nature portfolio

# Peer Review File

# A dynamic subset of network interactions underlies tuning to natural movements in marmoset sensorimotor cortex

Corresponding Author: Dr Nicholas Hatsopoulos

**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 this manuscript, the authors study how well neuronal activity can be predicted from behavior and from the activity of other neurons. It is nice that authors study here freely moving animals, as opposed to the vast majority of previous studies where behavior is restricted to a single task on which animals are (over)trained. However, despite that unique behavioral setup, the level of novelty in results is very limited here. To better describe it, I will follow the order of figures:
Fig. 1) It is an illustration of the experimental setup.
Fig. 2) It shows that neuronal activity in the motor/sensory cortex can be predicted from movement trajectories. It has been well-known for a long time that activity in the motor/sensory cortex is related to movement.
Fig. 3) Authors show that including information about the activity of other neurons can improve predictions from Fig. 2. This is also well known that neurons are correlated, thus it is obvious that including this information can improve predictions (see sample References below).
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Lin IC, Okun M, Carandini M, Harris KD. The nature of shared cortical variability. Neuron. 2015
Fig. 4) It is also obvious that permuting the strongest (most correlated) connections will have the largest effect on predictability, as those are the most predictive connections. It is a simple mathematical fact and not some surprising property of the brain networks.
Fig. 5) Showing similarities and differences between predictability in “reach” vs “spontaneous” condition is the most interesting part of this manuscript. However, it was shown already multiple times before that neuronal activity across tasks and states can be quite preserved. Such previously described similarities in activity are not exact, and there are some cells that can be defined as “context-specific” as called in this manuscript (see sample References below). Thus, this figure is also now that surprising.
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MacLean, J.N., Watson, B.O., Aaron, G.B., and Yuste, R. Internal dynamics determine the cortical response to thalamic stimulation. Neuron 2005.
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Fig. 6) Is a continuation of Fig. 5 showing more details.

Other points:
1) Manuscript is difficult to understand. It seems to be written for other lab members who already know details of the methods. I had to go to the Methods section multiple times to understand basic information about analyses. It should be much better/simpler explained in the Introduction/Results.
2) The functional network (FN) method used here is also not new and was published before by co-authors. Maybe it is explained in those other papers, but it is not clear to me what is the advantage of this method over using just population vectors for predictions. It seems that using population vectors would give very similar results and would be much easier to understand by a typical reader.
3) It is nice that the authors provided code to reproduce analyses, but there is no link to data in the manuscript.

4) In Fig 5, will you have new “context-specific” neurons if you would do training on reachFN, and test on spontaneousFN, instead?

Reviewer #2

(Remarks to the Author)
This study describes the factors influencing individual unit activity in the sensorimotor cortex of marmosets while they engage in natural behaviors. It offers valuable insights into both the unique behavioral context and the relationships between networks and unit tuning within this context. However, the analysis of behavior should be significantly improved to better contextualize some of the findings. Nonetheless, it is an interesting study that makes a significant contribution while presenting compelling results that pave the way for future research, promising to enhance our comprehension of cortical activity during naturalistic behavior.

The authors of this study examine the behavior of individual neurons in the sensorimotor cortex of marmosets during natural movements, including spontaneous activity and the task of prey capture. They employ encoding models to forecast neuron activity using both kinematic features and network features derived from pairwise spike-time statistics. They then compare the predictive power of network features extracted from spontaneous behavior to those from prey capture, evaluating the transferability of functional networks across contexts. Furthermore, they investigate the influence of neuron-specific connectivity on units sensitive to functional network (FN) activity through targeted perturbations to FN weight and connectivity features.

Overall, the study reveals that encoding models utilizing kinematic features, commonly employed to predict neural activity in constrained or trained settings, perform well in naturalistic settings too. Similarly, FN features contribute to prediction like their performance in trained tasks where FNs were previously used. Notably, units with stronger average functional inputs from other units tend to be better predicted by the kinematic model. Moreover, prediction accuracy depends on the specific functional group connecting to a neuron, rather than just the total strength of connections, echoing findings in the visual cortex of rodents.

Comparing the two contexts, most neuron models show similar improvements when using FN features extracted from spontaneous activity. However, a smaller subgroup of neurons exhibits significantly greater improvement with prey capture FN features. Intriguingly, units sensitive to prey capture FN features also demonstrate greater tuning to kinematics and stronger interconnections, correlating with preferred trajectories and showing connectivity reorganization across settings, as evidenced by meticulous perturbation tests of the models.

General conclusions:
Overall, the authors have compiled a valuable dataset using an exciting approach to record neural activity during both prey capture and spontaneous movements in marmosets. This work holds significant importance in the field as it marks a crucial step towards studying natural behavior, essential for advancing our understanding of the brain. The manuscript is well-written and the results are presented clearly. I applaud the authors for making it easily accessible even to readers unfamiliar with the models. The findings highlight the factors influencing single neuron activity in the sensorimotor cortex during untrained, natural behavior, demonstrating sensitivity to kinematic features and highlighting specific connectivity patterns within the local circuit. Furthermore, they suggest that these connectivity patterns may be influenced by the types of movements required within a given context.
The primary findings indicate that models previously used to predict neuronal activity in controlled and trained settings align well with natural behavior. This significant discovery implies that, fundamentally, neurons in this area are consistently attuned to kinematic features, irrespective of the experimental paradigm (trained or natural). This observation carries two implications: firstly, the generalizability of certain behavioral features to neuron activity in the motor cortex, and secondly, a potential oversight in the manuscript. Despite obtaining a unique dataset involving prey capture behavior, the authors have not provided detailed insights into this complex behavior, which involves tracking, planning, and rapid feedback-driven error correction. By fully leveraging the uniqueness of their dataset, the authors might uncover additional neural tuning factors related to higher-level aspects of behavior beyond those applicable in controlled or trained datasets. A more comprehensive quantification and analysis of prey capture behavior could potentially enhance the encoding models and reveal valuable insights into neural tuning. However, the outcome of such analysis remains uncertain, leaving it to the authors' discretion to determine its relevance to the manuscript. Nevertheless, improved quantification of prey capture behavior would bolster the validity of the results and aid readers in assessing their robustness.
The main novel findings center on the mapping between the spontaneous and prey capture settings. The surprising observation that most neurons maintain their functional connectivity structure while only a minority undergo reorganization holds significant implications for understanding this functional connectivity. However, due to the lack of behavioral data in the spontaneous setting, it remains challenging to ascertain the extent to which this result is influenced by behavioral repertoire or variability overlapping between the settings, as acknowledged by the authors in the discussion. Therefore, an effort to quantify behavior in the spontaneous setting, albeit constrained by camera setup limitations, could offer valuable insights.
My primary critique pertains to the need for more detailed behavioral analysis, which would enhance understanding of both the prey capture results and their relation to the spontaneous setting. Nonetheless, despite this criticism, I believe this work constitutes a substantial contribution and underscores the significance of investigating natural behavior in studying the brain, particularly the motor cortex.

Points to address:

Regarding prey capture: The authors utilize a novel behavioral paradigm involving prey capture in marmosets, facilitated by a specialized chamber enabling high-precision tracking of hand movements. While this approach is commendable, the description of the behavior lacks depth in several critical aspects. Enhancing the analysis of behavior statistics could significantly contribute to our understanding of what functional networks (FNs) capture. Moreover, identifying and describing capture strategies or predominant movement features related to prey identification, planning, tracking, and error correction would be highly informative. These insights could potentially be integrated into the encoding model to capture neural variability more effectively.

For instance, in the general quantification of prey capture behavior, additional information on the frequency and duration of visits, the number of reaches per visit, and potential FN changes between visits could offer valuable insights. Exploring the variability in reaching behavior between reaches and visits, such as distributions of velocity and position kinematic features used in the Generalized Linear Model (GLM), would provide a clearer understanding. Furthermore, investigating how these features co-vary and assessing whether their contribution to the model depends on the aforementioned behavior statistics would be insightful.

Additionally, a separate analysis focusing on higher-level features and strategies in prey capture is warranted. This exploration could reveal whether the behavior is predominantly random or if it offers opportunities for meaningful and useful descriptions. I encourage the authors to delve into this aspect, as it has the potential to uncover valuable insights beyond the current scope of the study.

Regarding the spontaneous vs pray capture. As noted above, it is challenging to determine the extent to which the observed results are influenced by the behavioral repertoire or overlapping variability between settings. The authors acknowledge limitations in the camera setup's ability to finely track the animals. However, there is potential to annotate and quantify candidate general behaviors, particularly directed hand movements such as reaching, object handling, and grooming. Even without identifying the finer details of these moments, the authors can utilize time stamps of annotated behaviors to construct functional networks (FNs) corresponding to each type and compare them to periods lacking clear directed hand movements. Examining the similarity or dissimilarity of these FNs among themselves and in comparison to prey capture behaviors could shed light on some of the unresolved questions posed by the authors themselves in the discussion section.

Reviewer #3

(Remarks to the Author)
I am not an expert on motor behavior or representations in M1. Nevertheless, I find the key findings in Moore et al. – that a minority of neurons form a context-specific functional group which increases its connectivity during reaches and is more tightly linked to kinematics than other neurons, and that the average in-weight and kinematics AUC are strongly correlated – interesting and potentially surprising. I have only 3 comments:

1. It is important to evaluate whether there might be something that distinguishes the context-specific functional group in spontaneous conditions. This gets at the question, raised by the authors in the Discussion, of whether: these cells likely represent a group that is related to extension movements, and that other analogous groups comprising different neurons exhibit context-specific reorganization of their functional interactions during other movements; or, these represent a distinct neuronal population (inhibitory interneurons?) that plays a unique role in network dynamics. I think the authors could address this question by looking at many factors related to the individual neurons e.g., firing rate, burstiness, etc., and their network connectivity, e.g., distribution of incoming and outgoing weights (strengths, skew towards positive vs. negative values, etc.), clustering of functional connections, etc. They could also compare the networks formed by the context-specific neurons vs. context-invariant neurons vs. all neurons under spontaneous conditions.
2. I found the paragraph at the bottom of page 10 (lines 249-258) pretty confusing and would recommend re-writing this to improve clarity.
3. Related to this, I find the interpretation of Fig. 4 confusing. Why is permuting a larger fraction of weights more likely to reveal a greater influence of stronger weights (panel b) whereas permuting a smaller fraction of target units is necessary to reveal a greater influence of stronger weights (panel c)? I think improving the clarity of the presentation and discussion of this entire section related to this type of analysis is necessary – I have not read the paper which this is based on so maybe some greater exposition is needed here.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
My main criticism was that the novelty of this paper is limited. The response from the authors did not change much my opinion that this is rather an incremental advancement.
For example, authors say that “novel aspects to the results ... we are working with a new primate model that remains under-studied relative to the immense body of work in macaques.” That’s true but the marmoset has a very similar organization of

sensorimotor system to macaques, thus providing more electrophysiological data for the marmoset is useful but it is not groundbreaking. A similar point can be made for most other responses provided by authors.
Note that since posting the preprint of this manuscript in Dec 2023, it did not get even a single citation. I understand that it is still very early, but this suggests that for a community, this research also does not seem to represent as important advancement.
I also have a problem with the way how this manuscript was revised, as most of my comments were only discussed in the response letter, and were not implemented in the manuscript. If a reviewer does not understand e.g. novelty of some results then likely other readers may have similar questions. The main role of a reviewer is to help to improve a manuscript, thus replaying to comments only in the letter is not a proper revision.

Reviewer #3

(Remarks to the Author)
I am satisfied with the authors' revisions.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors made multiple changes and improved this manuscript. Considering that other Reviewers had a much more favorable view of the importance of this work, I am OK with the current version. I guess, time will tell who was right.

## Response to Reviewers

We would like to thank the reviewers for their helpful comments. Addressing their critiques have made the manuscript much stronger. The reviewers' comments are denoted blue while our responses to the reviewers are in black text. Edits in the manuscript are presented in red text.

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors study how well neuronal activity can be predicted from behavior and from the activity of other neurons. It is nice that authors study here freely moving animals, as opposed to the vast majority of previous studies where behavior is restricted to a single task on which animals are (over)trained. However, despite that unique behavioral setup, the level of novelty in results is very limited here. To better describe it, I will follow the order of figures:

Fig. 1) It is an illustration of the experimental setup.

Fig. 2) It shows that neuronal activity in the motor/sensory cortex can be predicted from movement trajectories. It has been well-known for a long time that activity in the motor/sensory cortex is related to movement.

It is true that this broad finding – that activity in sensorimotor cortex relates to movement – has been well-known for a long time. There are, however, two novel aspects to the results we show here. First, we are analyzing more naturalistic behavior than previous work. Second, we are working with a new primate model that remains under-studied relative to the immense body of work in macaques. It was not guaranteed that an encoding model predicting spiking activity in macaque motor cortex based on well-trained 2D movements or reach-to-grasp movements would extend to predicting activity in marmoset sensorimotor cortex during a 3D, naturalistic prey-capture task. Additionally, establishing the encoding model as a useful description of the relationship between spiking and kinematics is foundational to the results presented in the rest of the paper.

Fig. 3) Authors show that including information about the activity of other neurons can improve predictions from Fig. 2. This is also well known that neurons are correlated, thus it is obvious that including this information can improve predictions (see sample References below).

Ref.:

Hatsopoulos et al. Information about movement direction obtained from synchronous activity of motorcortical neurons. PNAS 1998

Lin IC, Okun M, Carandini M, Harris KD. The nature of shared cortical variability. Neuron. 2015

The reviewer is correct in stating this improvement matched results of simpler models and behaviors and confirmed our hypothesis that prediction would be improved by incorporating the pairwise statistical interactions. However, to our knowledge nobody has looked at correlations between spike times in this way in motor cortex, and definitely not with behavior approaching this level of complexity. In Hatsopoulos et al 1998, the behavior is a 8-direction planar center-out task. In Lin et al 2015, the authors studied trial-long responses in visual cortex using a simple visual task in cats and mice, whereas here we examine the mutual information between spike trains at a fine timescale. Additionally, the result shown here is foundational to subsequent results - we have to show that incorporating the functional network improves the model before we can look more deeply into the details of the network. Furthermore, panels B and C of Fig. 3 provide novel context for understanding the relationship between kinematic tuning and functional interactions within the network.

Fig. 4) It is also obvious that permuting the strongest (most correlated) connections will have the largest effect on predictability, as those are the most predictive connections. It is a simple mathematical fact and not some surprising property of the brain networks.

We apologize for the unclear text that describes the results shown in Figure 4. We have revised this section and edited the figure to provide more clarity as to the purpose of each type of network permutation and the results. In short, the purpose of Figure 4 is to set a high bar for determining that the strongest connections are actually important at the level of specific connections and topology - that prediction of spiking doesn't depend on simply a large aggregate in-weight, but on the particular topology of the interactions. We also added text to highlight the differences in results for weight and edge permutations (Lines 281-284, 300-308).

Fig. 5) Showing similarities and differences between predictability in “reach” vs “spontaneous” condition is the most interesting part of this manuscript. However, it was shown already multiple times before that neuronal activity across tasks and states can be quite preserved. Such previously described similarities in activity are not exact, and there are some cells that can be defined as “context-specific” as called in this manuscript (see sample References below). Thus, this figure is also not that surprising.

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MacLean, J.N., Watson, B.O., Aaron, G.B., and Yuste, R. Internal dynamics determine the cortical response to thalamic stimulation. Neuron 2005..

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Levy, M., Sporns, O. & MacLean, J. N. Network Analysis of Murine Cortical Dynamics Implicates Untuned Neurons in Visual Stimulus Coding. Cell Rep (2020).

In MacLean et al (2005), the authors showed that synchronous activity patterns were similar between evoked and spontaneous responses in slice. They did not find any statistically significant differences between evoked and spontaneous responses. In Luczak, Bartho and Harris (2009), the authors study rodents under anesthesia and during passive sensory stimulation. The results demonstrate that possible sensory responses are constrained by the underlying structure of interactions within the population. However, the experimental setup differs from ours in key ways: we are studying a range of awake motor behaviors and comparing multiple behavior states rather than comparing artificial spontaneous states (i.e., slice without input or under anesthesia) to sensory inputs or behavior.

In Levy et al (2020), the authors show that units without significant tuning to the visual stimulus were involved in decoding the stimulus at the network level. Connectivity between tuned units was “context-specific” with dependence on the stimulus orientation. Connectivity between untuned units was “context-invariant.” However, the contexts span only the different stimulus orientations, which arguably is a much tighter range than the span of spontaneous motor behaviors such as rest and locomotion compared to prey-capture. We discuss the findings of this paper in our discussion and highlight similarities and differences with our results.

Overall, we contend that our findings in Figures 5 and 6 are novel for three reasons. First, preserved neural activity across behavioral contexts has not been shown in primate sensorimotor cortex. Second, prior work did not span the behavioral range used here. Third, prior work did not link the presence of conserved and context-specific modules to behavior, as we do in Figure 6. Thus, the findings presented in Figures 5 and 6 provide context and evidence for context-invariant and context-specific modules and activity structures in a new species and brain area and cover previously unexamined behaviors.

Fig. 6) Is a continuation of Fig. 5 showing more details.

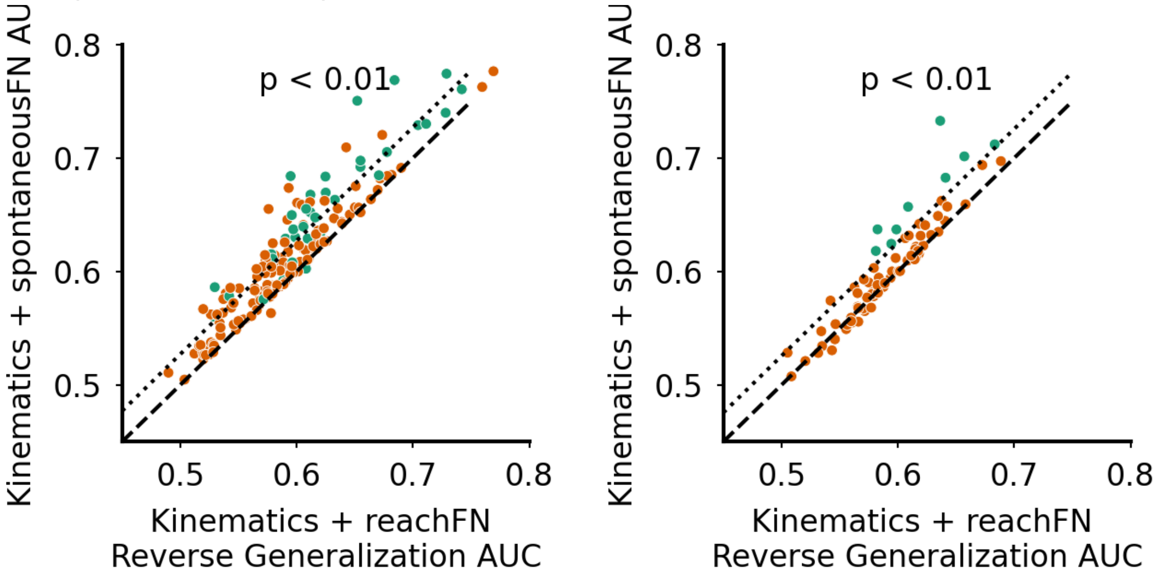
Other points:
1) Manuscript is difficult to understand. It seems to be written for other lab members who already know details of the methods. I had to go to the Methods section multiple times to understand basic information about analyses. It should be much better/simpler explained in the Introduction/Results.
Thank you for this note, as we seek to make the work approachable to every reader. Due to the word limit constraints for our submission, some details of the methods could not be fully explained within the Results section. Regardless, your point is well-taken and we have sought feedback from members of the lab with little exposure to these methods to identify key points of confusion. First, the many variations of the GLM kinematic encoding model are hard to follow without a visual guide. We have added a high-level flow chart (Supplementary Fig. 2) to aid understanding. Second, we have made substantial changes to the presentation of Figure 4, which was an issue for multiple readers and reviewers.

2) The functional network (FN) method used here is also not new and was published before by co-authors. Maybe it is explained in those other papers, but it is not clear to me what is the advantage of this method over using just population vectors for predictions. It seems that using population vectors would give very similar results and would be much easier to understand by a typical reader.
We appreciate this feedback and we want this rationale to be clear in the text. Thus, we have revised text in Results to clarify the rationale for using the FN method rather than fitting coupling coefficients to population vectors (originally lines 177-184, now lines 193-201). Critically, the use of the FN method is essential to the analyses producing Figures 5 and 6 - we could not have done these generalization experiments without the FN method.

3) It is nice that the authors provided code to reproduce analyses, but there is no link to data in the manuscript.
Unfortunately, we played a bit of email tag trying to get a link at which to provide the necessary data. We did prepare the data for sharing before the manuscript was sent to reviewers. The data are available now on Dandi (see link in Data Availability).

4) In Fig 5, will you have new “context-specific” neurons if you would do training on reachFN, and test on spontaneousFN, instead?

We have run this test and provide the result below. The results of this experiment are shown for marmoset TY on the left and MG on the right, with data colored according to the original context-specific and context-invariant functional groups from Fig. 5. Briefly, we do find a context-specific functional group which overlaps to some extent for TY and almost perfectly for MG. However, it is not clear to us what we learn from the result. In the experiment presented in Figs 5-6, the test on held-out data was conducted in both cases using the reachFN which was linked to prey-capture reaching behavior (the “correct” pairing). In the reverse, the test on held-out data is run using a mismatch of the spontaneousFN with reaching. This test would be most useful if we had a comparable encoding model for kinematics+FN during spontaneous behaviors (such as locomotion). Unfortunately, such a model is not readily available and beyond the scope of this manuscript.



Reviewer #1 (Remarks on code availability):

There is no link to data in the manuscript, thus I am not sure if code can be executed without data.

Please see our response to (3).

Reviewer #2 (Remarks to the Author):

This study describes the factors influencing individual unit activity in the sensorimotor cortex of marmosets while they engage in natural behaviors. It offers valuable insights into both the unique behavioral context and the relationships between networks and unit tuning within this context. However, the analysis of behavior should be significantly improved to better contextualize some of the findings. Nonetheless, it is an interesting study that makes a significant contribution while presenting compelling results that pave the way for future research, promising to enhance our comprehension of cortical activity during naturalistic behavior.

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Comparing the two contexts, most neuron models show similar improvements when using FN features extracted from spontaneous activity. However, a smaller subgroup of neurons exhibits significantly greater improvement with prey capture FN features. Intriguingly, units sensitive to prey capture FN features also demonstrate greater tuning to kinematics and stronger interconnections, correlating with preferred trajectories and showing connectivity reorganization across settings, as evidenced by meticulous perturbation tests of the models.

General conclusions:
Overall, the authors have compiled a valuable dataset using an exciting approach to record neural activity during both prey capture and spontaneous movements in marmosets. This work holds significant importance in the field as it marks a crucial step towards studying natural behavior, essential for advancing our understanding of the brain. The manuscript is well-written and the results are presented clearly. I applaud the authors for making it easily accessible even to readers unfamiliar with the models. The findings highlight the factors influencing single neuron activity in the sensorimotor cortex during untrained, natural behavior, demonstrating sensitivity to kinematic features and highlighting specific connectivity patterns within the local circuit. Furthermore, they suggest that these connectivity patterns may be influenced by the types of movements required within a given context.
The primary findings indicate that models previously used to predict neuronal activity in controlled and trained settings align well with natural behavior. This significant discovery implies that, fundamentally, neurons in this area are consistently attuned to kinematic features, irrespective of the experimental paradigm (trained or natural). This observation carries two implications: firstly, the generalizability of certain behavioral features to neuron activity in the motor cortex, and secondly, a potential oversight in the manuscript. Despite obtaining a unique

dataset involving prey capture behavior, the authors have not provided detailed insights into this complex behavior, which involves tracking, planning, and rapid feedback-driven error correction. By fully leveraging the uniqueness of their dataset, the authors might uncover additional neural tuning factors related to higher-level aspects of behavior beyond those applicable in controlled or trained datasets. A more comprehensive quantification and analysis of prey capture behavior could potentially enhance the encoding models and reveal valuable insights into neural tuning. However, the outcome of such analysis remains uncertain, leaving it to the authors' discretion to determine its relevance to the manuscript. Nevertheless, improved quantification of prey capture behavior would bolster the validity of the results and aid readers in assessing their robustness.

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We appreciate the detailed summary and helpful comments. We address them in detail below.

Points to address:
Regarding prey capture: The authors utilize a novel behavioral paradigm involving prey capture in marmosets, facilitated by a specialized chamber enabling high-precision tracking of hand movements. While this approach is commendable, the description of the behavior lacks depth in several critical aspects. Enhancing the analysis of behavior statistics could significantly contribute to our understanding of what functional networks (FNs) capture. Moreover, identifying and describing capture strategies or predominant movement features related to prey identification, planning, tracking, and error correction would be highly informative. These insights could potentially be integrated into the encoding model to capture neural variability more effectively.

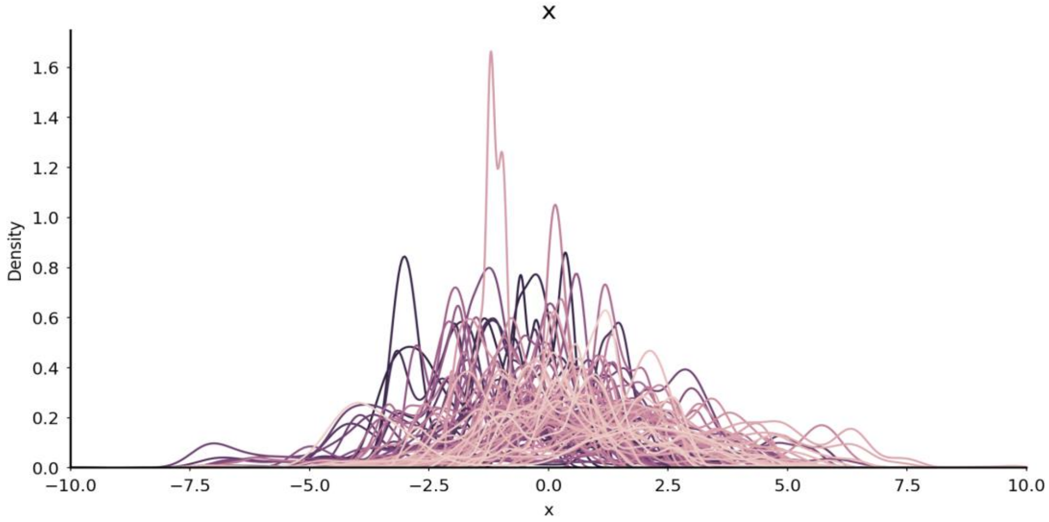
For instance, in the general quantification of prey capture behavior, additional information on the frequency and duration of visits, the number of reaches per visit, and potential FN changes between visits could offer valuable insights. Exploring the variability in reaching behavior between reaches and visits, such as distributions of velocity and position kinematic features used in the Generalized Linear Model (GLM), would provide a clearer understanding.

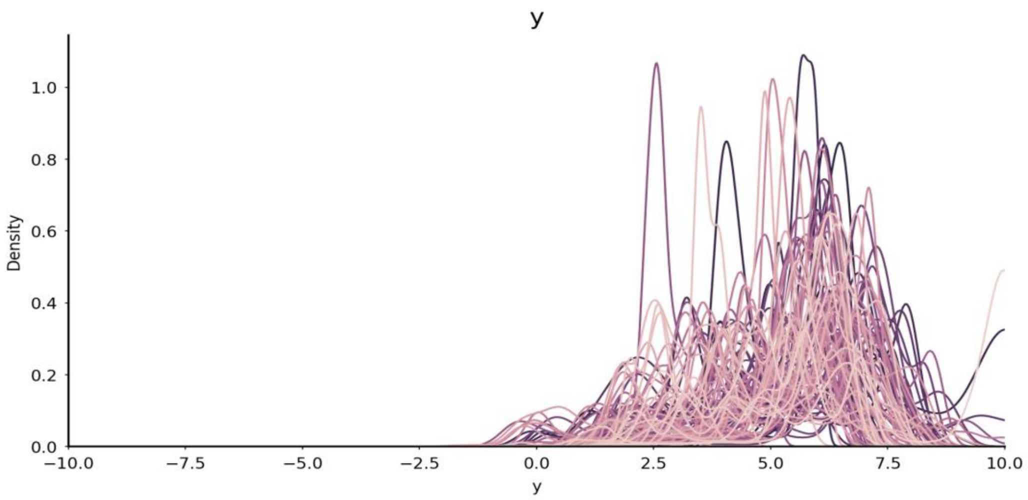
Furthermore, investigating how these features co-vary and assessing whether their contribution to the model depends on the aforementioned behavior statistics would be insightful.

Thank you for these comments. We have added basic information about the visits and reaches/visit to the methods section (Lines 557-568). In preparing this manuscript, we computed various kinematic features of the trajectories (such as mean speed, peak speed, and tortuosity) and attempted to incorporate these features into our investigation of the encoding model. For example, one might expect there to be 2 (or more) clusters of trajectories corresponding to ballistic movements (high peak speed, low tortuosity) vs corrective movements (low peak speed, high tortuosity). However, we did not find any actionable results from these kinematic metrics or a way to incorporate them into the model in any informative way.

We have now examined the distributions of velocity and position in each dimension as well as speed separately for each reach (darker for each consecutive reach) and examined any potential relationships between these features and FNs computed from individual reaches. We plotted distributions rather than as a function of time due to major differences in reach duration. We don't observe any clear progression or separation of groups of reaches by kinematics.

**Position**

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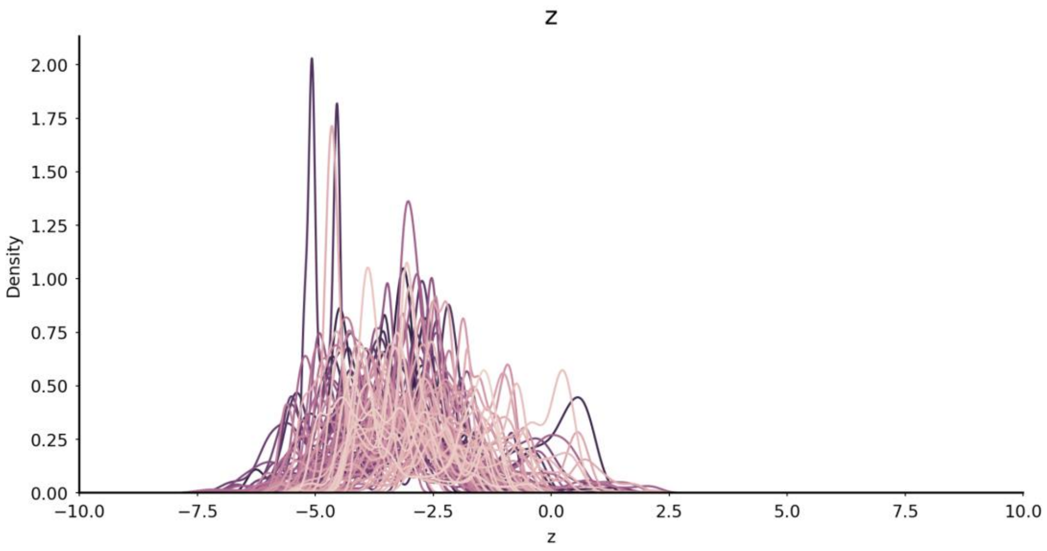
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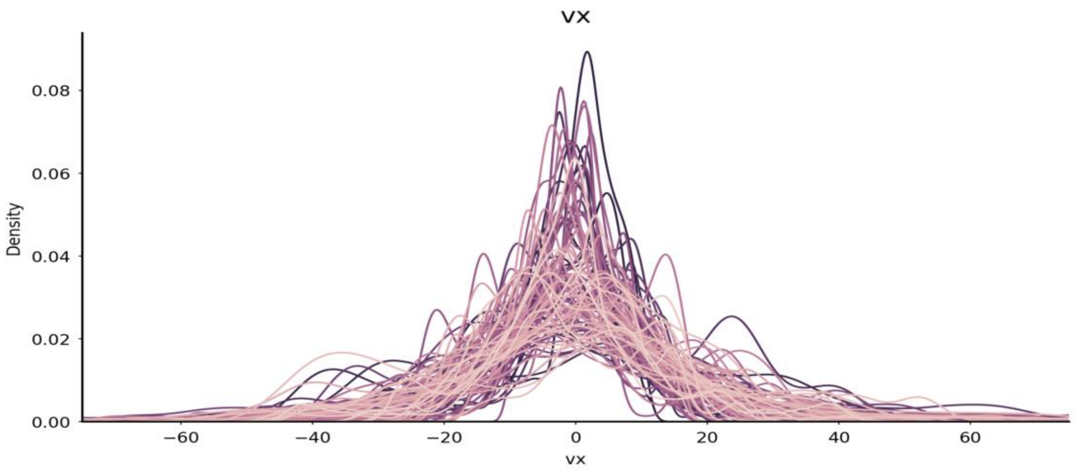
## **Velocity**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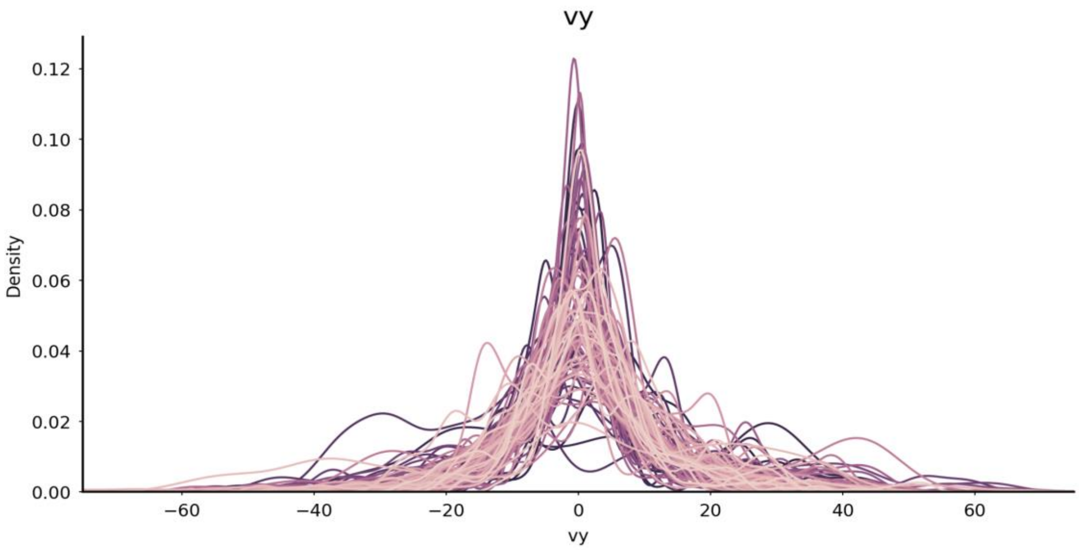
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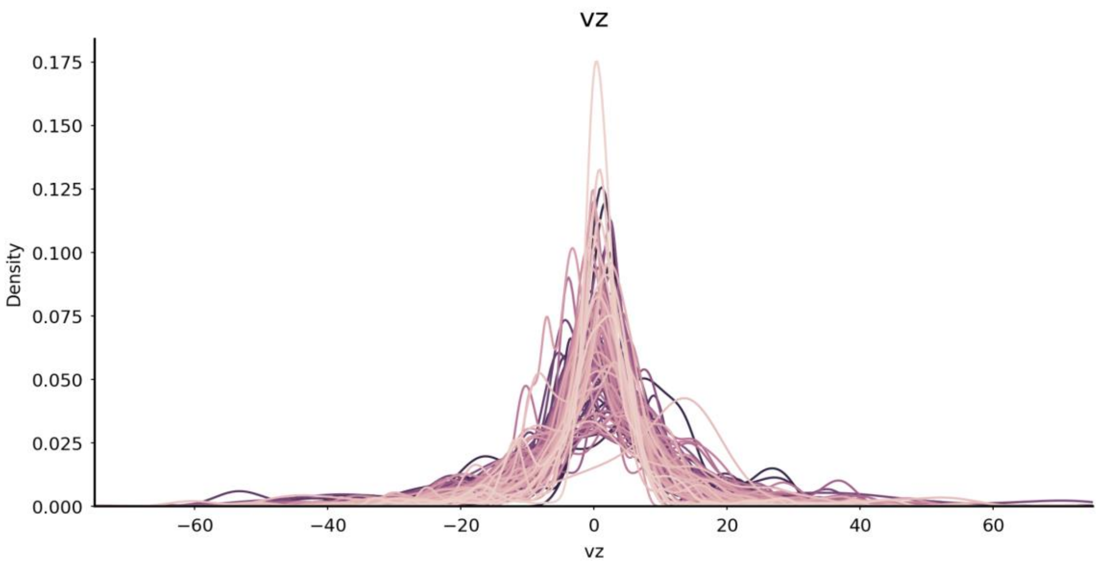
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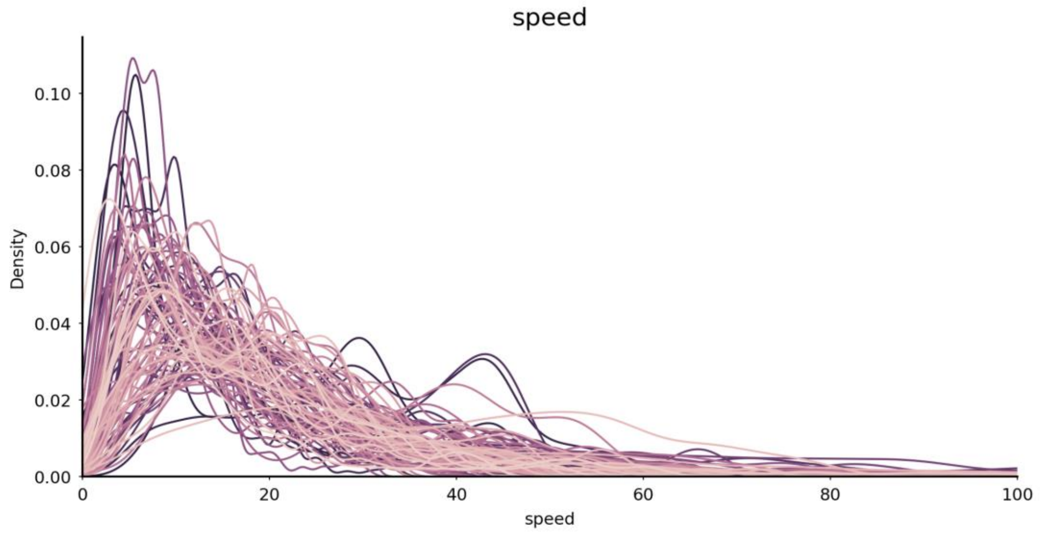
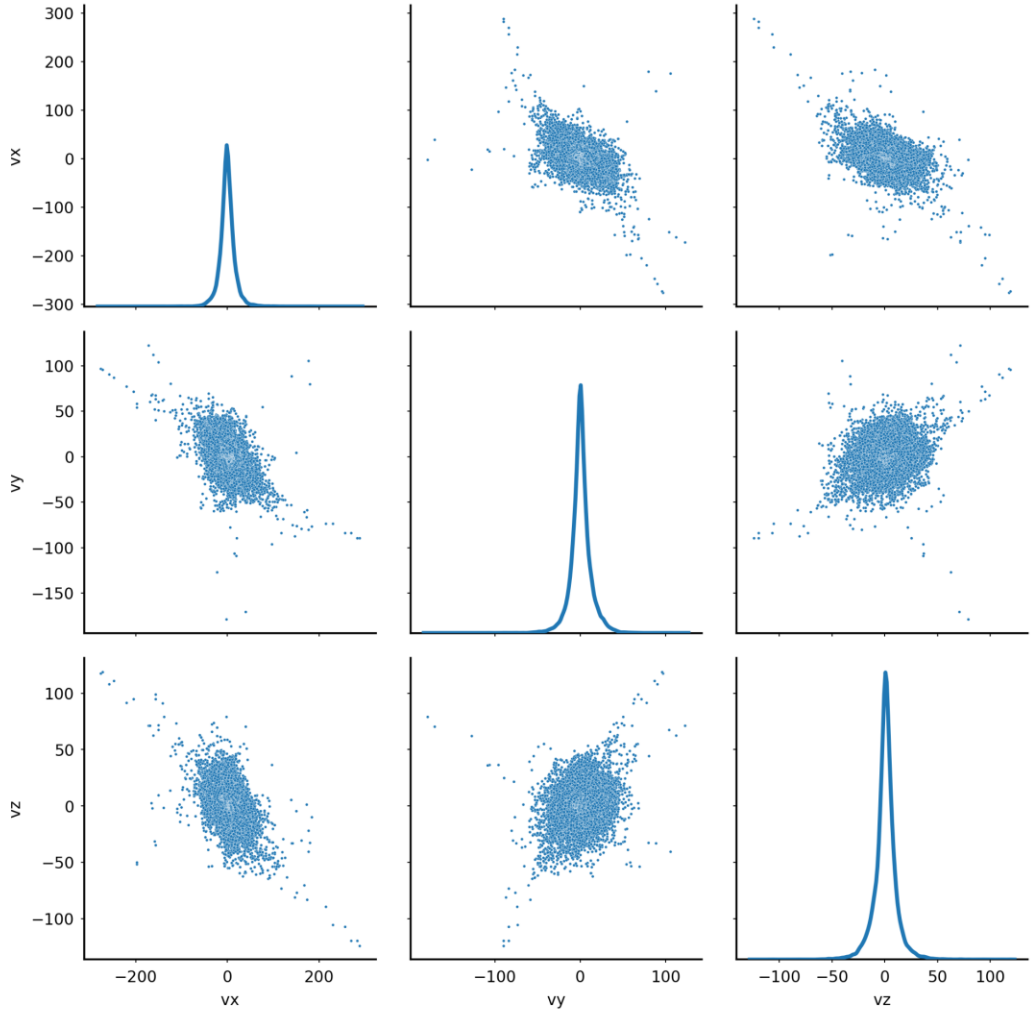

speed
Density
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
speed

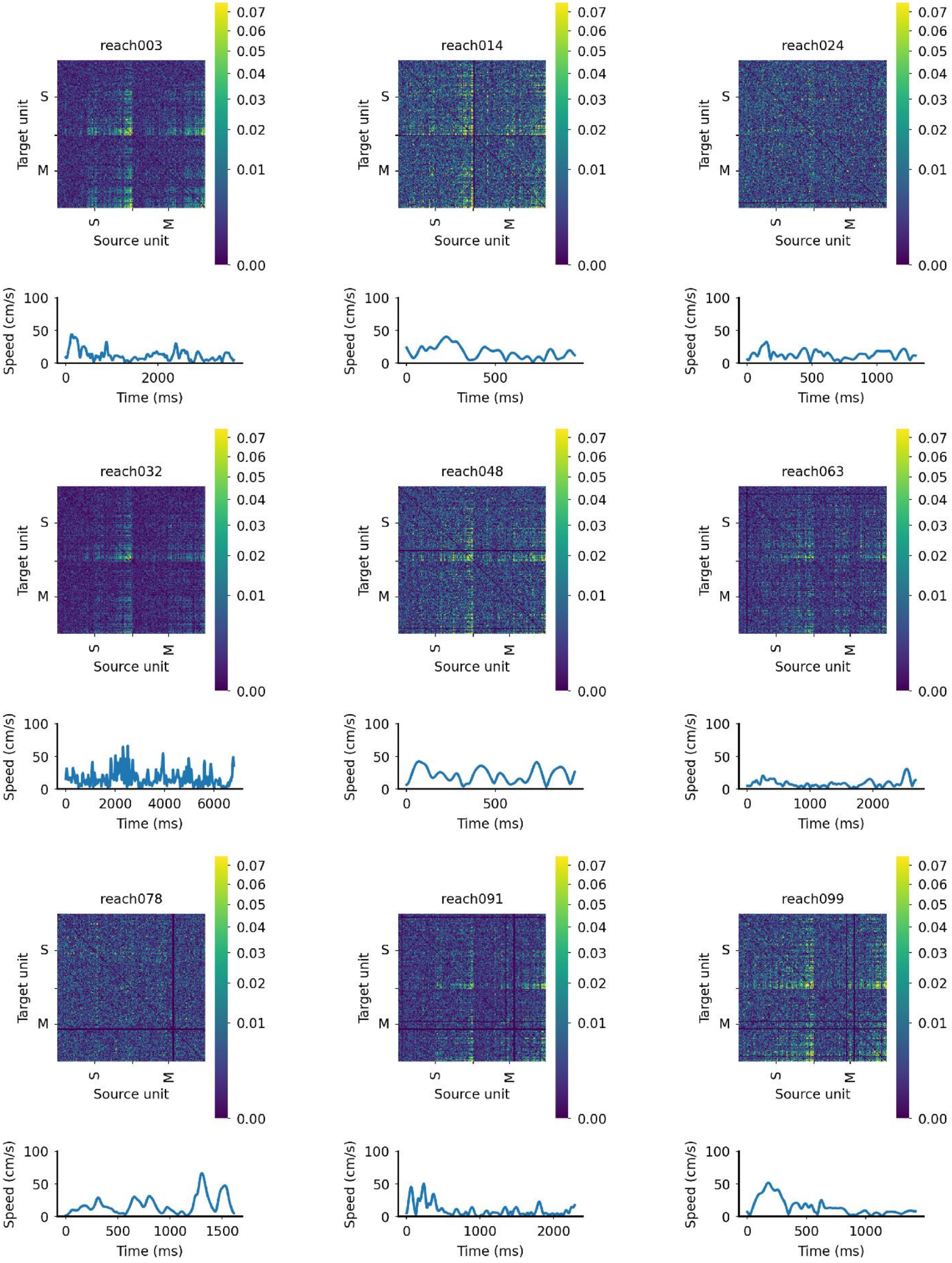
We also looked at covariance between behavior statistics – we found covariability particularly between velocities in the X and Y dimension which align with biomechanical constraints. However, we do not immediately see how to integrate these in the model differently from our previous attempts.

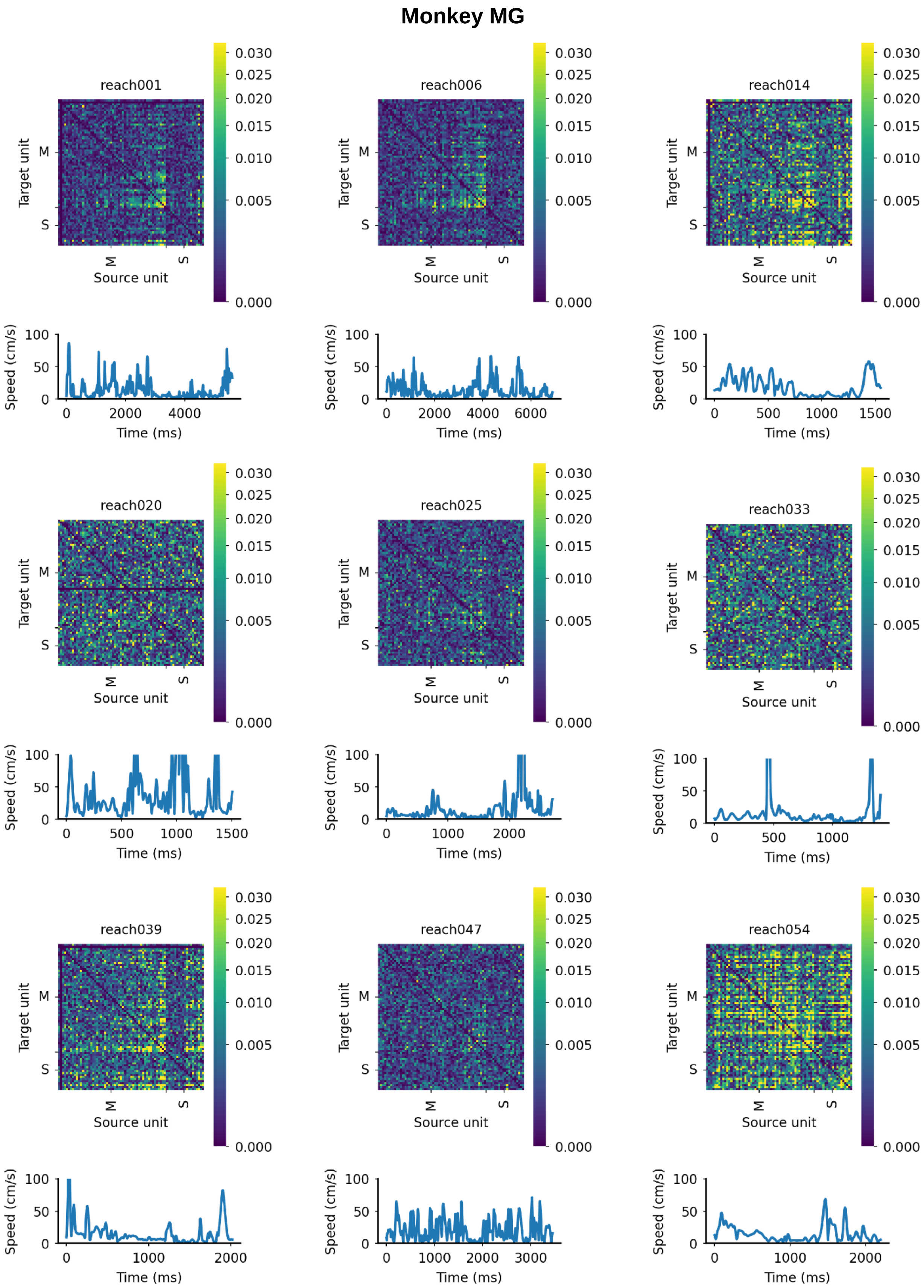


A analysis that proved fruitful is separating the extension and retraction movements within reaching and creating a functional network to describe activity patterns in each behavior. We have included these results in the revisions.

Regarding the evolution of the functional networks over the course of the experiment, we have computed FNs for each individual reach so that we can look at FNs and speed profiles together. We see no systematic changes in the FN over the course of the experiment. Furthermore, we see no relationship between the speed profile of each reach and its FN. Below are 9 example reaches chosen randomly for TY, followed by 9 reaches for MG. These single reaches largely reflect the same structured connectivity shown in the aggregate reachFNs.

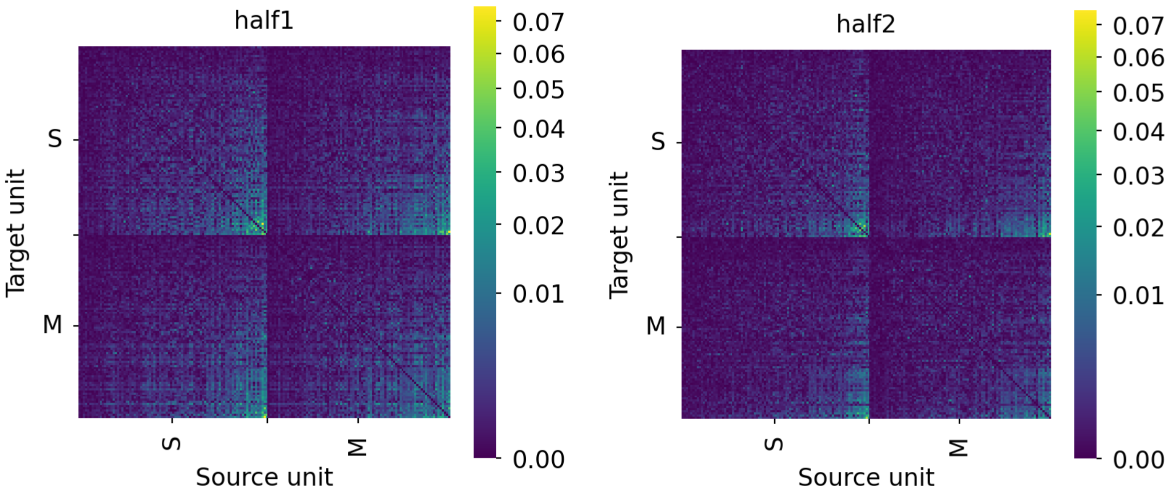
**Monkey TY**



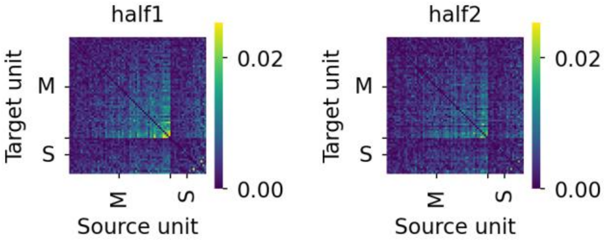

Monkey MG
reach001
reach006
reach014
reach020
reach025
reach033
reach039
reach047
reach054
Target unit
Source unit
M
S
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Speed (cm/s)
100
50
0
Time (ms)
0
2000
4000
6000
500
1000
1500
3000

We also looked at the FNs for the first and second half of all reaches to see if there were any systematic changes that could be detected when computed over longer periods. The FNs are qualitatively identical for the first half of reaches in the session compared to the second half.

**Monkey TY**



**Monkey MG**



Additionally, a separate analysis focusing on higher-level features and strategies in prey capture is warranted. This exploration could reveal whether the behavior is predominantly random or if it offers opportunities for meaningful and useful descriptions. I encourage the authors to delve into this aspect, as it has the potential to uncover valuable insights beyond the current scope of the study.

This is a great suggestion. In fact, a manuscript focused on the details of capture strategies and their neural basis is in preparation by an overlapping set of authors. This work is ongoing and beyond the scope of this manuscript, although the findings may inform future work with this model.

Regarding the spontaneous vs prey capture. As noted above, it is challenging to determine the extent to which the observed results are influenced by the behavioral repertoire or overlapping variability between settings. The authors acknowledge limitations in the camera setup's ability to finely track the animals. However, there is potential to annotate and quantify candidate general behaviors, particularly directed hand movements such as reaching, object handling, and grooming. Even without identifying the finer details of these moments, the authors can utilize time stamps of annotated behaviors to construct functional networks (FNs) corresponding to each type and compare them to periods lacking clear directed hand movements. Examining the similarity or dissimilarity of these FNs among themselves and in comparison to prey capture behaviors could shed light on some of the unresolved questions posed by the authors themselves in the discussion section.

We have completed this annotation and present the results in the revision (lines 383-393, 406-430), Supplementary Figs. 9-12). We updated the discussion to reflect these results as well. Please see the response to Reviewer #3 below for further detail.

Reviewer #3 (Remarks to the Author):

I am not an expert on motor behavior or representations in M1. Nevertheless, I find the key findings in Moore et al. – that a minority of neurons form a context-specific functional group which increases its connectivity during reaches and is more tightly linked to kinematics than other neurons, and that the average in-weight and kinematics AUC are strongly correlated – interesting and potentially surprising. I have only 3 comments:

1. It is important to evaluate whether there might be something that distinguishes the context-specific functional group in spontaneous conditions. This gets at the question, raised by the authors in the Discussion, of whether: these cells likely represent a group that is related to extension movements, and that other analogous groups comprising different neurons exhibit context-specific reorganization of their functional interactions during other movements; or, these represent a distinct neuronal population (inhibitory interneurons?) that plays a unique role in network dynamics. I think the authors could address this question by looking at many factors related to the individual neurons e.g., firing rate, burstiness, etc., and their network connectivity, e.g., distribution of incoming and outgoing weights (strengths, skew towards positive vs. negative values, etc.), clustering of functional connections, etc. They could also compare the networks formed by the context-specific neurons vs. context-invariant neurons vs. all neurons under spontaneous conditions.

These are all good suggestions. We have annotated spontaneous behavior, examined functional groups from multiple behaviors in relationship to the already presented spontaneous and reach FN, and completed generalization experiments for Rest and Locomotion FNs (just as we did with the inclusive spontaneousFN in Figs 5-6. We present the results in the revision (lines 392-427, Supplementary Figs. 9-12). We updated the discussion to reflect these results as well. We note that we annotated many spontaneous behaviors: rest, locomotion, climbing, non-prey-capture arm movements, and being groomed. We present FNs computed during rest and locomotion and completed generalization experiments using these FNs in place of the spontaneousFN. We chose rest and locomotion because they were both well-represented in the spontaneous behavior, with rest being very different from prey-capture and locomotion being more similar (both behaviorally and in the distribution of firing rates and weights vs. distance relationship). We expected non-prey-capture arm movements to be highly informative, but this class occurred rarely during spontaneous behavior to the point that the functional weights could not be reliably computed. These timepoints also exhibited firing rates and a weight-distance relationship more similar to rest than to reaching. These movements, which included movements before and after prey-capture in the apparatus as well as some reaching and grooming movements in the home enclosure, tended to be slow movements with small changes in position. Regarding climbing, for which we might expect results to be similar to locomotion: this was true for some results, but generalization experiments using the climbing FN produced opposite results for the two monkeys. We are not sure what to make of this, so we focus on locomotion instead.

In preparing the manuscript, we identified narrow-spiking (NS, putative inhibitory) and wide-spiking (WS, putative excitatory) units using the method published at https://journals.physiology.org/doi/full/10.1152/jn.00343.2016, and examined any relationship between these neuron types and the separate functional groups. We found that the ratio of NS to WS neurons was nearly identical between the context-specific group, context-invariant group, and the full FN in both monkey TY and monkey MG. We take this as a firm indicator that the context-specific group cannot be explained by differential membership of inhibitory interneurons. However, we did find that the context-specific functional group contains very few multi-units compared to the full network in both monkeys.

Regarding the skew towards positive vs. negative values, the confluent mutual information metric can only be positive so we are unable to identify inhibitory neurons in this way.

2. I found the paragraph at the bottom of page 10 (lines 249-258) pretty confusing and would recommend re-writing this to improve clarity.
See next response.

3. Related to this, I find the interpretation of Fig. 4 confusing. Why is permuting a larger fraction of weights more likely to reveal a greater influence of stronger weights (panel b) whereas permuting a smaller fraction of target units is necessary to reveal a greater influence of stronger weights (panel c)? I think improving the clarity of the presentation and discussion of this entire section related to this type of analysis is necessary – I have not read the paper which this is based on so maybe some greater exposition is needed here.
Thank you for these comments - the text and description of Figure 4 has been noted as confusing by multiple reviewers and readers. We have revised this section for clarity and added text to highlight the differences in results for weight and edge permutations (Lines 301-308). We hope the revised text and additional explanation answer all your questions raised here.

## Response to Reviewers (Second Revision)

We would like to thank Reviewer #1 for their commitment to improving the manuscript. Addressing their critiques has provided the opportunity to highlight the novelty of our findings more clearly. The reviewer's comments are denoted in blue while our response to the reviewer is in black text. Edits in the manuscript are presented in red text.

Reviewer #1 (Remarks to the Author):
My main criticism was that the novelty of this paper is limited. The response from the authors did not change much my opinion that this is rather an incremental advancement.
For example, authors say that "novel aspects to the results ... we are working with a new primate model that remains under-studied relative to the immense body of work in macaques." That's true but the marmoset has a very similar organization of sensorimotor system to macaques, thus providing more electrophysiological data for the marmoset is useful but it is not groundbreaking. A similar point can be made for most other responses provided by authors.
Note that since posting the preprint of this manuscript in Dec 2023, it did not get even a single citation. I understand that it is still very early, but this suggests that for a community, this research also does not seem to represent as important advancement.

Reviewers #2 and #3 expressed that our findings constitute a valuable, substantial, and potentially surprising contribution to the field. In response to the initial reviews, we made a concerted effort to clearly highlight the novelty and importance of our findings, and it is evident that the other reviewers agree that we were successful. Moreover, we have now made further revisions to the manuscript by adding a paragraph to the Discussion and revising lines in the Introduction that highlight the novelty of our findings.

I also have a problem with the way how this manuscript was revised, as most of my comments were only discussed in the response letter, and were not implemented in the manuscript. If a reviewer does not understand e.g. novelty of some results then likely other readers may have similar questions. The main role of a reviewer is to help to improve a manuscript, thus replaying to comments only in the letter is not a proper revision.

We believe we addressed many of the reviewer's original concerns in the first revision of the manuscript. We made substantial changes to descriptions of methodology within the Results section to improve clarity (primarily the text accompanying Figures 2 and 4 as well as the visualization of the analytical pipeline shown in Supplementary Fig. 2). We also added comments in multiple sections of the Introduction and Results to contextualize the findings and their significance, including a full paragraph to summarize the important takeaways of Figure 4. To further address the reviewer's concerns, we have made two revisions for the latest version of the manuscript. First, we added a full paragraph to the Discussion (lines 442-453) with references to MacLean et al. (2005) and Luczak et al. (2009), as suggested by the reviewer, as well as to Arieli et al. (2006), to compare our work with prior studies which in turn further highlights the novelty of our findings. The third reference suggested by the reviewer (Levy et al., 2020) is discussed separately within the manuscript. We also made minor changes to a portion of the Introduction (lines 34-37) to incorporate Hatsopoulos et al. (1998) which further highlights the novelty of our results in contrast to prior work. We chose not to reference Lin et al. (2015) because we are not making any claims about the underlying model governing cortical covariability. Finally, we thank the reviewer for prompting this revision, especially the textual additions to the Discussion, since it enabled us to improve the clarity with which we present our most novel findings.