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Learning twice the same motor task impairs its retention in a time- and dose-dependent manner

R. Hamel, L. Dallaire-Jean, É. De La Fontaine, J. F. Lepage and P. M. Bernier

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Original submission:	6 February 2020
1st revised submission:	16 October 2020
2nd revised submission:	7 December 2020
Final acceptance:	11 December 2020

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

Review History

RSPB-2020-0261.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?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
RSPB-2020-0261

The authors describe an intriguing and compelling series of experiments that document deficits in retention of a visuomotor adaptation reaching task that emerge after short, but not long, intervening delays between the same learning task. This is really interesting and may have implications about how we think about learning, interference, and the neural processes that underly both.

Typically interference in motor learning tasks is assessed using competing learning tasks, for example clockwise and then counterclockwise visuomotor rotations or in other studies, robot-imposed force fields. Here apparent anterograde interference is observed after learning the same visuomotor adaptation task, but only when the intervening delay is short (2 min). Retention impairments were not seen with longer delays (1 hour or 24 hours).

The authors conducted a second study in which retention after three learning sessions was compared to that after two, or one learning session, with 2 min intervening delays. They found a dose-dependent increase in the retention impairment as the number of learning sessions (of the same task) increased. A very nice built-in feature of the second experiment was a replication of (part of) the first experiment.

Taken together the experiments provide a compelling case that there may be a refractory period after initial learning, that impairs subsequent retention. The authors relate their results to evidence in the literature that document the potential neurophysiological and cellular/molecular bases of this effect.

The experiments are well designed, and the sample sizes are adequate. The paper is well written and the experimental approach is standard in the field. The analyses of the experimental data are well described and are appropriate. For the most part the interpretation of the results follows logically from the results (see below for some further comments on this point). The implications of the study are significant, to the extent that they may change the way we think about the neural bases of interference in motor learning, or even learning more generally.

Weaknesses/Comments/Suggestions

While repeated exposure to the same visuomotor rotation appeared to impair retention, it did not appear to affect the re-learning of the rotation itself (see Figs 2A vs 2B vs 2C, & Figure 3-B). I wonder if the authors have thought about this, and whether this is an important or informative aspect of the results.

The authors perform a number of analyses aimed at ruling out the possibility that some kind of fatigue, whether physical or attentional, might underlie the retention impairment after a 2 min interval. While these control tests are relatively convincing, what would be more convincing is a control group that performed an equivalent number of baseline reaches (no adaptation) followed by the same 2 min interval, followed by the experimental sequence (baseline/acquisition/retention).

There are a large number of cited references (75) I wonder if the authors could pare this down.

The primary effect of interest, the reductions in retention, may seem small in absolute terms, but they are statistically reliable, and what's more, the dose-dependent effect shown in the second experiment adds some degree of certainty that the experimental effect is reliable and reproducible. In the Results section the authors should report means and standard deviations or standard errors.

In Figure 3B, it is clear that on average, performance in the retention block drops off faster after the second session and even faster after the third session. What's strange however is it seems (by visual inspection) that after the third session, performance in the first half of the retention block (no visual feedback) drops and then rebounds just before the onset of the washout period, such that at the beginning of the washout period, performance is the same for all three curves. I wonder if the authors can comment on this.

In the Discussion the authors write that "Globally, the results of Experiment #1 suggest that learning twice the same task can induce temporally graded anterograde interference." I wonder if we really can conclude that it is *learning* the second time that is responsible for the retention decrement, versus, for example, the passage of time, or repeated arm movements of any kind for the equivalent time period. It would seem to me that in the absence of one or more control experiments it's difficult to really conclude that it was *learning* per se that is responsible here. I would be interested in the authors' thoughts on this point and whether there is an argument based on the current experimental design and the data in hand that can counter this concern.

Finally there is a paper by Hotermans et al (2006)—see below for reference—that would seem to be relevant to the present findings. In a finger sequencing task they found a transient *boost* in performance shortly after initial learning, an effect that was not present with longer intervals between initial learning and retention testing. There are many differences between their finger sequencing task and the reaching task tested here, but nevertheless it is likely worth discussing.

Hotermans, C., Peigneux, P., de Noordhout, A. M., Moonen, G., & Maquet, P. (2006). Early boost and slow consolidation in motor skill learning. Learning & memory, 13(5), 580-583.

Review form: Reviewer 2

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

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

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

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

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

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

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
Yes

Comments to the Author
In this study, the authors tried to demonstrate that the anterograde interference in motor adaptation is caused by a refractory period that occludes neuroplasticity. To investigate this hypothesis, they had participants perform the same motor adaptation experiment twice with various time intervals. They found that only when the two experiments were performed with a short interval (2 min), the retention of motor memory was weaker in the second experiment. Furthermore, the retention was further impaired when the 3rd experiment was performed with the short interval. I think that the authors' hypothesis is interesting, but I have several significant concerns mostly for the statistical test part.

Major points
1) The authors reported that the retention was impaired only when the second motor adaptation session was performed 2 min after the first session. I wondered how strongly the results supported this conclusion. As long as I looked at Figure 1, the data during the retention phase were largely overlapped for all three experimental conditions. I was also surprised by the results that the data in the acquisition phase was significantly different between 2 sessions for 1h and 24h groups. The difference in Figure 2 also looked quite small.
As a statistical test, the authors firstly demonstrated that there were significant interactions in 3-way and 2-way ANOVA. Then, they used an ordinary paired t-test between 1st and second sessions for each group. I do not believe this is a standard procedure. When there is a significant 3-way interaction, a simple 2-way interaction needs to be tested. Additionally, if there is a 2-way interaction, a simple or simple main effect should be calculated. The authors need to test if the impairment of retention for the 2min group (and the enhanced acquisition for 1h and 24h groups)

is still significant by following the standard statistical procedure.

2) If the authors' hypothesis was correct, the acquisition should have also been impaired. Any comment on why only the retention was impaired?

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Methods: It was hard to know how many subjects participated in Exp.1 and Exp. 2. I know the information is in the Introduction, but it should also be described in the Methods section.

Decision letter (RSPB-2020-0261.R0)

13-Mar-2020

Dear Mr Hamel:

I am writing to inform you that your manuscript RSPB-2020-0261 entitled "Initial learning anterogradely impairs retention of the same motor task in a time- and dose-dependent manner" has, in its current form, been rejected for publication in Proceedings B.

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

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

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

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2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.

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

Sincerely,
Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
We have now heard from two experts in the field. Although both reviewers recognize the importance of your study, they have raised significant concerns. Given their concerns, I am recommending rejection of your manuscript at this stage. I would encourage you, however, to re-submit a revised version that deals with the comments of the reviewers. There appears to be some concern with the statistical analysis -- and the need for a control group that deals with the issue of fatigue.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
RSPB-2020-0261

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Referee: 2

Comments to the Author(s)
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the short interval. I think that the authors' hypothesis is interesting, but I have several significant concerns mostly for the statistical test part.

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Methods: It was hard to know how many subjects participated in Exp.1 and Exp. 2. I know the information is in the Introduction, but it should also be described in the Methods section.

Author's Response to Decision Letter for (RSPB-2020-0261.R0)

See Appendix A.

RSPB-2020-2556.R0

Review form: Reviewer 2

Recommendation
Accept as is

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

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

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

Is the length of the paper justified?
Yes

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

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

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

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
Yes

Comments to the Author
I am very satisfied with the revision that the authors made. I also very like the additional experiment examining the effect of fatigue.

Review form: Reviewer 3

Recommendation
Reject – article is not of sufficient interest (we will consider a transfer to another journal)

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?
Marginal

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

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The novel idea of the experiments that are detailed in this manuscript is that anterograde interference may arise from an impairment in learning, one that is present not just in A-B scenarios in which B and A are opposed to each other, but even in A-A scenarios in which A is relearned following a passage of time after A. To test this idea, the authors trained people in the A-A protocol, and varied the amount of time between the two sessions. There were two main findings: performance gain from A1 to A2 was somewhat larger during re-learning when more time was allowed to pass between the two learning events. Furthermore, the decay in performance appeared to be greater in the re-learning episode when the time interval was short.
Major concerns
I am unsure about the strength of the main results. If learning is impaired with short passage of time, one should see an effect on the asymptote of performance during acquisition: the change from A1 (initial training) to A2 (re-training) should be small for small passage of time, and large as the time becomes longer. The data plotted in Fig. 2, left column, does not provide a clear indication of this. Furthermore, I think what is provided in the acquisition bar plot is an average over all the acquisition trials (though I am unsure of this). A better measure would be the performance during the asymptote phase of the experiment.
During the no-vision period (termed retention), performance decayed. The data on the right column of Fig. 2 suggest that retention was impaired at 2 min, but not at 1hr and 24hr. (I did not find the information on what trials are included in the bar plots). However, because the start of the curves may be different, what should be compared is the rate of this decay, and not the absolute value.
If indeed the decay rate is different, that would be an interesting finding, but I am unclear as to how this finding is related to the main hypothesis, which asks about the nature of the learning impairment during anterograde interference. If learning is impaired, then shouldn't the asymptote be different? On the other hand, if retention is impaired, then similarly the asymptote should again be smaller during re-training. However, the asymptote is not smaller during retraining.
The results of Exp. 2 are clear, demonstrating that repeated training in short temporal distance seems to accelerate the rate of decay during the retention period. This remains an interesting finding of the manuscript.

Decision letter (RSPB-2020-2556.R0)

27-Nov-2020

Dear Mr Hamel:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

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When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-

policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
We have now heard from two reviewers, one who read the original manuscript and a new reviewer. I am afraid that the some concerns have been raised by the new reviewer that I believe need to be addressed. This reviewer seems again concerned with the lack of an effect on the late stages of acquisition.

Reviewer(s)' Comments to Author:

Referee: 3
Comments to the Author(s).
The novel idea of the experiments that are detailed in this manuscript is that anterograde interference may arise from an impairment in learning, one that is present not just in A-B scenarios in which B and A are opposed to each other, but even in A-A scenarios in which A is relearned following a passage of time after A. To test this idea, the authors trained people in the

A-A protocol, and varied the amount of time between the two sessions. There were two main findings: performance gain from A1 to A2 was somewhat larger during re-learning when more time was allowed to pass between the two learning events. Furthermore, the decay in performance appeared to be greater in the re-learning episode when the time interval was short.
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Referee: 2
Comments to the Author(s).
I am very satisfied with the revision that the authors made. I also very like the additional experiment examining the effect of fatigue.

Author's Response to Decision Letter for (RSPB-2020-2556.R0)

See Appendix B.

Decision letter (RSPB-2020-2556.R1)

11-Dec-2020

Dear Mr Hamel

I am pleased to inform you that your manuscript entitled "Learning twice the same motor task impairs its retention in a time- and dose-dependent manner" 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.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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,
Dr Sasha Dall
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
Thank you for providing clear responses to the concerns raised by the reviewer. I enjoyed reading your revised manuscript. Congratulations.

Appendix A

13-Mar-2020

Dear Mr Hamel:

I am writing to inform you that your manuscript RSPB-2020-0261 entitled "Initial learning anterogradely impairs retention of the same motor task in a time- and dose-dependent manner" has, in its current form, been rejected for publication in Proceedings B.

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

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

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

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

Dr Sasha Dall
mailto: proceedingsb@royalsociety.org
Associate Editor
Board Member: 1
Comments to Author:
We have now heard from two experts in the field. **Although both reviewers recognize the importance of your study**, they have raised significant concerns. Given their concerns, I am recommending rejection of your manuscript at this stage**. I would encourage you, however, to re-submit a revised version that deals with the**

comments of the reviewers. There appears to be some concern with the statistical analysis -- and the need for a control group that deals with the issue of fatigue.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
RSPB-2020-0261

The authors describe an intriguing and compelling series of experiments that document deficits in retention of a visuomotor adaptation reaching task that emerge after short, but not long, intervening delays between the same learning task. This is really interesting and may have implications about how we think about learning, interference, and the neural processes that underly both.

Typically interference in motor learning tasks is assessed using competing learning tasks, for example clockwise and then counterclockwise visuomotor rotations or in other studies, robot-imposed force fields. Here apparent anterograde interference is observed after learning the same visuomotor adaptation task, but only when the intervening delay is short (2 min). Retention impairments were not seen with longer delays (1 hour or 24 hours).

The authors conducted a second study in which retention after three learning sessions was compared to that after two, or one learning session, with 2 min intervening delays. They found a dose-dependent increase in the retention impairment as the number of learning sessions (of the same task) increased. **A very nice built-in feature of the second experiment was a replication of (part of) the first experiment**.

Taken together the experiments provide a compelling case that there may be a refractory period after initial learning, that impairs subsequent retention. The authors relate their results to evidence in the literature that document the potential neurophysiological and cellular/molecular bases of this effect.

The experiments are well designed, and the sample sizes are adequate. **The paper is well written and the experimental approach is standard in the field**. The analyses of the experimental data are well described and are appropriate. For the most part the interpretation of the results follows logically from the results (see below for some further comments on this point). The implications of the study are significant, to the extent that they may change the way we think about the neural bases of interference in motor learning, or even learning more generally.

Weaknesses/Comments/Suggestions

While repeated exposure to the same visuomotor rotation appeared to impair retention, **it did not appear to affect the re-learning of the rotation itself** (see Figs 2A vs 2B vs 2C, & Figure 3-B). I wonder if the authors have thought about this, and whether this is an important or informative aspect of the results.

This aspect of the results had indeed been considered. It is also of our opinion that the unimpaired acquisition (2nd session) of the 2 min group is also informative that processes conceptually similar to the posited

occluded neuroplastic capabilities were at play. The following bit of discussion on this matter has been added to the discussion from lines 271 to 282:

"First, although the impairments were only observed during retention, the lack of acquisition enhancements when ISIs were of 2 min may constitute the flip side of the retention impairments, thus also suggestive of anterograde interference. Recently, Della-Maggiore et al. (2020; ref (6)) showed that the initial acquisition of A may interfere with the subsequent acquisition of B by transiently decreasing error sensitivity (< 1h), thus temporarily reducing the brain's subsequent learning capabilities. Moreover, human TMS studies have related such acquisition impairments of A over B to transiently (< 6h) occluded neuroplastic capabilities (7,25). Hence, an interesting contention is that the present lack of acquisition enhancements of A → A when the ISIs were of 2 min could be imputed to occluded neuroplastic capabilities. However, the notion of occlusion is unlikely to solely account for the present retention impairments, which indicate a reversal (forgetting) – rather than an occlusion (saturation) – of the induced neuroplastic changes during learning. Hence the current results extend these previous lines of work by showing that initial learning does not only occlude subsequent acquisition capabilities but also impairs the mechanisms of memory formation (see below for mechanistic explanations)."

Also see lines 283 to 288 for additional discussion of how the acquisition enhancements relate to the classic "savings effect":

"Second, despite controlling for acquisition rates with the gradual introduction of the deviation, the 1h and 24h Groups showed enhancements of subsequent acquisition (see (6) for similar results). Such a result is reminiscent of classic savings (defined as a faster relearning upon reexposure to a perturbation; ref (36)). However, since savings are thought to stem from explicit learning processes (37) and that none of the 100 participants consciously perceived the visual deviation – suggesting that the task was predominantly implicit – the similarity of the mechanisms that underlie the current results vs. those that mediate classic savings remains an open question."

The authors perform a number of analyses aimed at ruling out the possibility that some kind of fatigue, whether physical or attentional, might underlie the retention impairment after a 2 min interval. While these control tests are relatively convincing, **what would be more convincing is a control group that performed an equivalent number of baseline reaches** (no adaptation) followed by the same 2 min interval, followed by the experimental sequence (baseline/acquisition/retention).

The reviewer pointed out a highly relevant concern which, once answered, has the potential to greatly increase the convincingness of the current results. For that purpose, as the reviewer suggested, a third experiment was collected in which 20 participants took part in two experimental visits, separated by a week, at the same time of day, and in a counterbalanced order (see Figure 4 below). The rationale, results, and implications of this additional experiment are now part of the manuscript.

Briefly, the results suggest that the emergence of fatigue cannot account for the observed retention impairments since a Practice Session (comprising only baseline reaches) followed by a 2 min ISI did not influence subsequent retention capabilities (Practice-Preceded Session) as compared to a learning session that was not preceded by such a Practice Session (Single Session). These results suggest that an initial learning session is required to impair subsequent retention capabilities. This implies that the mechanisms mediating these impairments must interact with – or oppose – those involved in learning.

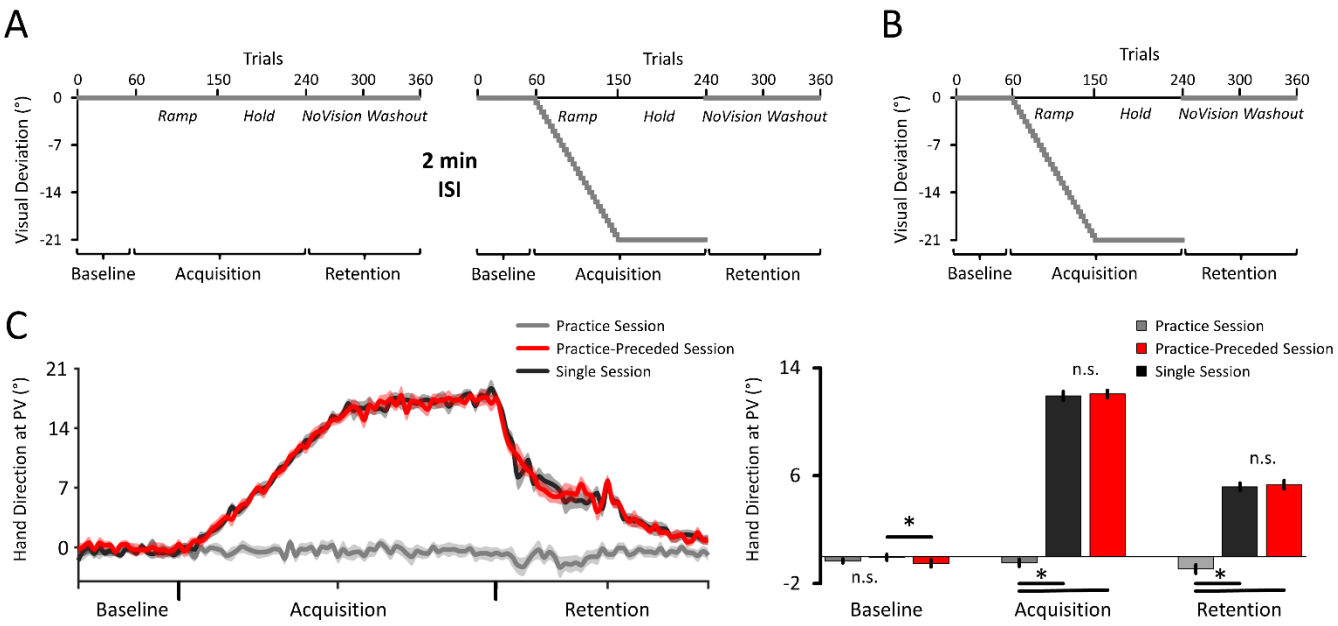

Figure 4. Procedures and reaching performance of Experiment #3. (A) Overview of the Practice (*left panel*) and Practice-Preceded Sessions (*right panel*). The sessions were identical to those of the previous experiments, with the exception that no visual deviation was introduced in the Practice Session to cause the accumulation of fatigue without concurrent learning. These sessions were separated by a 2 min ISI. (B) Overview of the Single Session. This session is also identical to those used in previous experiments. (C) *Left Panel:* Hand Direction at PV for the three sessions. *Right Panel:* Averaged Hand Direction at PV, shown separately for each phase of each session. Breakdown of a two-way interaction revealed that neither Acquisition nor Retention differed between the Practice-Preceded and Single Sessions (both Cohen's dz < 0.140; negligible effect sizes). For C, error bars represent within-subject 95% CIs. Asterisks (*) indicate significant differences ($p < 0.05$).

These results are now discussed from lines 323 to 333:

"The possibility that the accumulation of fatigue – whether physical or attentional – could also account for the present results was examined by a series of additional analyses (see Supplemental Material) and by a third experiment. In the three experiments, the additional analyses revealed no evidence of fatigue in RT, MT, and accuracy at movement endpoint, as these variables either remained stable or improved across sessions. Moreover, the additional results also revealed that similar levels of adaptation were reached by the end of the Acquisition phase in all experiments, which further suggests that fatigue did not emerge. More importantly, the results of the third experiment revealed that practicing in the absence of concurrent learning – with the intent of causing fatigue

accumulation – did not impair subsequent retention capabilities. While these results suggest that fatigue can be ruled out as an alternative interpretation, they also indicate that the retention impairments are learning-specific. This implies that the mechanisms mediating these impairments must interact with – or oppose – those involved in learning (see below)."

There are a **large number of cited references** (75) I wonder if the authors could pare this down.

The number of citations has been reduced to 58.

The primary effect of interest, the reductions in retention, may seem small in absolute terms, but they are statistically reliable, and what's more, the dose-dependent effect shown in the second experiment adds some degree of certainty that the experimental effect is reliable and reproducible. In the Results section the authors should **report means and standard deviations or standard errors**.

To answer this concern, we have added tables (one for each of the three Experiments) that report descriptive statistics of the data used in all Experiments to the manuscript. We reasoned that tables might be better suited than in-text statistics to keep the text as light as possible while remaining as thorough and transparent as possible concerning the data analyzed. To remain consistent with the data presented within the Figures, means ± 95% within-subject confidence intervals are now reported in the tables.

However, because the revised version of the manuscript exceeded the length authorized by the Journal, these tables – along with additional methodological details and results – had to be added to the Supplemental Material section. The reference to these tables is mentioned in the results section (lines 165 to 166 for Exp#1, lines 191 to 192 for Exp #2, and lines 228 to 229 for Exp #3). Moreover, the data set of all of our experiments has also been made freely available for others to replicate our analyses (https://gofile.io/d/qXd7u8). This data availability statement is now part of the manuscript on lines 160 to 161.

In Figure 3B, it is clear that on average, performance in the retention block drops off faster after the second session and even faster after the third session. What's strange however is it seems (by visual inspection) that **after the third session, performance in the first half of the retention block (no visual feedback) drops and then rebounds just before the onset of the washout period**, such that at the beginning of the washout period, performance is the same for all three curves. I wonder if the authors can comment on this.

This pattern of results is also something that came to our attention, and we are uncertain as to what it means. To the best of our knowledge, at least two non-exclusive possibilities may explain this pattern of results: (1) spontaneous recovery of the formed memory or (2) memory reemergence conditioned by contextual cues.

On the one hand, a spontaneous recovery of adapted reaching behaviors by the end of the third NoVision phase in Experiment #2 would resemble the spontaneous recovery of a previously extinguished pavlovian (Rescorla, 2004) or fear memory (Schiller et al., 2008). Assuming that sensorimotor memories share features of pavlovian memories, performance during the NoVision phase (extinction of adapted reaching behaviors) may be driven by inhibitory rather than "erasing" mechanisms (Rescorla, 2004). This suggestion implies that performance changes during NoVision do not reflect a passive forgetting of the memory but rather reflect an active mechanism

inhibiting the expression of the formed memory during Acquisition. As such, a possibility is that, as the NoVision phase proceeds, these active inhibitory mechanisms dissipate, thus allowing the formed memory to re-emerge. This memory reemergence may manifest as performance rebounds during the second half of the NoVision phase. However, if such a mechanism is really at play, then one might expect this pattern of result to be consistent across the 2nd and 3rd NoVision phases and not just during the 3rd one, which the results ostensibly fail to support. Moreover, this interpretation is hindered by the apparent lack of comprehension of the mechanisms driving deadaptation during NoVision trials. As a result, whether this interpretation can accommodate the present data remains a query for future studies.

On the other hand, nearing the end of the third NoVision phase may have acted as a contextual cue causing the emergence of an internal state that drove behavioral performance towards that which was acquired when vision was provided (during the Acquisition phase; see Hirashima and Nozaki, 2012 and Nozaki et al., 2016). Namely, participants relied on their cursor's vision to adapt to the visual deviation, which may have caused the newly formed memory to be tagged with vision. By the third session, participants may have been expecting vision of the cursor to be reinstated after ~40 to 60 trials (3 to 5 minutes) upon the onset of the first NoVision trial. As such, one possibility is that expecting the cursor's vision to soon be reinstated may have acted as a contextual cue which prompted the emergence of the memory tagged with the cursor's vision (memory of adapted reaching behaviors). This possibility could explain the performance rebound observed in the second half of the NoVision phase. Moreover, this interpretation would fit nicely with the data of Nozaki et al. (2016), which showed that modifying the neural state of the primary motor cortex with tDCS during acquisition can effectively act as a contextual cue prompting the flexible retrieval of sensorimotor memories. Whether this interpretation can accommodate the present data also remains a query for future studies.

Given the highly speculative nature of these possibilities, we elected not to add them to the paper.

- Rescorla, R. A. (2004). Spontaneous recovery. *Learning & Memory*, *11*(5), 501-509.
- Schiller, D., Cain, C. K., Curley, N. G., Schwartz, J. S., Stern, S. A., LeDoux, J. E., & Phelps, E. A. (2008). Evidence for recovery of fear following immediate extinction in rats and humans. *Learning & Memory*, *15*(6), 394-402.
- Hirashima, M. & D. Nozaki. 2012. Distinct motor plans form and retrieve distinct motor memories for physically identical movements. Curr. Biol. 22: 432–436.
- Nozaki, D., A. Yokoi, T. Kimura, et al. 2016. Tagging motor memories with transcranial direct current stimulation allows later artificially-controlled retrieval. eLife 5: e15378.

In the Discussion the authors write that "Globally, the results of Experiment #1 suggest that learning twice the same task can induce temporally graded anterograde interference." I wonder if we really can conclude that it is *learning* the second time that is responsible for the retention decrement, versus, for example, **the passage of time, or repeated arm movements of any kind for the equivalent time period.** It would seem to me that in the absence of one or more control experiments **it's difficult to really conclude that it was *learning* per se that is responsible here**. I would be interested in the authors' thoughts on this point and whether there is an argument based on the current experimental design and the data in hand that can counter this concern.

This is a very valid point made by the reviewer and this concern was the initial reason as to why we conducted analyses to carefully rule out the possibility that the emergence of fatigue (or the passage of time) can

account for the retention impairments we observed. We are now confident that the results from Experiment #3, which revealed that retention is not impaired when preceded by a Practice Session comprising only baseline reaches (no learning), allow us to conclude that fatigue cannot account for the present results, and thus that *learning* per se is responsible for the retention impairment. These ideas are now part of the discussion from lines 323 to 333 (same paragraph as above):

"The possibility that the accumulation of fatigue – whether physical or attentional – could also account for the present results was examined by a series of additional analyses (see Supplemental Material) and by a third experiment. In the three experiments, the additional analyses revealed no evidence of fatigue in RT, MT, and accuracy at movement endpoint, as these variables either remained stable or improved across sessions. Moreover, the additional results also revealed that similar levels of adaptation were reached by the end of the Acquisition phase in all experiments, which further suggests that fatigue did not emerge. More importantly, the results of the third experiment revealed that practicing in the absence of concurrent learning – with the intent of causing fatigue accumulation – did not impair subsequent retention capabilities. While these results suggest that fatigue can be ruled out as an alternative interpretation, they also indicate that the retention impairments are learning-specific. This implies that the mechanisms mediating these impairments must interact with – or oppose – those involved in learning (see below)."

Finally there is a paper by Hotermans et al (2006)—see below for reference—that would seem to be relevant to the present findings. In a finger sequencing task they found **a transient *boost* in performance shortly after initial learning**, an effect that was not present with longer intervals between initial learning and retention testing. There are many differences between their finger sequencing task and the reaching task tested here, but nevertheless it is likely worth discussing.

Hotermans, C., Peigneux, P., de Noordhout, A. M., Moonen, G., & Maquet, P. (2006). Early boost and slow consolidation in motor skill learning. Learning & memory, 13(5), 580-583.

We thank the reviewer for this relevant citation. This article is now part of the discussion from lines 288 to 298:

"Interestingly, the enhanced acquisition for both the 1h and 24h ISIs is not entirely incompatible with results from Hotermans et al. (2006; ref (38)), who revealed a short-lived performance boost – akin to savings – emerging after a break of 5 min, 30 min, and 24h – but not of 4h – following the explicit learning of a finger sequence press task. It should be noted, however, that since the mechanisms of memory formation between explicit sequence learning and implicit motor adaptation likely differ (39), the ISIs used by Hotermans et al. (2006; ref (38)) may not directly map onto the present ones. Furthermore, the occurrence of such boost has not been corroborated by others (40) and has yet to be replicated. Nevertheless, these evidence suggest that a sufficiently long ISI – which duration may depend on the task demand (task complexity and requirements, number of muscles involved, extent and overlap

of the neural structures involved, presence or absence of overlearning, etc.) – is crucial for performance to improve during a subsequent learning session."

Referee: 2

Comments to the Author(s)

In this study, the authors tried to demonstrate that the anterograde interference in motor adaptation is caused by a refractory period that occludes neuroplasticity. To investigate this hypothesis, they had participants perform the same motor adaptation experiment twice with various time intervals. They found that only when the two experiments were performed with a short interval (2 min), the retention of motor memory was weaker in the second experiment. Furthermore, the retention was further impaired when the 3rd experiment was performed with the short interval. I think that the authors' hypothesis is interesting, but I have several significant concerns mostly for the statistical test part.

Major points

1) The authors reported that the retention was impaired only when the second motor adaptation session was performed 2 min after the first session. I wondered how strongly the results supported this conclusion. As long as I looked at Figure 1, the data during the retention phase were largely overlapped for all three experimental conditions. I was also surprised by the results that the data in the acquisition phase was significantly different between 2 sessions for 1h and 24h groups. **The difference in Figure 2 also looked quite small**.

As a statistical test, the authors firstly demonstrated that there were significant interactions **in 3-way and 2-way ANOVA**. Then, they used an ordinary paired t-test between 1st and second sessions for each group. I do not believe this is a standard procedure. When there is a significant 3-way interaction, **a simple 2-way interaction needs to be tested**. Additionally, if there is a 2-way interaction, a simple or simple main effect should be calculated. The authors need to test if the impairment of retention for the 2min group (and the enhanced acquisition for 1h and 24h groups) is still significant by following the standard statistical procedure.

To inform the readers about the magnitude of the differences, the effect sizes of the key differences have been added to the Figure captions (where appropriate; the manuscript has been accordingly amended). Namely, a well-suited way to evaluate the magnitude of the differences is to consider the effect sizes rather than the absolute values (Lakens, 2013). Namely, when the effect sizes are integrated into the appreciation of the difference magnitudes, one can easily notice that the key differences sit somewhere between the medium to large benchmark values (Cohen's dz > 0.6). This suggests that the presented differences are closer to large magnitudes than small ones.

Concerning the second portion of the reviewer's concern, our approach appears to be standard according to Sawyer (2009). Moreover, as argued by Roberts and Russo (1999), higher-level interactions (three-way) supersede lower-level effects (both two-way interactions and main effects). We opted to break down the three-way interaction by conducting two-way ANOVAs on each level of the moderating factor (between-subject Group factor). Concerning this issue, clarifications have been brought to the results section on lines 165 to 170:

"Behavioral data from Experiment #1 are presented in Figure 2. The descriptive statistics of the data used in the following analyses are reported in Table 1 (see Supplemental Material). A three-way ANOVA conducted on Hand Direction at PV revealed a three-way interaction ($F_{(4,110)} = 2.652$, $p = 0.050$, $n_p^2 = 0.088$), which was broken down by conducting two-way ANOVAs on each level of the moderating Group factor. Namely, subsequent 2

Sessions X 3 Phases ANOVAs revealed two-way interactions for each level of the Group factor (all F > 7.414, all p < 0.002, all n_p^2 > 0.292)."

As encouraged by Sawyer (2009), each significant two-way interaction was broken down by conducting post-hoc t-tests corrected for multiple comparisons. Clarifications concerning this aspect and the citation of Sawyer (2009; ref 34) have been added to the manuscript (lines 151 to 152):

"Posthoc pairwise tests were conducted to break down significant two-way interactions and main effects (34)."

- Sawyer, S. F. (2009). Analysis of variance: the fundamental concepts. *Journal of Manual & Manipulative Therapy*, *17*(2), 27E-38E.
- Russo, R. (1999). *A student's guide to analysis of variance*. Routledge.
- Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology*, *4*, 863.

2) If the authors' hypothesis was correct, **the acquisition should have also been impaired**. Any comment on why only the retention was impaired?

This concern has also been raised by Reviewer #1. The following bit of discussion on this matter has been added to the discussion from lines 271 to 282:

"First, although the impairments were only observed during retention, the lack of acquisition enhancements when ISIs were of 2 min may constitute the flip side of the retention impairments, thus also suggestive of anterograde interference. Recently, Della-Maggiore et al. (2020; ref (6)) showed that the initial acquisition of A may interfere with the subsequent acquisition of B by transiently decreasing error sensitivity (< 1h), thus temporarily reducing the brain's subsequent learning capabilities. Moreover, human TMS studies have related such acquisition impairments of A over B to transiently (< 6h) occluded neuroplastic capabilities (7,25). Hence, an interesting contention is that the present lack of acquisition enhancements of A → A when the ISIs were of 2 min could be imputed to occluded neuroplastic capabilities. However, the notion of occlusion is unlikely to solely account for the present retention impairments, which indicate a reversal (forgetting) – rather than an occlusion (saturation) – of the induced neuroplastic changes during learning. Hence the current results extend these previous lines of work by showing that initial learning does not only occlude subsequent acquisition capabilities but also impairs the mechanisms of memory formation (see below for mechanistic explanations)."

3) The **absence of acquisition enhancement for the 2min group** did not contradict the saving effect that the motor adaptation is faster for the 2nd session?

This is a relevant concern. As stated above (Concern #2), we believe that the ostensible lack of classical savings (lack of acquisition enhancements) is in line with our interpretation of occluded neuroplastic capabilities (impaired subsequent learning capabilities).

Moreover, as can be read in the response to the above comment, the presence of savings in the present data is now part of the discussion from lines 282 to 288:

"Second, despite controlling for acquisition rates with the gradual introduction of the deviation, the 1h and 24h Groups showed enhancements of subsequent acquisition (see (6) for similar results). Such a result is reminiscent of classic savings (defined as a faster relearning upon reexposure to a perturbation; ref (36)). However, since savings are thought to stem from explicit learning processes (37) and that none of the 100 participants consciously perceived the visual deviation – suggesting that the task was predominantly implicit – the similarity of the mechanisms that underlie the current results vs. those that mediate classic savings remains an open question."

Minor points

Line 102: The radius of the outer annulus was correct? Considering the movement distance was 10 cm, **the radius of 5.15 cm seemed too large**.

We thank the reviewer for pointing out this mistake. The *diameter* (and not the radius) of the outer annulus was 5.15 cm. All the parameters concerning the experiment apparatus are now being reported in radiuses rather than in diameters to remain consistent with all the reported measures (see lines 13 to 16 of the Supplemental Material):

"The outer annulus had a radius of 2.575 cm, while that of the target was 0.63 cm. For all sessions, the three targets were located along a circular array of 10 cm radius at the following angles: 22.5°, 94.5°, and 130.5°. As a result, the required movement trajectories were of 10 cm in length. At the center of the workspace, a grey circle with a radius of 0.42 cm served as the starting point for every trial."

Lines 103-104: It would be helpful to describe the movement distance explicitly. The definition of angles of target positions is also necessary.

We agree this information would be helpful. This information has been added on lines 14 to 15 of the Supplemental Material:

"For all sessions, the three targets were located along a circular array of 10 cm radius at the following angles: 22.5°, 94.5°, and 130.5°."

Methods: It was hard to know how many subjects participated in Exp.1 and Exp. 2. I know the information is in the Introduction, **but it should also be described in the Methods section**.

We agree this is a relevant concern. The following information has been added to lines 91 to 93 of the methods in the manuscript:

“Specifically, three groups of 20 individuals and one group of 20 individuals took part in Experiment #1 and #2, respectively. An additional group of 20 individuals took part in Experiment #3.”

Appendix B

Referee: 3

Comments to the Author(s).

The novel idea of the experiments that are detailed in this manuscript is that anterograde interference may arise from an impairment in learning, one that is present not just in A-B scenarios in which B and A are opposed to each other, but even in A-A scenarios in which A is relearned following a passage of time after A. To test this idea, the authors trained people in the A-A protocol, and varied the amount of time between the two sessions. There were two main findings: performance gain from A1 to A2 was somewhat larger during re-learning when more time was allowed to pass between the two learning events. Furthermore, the decay in performance appeared to be greater in the re-learning episode when the time interval was short.

Major concerns

I am unsure about the strength of the main results. If learning is impaired with short passage of time, one should see an effect on the asymptote of performance during acquisition: the change from A1 (initial training) to A2 (re-training) should be small for small passage of time, and large as the time becomes longer. The data plotted in Fig. 2, left column, does not provide a clear indication of this.

We agree with the reviewer that anterograde interference would be expected to lead to a reduced capacity to re-acquire the task in the second exposure as compared to the first. While we did not see any difference in Acquisition between the first and second Sessions for the 2 min Group (Fig. 2A), it should be noted that in the 1h and 24h Groups there was a significant enhancement in Acquisition in the second session as compared to the first one (both $t > 2.845$, both $p < 0.017$, both Cohen's dz > 0.653; Fig. 2B and 2C). Hence when looking at the pattern of results across groups in Acquisition (i.e., Acquisition was enhanced for the 1h and 24h Groups, but not the 2 min Group), these data are fully consistent with the pattern of results we get for Retention (i.e., Retention was impaired in the 2 min Group, but not the 1h and 24h Groups), in that they both support the notion that the capacity for new learning was reduced in the 2 min Group.

We had tried to convey this message in the original discussion ("First, although the impairments were only observed during retention, the lack of acquisition enhancements when ISIs were of 2 min may constitute the flip side of the retention impairments, thus also suggestive of anterograde interference"). However, we admit that this point was not made clearly enough in the paper, and we have now strived to make this more explicit in the results section. We notably added this sentence in lines 181 to 184 of the results section:

"Hence, when taken together, the pattern of results for Acquisition (i.e., enhanced in the 1h and 24h Groups, but not the 2 min Group) is fully compatible with that for Retention (i.e., impaired in the 2 min Group, but not the 1h and 24h Groups), in that they both point toward the notion that the capacity for new learning was reduced in the 2 min Group."

Also, concerning the effect on the asymptote of performance during Acquisition, novel analyses testing this possibility have been included in the supplementary material (see next comment).

As for the strength of the main results, we would like to point out that although the magnitudes of the effects we report are indeed of modest size, as Experiment #2 reveals, the results are replicable and robust. The moderate effect sizes are likely to be due to the relatively important statistical power of the present design, which reduces the likelihood of overinflated effect sizes (Algermissen and Mehler, 2018). The replication with an independent dataset is an important aspect of the paper, strongly suggesting that the results are indicative of a meaningful phenomenon.

Algermissen, J., & Mehler, D. M. (2018). May the power be with you: are there highly powered studies in neuroscience, and how can we get more of them? *Journal of neurophysiology*, *119*(6), 2114-2117.

Furthermore, I think what is provided in the acquisition bar plot is an average over all the acquisition trials (though I am unsure of this). A better measure would be the performance during the asymptote phase of the experiment.

We had originally conducted the analyses suggested by the reviewer but opted not to include them in the manuscript. Globally, similar results are obtained when conducting the statistical analyses on the average of the asymptote (Hold Phase) or the whole Acquisition phase (both Ramp and Hold phases). Given the qualitatively similar results, we reasoned that reporting Acquisition as a whole was more representative than only the asymptote. Nonetheless, as suggested by the reviewer, the analyses conducted on the average of the asymptote (Hold Phase) have now been added to the supplemental material:

Experiment #1; Lines 81 to 91:

"**The 2 min ISI – but not the 1h and 24h ISIs – prevented performance improvements during the asymptote**

To determine if groups reached similar levels of adaptation during the performance asymptote, the averaged performance of the Hold Phase (90 trials; 2nd half of the Acquisition Phase) of each session and for each Group was submitted to a 3 Groups X 2 Sessions ANOVA. Results revealed a Groups X Sessions interaction ($F_{(2,55)} = 5.370$, $p = 0.007$, $n_p^2 = 0.163$), a main effect of Sessions ($F_{(1,55)} = 11.445$, $p = 0.001$, $n_p^2 = 0.172$), but no effect of Groups ($F_{(2,55)} = 0.620$, $p = 0.542$, $n_p^2 = 0.022$). Pairwise comparisons revealed that performance did not meaningfully improve across sessions for the 2 min Group ($t_{(18)} = 0.405$, $p = 0.691$, Cohen's dz = 0.093). However, performance improved for both the 1h ($t_{(19)} = 2.239$, $p = 0.056$, Cohen's dz = 0.501) and 24h Groups ($t_{(18)} = 3.903$, $p = 0.001$, Cohen's dz = 0.895), indicating that participants reached a higher level of adaptation during their second session at asymptote. Globally, these results suggest that a 2 min ISI prevented – whereas 1h and 24h ISIs allowed – meaningful performance improvements during the second session's asymptote as compared to the first one."

Experiment #2; Lines 122 to 132:

"Adaptation levels tended to decrease during performance asymptote as a function of sessions

To determine if participants reached similar levels of adaptation during the performance asymptote of each session, the averaged performance of the Hold Phase (90 trials; 2nd half of the Acquisition Phase) of each session was submitted to a 3 Sessions ANOVA. The results revealed a marginal effect of Sessions ($F_{(2,38)} = 3.243$, $p = 0.065$, $n_p^2 = 0.146$), indicating that adaptation levels tended to decrease as a function of sessions. Pairwise comparisons revealed that adaptation levels did not meaningfully differ between the First and Second Sessions ($t_{(19)} = 0.882$, corrected $p = 0.389$, uncorrected $p = 0.389$, Cohen's dz = 0.197), which replicates the results from the 2 min Group in Experiment #1. Although it did not reach significance, performance tended to modestly decrease in the Third Session as compared to both the First ($t_{(19)} = 2.024$, corrected $p = 0.171$, uncorrected $p = 0.057$, Cohen's dz = 0.453) and Second Sessions (Wilcoxon's signed rank test; W = 140.0, corrected $p = 0.303$, uncorrected $p = 0.202$, Cohen's dz = 0.474). Globally, these results suggest that cumulating 3 learning sessions in a short time period tends to decrease adaptation levels as a function of sessions."

Experiment #3; Lines 208 to 214:

"Similar levels of adaptation during the performance asymptote

To determine if participants reached similar levels of adaptation during the performance asymptote of the Practice-Preceded and Single Sessions, the averaged performance of the Hold Phase (90 trials; 2nd half of the Acquisition Phase) of each session was submitted to a dependent t-test. The results revealed no meaningful difference between the two sessions ($t_{(19)} = 0.814$, $p = 0.426$, Cohen's dz = 0.182), suggesting that participants reached similar adaptation levels during asymptote. This indicates that fatigue did not differ between the Practice-Preceded and Single Sessions. This also indicates that learning capabilities were similar between the two sessions."

During the no-vision period (termed retention), performance decayed. The data on the right column of Fig. 2 suggest that retention was impaired at 2 min, but not at 1hr and 24hr. (**I did not find the information on what trials are included in the bar plots**).

The information of what trials are included in the bar plots was provided in both the main text (Methods section; lines 126 to 127) and Figure 1C. However, to further clarify, we added the following information in the caption of Figure 1:

"The average of each phase (Baseline, 60 trials; Acquisition, 180 trials; Retention, 120 trials) was calculated for the statistical analyses."

However, because the start of the curves may be different, what should be compared is the rate of this decay, and not the absolute value.

The possibility that the "start of the curves" differed before participants proceeded to the NoVision phase was already evaluated for each Session and reported in the Supplemental Results (see below). This analysis had revealed that adaptation levels were similar (did not meaningfully or significantly differ) between the sessions of each group in each experiment. This rules out that the reported differences in retention be attributable to differing levels of adaptation reached before proceeding to Retention.

The following information was already present in the Supplemental Material:

Experiment #1; lines 92 to 99:

"**All groups were adapted to a similar extent before proceeding to the Retention phase**

To determine if groups differed in their extent of adaptation by the end of Acquisition, the averaged performance of the last 15 trials of Acquisition was submitted to a 3 Groups X 2 Sessions ANOVA. Results revealed no effect of Sessions ($F_{(1,55)} = 0.193$, $p = 0.662$, $n_p^2 = 0.004$), no effect of Groups ($F_{(2,55)} = 0.425$, $p = 0.656$, $n_p^2 = 0.015$) and no Groups X Sessions interaction ($F_{(2,55)} = 2.297$, $p = 0.110$, $n_p^2 = 0.077$). This indicates that the pattern of results during Retention cannot be accounted for by differing extents of adaptation late in the Acquisition phase. Similar

levels of adaptation by the end of Acquisition also argues against the possibility that participants were more fatigued during the second session as compared to the first one."

Experiment #2; lines 133 to 138:

"Similar extent of adaptation before proceeding to Retention across all sessions

To determine if participants differed in their extent of adaptation late during Acquisition across the three sessions, the averaged performance of the last 15 trials of Acquisition was submitted to a unifactorial repeated measures ANOVA. Results revealed no effect of Sessions ($F_{(2,38)}$ = 1.671, p = 0.202, n_p^2 = 0.081), which indicates that the pattern of results during Retention cannot be accounted for by differing extents of adaptation late in the Acquisition phase. Similar levels of adaptation by the end of Acquisition also argues against the accumulation of fatigue."

Experiment #3; lines 217 to 222:

"Similar extent of adaptation before proceeding to Retention

To determine if participants differed in their extent of adaptation late during Acquisition across the Practice-Preceded and Single Sessions, the averaged performance of the last 15 trials of Acquisition was submitted to a dependent pairwise comparison. Results revealed no difference between the two Sessions ($t_{(19)}$ = 0.971, p = 0.344, Cohen's dz = 0.217), which indicates that participants reached a similar level of adaptation before proceeding to the Retention phase. Similar levels of adaptation at the end of Acquisition also argues against the accumulation of fatigue."

Concerning modeling the decay rates in the Retention data, we had already tried to fit nonlinear least-squares curves to the data (not reported in the manuscript or supplemental material). The results were inconclusive as the models had poor to moderate goodness of fit on the data (R^2; see below), which gave us low confidence that this was an appropriate way to evaluate retention impairments. We reasoned that averaging the data was a more conservative and representative way of assessing retention than analyzing the decay rates of models that unsatisfactorily fit the data (see also Canaveral et al., 2017; Galea et al. 2011). To support out point, the R^2 values of the nonlinear least-squares curves we tried to fit on the Retention data of Experiment #2 are presented below:

R^2 values ; Nonlinear Least-Squares Fit			
Participant	1st Session	2nd Session	3rd Session
1	0,715	0,633	0,543
2	0,398	0,118	0,297
3	0,694	0,529	0,663
4	0,625	0,528	0,472
5	0,740	0,650	0,666
6	0,585	0,479	0,257
7	0,724	0,634	0,352
8	0,640	0,476	0,683
9	0,740	0,448	0,485
10	0,501	0,683	0,736
11	0,720	0,663	0,403
12	0,566	0,360	-0,017
13	0,439	0,336	0,257
14	0,510	0,530	0,537
15	0,716	0,717	0,621
16	0,787	0,735	0,648
17	0,730	0,385	0,038
18	0,566	0,216	0,017
19	0,814	0,760	0,348
20	0,778	0,541	0,757

Canaveral, C.A., Danion, F., Berrigan, F., Bernier, P-M. (2017). Variance in exposed perturbations impairs retention of visuomotor adaptation. *Journal of Neurophysiology.*118:2745-2754.

Galea JM, Vazquez A, Pasricha N, Orban de Xivry JJ, Celnik P. (2011). Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cereb Cortex* 21: 1761–1770.

If indeed the decay rate is different, that would be an interesting finding, but I am unclear as to how this finding is related to the main hypothesis, which asks about the nature of the learning impairment during anterograde interference. **If learning is impaired, then shouldn't the asymptote be different**? On the other

hand, if retention is impaired, **then similarly the asymptote should again be smaller during re-training**. However, the asymptote is not smaller during retraining.

This work hypothesized that anterograde interference would be observed in an A → A paradigm, would be temporally graded, and would be dose-dependent. To prevent conscious awareness and conduct a within-subject design allowing to optimize statistical power, we implemented a gradually introduced visual deviation. While this design was optimal to test for retention effects (which was our main goal), a ramp protocol arguably limited our capacity to observe performance decrements during Acquisition (unlike gradual protocols, see Lerner et al., 2020, for instance).

That being said, as mentioned in an earlier point, we still think that the pattern of results observed across groups in Acquisition is fully consistent with that observed in Retention, and supports the main claim of the paper that initial learning transiently perturbs the homeostasis of learning-related neuronal substrates and that introducing additional learning while homeostasis remains perturbed is counter-productive for memory formation.

As also mentioned before, we have strived to clarify this aspect in the results section on lines 181 to 184:

"Hence, when taken together, the pattern of results for Acquisition (i.e., enhanced in the 1h and 24h Groups, but not the 2 min Group) is fully compatible with that for Retention (i.e., impaired in the 2 min Group, but not the 1h and 24h Groups), in that they both point toward the notion that the capacity for new learning was reduced in the 2 min Group."

Lerner, G., Albert, S., Caffaro, P. A., Villalta, J. I., Jacobacci, F., Shadmehr, R., & Della-Maggiore, V. (2020). The origins of anterograde interference in visuomotor adaptation. *Cerebral Cortex*, *30*(7), 4000-4010.

The results of Exp. 2 are clear, demonstrating that repeated training in short temporal distance seems to accelerate the rate of decay during the retention period. This remains an interesting finding of the manuscript.

We thank the reviewer for this positive comment.

Referee: 2

Comments to the Author(s).
I am very satisfied with the revision that the authors made. I also very like the additional experiment examining the effect of fatigue.

We thank the reviewer for his/her positive evaluation of the revised manuscript.