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Peer Review File

Visual target and task-critical feedback uncertainty impair different stages of reach planning in motor cortex

Corresponding Author: Professor Alexander Gail

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In the manuscript entitled, "Visual target and task-critical feedback uncertainty impair different stages of reach planning in motor cortex", Amann, Casasnovas and A. Gail describe an experiment designed to assess the effect of visual uncertainty on the planning and execution of reaching behavior and neural activity recorded in the motor cortex. In the study, non-human primates are trained to make memory guided, point to point reaching movements with two separate effectors: one the handle of a planar manipulandum used for manual control and the other a visual cursor whose position is determined via a real time brain computer interface. The purpose of these two tasks is to experimentally differentiate the necessity of visual feedback in movement initiation/execution. They show that visual uncertainty regarding the location of the target modulates reaching performance both early and late in the reaches made under manual control. Feedback uncertainty, however, only modulated behavioral performance during the later stage of reaches for brain control not manual control. The effect of uncertatinty was also noted in both single/multi unit recordings and decoding of neural populations. Notably, population activity assessed with dimensionality reduction reflected a small but significant component of visual uncertatinty across all conditions. Overall, this is a well written paper that describes an interesting and potentially important set of experiments. The comparison of manual control and brain control is novel and interestng in this context. Enthusiasm for this work is dampened by potential confounds (outlined below) that are left unadressed.

Major Comments-

The exploration of Target Uncertainty under brain control conditions is conspicuously missing from the report and is a notable weakness and limitation of this study. Its exclusion is briefly mentioned in the methods secton with little rationale. It's absence could/should be addressed in the discussion section along with expectations based on the data presented for the other conditions. Would the expectation be that target uncertainty modulates behavior and neural activity similarly to manual control?

Movement time, as reported in figure 2, is quite variable between the brain control and manual control conditions. The relatively short times associated with manual control suggests that movements are predominantly feedforward in nature and have few, if any, corrective submovments. On the other hand, brain control, is inherently dependent on feedback for control. This raises a potential confound in the interpretation of the results. Specifically, that there are no differences in manual control under feedback uncertainty because proprioception is online. Instead, it seems that that the lack of difference noted during manual control under feedback uncertainty may arise from the fact that feedback control is unnecessary in the manual control condition as it is predominantly feedforward. Similarly, this might explain the lack of difference in the feedback uncertainty condition under brain control early in the reach. Here, it may be that visual feedback may not yet be available as it is known to take 200+ms to be reflected in action. This potential confound should be addressed with additional analysis of data (i.e. plots of kinematics including speed) for all conditions and additional discussion.

The choice of using a memory guided task for this study is poorly motivated and its implications are not well elaborated or considered. Is there an effect of the delay time on the amount of reach error present both early and late in the trials? This seems to be a potential confound as the certainty about the location of the target will likely deteriorate as the delay time increases, thereby causing incerased uncertainty in the location of target in the 'low uncertainty' condition.

Paragraph beginning on line 148. This text uses two terms "planning error" and "execution error" to describe the results of

the study. It is unclear what the authors mean by these two terms. Please define them with regard to the factors contributing to and implications of these different characterizations. Based on the current document, it is unclear how one would draw the conclusion that the results are due to one and not the other.

Minor Comments -

Please add a justificaton for use of a paired test for analysis of reach errors. I assume the paired sample is comparing measures of error obtained from trials with high and low uncertainty in a single session, but it is not clear.

Statistical notation is variable throughout the manuscript. Some tests report the test statistic and degrees of freedom but other don't. Please standardize all to include degrees of freedom such that the numbers of samples in each test can be assesed. For example, compare paragraphs begnning on line 103 and 212.

Details on the cluster based permutation test for the SVM decoder are lacking. Please provide more detail or an appropriate citation for the methodology.

There are some typos and errors throughout the manuscript. For example, Figure 4 (line 895) references to panels in figure are mislabeled. Line 163 apparently shows and error from reference manager.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
The authors explore how two sources of uncertainty (i.e., endpoint feedback uncertainty and reach goal uncertainty) impact motor preparation and execution in a reaching task and a BCI task. The BCI task nicely provides a condition in which the animal cannot rely on proprioceptive feedback when they receive uncertain visual feedback. This study contributes to a growing literature showing that non-motor signals are present in motor cortical activity and how the non-motor signals interact with motor signals to influence behavior. The work is solid, however throughout the manuscript it is difficult to appreciate the contributions because of the clarity of the writing. Clarity could be improved by addressing the following

1. Inconsistent and confusing terminology
a. I found the “target uncertainty” and “feedback uncertainty” terms difficult to keep straight in my mind as I read. In part because both are feedback uncertainty- the feedback is just about different aspects of the movement the endpoint or the path. What about “reach goal uncertainty” and “reach trajectory uncertainty”? These suggested terms are more descriptive of the source of the uncertainty. Having terms that are more descriptive will help the reader understand the questions addressed in this study and the interpretations.
b. Throughout the manuscript different terms to mean the same thing. For example, in setting up the motivating question both “visual uncertainty” (intro) and “spatial uncertainty” (discussion) are used. Really it is “visual uncertainty about spatial information”.
c. Sometimes “somatosensory inputs” is used and sometimes “proprioceptive feedback”. I think these terms are used to refer to the same signal. If so, I would choose one (probably proprioceptive) and use it consistently.

2. Aside from the discussion, the text is just reporting, it is not motivating choices or next steps or providing interpretation of the results.
a. For example, the BCI task is a nice way to focus on the influences of visual uncertainty about spatial information during reaching. However, the motivation could be presented more clearly. In part this choice could be justified with a clearer explanation of the proprioceptive feedback during BCI control. Throughout a BCI task, if the arm is lightly restrained, the animal is receiving incongruent proprioceptive and visual feedback. A BCI doesn’t remove proprioceptive feedback- it just removes task-related proprioceptive feedback. It’s not until Line 179/180 where you describe “no movement-contingent proprioception is available in BC”.

Relatedly, I understand the benefits of the BCI task for the feedback uncertainty question. Why didn’t you also test target uncertainty in the BCI task?
b. Another example is the paragraph that begins line 249. This paragraph describes Figure 5 but it would be more compelling to address how the results presented in the figure answer the question of if the activity is separated by uncertainty levels.

Specific concerns:

1. It is unclear to me if in the target uncertainty condition the reach goal could be any location around the circle or if the monkey was always reaching to the same 8 physical locations but with varying degrees of uncertainty in the cue of the rewarded location. This is a concern because if the monkeys are overtrained on the task (as most are) they could rely on “muscle memory” of the physical locations rather than relying on the visual feedback.

2. Line 96-97 Was the level of uncertainty varied each day or were there only two levels of uncertainty- certain and not certain- for all experiments?

3. Line 104 For assessing reach execution why limit yourself to absolute angular error at two time points? A more fine-grained, timestep-by-timestep assessment would provide more information about how the uncertainty about the endpoint is affecting the neural activity and the reach. Maybe something like progress toward the target at each timestep?

4. Were monkeys cued as to if the trial was certain/uncertain feedback condition during the planning period? Were the trial types blocked or interleaved?

5. Fig 2: It would be helpful to have the colors/conditions labeled in the figure (not just the caption and Fig 1). Also, just a stylistic preference but I find the color designation to be a bit of a Stroop test with the low uncertainty having the darker color and the high uncertainty having the lighter color.

6. Fig 3: Please label the scale bars in figure (not just in caption)

7. Line 224-226: What is the significance of the periods of low within-decoding corresponding to low generalization in cross-decoding?

8. Fig 5: It would be nice to indicate lower/higher uncertainty along the uncertainty axis. Can you plot all the trials on the same set of axes? Then you would see regardless of trial type that activity separates into a low uncertainty region and a high uncertainty region. In Fig 5a, are the projections time averaged and trial averaged? In Fig 5b, why are the feedback conditions already separated in baseline? What is the baseline window?

9. There is not a report of success rate on the tasks. It would be helpful to have a sense of how the uncertainty affects task performance. It would also be helpful in interpreting the decoding. It is not clear until the methods section if the decoding is of the executed reach or instructed reach? If the success rate is high, it probably doesn't matter too much but if it is not high it might be nice to add both. Regardless of if you include both, please clarify which is being decoding when the decoding is first described (paragraph starting line 157).

10. Line 487 Did monkey Y have any task constraints that discouraged him from moving his arm during the BC task?

11. Line 513 Why did you use a block design for BC and an interleaved design for MC?

12. Line 521 Did you use a different sorting procedure for BC task/analyses than for the MC task/analyses? Line 525: This reported number of units differs from the numbers reported above for the MC task. Is this because of the different sorting procedure?

13. Line 593 How many trials comprised each test set?

14. SF2: The open/closed symbols are missing from the legend to indicate which monkey is which. Also, the axis labels would be clearer if the labels were "within-decoder performance" and "cross-decoder performance"

15. SF3 I would like to see these panels added to Fig 5. If you do that, it'll be necessary to change the shading scheme because the saturation level indicates uncertainty level in all other figures. Is the average computed over dimension or experiments?

Minor concerns/typos:
1. Line 91, when the BCI is first mentioned it would be helpful to report parenthetically what the decoder you are using and point to the Methods for more details. Currently it's in the figure caption.

2. Line 94-95 "uncertainty types" -> "task conditions"

3. Line 151 suggest-> suggests

4. Line 203-205: the reader doesn't yet know the details of the task. Add "non-overlapping" windows

5. Line 233- Does "for MC target uncertainty" mean for the MC target uncertainty trials?

6. Line 238 "The variances related to uncertainty…" variances -> dimensions

7. Line 253 direction -> directions

8. Line 278 add "…front motor areas of monkeys"

9. Line 307 visible -> apparent

10. Line 373 taken -> given

11. Line 584 I'm not sure what this sentence should say but there is a typo somewhere.

12. Line 597 Is "significantly lower than zero" what you mean? Or "significantly different from zero"?

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
In this study, the authors investigate the behavioral data and neural correlates of reaching movements performed by non-human primates under different conditions of controlled effector and variability. They compare behavioral variables in conditions of variability or uncertainty related to the target or cursor aligned to the hand, and in a condition where neural data are used to control a cursor via the BCI. They reported different effects depending on whether the variability concerned the target or the hand position. They conclude that target information and neural variability are encoded in neural activities, but that uncertainty only affects behavior when no other modality can be used.

I have to say that I'm not an expert in BCI, so I'm not in a position to evaluate the technical details of the article related to this technique. On the other hand, I enjoyed reading the article, it is well written, easy to follow and the data seems of good quality. I am concerned about the description of SVM analysis and the lack of formalism for interpreting the data. The latter concern is important because I think the results are expected in the context of optimal state estimation, so the experiment presented could be a powerful test of the relatively well-accepted Bayesian integration (or Kalman filter) model, rather than a simple description of behavioral characteristics and neural data without theoretical grounds.

With regard to the SVM analyses, I missed a detailed description of how the results were presented: the chance level is 12.5% (p=1/8), so I assume that the panels in Figure 4 correspond to the frequency of well-classified targets, but there may be variability between targets. Do the shaded areas represent variability between targets or k-fold validation or both? The legend laconically states “95% CI for all sessions and monkeys”. I think this part needs to be explained in more detail. To continue, I think Figure 5 is misleading because all the targets seem to separate perfectly whereas (if I read the previous figure correctly), the accuracy of decoding at the start of the movement suggests that the targets are only well classified 50% of the time. Presumably, there are regions or variability around target location in Figure 5 that are not represented, or the session chosen is not representative of the variability in the data. Decoding and dimensionality analyses could benefit from better descriptions and illustrations.

My main concern is that these results may be entirely expected in the context of previously published models of dynamic estimates of limb movement to interpret multisensory integration during postural control and reaching and their neural correlates (Crevecoeur et al, Journal of Neuroscience 36 (33), 8598-8611; S Kasuga et al., Journal of Neurophysiology 127 (2), 354-372; Hoffmann and Crevecoeur, eNeuro, https://doi.org/10.1523/ENEURO.0262-24.2024; Cross et al., 2024, Eneuro 11 (2), cited). In this framework, the variability of visual feedback on limb movement is expected to have a minor impact when somatosensory feedback is available. It is also clear from simulations of this model that target uncertainty will have a greater impact, as somatosensory feedback cannot compensate for uncertainty about the movement target. I think this framework also explains Figure 5: the Kalman filter accumulates evidence, so the reduction in target variance between “early plan” and “late plan” (Figure 5c) is expected if the animal samples from estimates of target location and then commits to an estimated goal. In addition, the Kalman filter includes a term that weights the difference between actual and expected feedback. The weight is inversely proportional to the covariance of the feedback signal, so that if the variability of a signal is high, the weight is low, and vice versa. Thus, different variances of the sensory signals will have a direct impact on the variance of the internal estimates, predicting that along the main axis associated with variance, the estimate should separate. Taken together, the results are potentially a powerful validation of a theory that has been proposed (and tested) to understand exactly the type of experiments presented here.

I think that such an interesting dataset in a journal of the calibre of Nature Communications should not miss previous efforts made in the field to understanding multisensory integration during reaching. I think that with the addition of quantitative predictions from a model including a Kalman filter, the article would be much stronger and more suitable for the readership of this journal.

(Remarks on code availability)
Not applicable

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I thank the authors for their thoughtful responses to the reviewers concerns. I am satisfied with their responses and modifications the manuscript. I look forward to seeing the manuscript in the journal.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
I thank the authors for their careful responses to my feedback and their revisions to the manuscript. They have addressed my concerns and the changes have improved the clarity of the manuscript. I have only one minor comment:

I appreciate the addition of the new panels to Figure 2 (panels c and d). However, it is difficult to discern the 4 lines in each panel. The high uncertainty line gets lost in the error bars of the high uncertainty line. In part because of this, I end up comparing the two monkeys but I think the primary comparison is between the uncertainty levels.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The authors have addressed my previous comments. I did not understand their last point as indeed the Kalman filter is an estimation algorithm that captures how an optimal observer handles uncertainty across both perturbed and unperturbed movements. In this regard perturbations are not necessary to test the prediction of this estimation framework. In particular, the scaling of movement variance with sensory noise is a feature of the model that is testable without requiring perturbations. Likewise the possibility that the online (optimal) estimator samples sensory input continuously includes treatment of the target signal (see e.g. Ozawa and Shadmehr, JNS, 2008). Probably my own bias is to see much stronger links to Kalman filter theory than what the authors suggest but the connexion with the framework is developed fairly and I have no further recommendation.

(Remarks on code availability)
N/A

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We thank all three reviewers for their time in evaluating our manuscript and for their valuable feedback. Overall, the reviewers ask for more motivation and explanation of our study methods and results. Reviewers #1 and #2 suggest including additional behavioral results to allow for a more nuanced interpretation, which we are happy to provide in an updated result section with expanded figures. Reviewers #2 and #3 see the style of the manuscript on the "reporting"-side and reviewer #3 suggests to better link our study to previously established theoretical models of multisensory integration, which we also agree with and revised our manuscript where the scope of the study and the experimental design allow it. Below, we have provided point-to-point responses (in blue) to the reviewer's questions and comments (in black), making the corresponding changes in the manuscript (in *grey*). We believe that we have properly integrated the feedback, which has led to a substantially improved manuscript that is more comprehensible for the general audience.

REVIEWER #1

In the manuscript entitled, "Visual target and task-critical feedback uncertainty impair different stages of reach planning in motor cortex", Amann, Casasnovas and A. Gail describe an experiment designed to assess the effect of visual uncertainty on the planning and execution of reaching behavior and neural activity recorded in the motor cortex […]. Overall, this is a well written paper that describes an interesting and potentially important set of experiments. The comparison of manual control and brain control is novel and interestng in this context. Enthusiasm for this work is dampened by potential confounds (outlined below) that are left unadressed.

Major Comments:

The exploration of Target Uncertainty under brain control conditions is conspicuously missing from the report and is a notable weakness and limitation of this study. Its exclusion is briefly mentioned in the methods secton with little rationale. It's absence could/should be addressed in the discussion section along with expectations based on the data presented for the other conditions. Would the expectation be that target uncertainty modulates behavior and neural activity similarly to manual control?

We thank the reviewer for raising this important point. We agree that explaining the rationale behind our study design deserves additional attention in the manuscript. The study investigates two independent aspects of uncertainty and their effect on motor planning (uncertainty of the target instructed prior to planning versus uncertainty of the feedback during movement following planning). Both manipulations share the same "reference condition" (= low uncertainty in both target and feedback), against which we can compare the respective high uncertainty conditions. Additionally, the feedback uncertainty condition is complemented with a BCI condition in which we control for proprioceptive feedback. Please note that for reasons of feasibility, the study was NOT designed as a full 3-factor factorial design, in which all combinations of certainty (low/high) x task parameter (target/feedback) x execution mode (manual/BCI) are compared, which would have led to a total of eight conditions.

Using the BCI condition as a control for uncertain visual instructions, as suggested by the reviewer, would in principle still have been thinkable. Yet, we had no immediate research question associated with this condition, since we did not expect to observe meaningful differences between brain control

and manual control in the case of target uncertainty. This is because, in both cases, target information would be provided only visually during the cue period, i.e., long before movement onset. Removing movement-contingent proprioceptive inputs would likely have only minor influences on directional encoding during reach planning, if at all. Therefore, we considered the "Target BC" condition expendable, and gave it lower priority. In the end, practical reasons prevented us from adding such lower priority conditions. The animal research permit for this experiment in the meantime had expired and one of the animals was no longer available. We opted against a second, de novo study with a new animal to provide a Target BC condition.

To make our main argument more transparent, we expanded the description of the rationale behind our study design at the beginning of the Results section (p. 7, lines 152-159)

> *"Across daily sessions, we varied the type of uncertainty, target or feedback, to test for their respective effects on motor planning. Additionally, in the case of feedback uncertainty, we also varied the control mode, MC or BC, to vary the behavioral relevance of the visual feedback. This resulted in a total of three task conditions: target MC, feedback MC, and feedback BC. We did not test target uncertainty under BC because target information would be provided only visually during the cue period. Therefore, removing movement-contingent proprioceptive inputs would likely only have minor influences on directional encoding during reach planning."*

Movement time, as reported in figure 2, is quite variable between the brain control and manual control conditions. The relatively short times associated with manual control suggests that movements are predominantly feedforward in nature and have few, if any, corrective submovments. On the other hand, brain control, is inherently dependent on feedback for control. This raises a potential confound in the interpretation of the results. Specifically, that there are no differences in manual control under feedback uncertainty because proprioception is online. Instead, it seems that that the lack of difference noted during manual control under feedback uncertainty may arise from the fact that feedback control is unnecessary in the manual control condition as it is predominantly feedforward. Similarly, this might explain the lack of difference in the feedback uncertainty condition under brain control early in the reach. Here, it may be that visual feedback may not yet be available as it is known to take 200+ms to be reflected in action. This potential confound should be addressed with additional analysis of data (i.e. plots of kinematics including speed) for all conditions and additional discussion.

We thank the reviewer for drawing attention to this important discussion point. We agree that manual control (MC) and brain control (BC) trials are characterized by different movement times in our data and that this mandates a discussion in how far and during which time periods different modes of movement control could contribute to the observed dependencies from feedback uncertainty. We also agree that more than one factor may contribute to the task-relevance of the feedback and that these might be time-dependent, consistent with our results. We added this argument to the discussion (see below), emphasizing that this consideration is consistent with our conclusion that feedback needs to be task-critical for uncertainty to have an effect.

To address this point quantitatively, we now additionally show speed profiles and traveled distance as function of time. Both measures indicate, as expected, that MC reaches were composed by a fast initial component (putatively feedforward) bringing the cursor close to the target, followed by smaller

homing-in sub-movements (putatively feedback-based). The abstract (lines 12-16), results (lines 198-211) and discussion (lines 495-510) sections have been updated accordingly, considering the additional argument in support of our conclusion:

Abstract (p. 2, lines 12-16):

> *"Our findings demonstrate that while both target and feedback uncertainty always reflect in neural activity, uncertain feedback only deteriorates neural reach goal information and behavior when it is task-critical, i.e., when having to rely on the sensory feedback and no other more reliable sensory modalities are available."*

Results (p. 8-9, lines 198-211):

> *"Second, we evaluated speed profiles aligned to the go cue (Fig. 2c). For MC conditions, reaches started with a steep, transient speed increase, followed by a much smaller increase and decrease in speed. This suggests that reaches were composed by an initial "ballistic" component to bring the hand close to the target, likely in a feedforward manner. If the monkey did not directly enter the target area after this initial component, smaller homing-in movements were made, putatively guided by incoming sensory feedback. This observation also reflects in the traces of the traveled distance, which show a steep increase followed by a plateau (Fig. 2d). In contrast to MC, speed and distance for BC reaches changed more gradually, suggesting that movements were predominantly feedback-based throughout the movement. Moreover, we observed that the reduction between early and late reach errors was larger for feedback BC compared to MC (Fig. 2b), indicating that feedback-based corrections were more prominent under BC. There were no evident differences between uncertainty levels regarding speed and traveled distance-from-the-center as a function of time."*

Discussion (p. 18, lines 496-511):

> *"We additionally observed differences in the way reaches were performed between MC and BC. MC reaches were composed by a large initial feedforward component, followed by smaller corrective sub-movements to enter the target area, if necessary. This entails that monkeys did not need to rely on sensory feedback to control the movement during the initial component, as this could be majorly based on internal predictions of the limb's state[51,58]. In contrast, BC reaches were predominantly feedback-based, as proper BC requires of continuous monitoring of visual feedback. Thus, the nature of movement (feedforward vs. feedback-based) was also different between MC and BC, which could have contributed for the differences in effects of visual feedback uncertainty."*

Regarding the time lag in the availability of visual feedback, we purposefully contrasted an early (25% eccentricity) with a later (75%) time point along the movement to compare planning errors (feedback independent) with feedback dependent execution errors. In our behavioral data, the 25% eccentricity was reached 140-190ms after movement onset, preceding the time visual feedback can be considered for action. To emphasize this rationale more, we complemented the previous statement in the Results section (p. 8, line 179-185):

> *"First, we used early (25% eccentricity) and late (75%) absolute angular reach errors to characterize reach planning and execution, respectively (Fig. 2a). Large early reach errors*

indicate that the planned reach direction is incorrect (planning error). This is because movement corrections at that time are not possible due to slow visual feedback processing. In contrast, late reach errors do not only capture an incorrectly planned reach direction, but also an inability to use effector feedback to properly guide reach execution (execution error).”

The choice of using a memory guided task for this study is poorly motivated and its implications are not well elaborated or considered. Is there an effect of the delay time on the amount of reach error present both early and late in the trials? This seems to be a potential confound as the certainty about the location of the target will likely deteriorate as the delay time increases, thereby causing incerased uncertainty in the location of target in the 'low uncertainty' condition

We thank the reviewer for the opportunity to elaborate more on the implications of a memory-guided task design. The main reason why we employed a memory-guided task was that we wanted to understand the impact of different forms of spatial uncertainty on motor goal encoding/motor-preparatory activity in movement planning areas of the brain. To stay compatible with a large number of previous studies on this broader topic from other labs and our own lab (e.g., Cisek and Kalaska 2005; Klaes et al. 2011; Taghizadeh et al. 2024), we opted for a memory task in order to minimize possible influences of pure visual processing of the target cue on the neural activity during planning. We have expanded the Methods section accordingly (p. 22, lines 650-651).

“Having a delay period in which the target was not visible allowed us to remove influences of immediate processing of the visual stimulus on neural activity.”

We also directly tested the impact of delay period duration on reach errors in target uncertainty sessions, grouping trials into those with short (400-800ms) or long (800-1200ms) delays. This median split neither indicated an effect of memory duration on early nor on late reach errors in trials with high target uncertainty. We found small differences for low target uncertainty, but with opposite-side effects between animals, while our reported main findings were consistent between animals. Overall, since we do not observe systematic effects of the duration of the delay on reach errors, we think that memory decay does not confound our conclusions.

Paragraph beginning on line 148. This text uses two terms "planning error" and "execution error" to describe the results of the study. It is unclear what the authors mean by these two terms. Please define them with regard to the factors contributing to and implications of these different characterizations. Based on the current document, it is unclear how one would draw the conclusion that the results are due to one and not the other.

Thank you for asking for clarification. We now explain these terms in more detail when introducing them in the behavioral results (p. 8, line 179-185; see also the answer to the comment about early and late error above).

“First, we used early (25% eccentricity) and late (75%) absolute angular reach errors to characterize reach planning and execution, respectively (Fig. 2a). Large early reach errors indicate that the planned reach direction is incorrect (planning error). This is because movement corrections at that time are not possible due to slow visual feedback processing. In contrast, late reach errors do not only capture an incorrectly planned reach

direction but also an inability to use effector feedback to properly guide reach execution (execution error).”

Additionally, we have adjusted the paragraph mentioned by the reviewer to provide more detail (p.10, lines 255-266).

> *“Observing larger behavioral reach errors for target MC and feedback BC at different states stages of the reach is in line with our hypothesis that the two types of uncertainty affect neural activity for movement planning and execution in distinct ways. Specifically, larger reach errors during early movement, as observed with target uncertainty (Fig. 2b), are linked to increased planning errors, which we expect to be the consequence of less informative neural motor planning activity. Therefore, we predict that neural representations of reach directions are less well separated in neural space. Larger behavioral reach errors during late movement, instead, as observed in target MC and feedback BC indicate increased execution errors. These errors could result from the cumulative effect of less informative neural planning activity and/or less informative neural movement-associated activity.”*

Minor Comments:

Please add a justificaton for use of a paired test for analysis of reach errors. I assume the paired sample is comparing measures of error obtained from trials with high and low uncertainty in a single session, but it is not clear.

The reviewer is correct in this assumption. To better explain our choice, we have rewritten the lines where we present the test in the methods section, adding more information (p. 27, lines 785-789).

> *“To test for behavioral differences between uncertainty levels for each uncertainty type and monkey, we used paired t-tests with a significance threshold of α=0.05. This means that the average measures from the low and from the high uncertainty levels, respectively, were paired for each session.”*

Statistical notation is variable throughout the manuscript. Some tests report the test statistic and degrees of freedom but other don't. Please standardize all to include degrees of freedom such that the numbers of samples in each test can be assesed. For example, compare paragraphs begnning on line 103 and 212.

Thank you for catching this inconsistency. We have now included the test statistic and the corresponding degrees of freedom for the applicable tests throughout the results.

Details on the cluster based permutation test for the SVM decoder are lacking. Please provide more detail or an appropriate citation for the methodology.

We now additionally provide the citation in the main text (p. 11, line 286), not just when the test is presented in the methods section (p. 28, line 826).

There are some typos and errors throughout the manuscript. For example, Figure 4 (line 895) references to panels in figure are mislabeled. Line 163 apparently shows and error from reference manager.

We thank the reviewer for pointing out the need for further proofreading. We have fixed the errors mentioned above and checked for remaining typos.

REVIEWER #2

The authors explore how two sources of uncertainty (i.e., endpoint feedback uncertainty and reach goal uncertainty) impact motor preparation and execution in a reaching task and a BCI task. The BCI task nicely provides a condition in which the animal cannot rely on proprioceptive feedback when they receive uncertain visual feedback. This study contributes to a growing literature showing that non-motor signals are present in motor cortical activity and how the non-motor signals interact with motor signals to influence behavior. The work is solid, however throughout the manuscript it is difficult to appreciate the contributions because of the clarity of the writing. Clarity could be improved by addressing the following

1. Inconsistent and confusing terminology

a. I found the "target uncertainty" and "feedback uncertainty" terms difficult to keep straight in my mind as I read. In part because both are feedback uncertainty- the feedback is just about different aspects of the movement the endpoint or the path. What about "reach goal uncertainty" and "reach trajectory uncertainty"? These suggested terms are more descriptive of the source of the uncertainty. Having terms that are more descriptive will help the reader understand the questions addressed in this study and the interpretations.

We appreciate the reviewer's suggestion and agree that terminology should be consistent and intuitive for easy understanding. We tend to disagree that every form of sensory input should be considered as feedback. In our context, "feedback" implies a closed-loop system in which the input to the system (here: sensory information) is contingent on its output (here: arm movement), thereby feeding back information about the effect that the output has. Target uncertainty in our task is independent of the arm movement. Hence, we contrast it with uncertainty about the current state of the effector, the estimation of which depends on the cursor/cloud, which represents the arm movement, and which we therefore call feedback uncertainty. In selecting these terms, we aimed to be descriptive, maintain consistency with prior work, avoid overly lengthy names, and minimize potential confusion. For example, we avoided "hand uncertainty," as used in previous work, since it does not apply to our brain control condition.

We agree with the reviewer that the terminology should be as clear as possible to the reader. We have rephrased the introduction of the terms in the Introduction section to better clarify our working definitions for target and feedback uncertainty (p. 3, lines 27-30).

> *"When performing a reach towards a visual target, uncertainty can affect both sensory information about the current, time-varying position of the effector (feedback uncertainty) and the instructed movement endpoint (target uncertainty)."*

b. Throughout the manuscript different terms to mean the same thing. For example, in setting up the motivating question both “visual uncertainty” (intro) and “spatial uncertainty” (discussion) are used. Really it is “visual uncertainty about spatial information”.

Thank you for catching this. We have revised the wording and made adjustments to be more precise and consistent.

c. Sometimes “somatosensory inputs” is used and sometimes “proprioceptive feedback”. I think these terms are used to refer to the same signal. If so, I would choose one (probably proprioceptive) and use it consistently.

We thank the reviewer for pointing out this inconsistency. Indeed, we sometimes use these terms interchangeably. We have harmonized the terms and are now using proprioceptive feedback. However, in some instances, we purposefully chose ‘somatosensory’ to include relevant literature.

2. Aside from the discussion, the text is just reporting, it is not motivating choices or next steps or providing interpretation of the results.

We appreciate the reviewer’s input on the overall writing style of the manuscript. We agree that our results text is on the reporting side, as we reserve most interpretations for the discussion section. We also kept the motivation for the most specific aspects of the design and analysis in the methods section, close to the description of the method itself. Additional to this, as laid out in the following, we overall modified our results section for more comprehensive readability and embedding of the presented data into the bigger concept.

a. For example, the BCI task is a nice way to focus on the influences of visual uncertainty about spatial information during reaching. However, the motivation could be presented more clearly. In part this choice could be justified with a clearer explanation of the proprioceptive feedback during BCI control. Throughout a BCI task, if the arm is lightly restrained, the animal is receiving incongruent proprioceptive and visual feedback. A BCI doesn’t remove proprioceptive feedback- it just removes task-related proprioceptive feedback. It’s not until Line 179/180 where you describe “no movement-contingent proprioception is available in BC”.

We thank the reviewer for pointing out that our previous motivation for the use of BCI in the Introduction section was not clear enough. We adjusted the wording in the second paragraph of the Introduction section to expand on our motivation for use of a BCI (p. 4-5, lines 75-86).

> *“[…] To prevent cross-modal compensation, we leverage a brain-computer interface (BCI) BCI-controlled movements allow to experimentally determine the sensory feedback the user receives. BCIs translate recorded neural activity directly into a behavioral readout, such as movement of an external effector[21–24]. As this movement is controlled through the BCI and not the physical arm, movement-contingent proprioceptive feedback becomes unavailable. Therefore, by only providing visual feedback about the BCI-controlled movement, the effects of visual feedback uncertainty on neural activity can be isolated[25] and compared to those during physical arm reaches. At the same time, BCIs have also been successfully employed to restore lost motor function in patients[26–32]. From this clinical perspective, the lack of relevant proprioceptive inputs could make BCI users*

particularly vulnerable to visual feedback uncertainty. Thus, studying this condition under BCI control also provides insights for optimizing its clinical application."

Relatedly, I understand the benefits of the BCI task for the feedback uncertainty question. Why didn't you also test target uncertainty in the BCI task?

We thank the reviewer for the question, which was also asked by reviewer #1 (please see also our answer there). Our study focused on the comparison between target and feedback uncertainty, because we expected them to contribute differently to motor planning. We did not include the "Target BC" condition, as we expected no or only minimal differences between MC and BC conditions in response to target uncertainty. This is because target information is provided only visually (in which regard MC and BC do not differ) and only before the movement (at a time when MC and BC also do not differ yet). Removing movement-contingent proprioceptive inputs after a delay period would likely have only minor influences on directional encoding during visual target processing before the delay period, if at all. Since we had no immediate research question associated with such comparison, we deprioritized the "Target BC" condition. In the end, as the animal research permit for this experiment in the meantime expired and one of the animals was no longer available, we opted against a second study to provide this condition.

We expanded our rationale at the beginning of the Results section to better emphasize the focus of our study (p. 7, lines 153-159).

"Additionally, in the case of feedback uncertainty, we varied also the control mode, MC or BC, to vary the behavioral relevance of the visual feedback. This resulted in a total of three task conditions: target MC, feedback MC, and feedback BC. We did not test target uncertainty under BC because target information would be provided only visually during the cue period. Therefore, removing movement-contingent proprioceptive inputs would likely only have minor influences on directional encoding during reach planning."

b. Another example is the paragraph that begins line 249. This paragraph describes Figure 5 but it would be more compelling to address how the results presented in the figure answer the question of if the activity is separated by uncertainty levels.

We thank the reviewer for highlighting this point. First, we now better motivate the use of the dPCA method (p. 12, lines 316-319):

"At the same time, uncertainty might affect neural activity irrespectively of reach direction. Thus, second, we analyzed neural state spaces between both uncertainty levels to further specify the test for potential non-alignment of neural dynamics (Fig. 5)."

Second, we have added more interpretation to the mentioned results. For instance (p. 14, lines 375-378):

"At the same time, states for all reach directions were separated along the uncertainty dPC in all task conditions. This indicates that uncertainty itself is also reflected in neural population activity, regardless of whether it affects the target or effector location."

And (p. 15, lines 423-430):

"Thus, uncertainty in target MC has the largest effect during early planning, when the monkey starts to integrate target information and selects its reach direction, even though target uncertainty persists throughout the remainder of the trial until the end of the movement. Conversely, uncertainty in feedback BC becomes more prominent towards the start of the movement, when the monkey begins to integrate visual effector information for control, even though uncertainty about the hand position exists already from the beginning of the trial prior to movement planning."

Specific concerns:

1. It is unclear to me if in the target uncertainty condition the reach goal could be any location around the circle or if the monkey was always reaching to the same 8 physical locations but with varying degrees of uncertainty in the cue of the rewarded location. This is a concern because if the monkeys are overtrained on the task (as most are) they could rely on "muscle memory" of the physical locations rather than relying on the visual feedback.

We thank the reviewer for pointing out this important aspect of the task design. In fact, the target uncertainty condition included 16 target directions (see Methods section), resulting in only 22.5° target separation. Behaviorally, we thereby achieved a quasi-continuous sampling and minimized the risk of motor memory. We still opted for a discrete set of directions to facilitate grouping for analysis and ensure sufficient trials per group. The target separation was small enough that under high target uncertainty, the visual cue span multiple of the underlying (invisible) discrete set of 16 potential targets. Importantly, the observed high reach errors across target directions under high target uncertainty show a continuous distribution (data not shown) and indicate that the monkeys relied on the visual cue to plan their reaches rather than solely on the remembered locations of potential targets. In our analyses, though, we only present 8 of the 16 targets for better comparability with the feedback uncertainty sessions. When analyzing the datasets including all 16 targets (data not shown), we arrive at the same conclusions as for the presented 8-target data. We have added a more detailed explanation for using 8 discrete target locations in the Methods section (p. 22, lines 641-646):

"We chose a discrete set of target directions to ensure a sufficient number of trials for later analyses, while maintaining spatial proximity between them to cover the whole circular space. Reach errors were not clustered around specific directions but showed continuous distributions in both monkeys when target uncertainty was high (data not shown), suggesting that the monkeys did not reach to overtrained target locations."

And (p. 26, lines 755-758):

"In MC sessions with 16 targets, we only analyzed eight target directions (cardinal and oblique, spaced 45°) to be comparable to BC sessions. Including all 16 directions in our analysis did not change our conclusions (data not shown)."

2. Line 96-97 Was the level of uncertainty varied each day or were there only two levels of uncertainty- certain and not certain- for all experiments?

We used the same two levels of uncertainty (same parameters for the visual stimuli) for all sessions. We have adjusted this explanation to be very explicit about this (p. 7, lines 152-169).

"Across daily sessions, we varied the type of uncertainty, target or feedback, to test for their respective effects on motor planning. Additionally, in the case of feedback uncertainty, we also varied the control mode, MC or BC, to vary the behavioral relevance of the visual feedback. This resulted in a total of three task conditions: target MC, feedback MC, and feedback BC. We did not test target uncertainty under BC because target information would be provided only visually during the cue period. Therefore, removing movement-contingent proprioceptive inputs would likely only have minor influences on directional encoding during reach planning.

Within each task condition, we tested two fixed uncertainty levels (low and high). The low level of target uncertainty was implemented by presenting the true target, the high level by showing an array of five bars distributed along a ring (high; Fig. 1c, left panel). The low level of feedback (effector position) uncertainty was implemented by having monkeys control a focal cursor, the high level by controlling a cloud of five moving dots (Fig. 1c, middle and right panels)."

3. Line 104 For assessing reach execution why limit yourself to absolute angular error at two time points? A more fine-grained, timestep-by-timestep assessment would provide more information about how the uncertainty about the endpoint is affecting the neural activity and the reach. Maybe something like progress toward the target at each timestep?

We appreciate the reviewer's interest in a more nuanced behavioral analysis, as also indicated by reviewer #1. We now have added time-resolved behavioral measures (speed and distance from the center in Fig. 2c,d) to provide more information on the time course of the reach. For the discussion of these data, please see our response to reviewer #1.

4. Were monkeys cued as to if the trial was certain/uncertain feedback condition during the planning period? Were the trial types blocked or interleaved?

In each trial and condition, monkeys did know which uncertainty level was going to be used from before the beginning of the planning period. In feedback trials, since the visual feedback stimulus switched between cursor and cloud at the beginning of the feedback trials (p. 23, lines 679-680), the information about the feedback uncertainty level was present throughout the entire trial. In target trials, the uncertainty level was evident with the presentation of the target cue before planning and remained relevant for the rest of the trial. In target MC and feedback MC conditions, uncertainty levels were interleaved on a trial-by-trial basis. In BC trials, uncertainty levels were blocked and they switched every 48 trials (p. 25, lines 729-731).

5. Fig 2: It would be helpful to have the colors/conditions labeled in the figure (not just the caption and Fig 1). Also, just a stylistic preference but I find the color designation to be a bit of a Stroop test with the low uncertainty having the darker color and the high uncertainty having the lighter color.

We thank the reviewer for pointing out the missing color labels. We have included the corresponding labels in Figure 2. We also appreciate the comment on our color scheme. Our intention was to use the darkness of the color to reflect the amount of information contained in the signal—darker colors representing higher information and lighter colors representing lower information. We understand

how this could be interpreted as counterintuitive and considered alternative options. However, inverting the color scheme might introduce similar challenges, and adopting completely different colors could reduce the ease of distinguishing between tasks. Therefore, unless this is deemed critical, we would prefer to retain the current color scheme.

6. Fig 3: Please label the scale bars in figure (not just in caption)

We have accordingly labeled all scale bars.

7. Line 224-226: What is the significance of the periods of low within-decoding corresponding to low generalization in cross-decoding?

We appreciate the reviewer's question. We consider these two analyses as complementary. Within-level decoding assesses how neural representations differ between reach directions within each uncertainty level, while cross-level decoding evaluates how neural representations of reach direction differ across uncertainty levels. Periods of correspondence could mean that within-level decoding differences contribute to overall neural representations being different between uncertainty levels. However, it is possible to observe no within-level decoding differences but still deficits in cross-level decoding, as we actually see in our data. For instance, we observe no within-level decoding differences during late planning (Figure 4c), yet cross-level decoding is reduced during the same epoch (Figure 4f). We have revised the Results section to clarify the motivation for these two analyses. Specifically:

First, on page 11 in lines 272-274:

> *"In a first approach, we trained decoders on trials with different uncertainty levels independently to assess the separability of directional representations in each uncertainty level (Fig. 4b, 'within-level')."*

Second, on page 12 in lines 309-316:

> *"The above within-level decoding analyses showed that high uncertainty can lead to less distinct representations of reach directions. Yet, similar within-level decoding performances do not mean that directional representations are conserved between uncertainty levels. In fact, we observed single neuron response differences (Fig. 3c) between uncertainty levels in feedback MC despite similar within-level decoding results. Therefore, in a second analysis approach, we conducted cross-level decoding to test for generalization of reach direction coding between uncertainty levels (Fig. 4f-h)."*

Third, on page 12 in lines 327-328:

> *"We expect this to occur at least during intervals with different within-level performances."*

Finally, we added an interpretation of the results highlighting the epochs of non-correspondence (p. 13, lines 354-358):

> *"As expected, reduced within-decoding performances for high uncertainty (Fig. 4c-e) are accompanied by reduced generalization of direction decoding between uncertainty levels (Fig. 4f-h). However, we also found periods of non-correspondence (i.e., during late*

planning for target uncertainty and feedback uncertainty BC), suggesting that visual uncertainty can affect neural activity without impairing reach goal representations."

8. Fig 5: It would be nice to indicate lower/higher uncertainty along the uncertainty axis. Can you plot all the trials on the same set of axes? Then you would see regardless of trial type that activity separates into a low uncertainty region and a high uncertainty region. In Fig 5a, are the projections time averaged and trial averaged? In Fig 5b, why are the feedback conditions already separated in baseline? What is the baseline window?

We thank the reviewer for these suggestions. We computed the dPCA for each session individually due to variability in the number of recorded neurons. This results in unique axes for each session. While we assume comparability of axes within a given uncertainty type, we opted for presenting the different uncertainty types in separated plots but with consistent axis scaling.

Projections in Fig 5a are indeed time- and trial-averaged. We added this information to the figure caption. It is to be expected that projections for feedback uncertainty levels are separated already during baseline (-100 to 0 ms from target cue onset), as the uncertainty level is already known at this time (visual cursor/cloud representation is switched at the start of the trial). We have added an explanation of this on page 15 in lines 413-416.

"In case of feedback uncertainty, the separation was already present during the baseline epoch, following that the uncertainty level was already known at that time due to the visual appearance of the cursor/cloud."

9. There is not a report of success rate on the tasks. It would be helpful to have a sense of how the uncertainty affects task performance. It would also be helpful in interpreting the decoding. It is not clear until the methods section if the decoding is of the executed reach or instructed reach? If the success rate is high, it probably doesn't matter too much but if it is not high it might be nice to add both. Regardless of if you include both, please clarify which is being decoding when the decoding is first described (paragraph starting line 157).

We appreciate the reviewer's suggestions. We have now included a paragraph reporting the general success rates before presenting the more detailed behavioral measures that we previously had focused on exclusively (p. 7-8, lines 171-177):

"Monkeys were able to perform the task in all three task conditions. Average success rates were reduced for high compared to low uncertainty levels, especially for target MC (monkey Y: low: 62%, high: 31%; monkey Z: low: 77%, high: 38%). For feedback MC (monkey Y: low: 94%, high: 84%; monkey Z: low: 94%, high: 89%) and BC (monkey Y: low: 95%, high: 87%; monkey Z: low: 94%, high: 82%) performance reductions were less pronounced. To evaluate the behavioral consequences of uncertainty in more detail, we analyzed the monkeys' reach trajectories (Fig. 1d)."

We decoded the executed reach direction and not the instructed direction precisely because of the differences in reach errors. When decoding the instructed direction, performances are even lower for highly uncertain targets. This decrease likely results from grouping reaches with different kinematics, not from less well-represented reach goals. Therefore, we think that the currently reported difference

in decoding performance between uncertainty levels, which shows even when using kinematics that are as similar as possible, is a more meaningful finding. When referring to the decoding results, we now state more clearly that by reach direction in we mean the executed, not the instructed reaches (p. 10-11, lines 268-271).

> *"To decide between these two possibilities, we trained a series of support vector machines (SVMs) and decoded the reach direction based on spike densities over the course of the trial (Fig. 4a). We decoded the executed reach direction, as compared to the instructed target direction, to account for large deviations between these two in the case of target uncertainty."*

10. Line 487 Did monkey Y have any task constraints that discouraged him from moving his arm during the BC task?

Yes, monkey Y was required to hold the manipulandum handle. We have added the missing explanation in the Methods section (p. 24, lines 698-703):

> *"The arm contralateral to the implantation hemisphere was additionally lightly restrained for monkey Z, while monkey Y was required to hold the haptic manipulandum handle without attempting to move it. To ensure this, the haptic manipulandum position was fixed, which allowed us to measure any isometric forces applied to the handle. As soon as these forces exceeded a threshold (force at the start of the trial $\pm$ 0.4N), the trial was aborted."*

11. Line 513 Why did you use a block design for BC and an interleaved design for MC?

We thank the reviewer for this question. Our rationale for using a block design in the BC task was to minimize task difficulty for the monkeys, as the BC task is inherently more challenging. We note that feedback uncertainty was introduced at the start of each trial, even before the cursor was moved to the fixation point (see also our response to comment #4). Therefore, the monkeys were informed about the uncertainty level during the baseline period in both the block and interleaved designs. As a result, we believe this the design choice (block/interleaved) did not influence our results and conclusions. We added our rationale to the Methods section (p. 25, lines 729-731):

> *"Different to MC, in BC we employed a block design in which the uncertainty level was switched every 48 trials (six trials per target) to reduce task difficulty."*

12. Line 521 Did you use a different sorting procedure for BC task/analyses than for the MC task/analyses? Line 525: This reported number of units differs from the numbers reported above for the MC task. Is this because of the different sorting procedure?

We thank the reviewer for pointing this out. We used the same sorting procedure for offline analyses for MC and BC. The reported numbers in lines 235-237 consider both MC and BC sessions. The online sorting procedure used to identify units for input into the BCI decoder (at the beginning of each session) was slightly different from the offline sorting, as during online sorting we only considered the PCA waveform space and no other metrics. Unit identification was done more coarsely in the online situation to reduce the overall setup time for the animals before they could engage in the task.

Moreover, in the online protocol we did not apply any additional inclusion criteria, like minimum firing rate. Therefore, the reported unit numbers for offline analysis are slightly different from those for BCI input. We added this explanation in the Methods section (p. 25, lines 741-743).

> *"This offline sorting procedure was more conservative than the one used for online sorting during the BC sessions, leading to slightly different unit counts for the analyses."*

13. Line 593 How many trials comprised each test set?

We thank the reviewer for noticing the missing information. The number of trials used for the decoding analyses depended on the minimum number of repetitions per reach direction. On average, across monkeys and task conditions, each fold included 105 ± 6 (mean ± std) training trials and 26 ± 3 testing trials. We added this information to the Methods section of the manuscript (p.28, lines 818-821):

> *"The number of trials used for the decoding analyses depended on the minimum number of repetitions per reach direction. On average, across monkeys and task conditions, each fold included 105 ± 6 (mean ± std) training trials and 26 ± 3 testing trials."*

Additionally, we have included detailed information about the total number of trials for each uncertainty level per session in the new Supplementary Table S1.

14. SF2: The open/closed symbols are missing from the legend to indicate which monkey is which. Also, the axis labels would be clearer if the labels were "within-decoder performance" and "cross-decoder performance"

We apologize for the oversight; we have added the monkey labels. We also followed the reviewer's recommendation and updated the axis labels for clarity.

15. SF3 I would like to see these panels added to Fig 5. If you do that, it'll be necessary to change the shading scheme because the saturation level indicates uncertainty level in all other figures. Is the average computed over dimension or experiments?

We are grateful for this suggestion. We agree that including the explained variance provides a more comprehensive view of the dPCA results. We have now added these plots to Figure 5b. We have clarified further in the text how variance explained is computed (p. 29, lines 860-863):

> *"The variance captured for each task parameter was calculated by the sum of the variances of all components assigned to that parameter, divided by the total variance in the data. We report the average variance captured across sessions for each monkey for the uncertainty and interaction parameters in the main text."*

Minor concerns/typos:

1. Line 91, when the BCI is first mentioned it would be helpful to report parenthetically what the decoder you are using and point to the Methods for more details. Currently it's in the figure caption.

Done.

2. Line 94-95 “uncertainty types” -> “task conditions”

Done.

3. Line 151 suggest-> suggests

Done.

4. Line 203-205: the reader doesn’t yet know the details of the task. Add “non-overlapping” windows

Done.

5. Line 233- Does “for MC target uncertainty” mean for the MC target uncertainty trials?

We clarified the corresponding line.

6. Line 238 “The variances related to uncertainty…” variances -> dimensions

Thanks for catching that. We corrected it accordingly.

7. Line 253 direction -> directions

Done.

8. Line 278 add “…front motor areas of monkeys”

Done.

9. Line 307 visible -> apparent

Done.

10. Line 373 taken -> given

Done.

11. Line 584 I’m not sure what this sentence should say but there is a typo somewhere.

We thank the reviewer for noticing these errors and the careful attention. We have corrected them accordingly.

12. Line 597 Is “significantly lower than zero” what you mean? Or “significantly different from zero”?

For the cross-decoding results, we are using a one-tailed test, as our hypothesis was that there would be a decrease in performance between testing sets. Therefore, we are testing for a significantly lower than zero decrease.

REVIEWER #3

In this study, the authors investigate the behavioral data and neural correlates of reaching movements performed by non-human primates under different conditions of controlled effector and variability. They compare behavioral variables in conditions of variability or uncertainty related to the target or cursor aligned to the hand, and in a condition where neural data are used to control a cursor via the BCI. They reported different effects depending on whether the variability concerned the target or the hand position. They conclude that target information and neural variability are encoded in neural activities, but that uncertainty only affects behavior when no other modality can be used.

I have to say that I'm not an expert in BCI, so I'm not in a position to evaluate the technical details of the article related to this technique. On the other hand, I enjoyed reading the article, it is well written, easy to follow and the data seems of good quality. I am concerned about the description of SVM analysis and the lack of formalism for interpreting the data. The latter concern is important because I think the results are expected in the context of optimal state estimation, so the experiment presented could be a powerful test of the relatively well-accepted Bayesian integration (or Kalman filter) model, rather than a simple description of behavioral characteristics and neural data without theoretical grounds.

We thank the reviewer for the effort in assessing our work and the valuable suggestions. We agree that a more explicit link to previously developed theoretical work should be provided (see below).

With regard to the SVM analyses, I missed a detailed description of how the results were presented: the chance level is 12.5% (p=1/8), so I assume that the panels in Figure 4 correspond to the frequency of well-classified targets, but there may be variability between targets. Do the shaded areas represent variability between targets or k-fold validation or both? The legend laconically states “95% CI for all sessions and monkeys”. I think this part needs to be explained in more detail.

We appreciate the request for a more detailed explanation of the plotted variability. Within each session and uncertainty level, decoding performances were averaged across the five folds. We then averaged these average performances across all sessions pooled across both monkeys. The shaded areas in Fig. 4c-e represent the variability across sessions and monkeys. Since we did not systematically vary the induced uncertainty for the different reach directions, we did not individually compute decoding performance for the reach directions.

We rephrased the description of the SVM decoding in the Methods section and updated the figure caption accordingly to be clearer about the approach.

The description of the decoding analysis in the Methods section now reads (p. 28, lines 813-818):

> *“We performed 5-fold cross-validation by randomly grouping trials of one session into five groups, matching the number of trials per uncertainty level and reach direction. Each of*

these groups was then used once as testing set while the remaining four were used to train the decoder.

And: (p. 28, lines 821-824):

For each session we then averaged the decoding performance across the five folds. In Fig. 4c-e we plotted the average of these average within-session performances across all sessions, pooling both monkeys, separately for each uncertainty level."

The caption of Fig. 4 now reads:

"Performance was computed for each session and uncertainty level independently using 5-fold cross-validation. Performance was then averaged across folds for each session. Shaded areas depict 95% CIs for the average performance across sessions and monkeys."

To continue, I think Figure 5 is misleading because all the targets seem to separate perfectly whereas (if I read the previous figure correctly), the accuracy of decoding at the start of the movement suggests that the targets are only well classified 50% of the time. Presumably, there are regions or variability around target location in Figure 5 that are not represented, or the session chosen is not representative of the variability in the data. Decoding and dimensionality analyses could benefit from better descriptions and illustrations.

We are grateful for the reviewer's point, which allows us to clarify further details. Figure 5a is intended primarily for visualizing the neural activity's general structure in the dPCA space, as done also in previous work (e.g., Gallego et al. 2020; Smoulder et al. 2024). Namely, it highlights the low-dimensional orderly arrangement of the reach goals, plus the independent uncertainty dimension. We agree that adding information about the reliability of the plotted mean neural states for each reach goal allows better linking of these data to the decoding accuracies reported in figure 4. Making this link requires an assessment of the target separability in neural state space, which can be better visualized in 2D projections onto the direction plane than in the 3D plots (to be able to put the confidence intervals quantitatively in relation to the target separation). We added now the 95% confidence intervals to the cluster means and show them for different epochs during the trial in Supplementary Figure S4. We plotted individual trials together with the trial averages for the first two direction dPCs.

My main concern is that these results may be entirely expected in the context of previously published models of dynamic estimates of limb movement to interpret multisensory integration during postural control and reaching and their neural correlates (Crevecoeur et al, Journal of Neuroscience 36 (33), 8598-8611; S Kasuga et al., Journal of Neurophysiology 127 (2), 354-372; Hoffmann and Crevecoeur, eNeuro, https://doi.org/10.1523/ENEURO.0262-24.2024; Cross et al., 2024, Eneuro 11 (2), cited). In this framework, the variability of visual feedback on limb movement is expected to have a minor impact when somatosensory feedback is available. It is also clear from simulations of this model that target uncertainty will have a greater impact, as somatosensory feedback cannot compensate for uncertainty about the movement target. I think this framework also explains Figure 5: the Kalman filter accumulates evidence, so the reduction in target variance between "early plan" and "late plan" (Figure 5c) is expected if the animal samples from estimates of target location and then commits to an estimated goal. In addition, the Kalman filter includes a term that weights the difference between

actual and expected feedback. The weight is inversely proportional to the covariance of the feedback signal, so that if the variability of a signal is high, the weight is low, and vice versa. Thus, different variances of the sensory signals will have a direct impact on the variance of the internal estimates, predicting that along the main axis associated with variance, the estimate should separate. Taken together, the results are potentially a powerful validation of a theory that has been proposed (and tested) to understand exactly the type of experiments presented here.

I think that such an interesting dataset in a journal of the calibre of Nature Communications should not miss previous efforts made in the field to understanding multisensory integration during reaching. I think that with the addition of quantitative predictions from a model including a Kalman filter, the article would be much stronger and more suitable for the readership of this journal.

We thank the reviewer for highlighting the relevance of Kalman-filter based models in multisensory integration during reaching. We agree that optimal integration theory is an important foundation for our work and that it very nicely adds to framing the narrative of our study. We systematically revised the Introduction and Discussion sections to include important arguments and references to previous studies laid out by the reviewer.

For example, we have added a paragraph to the Discussion section highlighting possible mechanisms underlying our findings and suggesting potential adaptations of our task design in future research, such as introducing perturbations to directly test feedback-based corrections (p. 17-18, lines 478-494).

> *"Humans and monkeys have been shown to integrate inputs from two sensory modalities by adjusting the sensory weights according to the relative reliability of the signals[13,14,19,20]. Furthermore, experimental and theoretical work demonstrated that corrective responses to mechanical perturbations initially exhibit similar dynamics regardless of visual feedback availability[53,54]. These studies, grounded in optimal feedback control theory, proposed that motor control relies on an optimal estimate of the moving limb's state, accounting for both sensory uncertainty and long visual delays[53–55]. Extending these behavioral and modeling findings, a recent study demonstrated that overlapping populations of motor cortex neurons respond to both visual and mechanical perturbations[4]. This suggests that motor cortex might receive the final result of a state estimator located in upstream regions such as the parietal cortex, where sensory inputs are suggested to be optimally integrated[56,57]. In our case, high feedback uncertainty under MC likely shifted sensory weights towards proprioception. In contrast, only uncertain visual information was available for state estimation under BC, resulting in behavioral and neural impairments. Also, while state estimation for movement control could make use of cross-modal integration, in our experiment, target information was exclusively available in the visual domain. Thus, there were no alternative sources of information to compensate for high target uncertainty, leading to the most prominent impairments."*

While we acknowledge the importance of the optimal integration framework, we believe that direct modelling of the data in the suggested way does not align with the specific experimental design of our study. Here, unlike studies designed to probe corrective movement dynamics, we examine how visual target and feedback uncertainty modulate motor goal encoding during the planning and initiation of reaching movements, i.e., at a time when corrective feedback control is not yet happening, and in a

setting without any movement perturbations. Our results do not directly test predictions from Kalman filter models, which are typically used to model feedback-based corrections to deviations from expected states. We recognize that Kalman filter models could still be applied in a more general framework of sensory integration, even in the absence of explicit perturbations. For example, adjusting the Kalman gain could potentially account for varying levels of feedback reliance between manual control and brain control. Our data indeed suggest a change in control policy between MC and BC conditions (see reply to reviewer #1). Therefore, we have expanded the paragraph discussing the potential change in control modes (p. 18, lines 504-511):

> *"Numerous studies have demonstrated that movement control can be effectively modeled using a Kalman filter, which combines the internal prediction of the limb's state with sensory evidence[1,54,59,60]. Consequently, the shift in control policy between MC and BC may reflect a change in the Kalman gain, altering the degree to which the final state estimate relies on sensory input. Future studies could use a BCI paradigm incorporating controlled perturbations[23,24,61,62] to investigate how motor cortical activity reflects state estimation processes under uncertainty and varying access to sensory information."*

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We again thank all three reviewers for their time in evaluating our manuscript. Below, we have provided point-to-point responses (in blue) to the reviewer's comments (in black), with references to the manuscript (in *grey*).

REVIEWER #1

I thank the authors for their thoughtful responses to the reviewers concerns. I am satisfied with their responses and modifications the manuscript. I look forward to seeing the manuscript in the journal.

We thank the reviewer for the positive assessment and support.

REVIEWER #2

I thank the authors for their careful responses to my feedback and their revisions to the manuscript. They have addressed my concerns and the changes have improved the clarity of the manuscript.

We thank the reviewer for the positive assessment and support.

I have only one minor comment: I appreciate the addition of the new panels to Figure 2 (panels c and d). However, it is difficult to discern the 4 lines in each panel. The high uncertainty line gets lost in the error bars of the high uncertainty line. In part because of this, I end up comparing the two monkeys but I think the primary comparison is between the uncertainty levels.

We agree with the reviewer that the way how the data is presented in Fig. 2c and d might encourage comparing between monkeys instead of uncertainty levels. At the same time, we want to present results from both animals simultaneously and keep the same color scheme to be consistent with the other panels of figure 2 and the rest of the manuscript. Therefore, we added a sentence to the figure caption to highlight the intended primary comparison:

> *"Please note that the lines for high and low uncertainty levels mostly overlap, indicating little to no difference between uncertainty conditions in (**c**) and (**d**)."*

REVIEWER #3

The authors have addressed my previous comments. I did not understand their last point as indeed the Kalman filter is an estimation algorithm that captures how an optimal observer handles uncertainty across both perturbed and unperturbed movements. In this regard perturbations are not necessary to test the prediction of this estimation framework. In particular, the scaling of movement variance with sensory noise is a feature of the model that is testable without requiring perturbations. Likewise the possibility that the online (optimal)

estimator samples sensory input continuously includes treatment of the target signal (see e.g. Ozawa and Shadmehr, JNS, 2008). Probably my own bias is to see much stronger links to Kalman filter theory than what the authors suggest but the connexion with the framework is developed fairly and I have no further recommendation.

We thank the reviewer for the positive assessment and valuable insights. While we agree that the Kalman filter framework can be assessed without the need for perturbations, we aimed to provide an example of a potential future study to further explore this.