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PROCEEDINGS B

The smell of cooperation: rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task

Nina Gerber, Manon K. Schweinfurth and Michael Taborsky

Article citation details
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Review timeline

Original submission:	23 March 2020
1st revised submission:	24 September 2020
2nd revised submission:	18 October 2020
Final acceptance:	23 October 2020

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

Review History

RSPB-2020-0654.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?
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?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is a fantastic study investigating proximate mechanisms of cooperation among rats with an ingenious experimental design. This is a very timely topic that should be of interest to a wide variety of researchers. In particular, the authors show that odor cues from a cooperating rat are sufficient to trigger reciprocal cooperation. This is true even if the rat right next to the focal rat was not actually cooperating, and the odor came from a different rat invisible to the focal rat. This study follows in the wake of a similar recent paper by the same group (Schneeberger et al 2020 PLoS Biology) in which they show that the smell of a hungry rat triggers more helping, indicating that rats are sensitive to a recipient's hunger state; moreover, in that paper they also analyzed the "hunger smell" and identified specific compounds that are physiologically linked to hunger states and make this an honest signal. In the present study such an analysis is lacking, leaving me to wonder what could possibly produce this "i'm a helpful, cooperative rat" smell, and how it could be an honest signal. If it's not an honest signal, why should it trigger helping? This is my major concern with the present paper, and I would love to see a more in-depth analysis of the actual odor cues, and a more convincing discussion of how this whole signaling system can possibly be robust against cheating - a discussion of the naturalistic contexts in which rats cooperate (outside of this contrived experimental situation) would also be highly illuminating in this context. As it is, I'm reminded of the old saying that exceptional claims require exceptional evidence, and it seems to me that a "cooperation smell" that can trigger helping even when the focal rat hasn't actually received any food is an exceptional claim - but we have yet to see the exceptional evidence.

Minor comments:
Lines 202-211: Unlike the previous experiment, this experiment has no experience phase, rather than an experience phase without odor cues. I wondered why, and how the experimenter recording the rats' behavior could possibly be blind to the condition when there is such a clear difference (experience phase yes or no, with corresponding noise from odor machine)?

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physical activity smell, that's not a disaster - on the contrary, this would actually provide more credible evidence that there's an honest signal here. And it would make it all the more important to discuss these experiments in light of naturalistic rat cooperation - why would honest cues of physical activity be used in decisions to reciprocate? Could it be that physical activity acts as a proxy for "work effort", and would work effort be something that is rewarded in rat colonies? I'm just speculating here, but this is the kind of discussion I would like to see. Also, it's worth noting that a physical activity smell couldn't just result in social contagion since pulling by the focal rat was higher when there was a partner compared to an empty cage.

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253-255 not significant does not mean "did not differ". if you look at the figure, the two lines clearly differ - predicted probability of puling at 400s seems to be about 50% for cooperator but only 25% for non-cooperator -, just not significantly so. sorry for the rant, but i really dislike the false dichotomy of null-hypothesis significance testing. Not a big issue, but I would prefer a more nuanced statement

365-372 Here I would like to see more discussion of why rats should have a communication system for such mutualistic cooperation. What is it that rats do in the wild that would select for such mechanisms? Or did the mechanisms perhaps evolve for some entirely different purpose but could be co-opted in the context of this contrived experimental situation (wild rats don't pull sticks in cages) that these particular rats were trained to succeed in?

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Review form: Reviewer 2

Recommendation
Reject – article is scientifically unsound

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

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

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

Is the length of the paper justified?
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Should the paper be seen by a specialist statistical reviewer?
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Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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This study addresses whether olfactory cues associated with different behavioural states can influence another individual's tendency to perform a trained task for a delayed reward. I was very interested to read this study and I find the paradigm to be quite fascinating. If the experimental paradigm can be properly justified, I think the results of this study would make an interesting contribution to the literature. However, a major issue in the manuscript is that the procedures here are so poorly described that it was almost impossible for me to understand what was done, and why. The gaps in the text, and the data shown in Figure 2 raised concerns for me as to whether this paradigm really shows what is claimed. Below, I make some specific suggestions for how the transparency of this study could be improved.

Title
The wording in the latter half of this title strongly suggests an identity signal (i.e., "helpful conspecifics" connotes helpful individuals, not individuals who are helping). This appears to be contrary to your main claim. Assuming your results do indeed show an effect of an olfactory signal of partner behaviour, how about something like this instead: "The smell of cooperation: rats reciprocate more when they receive odour cues of a partner performing help"

Abstract
Line 15: "is triggered solely by" is not correct, because no behavioural experiment can prove a universal like that. How can you be sure that the behaviour is not triggered by things you did not test? I suggest rewriting this to be something like: "odour cues associated with… are sufficient to trigger… xxx behaviour"

Lines 19-20: "do not represent individual identity" I find that phrasing odd and hard to understand. How about "are not identity-specific" instead?

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only seems "direct" because vision is what we humans rely on.

Line 20: Is the unimportance of the visual cues really that surprising? As you explain in the Introduction, visual cues are not particularly useful for a nocturnal fossorial animal, and we already know rats use olfactory cues for so many important functions. So, is this really the most remarkable aspect of your results?

Introduction
Lines 52-53: This is too vague to understand. Please explain how these previous experimental results "suggest" this. Can you elaborate what was shown, what the specific conclusion is, and why it's only suggested (in italics)? All of this is opaque to the reader.

Line 54: "signal their helping effort" to whom?

Lines 76: Please explain how your experimental paradigm is a sequential iterated prisoner's dilemma. This seems to be complicated by the fact that the rats are first trained to pull the stick for an individual (self) reward, that gets progressively delayed through further training. So how does the expression of this trained behaviour in the social paradigm qualify as a PD game? I think this whole paradigm needs to be explicitly justified as a PD (by explaining the costs and benefits to each player and how it meets the game theoretic definition), or you should change your terminology to call it costly helping behaviour, or motivation for a delayed reward, or something else.

Methods
Line 79: How many? Provide overall sample size of individuals here. The exact sample size for each experiment should also be provided in the appropriate places further down.

Line 90: Revise the phrase "trained to get used to"…

How about: "habituated to" or "trained to be familiar with"?

Line 90: At this point, the reader does not know what "pull the stick" refers to. This is a consistent problem throughout the Methods (i.e., the order consistently lacks background needed for the reader to make sense of it). My general suggestion is to rewrite the Methods to start with a section that explains overall goals of the study, and then explain the apparatus that allowed you to achieve it, and then define the "types" of individuals that were created and why (stooge, cooperator etc). Finally, explain how you used the training paradigm to create those individual types.

Line 94: The reader doesn't yet know what "partner" means here in terms of the physical set-up of the apparatus. Again, this would make more sense if the apparatus was explained first, before the training section.

Line 96: What does it mean to be "a cooperator"? This is not defined until much later, making it impossible to understand.

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Lines 101-102: What were the signs of stress and anxiety? Please define so that this is reproducible by others.

Line 117: The reader doesn't know what you mean by "stooges", because it has not yet been defined. As suggested above, it would be best to have this explained much earlier.

Lines 117-118: What distinguishes a focal rat from a cooperator? Need to clarify this

Line 117: "all experiments" Confusing because reader doesn't yet know that there are multiple experiments

Lines 133-134: It doesn't make sense to mention "experiments one and two" here before the reader even knows that there were multiple experiments. This could be resolved by adding a sentence (perhaps at the beginning of the Methods as suggested above), "In total, we performed X experiments with the goals of…"

Line 139: Add an explanation of the purpose to the beginning of this section: "To determine if… we…"

Lines 142-143: This section suffers from a lack of logical order. Here, you mention the "direct reciprocity" and "generalized reciprocity" paradigms, but they are not defined until further below.

Line 151: What is meant here by "the importance of social context"? I think (based on reading further) that you mean presence vs. absence of a social partner in the neighbouring chamber, but I'm not sure. Please clarify by saying exactly what you manipulated; "context" doesn't tell us.

Line 228: I think by "n.d." you are indicating no date, but R package versions do indeed have publication dates. You can get that information by looking at the package info within your installation of R, or by going to the CRAN website for a given package. E.g.:
https://cran.r-project.org/web/packages/survival/
and
https://cran.r-project.org/src/contrib/Archive/survival/
Please add the date and version information for each package that you used. This applies to lme4 as well, you should cite the package with its version, not just a 6-year old preprint by a subset of the package authors. The actual package has far more authors than those listed.

Line 242: "if models were overdispersed, an individual based random factor was included to deal with overdispersion."
This is incorrect. If you have repeated measures in these models, a random effect of individual ID should always be included to properly account for non-independence, i.e., to avoid inflating your power due to pseudoreplication. What you seem to be referring to here is the strategy of including an observation-level random effect to deal with overdisperson in some types of GLMMs (i.e., this means implementing a random effect with a different level for each row or observation in the dataset, NOT each individual). Individual ID needs to be accounted for as a random effect in any of your models that have repeated measures of individual, regardless of dispersion. This needs to be corrected in your statistical analysis and in the text here.
Line 237: This is incorrect, the use of Poisson distribution in GLMMs is not automatic when the data are count data. Often, count data do have Poisson-distrubted errors, but not necessarily! And the choice of error distribution should be based on the actual error distribution of the fitted model. You could easily have count data that have Gaussian-distributed errors, and then a regular Gaussian model would be appropriate. Looking at your data in Figures 3 and 4, I strongly suspect that is what you have. In other words, an overdispersed Poisson appears to be incorrect here, because the error structure of these data does not appear to follow a Poisson distribution at all. I would suggest checking a Gaussian model first, and examining whether the assumptions of that model are met (look at distribution of the residuals).

Results
Line 251: Information was available to whom?

Same comment lines 254, previously cooperative to whom? Any other rat, or the focal rat?

Lines 251-252: I am concerned that this interpretation (and the comparisons on the figure) is missing the point. E.g., "focal rats pulled earlier for a formerly cooperative social partner than for a formerly non-cooperative partner when olfactory information was available" Perhaps I'm missing something here, but wouldn't this simply be explained by the continuation of (greater) reward in one condition, but not the other? i.e., isn't this simply recapitulating what we already know about operant conditioning?

I would think it would be far more interesting in Figure 2a to compare the solid green condition to the dotted green condition, and to compare the solid blue condition to the dotted blue condition, respectively. This is like examining the independent effect of neighbour odour in the analysis. Based on the data in the figure, the perception of neighbour odour appears to have a positive effect on the focal rat's performance, regardless of what the neighbour was actually doing. This raises serious questions about the general interpretation of a "smell of cooperation". In other words, Figure 2a appears to show additive positive effects of (i) having an actively reciprocating neighbour (or reward magnitude, already known to have an effect on operant behaviour), and (ii) olfactory perception of a neighbour, regardless of that neighbour's behaviour. Neighbour odour may have a similarly sized positive effect on the focal rat, regardless of whether the neighbour was cooperating or a stooge. If so, this would contradict the later interpretation of "a smell of cooperation". Instead, it suggests that the smell of a social neighbour has a more general motivating effect.

Lines 283-295: This section explains the results of experiment 2, but I can't understand the purpose of this experiment. As far as I can tell, experiment 2 is not presented in any figure, and not mentioned in the Abstract. Is it necessary to include in the main text? Notably, experiment 1 already shows us that olfactory information affects the focal rat's behaviour (e.g., by comparing solid green to the dotted green line in Figure 2a). Perhaps you could remove experiment 2 from the main text and use that space to improve the transparency of other aspects of this study.

Line 313: What makes it the smell of cooperation per se? How do you know it's not the smell of social awareness, or the smell of reward anticipation in another individual? If there is justification for operationalizing something as "the smell of cooperation", it should be defined much earlier, within the Methods. As it stands, this value-laden interpretation is introduced for the first time down in the Results. It's not clear to me what the justification for that interpretation would be.

Discussion
Line 325: Same as the comment above, is it really the "odour cues from a helpful act"? Or could it be odour cues of conspecific reward anticipation?

Line 392: Needs a comma after "in other animals"

Figures
There are some issues with Figures 2-4 that need to be improved. First, the font size within the figures (e.g., legends) is way too small.

Secondly, in the captions, it would be helpful to say which experiment is shown. This is done for Figure 1 (referring to experiments 1, 2, 3, and 4 within the caption), but it is not done for Figures 2-4. Adding this info would make it much easier for the reader to keep track.

It would also be very helpful to add a Table that briefly defines each experiment, its purpose, and its sample size of focal individuals.

Figure 2
The caption should explain what is meant by direct vs. generalized reciprocity. What is the purpose of each panel? Explain briefly what this means within the caption.

I notice that the rapidity of the first pull is much more rapid, in all treatments, in Figure 2b as compared to Figure 2a. In other words, the focal rats performed the action nearly twice as fast in the generalized reciprocity conditions as compared to direct reciprocity. Why? What is it about that treatment that prompted faster responses? Is that surprising? Were those experiments different in some way that affected general motivation? Please explain within the main text.

Figure 3
It would be helpful to explain in the caption and main text why the animals delayed performing the action in the test phase, as compared to the experience phase. i.e., Why is the first pull so much more rapid in the experience phase in this figure?

The caption for Figure 3 also needs extensive editing to make it clear what was actually tested. According to lines 306-309 in the Results, Figure 3 is showing the results of experiment 3. If so, the focal rat was pulling for a neighbour that was always uncooperative (as per line 181 in the Methods), but that seems to contradict the wording within the caption. This discrepancy is quite confusion. For example, if this is experiment 3, the caption should not say “focal rats provided food to a cooperative partner” because the neighbour was always a stooge. Instead, it should say, “focal rats provided food to a stooge” or something along those lines. Also, the legend conditions should be labelled as: “olfactory information from an unseen cooperator” vs. “olfactory information from an unseen non-cooperator”.

Decision letter (RSPB-2020-0654.R0)

30-Apr-2020

Dear Miss Gerber:

Thank you for your submission of manuscript RSPB-2020-0654 entitled "The smell of cooperation: rats reciprocate help based on odour cues of helpful conspecifics". The paper has now been peer-reviewed, and the reviews have been assessed by an Associate Editor. Based on their reviews, the paper has been rejected for publication in Proceedings B in its current form.

This action has been taken on the referees' indications 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. We are all agreed that this is a fascinating and potentially very valuable paper, but the reviewers have raised several important points with regards to the manuscript which need to be addressed. With regard to one of these, Reviewer 1 asks for a chemical analysis of the hypothesised 'smell of cooperation'. I agree that this was be a very nice addition to the manuscript, but I leave it to your discretion as to whether to include it. Please also pay attention to the need for data archiving.

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.

To upload a resubmitted 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 Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Finally, I hope you and your co-authors are well in this challenging times.

Yours sincerely,
Professor Loeske Kruuk
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Two expert reviewers have seen your manuscript, both of whom found your study to be fascinating and potentially exciting. However both reviewers had significant difficulties with the manuscript in its current form. Reviewer 1 for instance felt it will be necessary to identify the actual odour involved in cooperation before publication (as you have done in your previous study on hunger), and both reviewers feel that the bold claim you make in the manuscript - i.e. that there is a "smell of cooperation" - is currently not backed up by your data. Reviewer 2 also felt that the rationale and the methods are simply not clear enough to repeat the experiment, and also worries that the data presented in Figure 2a call into question your main interpretation, because that figure appears to show a positive effect perceiving a neighbour's smell, independent of what the neighbour was doing. Both reviewers also have quite a number of smaller points that need to be addressed prior to publication.

Reviewer(s)' Comments to Author:
Referee: 1

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Lines 142-143: This section suffers from a lack of logical order. Here, you mention the "direct reciprocity" and "generalized reciprocity" paradigms, but they are not defined until further below.

Line 151: What is meant here by "the importance of social context"? I think (based on reading further) that you mean presence vs. absence of a social partner in the neighbouring chamber, but I'm not sure. Please clarify by saying exactly what you manipulated; "context" doesn't tell us.

Line 228: I think by "n.d." you are indicating no date, but R package versions do indeed have publication dates. You can get that information by looking at the package info within your installation of R, or by going to the CRAN website for a given package. E.g.:
https://cran.r-project.org/web/packages/survival/
and
https://cran.r-project.org/src/contrib/Archive/survival/
Please add the date and version information for each package that you used. This applies to lme4 as well, you should cite the package with its version, not just a 6-year old preprint by a subset of the package authors. The actual package has far more authors than those listed.

Line 242: "if models were overdispersed, an individual based random factor was included to deal with overdispersion."
This is incorrect. If you have repeated measures in these models, a random effect of individual ID should always be included to properly account for non-independence, i.e., to avoid inflating your power due to pseudoreplication. What you seem to be referring to here is the strategy of including an observation-level random effect to deal with overdisperson in some types of GLMMs (i.e., this means implementing a random effect with a different level for each row or observation in the dataset, NOT each individual). Individual ID needs to be accounted for as a random effect in any of your models that have repeated measures of individual, regardless of dispersion. This needs to be corrected in your statistical analysis and in the text here.

Line 237: This is incorrect, the use of Poisson distribution in GLMMs is not automatic when the data are count data. Often, count data do have Poisson-distrubted errors, but not necessarily! And the choice of error distribution should be based on the actual error distribution of the fitted model. You could easily have count data that have Gaussian-distributed errors, and then a regular Gaussian model would be appropriate. Looking at your data in Figures 3 and 4, I strongly suspect that is what you have. In other words, an overdispersed Poisson appears to be incorrect here, because the error structure of these data does not appear to follow a Poisson distribution at all. I would suggest checking a Gaussian model first, and examining whether the assumptions of that model are met (look at distribution of the residuals).

Results
Line 251: Information was available to whom?

Same comment lines 254, previously cooperative to whom? Any other rat, or the focal rat?

Lines 251-252: I am concerned that this interpretation (and the comparisons on the figure) is missing the point. E.g., "focal rats pulled earlier for a formerly cooperative social partner than for a formerly non-cooperative partner when olfactory information was available" Perhaps I'm missing something here, but wouldn't this simply be explained by the continuation of (greater) reward in one condition, but not the other? i.e., isn't this simply recapitulating what we already know about operant conditioning?

I would think it would be far more interesting in Figure 2a to compare the solid green condition to the dotted green condition, and to compare the solid blue condition to the dotted blue condition, respectively. This is like examining the independent effect of neighbour odour in the analysis. Based on the data in the figure, the perception of neighbour odour appears to have a positive effect on the focal rat's performance, regardless of what the neighbour was actually doing. This raises serious questions about the general interpretation of a "smell of cooperation". In other words, Figure 2a appears to show additive positive effects of (i) having an actively reciprocating neighbour (or reward magnitude, already known to have an effect on operant behaviour), and (ii) olfactory perception of a neighbour, regardless of that neighbour's behaviour. Neighbour odour may have a similarly sized positive effect on the focal rat, regardless of whether the neighbour was cooperating or a stooge. If so, this would contradict the later interpretation of "a smell of cooperation". Instead, it suggests that the smell of a social neighbour has a more general motivating effect.

Lines 283-295: This section explains the results of experiment 2, but I can't understand the purpose of this experiment. As far as I can tell, experiment 2 is not presented in any figure, and not mentioned in the Abstract. Is it necessary to include in the main text? Notably, experiment 1 already shows us that olfactory information affects the focal rat's behaviour (e.g., by comparing solid green to the dotted green line in Figure 2a). Perhaps you could remove experiment 2 from the main text and use that space to improve the transparency of other aspects of this study.

Line 313: What makes it the smell of cooperation per se? How do you know it's not the smell of social awareness, or the smell of reward anticipation in another individual? If there is justification for operationalizing something as "the smell of cooperation", it should be defined much earlier, within the Methods. As it stands, this value-laden interpretation is introduced for the first time down in the Results. It's not clear to me what the justification for that interpretation would be.

Discussion
Line 325: Same as the comment above, is it really the "odour cues from a helpful act"? Or could it be odour cues of conspecific reward anticipation?

Line 392: Needs a comma after "in other animals"

Figures
There are some issues with Figures 2-4 that need to be improved. First, the font size within the figures (e.g., legends) is way too small.

Secondly, in the captions, it would be helpful to say which experiment is shown. This is done for Figure 1 (referring to experiments 1, 2, 3, and 4 within the caption), but it is not done for Figures 2-4. Adding this info would make it much easier for the reader to keep track.

It would also be very helpful to add a Table that briefly defines each experiment, its purpose, and its sample size of focal individuals.

Figure 2
The caption should explain what is meant by direct vs. generalized reciprocity. What is the purpose of each panel? Explain briefly what this means within the caption.

I notice that the rapidity of the first pull is much more rapid, in all treatments, in Figure 2b as compared to Figure 2a. In other words, the focal rats performed the action nearly twice as fast in the generalized reciprocity conditions as compared to direct reciprocity. Why? What is it about that treatment that prompted faster responses? Is that surprising? Were those experiments different in some way that affected general motivation? Please explain within the main text.

Figure 3
It would be helpful to explain in the caption and main text why the animals delayed performing the action in the test phase, as compared to the experience phase. i.e., Why is the first pull so much more rapid in the experience phase in this figure?

The caption for Figure 3 also needs extensive editing to make it clear what was actually tested. According to lines 306-309 in the Results, Figure 3 is showing the results of experiment 3. If so, the focal rat was pulling for a neighbour that was always uncooperative (as per line 181 in the Methods), but that seems to contradict the wording within the caption. This discrepancy is quite confusion. For example, if this is experiment 3, the caption should not say "focal rats provided food to a cooperative partner" because the neighbour was always a stooge. Instead, it should say, "focal rats provided food to a stooge" or something along those lines. Also, the legend conditions should be labelled as: "olfactory information from an unseen cooperator" vs. "olfactory information from an unseen non-cooperator".

Author's Response to Decision Letter for (RSPB-2020-0654.R0)

See Appendix A.

RSPB-2020-2327.R0

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list 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?
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?
Yes

Is it clear?
No

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have responded to all my comments in great detail and argued their points well. I didn't realize that the chemical analysis would take so long, and I would actually suggest that the authors mention this in the paper (Line 512 would be the obvious place to do so). I also appreciate the new paragraphs discussing the physical activity explanation and the synergistic mutualism game. I think the paper now stands well on its own, and even though the "smell of cooperation" is still an exceptional claim, I would agree that this study could inspire many others to follow up on these results and focus more on the role of communication in cooperation more broadly. In that sense, the results of the experiments are well worth publishing, and I would close with another maxim: "the product of scientific research is the data, the paper is merely an advertisement"

minor comments:
isn't "sequential iterated prisoner's dilemma" a bit redundant? i would think iterated alone is sufficient?
line 304: "frailty term" = random effect

Decision letter (RSPB-2020-2327.R0)

12-Oct-2020

Dear Miss Gerber

I am pleased to inform you that your manuscript RSPB-2020-2327 entitled "The smell of cooperation: rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task" has been accepted for publication in Proceedings B.

The Associate Editor and a referee have recommended publication, but have also suggested some minor revisions to your manuscript, and pointed out the need to clarify the supporting data. Therefore, I invite you to respond to their comments and revise your manuscript/data files. 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 us know.

To revise your manuscript, log into https://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, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

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. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. 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.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository (https://royalsociety.org/journals/authors/author-guidelines/#data).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
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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=(Document not available) 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. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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

Sincerely,
Professor Loeske Kruuk
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Your revised manuscript has now been seen by the original reviewer and as you will see the reviewer is very positive to the way you have responded to their specific criticisms and suggestions. The reviewer has only two minor suggestions which you should consider.

Thanks you for making your data accessible but more metadata would be useful. For instance, some of the variable names are in German, and the experiment numbers differ from the ones reported in the paper (e.g. there is one file labelled Experiment5_results even though there are

only 4 experiments in the paper). It would not only be wise to consider the replicability of your analyses, but also the potential use of your data for future scientists wanting to test different hypotheses. For this good metadata documentation is essential.

Reviewer(s)' Comments to Author:
Referee: 1

Comments to the Author(s).
The authors have responded to all my comments in great detail and argued their points well. I didn't realize that the chemical analysis would take so long, and I would actually suggest that the authors mention this in the paper (Line 512 would be the obvious place to do so). I also appreciate the new paragraphs discussing the physical activity explanation and the synergistic mutualism game. I think the paper now stands well on its own, and even though the "smell of cooperation" is still an exceptional claim, I would agree that this study could inspire many others to follow up on these results and focus more on the role of communication in cooperation more broadly. In that sense, the results of the experiments are well worth publishing, and I would close with another maxim: "the product of scientific research is the data, the paper is merely an advertisement"

minor comments:
isn't "sequential iterated prisoner's dilemma" a bit redundant? i would think iterated alone is sufficient?
line 304: "frailty term" = random effect

Author's Response to Decision Letter for (RSPB-2020-2327.R0)

See Appendix B.

Decision letter (RSPB-2020-2327.R1)

23-Oct-2020

Dear Miss Gerber

I am pleased to inform you that your manuscript entitled "The smell of cooperation: rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task" 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

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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.

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,
Professor Loeske Kruuk
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear Professor Kruuk,

Thank you for your time and effort handling our MS. We are grateful to you and the two reviewers for their useful comments. Below we detail point-by-point how we have used them to improve our manuscript. We hope that this will meet with your approval.

With kind regards,

Nina Gerber, Manon Schweinfurth & Michael Taborsky

Suggestions by reviewers are numbered and marked by “Comment”, followed by our responses using the same numbers and marked as “Response”.

Associate Editor
Board Member: 1
Comments to Author:

Two expert reviewers have seen your manuscript, both of whom found your study to be fascinating and potentially exciting. However, both reviewers had significant difficulties with the manuscript in its current form. Reviewer 1 for instance felt it will be necessary to identify the actual odour involved in cooperation before publication (as you have done in your previous study on hunger), and both reviewers feel that the bold claim you make in the manuscript - i.e. that there is a "smell of cooperation" - is currently not backed up by your data. Reviewer 2 also felt that the rationale and the methods are simply not clear enough to repeat the experiment, and also worries that the data presented in Figure 2a call into question your main interpretation, because that figure appears to show a positive effect perceiving a neighbour’s smell, independent of what the neighbour was doing. Both reviewers also have quite a number of smaller points that need to be addressed prior to publication.

Thank you for your assessment and the summary of the major points of critique. We detail below how we have attempted to improve our manuscript based on the reviewers’ comments.

We now provide more details on our protocol and clearly explain why we took each respective step, and which conclusions are prompted by the results. We think that this has clarified how our data and analyses support our claim of a “smell of cooperation” instead of merely a “neighbour’s smell”, irrespective of their behaviour. Nevertheless, we also discuss the possibility to interpret the results differently. The suggestions by the reviewers were very helpful also to improve the text to enable future replications.

To identify the chemical compounds responsible for the rats’ responses to the behaviour of partners they can only smell is an intriguing challenge for future studies. This is clearly beyond the scope of the present study, as it would require an entirely different experiment and involve the collaboration of chemical ecologists experienced with the type of molecules that one might expect. Indeed, we conducted a pilot study that revealed the difficulty of extracting promising controlling compounds of the smell. A completely new study of this kind would delay the publication of the results of the current study for several years. We think that the results of our study clearly reveal that chemical information from a social behaviour triggers the cooperative response of experimental subjects receiving that smell, which in our view is valuable information even if the chemical compounds are yet unknown. Thereby, our results can inspire further work in this

direction. Nevertheless, we now discuss the role of odour compounds and the apparent smell of cooperation in a more balanced form and in more detail in our manuscript.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

Comment 1: This is a fantastic study investigating proximate mechanisms of cooperation among rats with an ingenious experimental design. This is a very timely topic that should be of interest to a wide variety of researchers. In particular, the authors show that odor cues from a cooperating rat are sufficient to trigger reciprocal cooperation. This is true even if the rat right next to the focal rat was not actually cooperating, and the odor came from a different rat invisible to the focal rat. This study follows in the wake of a similar recent paper by the same group (Schneeberger et al 2020 PLoS Biology) in which they show that the smell of a hungry rat triggers more helping, indicating that rats are sensitive to a recipient's hunger state; moreover, in that paper they also analyzed the "hunger smell" and identified specific compounds that are physiologically linked to hunger states and make this an honest signal. In the present study such an analysis is lacking, leaving me to wonder what could possibly produce this "i'm a helpful, cooperative rat" smell, and how it could be an honest signal. If it's not an honest signal, why should it trigger helping? This is my major concern with the present paper, and I would love to see a more in-depth analysis of the actual odor cues, and a more convincing discussion of how this whole signaling system can possibly be robust against cheating - a discussion of the naturalistic contexts in which rats cooperate (outside of this contrived experimental situation) would also be highly illuminating in this context. As it is, I'm reminded of the old saying that exceptional claims require exceptional evidence, and it seems to me that a "cooperation smell" that can trigger helping even when the focal rat hasn't actually received any food is an exceptional claim - but we have yet to see the exceptional evidence.

Response 1: Thank you very much for your encouraging appraisal of our work and the constructive comments and criticism. We agree that an analysis of the chemical compounds would be a very interesting addition to our work, but this is clearly beyond the scope of this study. We have actually performed pilot experiments to clarify technical and analytical requirements for identifying the chemical compounds causing the observed responses. This pilot study revealed that it will not be as straightforward as identifying the food- and digestion related compounds studied in the mentioned study of Schneeberger et al. 2020. There was a great number of potential molecules and so many differences in the volatiles collected from cooperating and defecting individuals that solving this question would require a much bigger study, probably involving hundreds of potentially informative compounds and the respective tests demonstrating corresponding effects on the behaviour of rats.

We feel that our study provides compelling evidence that rats respond to the isolated odour of an otherwise imperceptible rat. While we agree that chemical analyses would be a valuable addition to our study, we think they concern a different level of explanation. In addition, since the presented paper already includes results of four separate experiments, we fear that it would be difficult to include another extensive study into one manuscript, where all experimental details can be explained in sufficient detail to be fully comprehensible. Furthermore, waiting for the results of such a study would probably mean a delay of several years according to the information of the chemical ecologists involved in our pilot study, which would unduly postpone the publication of the results presented here. Therefore, we think that the chemical analyses should be the subject of future research while the focus of our manuscript should remain on the behavioural level.

Nevertheless, we accept your criticism that our conclusions from the behavioural data should be discussed more carefully until the compounds responsible for the demonstrated response are identified. Therefore we now discuss in detail alternative possibilities which information rats might convey, intentionally or inadvertently lines 465 ff. and lines 497 ff. We agree that the smell of cooperation might be an exceptional claim. We think that our four experiments stepwise build up and strengthen evidence for such claim. Our new discussion on potential alternative explanations should enable readers to evaluate the evidence and hopefully spark interest in elucidating the underlying chemical compounds. Finally, our results may not explain the whole story, but we hope our results inspire other research groups to perform research in this direction and develop a diverse set of studies which then altogether eventually provide exceptional evidence.

Minor comments:

Comment 2: Lines 202-211: Unlike the previous experiment, this experiment has no experience phase, rather than an experience phase without odor cues. I wondered why, and how the experimenter recording the rats' behavior could possibly be blind to the condition when there is such a clear difference (experience phase yes or no, with corresponding noise from odor machine)?

Response 2: Thanks for pointing to our ambiguous wording. The experimenter was only blind to the treatment within the experiments (with smell or without smell), but as you pointed out was knowing of the experiment (a or b) that was performed. We have changed the wording on line 278-280 to:

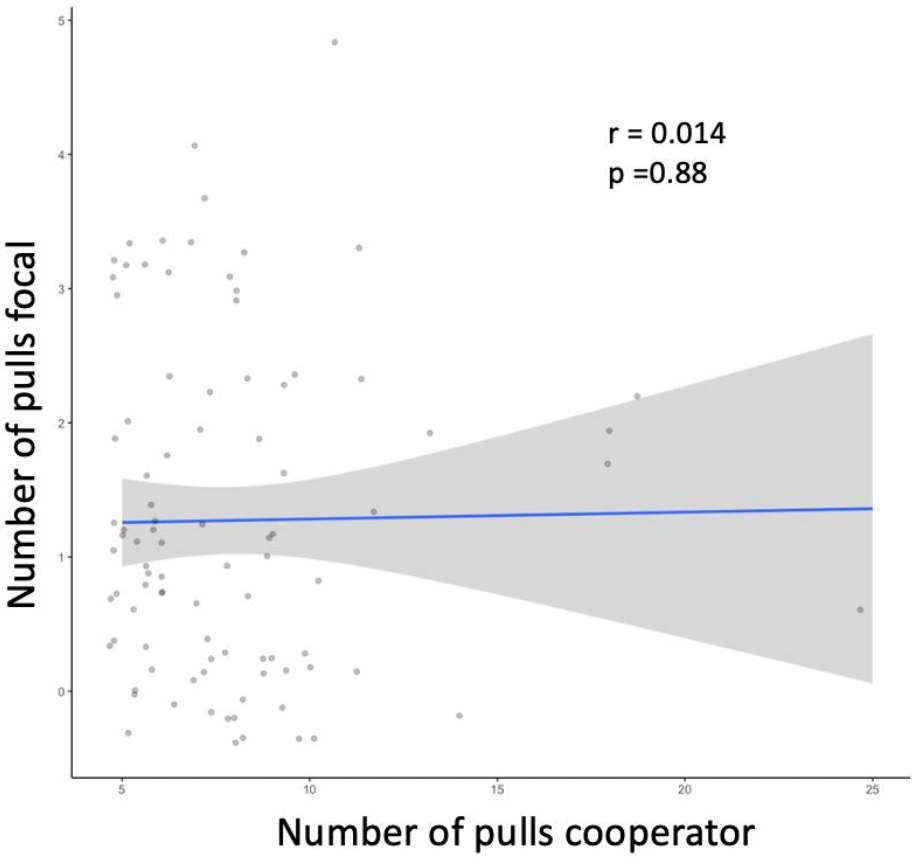
> "For both experiments (3a and 3b), the experimenters recording the focal rats' behaviour were blind to the experimental treatments (with or without odour transmission) but informed about the type of experiment (3a or 3b)."

Comment 3: 220-222: Starting here I was wondering whether the odor provider was simply more physically active in the cooperation condition, and the "smell of cooperation" could therefore simply be a smell of physical activity? The authors bring this up in the discussion (358-360), stating that a previous study from 2007 had shown no difference in activity levels between rats that pull a stick and those that do not. I would really like to see such a comparison repeated for the present study. Again, this would be crucial to support the exceptional claim of a "cooperation smell". Should it turn out that there is a difference in physical activity and that the "cooperation smell" really is a physical activity smell, that's not a disaster - on the contrary, this would actually provide more credible evidence that there's an honest signal here. And it would make it all the more important to discuss these experiments in light of naturalistic rat cooperation - why would honest cues of physical activity be used in decisions to reciprocate? Could it be that physical activity acts as a proxy for "work effort", and would work effort be something that is rewarded in rat colonies? I'm just speculating here, but this is the kind of discussion I would like to see. Also, it's worth noting that a physical activity smell couldn't just result in social contagion since pulling by the focal rat was higher when there was a partner compared to an empty cage.

Response 3: Thanks for stressing the point of a potential effect of physical activity. We have not seen any clear difference between cooperative and non-cooperative partners, but the reviewer is right that there might be a difference in activity level, since one is pulling and the other one not. As we have not measured activity levels during our experiment, we checked for a potential correlation between the number of pulls of cooperative rats and the number of pulls of focal individual as a proxy for the effect of "activity" or "effort" differences since pulling more often reflects greater activity. To obtain maximal power for this analysis, we have pooled the results of all experiments to check for such correlation (N=159). Nevertheless, we did not find a positive relationship between the number of pulls of a cooperative partner and the number of pulls of the focal rat (see result in the graph below). This does not point towards an important effect of activity, even if this proxy

may not fully represent total activity of the odour provider. In connection with the results of the mentioned previous study, we therefore conclude that the smell of a cooperative act seems to better explain the test rat's response than differences in activity levels. To summarize, if activity was related to work effort and the "smell of activity" was a key scent for triggering helpful behaviour, we would expect higher activity levels to be rewarded more, which we did not find. These results also indicate that the response to a smell of cooperation seems to be of qualitative rather than quantitative nature.

We followed the reviewer's suggestion and are now expanding on this in the Discussion at lines 469 ff, and added the below figure to the supplement.



Comment 4: 233-234 I don't think there's any need to do non-parametric statistics - a Wilcoxon is just an old, outdated approach to data that really call for GLM(M)s.

Response 4: We have used GLMMs in situations where multiple factors predict a single outcome and the conditions for this type of analysis are met. In situations with a single predictor (two treatments), however, we have decided to use a conservative statistical test that does not rely on variance assumptions. As our data required a non-parametric procedure and the sample size was sufficiently large, the Wilcoxon test seems an appropriate choice.

Comment 5: 242-243 Surely you mean observation-level random effect, not "individual based random factor"? An OLRE is the typical approach to overdispersion as it acts like an error term that captures each data points deviation from the predicted fit, and you already have individual-level random factor (line 239)

Response 5: This is correct. We of course meant here that we included a random factor for individual observations. We agree that the term "individual based random factor" was misleading, and we changed the wording accordingly (line 317).

Comment 6: 253-255 not significant does not mean "did not differ". if you look at the figure, the two lines clearly differ - predicted probability of puling at 400s seems to be about 50% for cooperator but only 25% for non-cooperator -, just not significantly so. sorry for the rant, but i really dislike the false dichotomy of null-hypothesis significance testing. Not a big issue, but I would prefer a more nuanced statement

Response 6: We fully agree with your statement and have changed the wording accordingly (line 330). It now reads:

> "In contrast, when no olfactory information was provided, there was no significant difference in the latencies to pull for the partner between the previously cooperative and non-cooperative partners (latency: Cox. prop. haz., n= 23, p> 0.05, Tables S2a&b)"

Comment 7: 365-372 Here I would like to see more discussion of why rats should have a communication system for such mutualistic cooperation. What is it that rats do in the wild that would select for such mechanisms? Or did the mechanisms perhaps evolve for some entirely different purpose but could be co-opted in the context of this contrived experimental situation (wild rats don't pull sticks in cages) that these particular rats were trained to succeed in?

Response 7: Rats were shown to exchange different commodities among each other reciprocally, which also involves natural behaviour such as allogrooming (Schweinfurth & Taborsky 2018, Current Biology 28, 594–599). We therefore think that the ability to recognize the cooperative propensity of a social partner can be very useful for rats interacting in a natural setting. We now briefly refer to these possibilities in the Discussion (lines 482ff).

Comment 8: 374-376 This seems like a far-fetched claim. A rat is pulling on a stick. Why should there be an inevitable odor cue that says "hey, i just pulled a stick, i'm a helpful rat"? unless it's a general physical activity cue (see above).

Response 8: We agree that at first sight pulling a stick may not seem to unambiguously denote cooperation. However, the rats in our experiments have learned over a long period that in this situation pulling the stick is a cooperative behaviour only rewarding their partner. After a short initial training phase where they learned to pull the stick in the first place, the rats have never been rewarded for pulling the stick by themselves; the only reward they received came from reciprocating partners. We clarify this now at lines 159-162. Further, this trained cooperative behaviour was shown to be transferred also to cooperative natural behaviour (allogrooming, as mentioned above; see Schweinfurth & Taborsky 2018, Curr Biol 28, 594–599). Therefore, if rats realize that their effort is "generous", i.e. supporting a conspecific partner, it does not seem to be far-fetched to assume that this might be associated with releasing a specific odour.

But we now also discuss more extensively the possibility that the released odour might be a general activity cue, even if we did not find evidence for this interpretation (see line 469 ff.).

Comment 9: 357-378 in general, this section falls short of convincing me that what we are seeing in these experiments is communication of cooperative intent using honest signals evolved for a mutually beneficial context (see major comment above)

Response 9: In response to your helpful comments we have restructured the Discussion to put more emphasis on the potential role of such cooperative smell in social interactions, and how this might reflect an honest signal or an inadvertent cue.

Referee: 2

Comments to the Author(s)
This study addresses whether olfactory cues associated with different behavioural states can influence another individual's tendency to perform a trained task for a delayed reward. I was very interested to read this study and I find the paradigm to be quite fascinating. If the experimental paradigm can be properly justified, I think the results of this study would make an interesting contribution to the literature. However, a major issue in the manuscript is that the procedures here are so poorly described that it was almost impossible for me to understand what was done, and why. The gaps in the text, and the data shown in Figure 2 raised concerns for me as to whether this paradigm really shows what is claimed. Below, I make some specific suggestions for how the transparency of this study could be improved.

Thank you for your appreciation of our work and for your very detailed and constructive feedback. Your suggestions really helped us to clarify details of our study and improve the manuscript in order to show how our data support our claim. We explain in detail below how we have dealt with your questions and points of criticism and hope to have clarified the experimental procedure in a way that enables a better understanding of what was done, and why. We also explain in detail why an increase in the pulling behaviour in response to the presence of odour cues in general (as shown in figure 2) does not exclude the existence of a smell of cooperation.

Title

Comment 1: The wording in the latter half of this title strongly suggests an identity signal (i.e., "helpful conspecifics" connotes helpful individuals, not individuals who are helping). This appears to be contrary to your main claim. Assuming your results do indeed show an effect of an olfactory signal of partner behaviour, how about something like this instead: "The smell of cooperation: rats reciprocate more when they receive odour cues of a partner performing help"

Response 1: Thank you for this suggestion. We have now adopted the title accordingly, which now reads: "The smell of cooperation: rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task".

Abstract
Comment 2: Line 15: "is triggered solely by" is not correct, because no behavioural experiment can prove a universal like that. How can you be sure that the behaviour is not triggered by things you did not test? I suggest rewriting this to be something like: "odour cues associated with… are sufficient to trigger… xxx behaviour"

Response 2: You are right that our wording can lead to wrong conclusions. We have rewritten this part of the abstract to match what we actually meant on lines 15-17. It now states:

> "In a sequence of four consecutive experiments, we show that odour cues from a cooperating conspecific are sufficient to induce altruistic help of rats in a food-exchange task"

Comment 3: Lines 19-20: "do not represent individual identity" I find that phrasing odd and hard to understand. How about "are not identity-specific" instead?

Response 3: Thank you, we have adopted the suggested wording on line 20:

> "We further show that the cues inducing altruistic behaviour are released during the act of cooperation and do not depend on the identity of the cue provider."

Comment 4: Lines 20-21: What do you mean by "direct behavioural cues"? Perhaps you mean that the rats are not influenced by the visual appearance of what is going on in the neighbouring chamber. But why are visual cues necessarily "direct", whereas olfactory cues are not? Visual perceptions can also be incorrect and even deceived, so I don't understand the use of "direct" here. Perhaps it only seems "direct" because vision is what we humans rely on.

Response 4: There seems to be a misunderstanding with regard to what we meant by direct vs. indirect cues. With 'direct behavioural cues' we did not refer to *visual* cues but to experiencing cooperative behaviour instead of "just" receiving cues of an act. We have made this clearer in the main text at line 22 See also the comment below.

> "Remarkably, olfactory cues seem to be more important for cooperation decisions than experiencing a cooperative act per se."

Comment 5: Line 20: Is the unimportance of the visual cues really that surprising? As you explain in the Introduction, visual cues are not particularly useful for a nocturnal fossorial animal, and we already know rats use olfactory cues for so many important functions. So, is this really the most remarkable aspect of your results?

Response 5: As you point out correctly, it is not surprising that visual cues might not be the most useful cues for nocturnal animals, as was shown also by a previous study (Dolivo & Taborsky 2015, Ethology 121, 1071-1080). However, we were not referring to visual cues here but to actually experiencing cooperative behaviour, i.e. the experience of receiving food from a social partner. Our results indicate that olfactory cues (indirectly informing the rat about the cooperativeness of its partner) are more important for cooperative decisions than directly experiencing cooperative behaviour. As this was apparently not clear we changed this at line 22:

> "Remarkably, olfactory cues seem more important for cooperation decisions than experiencing a cooperative act per se."

Introduction

Comment 6: Lines 52-53: This is too vague to understand. Please explain how these previous experimental results "suggest" this. Can you elaborate what was shown, what the specific conclusion is, and why it's only suggested (in italics)? All of this is opaque to the reader.

Response 6: We have rewritten this sentence on line 59, which now states:

> "Experimental results in rats have shown that individuals communicate their need for help to social partners by behaviours such as reaching towards a potential reward and emitting ultrasonic calls"

Line 54: "signal their helping effort" to whom?

We refer to signalling the helping effort to a conspecific partner and write:

> "However, do individuals also signal their helping effort to their partner in order to increase their partner's propensity to pay back help in the future?"

Comment 7: Lines 76: Please explain how your experimental paradigm is a sequential iterated prisoner's dilemma. This seems to be complicated by the fact that the rats are first trained to pull the stick for an individual (self) reward, that gets progressively delayed through further training. So how does the expression of this trained behaviour in the social paradigm qualify as a PD game? I think this whole paradigm needs to be explicitly justified as a PD (by explaining the costs and benefits to each player and how it meets the game theoretic definition), or you should change your terminology to call it costly helping behaviour, or motivation for a delayed reward, or something else.

Response 7: It is important to note that in the training phase preceding our experiments the rats have learned that pulling the stick is a cooperative behaviour rewarding only their partner. After a short initial training phase where they learned to pull the stick at all, the rats have never been rewarded by themselves for pulling the stick; the only reward they received came from reciprocating partners after some delay. We clarify this now at lines 159-164.

Thus, pulling the stick reflected a cooperative and costly act for the rats, because the energy expenditure and opportunity costs (loss of time) were not compensated by an immediate reward of the act. Previous work has shown that reciprocity in rats depends on the cost to the donor and benefit to the recipient (Schneeberger et al. 2012), revealing that the rats are sensitive to the costs of the act. We now refer to our experimental situation as a sequential IPD and point towards the costs involved for the players at lines 64ff.

Methods

Comment 8: Line 79: How many? Provide overall sample size of individuals here. The exact sample size for each experiment should also be provided in the appropriate places further down.

Response 8: Throughout our experiments we have trained and housed many rats. We think that mentioning the total number of trained rats here would be rather confusing instead of providing important information. We do provide the sample size of each experiment, e.g. at lines, 191, 203 etc., as well as in the supplementary figures and tables. To further increase clarity, we have added a Table S1 with an overview of all experiments including sample sizes in the supplementary material.

Comment 9: Line 90: Revise the phrase "trained to get used to"…
How about: "habituated to" or "trained to be familiar with"?

Response 9: Done, we have rephrased this sentence accordingly.

Comment 10: Line 90: At this point, the reader does not know what "pull the stick" refers to. This is a consistent problem throughout the Methods (i.e., the order consistently lacks background needed

for the reader to make sense of it). My general suggestion is to rewrite the Methods to start with a section that explains overall goals of the study, and then explain the apparatus that allowed you to achieve it, and then define the "types" of individuals that were created and why (stooge, cooperator etc). Finally, explain how you used the training paradigm to create those individual types.

Response 10: Thank you for this excellent suggestion. We have now rewritten and restructured the *Methods* section accordingly, starting with an explanation of the apparatus and experimental setup, which is followed by the sections on training and housing the rats before we explain the detailed methods of each experiment.

Comment 11: Line 94: The reader doesn't yet know what "partner" means here in terms of the physical set-up of the apparatus. Again, this would make more sense if the apparatus was explained first, before the training section.

Response 11: We have restructured the Methods section as outlined above and hence the meaning of "partner" is now explained earlier.

Comment 12: Line 96: What does it mean to be "a cooperator"? This is not defined until much later, making it impossible to understand.

Response 12: Thanks. Again, through restructuring the Methods the meaning of being a "cooperator" is now explained earlier.

Comment 13: Lines 99-101: Explain the procedure. It is not sufficient to merely cite the previous work "see xxx for a description of the procedure"). Please add a brief explanation so the reader can understand what was done and evaluate it.

Response 13: We used exactly the same training protocol as in the cited reference. We have now added a paragraph to explain (i) that rats have not been self-rewarded after the first training step, and (ii) how they learnt to reciprocate food donations (lines 159 ff).

Comment 14: Lines 101-102: What were the signs of stress and anxiety? Please define so that this is reproducible by others.

Response 14: We wrote in our original manuscript "The experimental rats did not show any signs of stress or anxiety." With this statement we aimed to emphasise that in our experiments we have only observed normal behaviour of our rats including grooming, exploring the environment, sniffing, eating (if food was provided). We have not observed any escape or avoidance behaviours, increase in ultrasonic calls, scratching on the cage, wagging the tail on the ground, or any other behaviours that would indicate discomfort.

Comment 15: Line 117: The reader doesn't know what you mean by "stooges", because it has not yet been defined. As suggested above, it would be best to have this explained much earlier.
Lines 117-118: What distinguishes a focal rat from a cooperator? Need to clarify this
Line 117: "all experiments" Confusing because reader doesn't yet know that there are multiple experiments

Response 15: As outlined above, we have now rewritten and restructured the Methods section according to your suggestion. Thus, the reader has now the required information about the different roles at this point.

Comment 16: Lines 133-134: It doesn't make sense to mention "experiments one and two" here before the reader even knows that there were multiple experiments. This could be resolved by adding a sentence (perhaps at the beginning of the Methods as suggested above), "In total, we performed X experiments with the goals of…"

Response 16: We now mention the number of experiments at the beginning of the Methods section (line 99).

> "We performed a series of four consecutive experiments…"

Comment 17: Line 139: Add an explanation of the purpose to the beginning of this section: "To determine if… we…"

Response 17: We have added such statement at the beginning of this section (line 178)

> "To test for the importance of olfactory information in cooperation decisions…"

Comment 18: Lines 142-143: This section suffers from a lack of logical order. Here, you mention the "direct reciprocity" and "generalized reciprocity" paradigms, but they are not defined until further below.

Response 18: At lines 142-143 (Now 183) we had aimed to give an overview of the experiment, the details of which we provided later on (e.g. at lines 191 ff). We have now added an introductory sentence at line 178 and define direct and generalized reciprocity at lines 184-188.

> "In the direct reciprocity paradigm, we tested whether a rat increases cooperative behaviour towards an individual that was helpful to them, whereas in the generalized reciprocity paradigm we tested whether rats generally increase their cooperation propensity if they experienced cooperative behaviour from any other conspecific."

Comment 19: Line 151: What is meant here by "the importance of social context"? I think (based on reading further) that you mean presence vs. absence of a social partner in the neighbouring chamber, but I'm not sure. Please clarify by saying exactly what you manipulated; "context" doesn't tell us.

Response 19: You are right that by social context we meant the presence or absence of a social partner, as we explain at line 181. To make this clearer we have also added this information at line 198.

> "In addition to these four treatments, again the control treatment served to test for the importance of a social context (i.e., the presence of a social partner)."

Comment 20: Line 228: I think by "n.d." you are indicating no date, but R package versions do indeed have publication dates. You can get that information by looking at the package info within your installation of R, or by going to the CRAN website for a given package. E.g.:
https://cran.r-project.org/web/packages/survival/
and
https://cran.r-project.org/src/contrib/Archive/survival/
Please add the date and version information for each package that you used. This applies to lme4 as well, you should cite the package with its version, not just a 6-year old preprint by a subset of the

package authors. The actual package has far more authors than those listed.

Response 20: Thanks for checking those details. We have added the package version where it was missing, as suggested. The lme4 citation is the official citation from CRAN. Thus, we cite the lme4 package with the authors listed in this citation.

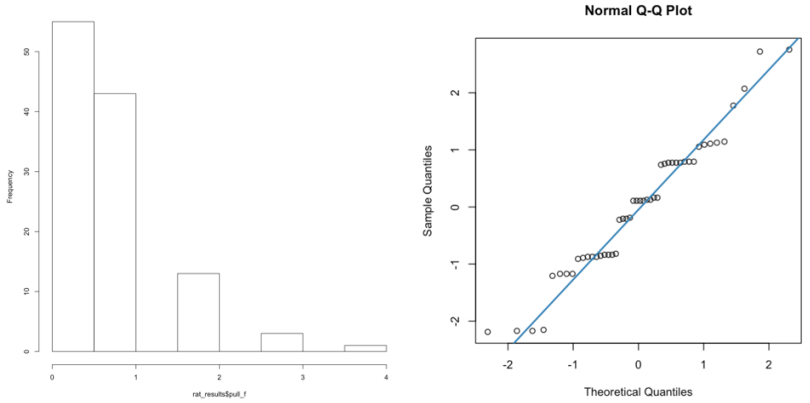
Comment 21: Line 242: "if models were overdispersed, an individual based random factor was included to deal with overdispersion."
This is incorrect. If you have repeated measures in these models, a random effect of individual ID should always be included to properly account for non-independence, i.e., to avoid inflating your power due to pseudoreplication. What you seem to be referring to here is the strategy of including an observation-level random effect to deal with overdisperson in some types of GLMMs (i.e., this means implementing a random effect with a different level for each row or observation in the dataset, NOT each individual). Individual ID needs to be accounted for as a random effect in any of your models that have repeated measures of individual, regardless of dispersion. This needs to be corrected in your statistical analysis and in the text here.

Response 21: Thanks for pointing out this sloppy phrasing. Of course, we meant that in case of overdispersion we included a random factor for individual observations (in addition to a random effect term accounting for non-independence among individuals). Obviously, this was not a problem with the statistical analysis but with the wording. We had outlined that "The individual identity of the focal animals was included as a random factor to account for repeated measures" in our GLMMs, and we have now amended the text to clarify how we have dealt with overdispersion on line 316. This now reads:

> "The model assumptions were tested and if models were overdispersed, an observation-level random effect was included to deal with overdispersion."

Comment 22: Line 237: This is incorrect, the use of Poisson distribution in GLMMs is not automatic when the data are count data. Often, count data do have Poisson-distrubted errors, but not necessarily! And the choice of error distribution should be based on the actual error distribution of the fitted model. You could easily have count data that have Gaussian-distributed errors, and then a regular Gaussian model would be appropriate. Looking at your data in Figures 3 and 4, I strongly suspect that is what you have. In other words, an overdispersed Poisson appears to be incorrect here, because the error structure of these data does not appear to follow a Poisson distribution at all. I would suggest checking a Gaussian model first, and examining whether the assumptions of that model are met (look at distribution of the residuals).

Response 22: We agree that count data can have non-Poisson distributed errors. However, this is usually the case with data with large mean values. In our case the data have a low mean and thus are truncated at 0 (see histogram below), which reflects a skewed distribution. While we could fit models assuming a normal error distribution, the normality of the error distribution is questionable in our opinion (see qq plot below). In any case, using a Gaussian LMM does not qualitatively change any of our results. Given the discrete nature and zero inflation of our data we think that a Poisson model is appropriate. However, we agree that the wording was wrong and changed it accordingly (line 310).



Results

Comment 23a: Line 251: Information was available to whom?

Response 23a: We have made this clearer by writing:

> "In addition, focal rats pulled earlier for a formerly cooperative social partner than for a formerly non-cooperative partner when they had access to olfactory information of their partner"

Comment 23b: Same comment lines 254, previously cooperative to whom? Any other rat, or the focal rat?

Response 23b: We have edited the text to make this clearer.

Comment 24: Lines 251-252: I am concerned that this interpretation (and the comparisons on the figure) is missing the point. E.g., "focal rats pulled earlier for a formerly cooperative social partner than for a formerly non-cooperative partner when olfactory information was available" Perhaps I'm missing something here, but wouldn't this simply be explained by the continuation of (greater) reward in one condition, but not the other? i.e., isn't this simply recapitulating what we already know about operant conditioning?

Response 24: Here we aimed to find out whether rats reciprocate help also when olfactory information is blocked. It was already shown in previous studies that rats reciprocate help when olfactory information is available (e.g. Rutte & Taborsky 2007, 2008; Schneeberger et al. 2012; Dolivo & Taborsky 2015a, b; Schweinfurth & Taborsky 2018). Still, it was important to reproduce this result with our experimental setup, which differs from previous studies where no manipulation of airflow was involved. Thus, even if this result is not surprising it still needs to be reported. We should like to point out that the behaviour cannot be explained by operant conditioning, as the action was not directly rewarded. Instead, the decision rule underlying this behaviour reflected direct reciprocity (cf. Dolivo et al. 2016).

Comment 25: I would think it would be far more interesting in Figure 2a to compare the solid green condition to the dotted green condition, and to compare the solid blue condition to the dotted blue condition, respectively. This is like examining the independent effect of neighbour odour in the analysis. Based on the data in the figure, the perception of neighbour odour appears to have a positive effect on the focal rat's performance, regardless of what the neighbour was actually doing. This raises serious questions about the general interpretation of a "smell of cooperation". In other words, Figure 2a appears to show additive positive effects of (i) having an actively reciprocating neighbour (or reward magnitude, already known to have an effect on operant behaviour), and (ii)

olfactory perception of a neighbour, regardless of that neighbour's behaviour. Neighbour odour may have a similarly sized positive effect on the focal rat, regardless of whether the neighbour was cooperating or a stooge. If so, this would contradict the later interpretation of "a smell of cooperation". Instead, it suggests that the smell of a social neighbour has a more general motivating effect.

Response 25: We agree that the smell of a social partner may have a generally positive effect on the propensity of cooperation, as suggested by the solid lines exceeding the dotted lines in Figure 2a. Access to the smell of a partner might be important to recognise the situation as a social situation (see Table S7 and Figure S5). We show the results of all comparisons from Figure 2 in Table S7. However, there is no significant difference between a non-cooperative rat without olfactory information and a non-cooperative rat with olfactory information (comparison of the two blue lines, as suggested by the referee). In contrast, there is a significant difference between a cooperator with smell to a cooperator without smell (comparison of the two green lines). The crucial comparison is the one between the two treatments where olfactory information is available (between the two solid lines in Figure 2). If the mere presence of odour of a conspecific were triggering helpful behaviour, there should be no significant difference between these conditions. But as illustrated in Figure 2 and shown in Table S7 we did find a highly significant difference, indicating that the neighbour's behaviour is crucial. To point to the provided information on all comparisons more explicitly we now refer to Table S7 in the figure legend of Figure 2.

A potential effect of the access to smell of a conspecific does not call into question the validity of our interpretation of a "*smell of cooperation"*. Importantly, our interpretation of a "*smell of cooperation*" comes from a series of experiments and not just the experiment presented in Figure 2. The subsequent experiments clearly showed that the smell of a rat performing a cooperative act raised the levels of cooperation compared to the smell of a rat that was not performing a cooperative task. The potential existence of a motivating effect of a social partner does not exclude the existence of an effect of the behaviour of that partner, i.e. the "*smell of cooperation*".

Comment 26: Lines 283-295: This section explains the results of experiment 2, but I can't understand the purpose of this experiment. As far as I can tell, experiment 2 is not presented in any figure, and not mentioned in the Abstract. Is it necessary to include in the main text? Notably, experiment 1 already shows us that olfactory information affects the focal rat's behaviour (e.g., by comparing solid green to the dotted green line in Figure 2a). Perhaps you could remove experiment 2 from the main text and use that space to improve the transparency of other aspects of this study.

Response 26: The purpose of this experiment was apparently not described sufficiently clearly, thank you for pointing this out. Experiment 2 was performed to test in which of the phases olfactory information is important, i.e. while experiencing cooperative behaviour or while performing it. Thus, in contrast to experiment 1 where olfactory information was available in both phases, in experiment 2 olfactory information was blocked in one phase only. We aimed to discriminate between a situation in which rats can communicate their demand to a potential donor from a situation in which they might be able to communicate their cooperative behaviour. The results show that in both situations, the availability of olfactory information increases the propensity to cooperate. Thus, removing this experiment from the main text would not allow us to make conclusions about the phase in which olfactory information is important, while performing or receiving a cooperative act. To clarify the purpose of experiment 2 we now write (lines 211-219):

> "To investigate whether cooperative behaviour is triggered by the transmission of olfactory information either during the experience or during the test phase of the experimental interaction, in experiment 2 focal rats got access to the smell of their partner in one of

these phases only. If the cooperation propensity of focal rats was enhanced when receiving olfactory information only in the experience phase, this would indicate that cooperative partners may have communicated their helping effort. On the other hand, if the cooperation propensity of focal rats was enhanced when receiving information only in the test phase, this would indicate that partners may communicate a demand for help."

We provide the statistical results of this experiment in the two tables (S3a and S3b), but if requested we could also add a graph.

Comment 27: Line 313: What makes it the smell of cooperation per se? How do you know it's not the smell of social awareness, or the smell of reward anticipation in another individual? If there is justification for operationalizing something as "the smell of cooperation", it should be defined much earlier, within the Methods. As it stands, this value-laden interpretation is introduced for the first time down in the Results. It's not clear to me what the justification for that interpretation would be.

Response 27: This interpretation was based on the results of experiment 3, which showed that the decision of focal rats to help a social partner was significantly affected by olfactory cues received from a cooperative or uncooperative conspecific without receiving any other cues or experiencing cooperative behaviour themselves. Therefore, neither "social awareness" nor "reward anticipation" alone could explain the results of this experiment. But we followed your advice and now introduce the concept already in the Methods (lines 237-240).

Discussion

Comment 28: Line 325: Same as the comment above, is it really the "odour cues from a helpful act"? Or could it be odour cues of conspecific reward anticipation?

Response 28: As explained above, we have shown in experiment 2 that odour cues are important in both, the experience *and* the test phases of the experimental interaction. Furthermore, in experiment 3a the rats received odour information from the remote experimental partner acting in a different room *only* during the experience phase, which triggered a helpful response of the focal individual in the subsequent test phase during which no olfactory information was conveyed to her. Thus "*conspecific reward anticipation"* alone cannot explain the change in behaviour of the focal animal, because apparently the olfactory information the focal rat receives while its partner is performing a cooperative act has an important influence on her helping propensity in a subsequent experimental period (the test phase). As olfactory cues from a helpful act can apparently trigger cooperative behaviour, it seems justified to infer a smell of cooperation released during a helpful act. We hope that our results inspire research into the chemical components responsible for this intriguing behavioural response.

Comment 29: Line 392: Needs a comma after "in other animals"

Response 29: Comma was added

Figures

Comment 30: There are some issues with Figures 2-4 that need to be improved. First, the font size within the figures (e.g., legends) is way too small.

Response 30: Thank you, we have increased the font size in Figures 2-4 accordingly.

Comment 31: Secondly, in the captions, it would be helpful to say which experiment is shown. This is done for Figure 1 (referring to experiments 1, 2, 3, and 4 within the caption), but it is not done for

Figures 2-4. Adding this info would make it much easier for the reader to keep track.

Response 31: Thank you for this suggestion, we have now added this information to the titles of the figure legends.

Comment 32: It would also be very helpful to add a Table that briefly defines each experiment, its purpose, and its sample size of focal individuals.

Response 32: We have added Table S1 to provide such overview of all experiments. For details of the experimental design of each experiment we provide supplementary Figures S1-S4.

Figure 2
Comment 33: The caption should explain what is meant by direct vs. generalized reciprocity. What is the purpose of each panel? Explain briefly what this means within the caption.

Response 33: Given limited space in captions, we fear that we cannot provide a detailed explanation of the two paradigms in the figure captions. Nevertheless, we added the decision rules in the caption as a reminder, as these two forms of reciprocity are explained in detail already in the Introduction (lines 40-42) and in the Methods section (lines 184-189).

Comment 34: I notice that the rapidity of the first pull is much more rapid, in all treatments, in Figure 2b as compared to Figure 2a. In other words, the focal rats performed the action nearly twice as fast in the generalized reciprocity conditions as compared to direct reciprocity. Why? What is it about that treatment that prompted faster responses? Is that surprising? Were those experiments different in some way that affected general motivation? Please explain within the main text.

Response 34: The slopes denoting the response speed apparently differ between the two situations. It seems that in the generalized reciprocity situation the rats provided help earlier and the proportion of rats providing help was also higher. This difference was most pronounced in the treatments where olfactory information was blocked (dotted lines), however, whereas the difference in the response to a cooperator from which olfactory information was obtained was rather small (green solid lines). This might indicate that olfactory information is more important for direct reciprocity. Given that individual information is crucial for direct reciprocity, and in rats such information is obviously of olfactory nature, this interpretation may seem plausible. It is however difficult to compare the two paradigms directly, since in the direct reciprocity situations rats experienced one partner in the morning while the test phase took place in the afternoon of the same day. In contrast, in the generalized reciprocity paradigm the focal rats experienced three different partner rats on three consecutive days while then being tested on the fourth day. Thus, this difference could also be confounded by differences in the timing of the experimental procedure. It might be interesting to investigate these differences more closely while controlling the timing of the experimental procedures for both paradigms.

Figure 3
Comment 35: It would be helpful to explain in the caption and main text why the animals delayed performing the action in the test phase, as compared to the experience phase. i.e., Why is the first pull so much more rapid in the experience phase in this figure?

Response 35: Thanks for pointing to this interesting difference, which again mainly exists in the "co-operator with olfactory information" treatment. This can be plausibly explained by the experimental design. In the "experience phase treatment" (3a), the rats received information about their partner before being tested, i.e. in the experience phase of the experiment, so they had time to process information obtained during this phase before being tested subsequently (i.e., in the

test phase). In the "test phase treatment" (3b), the olfactory information from the remote partner was only available during the test phase. It is conceivable that it takes some time for the olfactory information to be processed, which would then cause a delay in the behavioural response.
We now refer to this in the manuscript (lines 399-406):

> "When comparing Figures 3a & 3b, it seems that the response of the focal rats was somewhat delayed when they received the olfactory information from the remote partner in the different room only during the test phase (3b) as compared to the situation when this information was available during the experience phase (3a). This delay might be explained by the fact that when olfactory information was available already before the test (because it was provided during the experience phase; condition 3a), the rats had more time to process this information than when it was only provided in the test phase itself (condition 3b)."

Comment 36: The caption for Figure 3 also needs extensive editing to make it clear what was actually tested. According to lines 306-309 in the Results, Figure 3 is showing the results of experiment 3. If so, the focal rat was pulling for a neighbour that was always uncooperative (as per line 181 in the Methods), but that seems to contradict the wording within the caption. This discrepancy is quite confusion. For example, if this is experiment 3, the caption should not say "focal rats provided food to a cooperative partner" because the neighbour was always a stooge. Instead, it should say, "focal rats provided food to a stooge" or something along those lines. Also, the legend conditions should be labelled as: "olfactory information from an unseen cooperator" vs. "olfactory information from an unseen non-cooperator".

Response 36: Thanks for pointing this out. We have edited the legend to better reflect the experimental procedure. It now reads:

> "Focal rats provided food to a stooge earlier (a, b) and more often (c, d) while having access to smell of a cooperative individual (green lines and symbols) than they did to a stooge while receiving olfactory information of a non-cooperative individual (blue lines and symbols). This applies in the experience phase (a, c) and in the test phase (b, d)."

Appendix B

Dear Professor Loeske Kruuk

Thank you for accepting our manuscript for publication. We are grateful to you and the two reviewers for helping us improving the manuscript. We have clarified the supporting data and detail below our response to their comments.

Kind regards,

Nina Gerber, Manon Schweinfurth & Michael Taborsky

Original text in *italics* followed by our responses

Associate Editor:

Thanks, you for making your data accessible but more metadata would be useful. For instance, some of the variable names are in German, and the experiment numbers differ from the ones reported in the paper (e.g. there is one file labelled Experiment5_results even though there are only 4 experiments in the paper). It would not only be wise to consider the replicability of your analyses, but also the potential use of your data for future scientists wanting to test different hypotheses. For this good metadata documentation is essential.

Thanks for checking the data files. We have now cleaned the data so it only includes variables explained in the manuscript and renamed the files to match the experiment numbers from the main text. We have also added a readme file with contact details as well as a short description of the variable names, which are all in English now. Further, the data is now published on Dryad doi: https://doi.org/10.5061/dryad.h18931zjd

Referee: 1

Comments to the Author(s).
The authors have responded to all my comments in great detail and argued their points well. I didn't realize that the chemical analysis would take so long, and I would actually suggest that the authors mention this in the paper (Line 512 would be the obvious place to do so). I also appreciate the new paragraphs discussing the physical activity explanation and the synergistic mutualism game. I think the paper now stands well on its own, and even though the "smell of cooperation" is still an exceptional claim, I would agree that this study could inspire many others to follow up on these results and focus more on the role of communication in cooperation more broadly. In that sense, the results of the experiments are well worth publishing, and I would close with another maxim: "the product of scientific research is the data, the paper is merely an advertisement"

Thank you very much for your positive feedback, we are grateful to your comments which really helped to improve the quality of the advertisement of our data! We have added a sentence on line 513:

> “Identifying these chemical components is not trivial but will help to shed light on the exact mechanisms in the behavioural response we observe.”

minor comments:
isn't "sequential iterated prisoner's dilemma" a bit redundant? i would think iterated alone is sufficient?

The term *"sequential iterated prisoner's dilemma"* seems redundant, but since we refer to a sequential game that is iterated, we think that the term is correct.

line 304: "frailty term" = random effect

Thanks! We agree that the term random effect will be easier to understand.