and included the spike rates per single trials in the figure (please see the figures below, note that we used "beeswarm" plots instead than violin plots, each point is a spike rate per trial). However, we think they are visually overloaded. We therefore would prefer to stick to the original figures (Figure 2D in the actual manuscript). In case we misunderstood your suggestion, we are happy to revise the figure.



Referee: 2

Comments to the Author(s)

Popov and Szyszka present an interesting paper documenting a novel oscillation observed in the mushroom body of the honey bee. Their principal finding is that this novel oscillation exhibits characteristics that are strikingly similar to alpha oscillations observed in primates. This has broad implications as it promotes the honey bee as a model system for understanding oscillatory brain activity, connecting a substantial body of work using surface recordings in humans (EEG, MEG) to more mechanistic insights obtainable in animals. As such this work has broad interest.

The study is also methodologically well-conducted and includes all major analyses that I would expect to substantiate the core claim that the observed phenomenon is 'alpha-like'. I have one major comment on the conclusions and a few minor comments.

Major comment:

1. Alpha oscillations in primates are predominant around 10Hz, whereas this oscillation is observed at 18Hz. Primate alpha oscillations are associated with the inhibition of spikes - spike rate tends to be higher at the trough than the peak - whereas this oscillation shows the opposite effect. Is it truly sensible to call this an 'alpha' oscillation given these differences which are not trivial? At a minimum this should be clarified in the discussion; it should be as clear as possible to what degree this phenomenon is comparable with primate alpha and to what degree it is different.

Response 1:

Thank you for your constructive review, and thank you for pointing out this important issue. We have revised the manuscript to reflect your suggestion. We have now clarified (Line 233 in the "clean" version of the revised manuscript, we also provide a version with "Track Changes"):

"Here we demonstrate that the honey bee brain generates oscillations (around 18 Hz) which share characteristics of alpha oscillations in the primate brain. We termed these 18 Hz oscillations "alpha" based on their similarity with alpha oscillations in humans (around 10 Hz) and alpha/beta oscillations in non-human primates (ranging between 10 - 20 Hz) (Buffalo et al., 2011; Haegens et al., 2011; van Kerkoerle et al., 2014; Bastos et al., 2015). Similar as primate's alpha/beta oscillations, honey bees' 18 Hz oscillations were spontaneously generated, reduced in amplitude during sensory stimulation, and biased spike timing and higher frequency neuronal activity. We believe that it is adequate to term honey bees' 18 Hz oscillations "alpha" rather than "beta", because the distinction between human "alpha" and monkey "beta" oscillations is based on frequency bands rather than on function. However, human alpha and monkey alpha/beta oscillations carry out similar functions. In monkeys for example, 10 - 20 Hz oscillations regulate communication between cortical modules in the visual system (e.g. V4 to V1) (van Kerkoerle et al., 2014; Bastos et al., 2015). This mechanism of top-down control of alpha/beta oscillations within an anatomically defined cortical hierarchy also occurs in the human visual cortex (Michalareas et al., 2016), strengthening the notion that alpha/beta oscillations serve similar functions across phyla. The present finding of asymmetric, alpha oscillation-mediated functional connectivity between the brain hemispheres (Figure 2) is in line with this view. We therefore

believe that it is reasonable to subsume honey bees' 18 Hz, monkeys' 10 - 20 Hz and humans 10 Hz oscillation under the term "alpha"."

Concerning the difference in spike-LFP phase preference between our recordings and recordings in monkeys:

In our recordings, the recurrence of spikes was larger during the peak of the alpha oscillation (Figure 2A, B). In contrast, in monkeys the recurrence of spikes is smaller during the peak of the alpha LFP oscillation and this has been interpreted as evidence of an inhibitory effect of alpha oscillations on spike activity (Haegens et al., 2011). However, we believe that this peak/trough-to-spike relationship is arbitrary since the polarity of LFP oscillations depends on the position of the recording and reference electrodes (https://doi.org/10.3389/fncir.2016.00101). Thus, the seemingly contradictory phase preference of spikes in honey bees should be taken with caution. Whether the peak or trough regulate spike inhibition cannot be deduced from the present data. What matters is that firing rates are biased within the entire oscillatory cycle suggesting the presence of "opportunity windows" as reported in the human and non-human literature (Jacobs et al., 2007; Rutishauser et al., 2010; Haegens et al., 2011).

Minor comments:

Methods section/Animals: Please report number of animals and number of recording sessions per animal. Were recording sessions concatenated averaged for stats purposes or was each session treated as a separate observation (fixed effects analysis)?

Response 2:

Thank you for spotting this lack of information. We have added this information now.

Line 88: "We used 9 female forager honey bees (Apis mellifera) in an in vivo preparation."

Line 105: "Each bee received between 16 and 108 odorant stimuli (mean = 57, SD = 28 stimuli) during a 3 to 37 minutes long recording session (mean = 19, SD = 11 minutes)."

And we have indicated the number of animals in the figure legends.

Please note that while checking the analyses we realized that we erroneously assigned one recording to two different honey bees. We have corrected this now and re-done the analyses. The results do not change qualitatively.

Fig 1B: 'raw power' should have a unit.

Response 3:

Thank you. We have corrected Fig 1B.

Data availability: Authors indicate data are available at https://apc01.safelinks.protection.outlook.com/?url=www.fieldtriptoolbox.org&data=02%7C01%7Cpaul.szyszka%40otago.ac.nz%7C4fab3a0086f347decff808d751753b74%7C0225efc578fe4928b1579ef24809e9ba%7C1%7C0%7C637067436305407558&sdata=56u7GULL1LUtX48Gg9Z6yY2cn7x9pRjkNvI9XoGYbio%3D&reserved=0, however this seems to be the home page of a data analysis package. Authors should provide a link to a specific page so the data can be found.

Response 4:

Thank you for spotting this error. We have now uploaded the data on https://osf.io/523tk/.