Peer Review File

Manuscript Title: Cortical preparatory activity indexes learned motor memories

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

This is a review of “Skill-specific changes in cortical preparatory activity during motor learning” by Sun and colleagues. The paper asks an important and long-standing issue in arm motor control and motor learning, specifically the neuronal basis of learning a new dynamical load, in this case the canonical curl-field which has been studies in hundreds of human studies.

The actual experimental approach is straightforward and logical. Two rhesus monkeys were trained to reach while holding a joystick attached to a robot the movement of which veridically controlled the movement of a cursor on a screen that the animal could see. The reach targets were presented to the animal on the same screen. Initially no loads were applied. After some number of baseline reaches, a load was introduced for only one target that pushed the animal's hand perpendicular to its movement direction with a magnitude proportional to the movement speed (the curl field). This initially displaced the hand but the animals learned to compensate for the load and returned to roughly straight reaches, evidence of learning the applied load. After some amount of learning, the animal started reaching to all 12 potential targets but the other 11 had no curl field. For all these reaches the animal also experienced so-called clamp trials where the robot forced the animal to reach straight through a virtual channel and measured how hard the animal pushed against the channel, providing a direct measure of the forces the animal thought it needed to generate to counter the load. After these trials, the loads were turned off and the animal returned to its baseline behavior. This is essentially the standard approach to this kind of study with the exception of having the curl field in place for only one target location.

In terms of results. First, the animals roughly behave as expected (though see #4 below). They show initial errors after the field is introduced, which is slowly reduced over trials. They generalize this learning to targets nearby but not far away. They show kinematic after-effects (errors in the opposite direction as the load) in the washout epoch and they eventually return to baseline performance again. The core results, and what is to be seen as the major advance here, is a dynamical systems description of the population level neural activity in PMd-M1 (acquired at the single cell level) that is correlated to this behavior. In my view, there are two key findings. First, there exists a neural subspace where shifts in the preparatory state closely reflect the behavior. As expected, therefore, these shifts only apply to those reaches that demonstrate learning. Second, there exists another shift in neural states along a different dimension that applies to all reaching targets uniformly, which implies the formation of some new set of neural activity. When the field is removed and the animal returns to baseline, the uniform shift does not return to baseline rather another uniform shift is introduced. This uniform shift may tag force field environment, and these tags may underlie so-called savings where re-learning a field is faster the second time than the first time.

My general assessment is that this a substantive effort on many fronts and one that usefully drives the field forward. On the other hand, I don't think the scope of the advance presented here is sufficient given the past literature and the desired venue. There are several critical empirical details missing that could get it there but the amount of work needed is substantial given the animal model used being used.

1. Of the key findings, the first one (shifts in neural state that predict behavior) seems a minor advance relative to Bizzi (2001) as well as others (as cited) who demonstrated much of this before. The demonstration of generalization function is nice but rather expected and certainly not
a shift in thinking for the field. The authors recognize this I think and spend most of their time on
the second claim about the uniform shift. But is this new? There is a paper by Rokni and
colleagues (Neuron, 2007) that not only gets to a similar point but provides a conceptual
underpinning for why it may be so – which I note that the authors don’t cite. Now, I don’t think
these are identical claims and I think the present paper provides a much stronger and more
nuanced analysis empirically. At the very least the authors need make it clear if and how the
present findings relate to this work and what precisely is different and new conceptually.

2. Let’s take for granted for the moment that the second key finding about uniform shifts is
completely new. Well, I agree with the authors that we are left with a putative mechanism that
underlies savings and a mechanism that can underlie the ability to learn multiple force
environments. And I agree that’s exciting. Unfortunately, the authors leave it at essentially
description and don’t nail the point. They have simply not robustly tested that idea empirically and
not demonstrated its validity beyond the very rudimentary results described at line 224-242. There
are interesting ways of getting at it empirically without complex shifts in the neural state directly
(though I agree with the authors that would be awesome). I wouldn’t expect this dataset in
general but given the desired venue for this work it seems a fair enough ask to empirically address
the prediction in a robust manner.

3. I am worried about subtle changes in behavior and how they were measured and controlled. For
example, I would like to understand how the authors control the overall geometry of the animal’s
arm and body in their apparatus? From what I can see, it seems to me that a lot about the general
state of the animal goes unmeasured or unreported. Can the authors rule out that the key results
here (trivially) reflect things like changes in the animal’s posture as a function of trials or
conditions?

4. The monkeys show quite disparate behavior, certainly in terms of learning rates. I appreciate
that the authors show us both animals for some analyses but they do not do it for all of them. And
sometimes unclear how they are lumped together and what effect it has on the results.

Referee #2 (Remarks to the Author):

Sun and colleagues examined neural population dynamics in motor and premotor cortex while
monkeys learned to adapt their reaches to a force field. This manipulation holds particular interest
because it is well-studied in humans, but there is relatively little understanding of the neural basis
of how we learn even this simple motor skill. Thus, this behavior is the right place to start in our
efforts to understand the neural basis of skill, and in fact, this paper seems likely to stand as one
of the first to reveal the neural-population principles of motor learning. As the authors allude to,
efforts to tie motor learning to neural changes have in the past largely focused on single neurons,
where organized, rational changes were hard to see. To add to the potential impact of the work, it
is one of the first to perform high-density recordings (here, neuropixels), and crucially, be able to
interpret and make sense of those population signals, rather than simply show them off. The main
conceptual advance of this study is to help show how the brain uses task-irrelevant dimensions of
neural population activity space to perform useful computations. They put forth an interesting new
proposal - that high-dimensional spaces are used to sequester motor memories, to prevent them
from interfering. For these reasons, I see this as a high-impact, broad-interest finding, worthy of
publication in Nature. However, the paper will improve if the positive aspects of the paper are
brought out more impactfully in the writing and analyses. (This review is lengthy, but that's
because the paper is good.)

Major Suggestions:
1) Concerns about the text.
The novel claims of the paper should be emphasized. This paper should add clarity to the
terminology in the field. Here are some specific instances where clarity in the presentation could be enhanced, for the betterment of this study and the field.

1.1. As written, the key findings for which the paper seems likely to become known are just below the surface, and need to be brought to the top. The paper as written details a voyage of discovery, but it would be more effective and impactful to present it in a hypothesis-driven manner. That is, "here is our vision, and look! The data support it." For example, the second-to-last sentence of the abstract says "distinct uniform shifts can separate motor memories" but that would be better as the second sentence! "How does the brain keep track of different motor skills? We hypothesize that motor memories are stored by using uniform shifts in neural population activity space to keep distinct motor memories separated. Our data support this hypothesis." Maybe the authors would like to give this concept a name? "memory indexing" seems like a good candidate. And, the learning axis also deserves a name.

This comes up again in the third paragraph of the Introduction. The phrasing, "Here we asked..." is weaker than the data could support. "Here we show that there are dynamics that occur with learning but do not directly support the new behavior. We propose that these dynamics serve an "indexing" function that enables different motor skills to be stored in a population space. This could provide a mechanism for preventing them from interfering, and rapidly re-accessing them."

This key conceptual advance is again buried in the discussion, only appearing in paragraph four.

I consider figure 2f to be the most significant (sensible and surprising at the same time) finding of the study. As structured, this result sneaks up on the reader, and if you blink, you miss it. Perhaps figure 1 should be reformulated to show the hypothesized neural outcome. That way, when we arrive at the data that support it, it will stand out more, and be more memorable.

Perhaps figs 2f-j should be a separate figure from 2a-e? Fig 2d is a nice demonstration of a neural correlate of a known behavioral effect. It is novel, and it is reassuring to see, and a nice validation of the use of the TDR approach here, but it is not the main accomplishment of this study. A simple split into two figures will prevent this material from overshadowing the effect in fig 2f.

1.2. Everybody is excited about neural population dynamics, due in large part to the senior author’s previous work. However, the term runs the risk of becoming an amorphous catch-all. I suggest using “neural population dynamics” to refer only to short-timescale changes in neural population state — that is, the types of effects captured by jPCA or GPFA, and here in fig 4j. To use the same term to also refer to changes over timescales of minutes only dilutes its meaning and creates some potential for confusion.

1.3. Are the effects in fig 2D "reaiming" or "reassociation"? As written, the manuscript seems agnostic on the issue (e.g., end of "reassociation-like changes..." section.) Those terms are used here without a lot of definition or explanation, beyond the relevant citations. Shall we think of reaiming as the neural correlate of a behavioral strategy that can be implemented entirely in the workspace? And, reassociation as a strategy that extends reaiming to include dimensions of neural activity that are not isomorphic with the workspace? Whatever determination the authors make, please don’t pass up the opportunity to define these terms clearly.

1.4. How about the effects in fig 2F? Are those really "new patterns", or something simpler, like reassociation, or a neural correlate of force? Oby et al (2019) were careful to define "new patterns" as patterns of neural activity that were demonstrably not present during the first day of behavior, prior to learning. That paper made note of the caveat that it was possible that the capacity to exhibit those patterns may have already been present prior to learning, and the animals simply did not express them. Here, that caveat is at least as much of a concern. That is, it seems a viable possibility here that the capacity to exhibit the after-learning repertoire was present prior to learning. Evidence for this is that the repertoire shift illustrated in fig 2f seems to
have happened already in the first day. Could it have happened simply by changing the forces the
animal exerted? The learning-related changes in behavior probably closely track forces; if the
animal can exhibit a shift in neural activity simply by exerting different forces without any learning,
then it would be an overstatement to call those "new patterns." One can envision a control task
where the animals simply have to resist a force to keep their hand centered. That seems likely to
shift neural activity in a dimension unrelated to kinematics, and we would not call those "new
patterns", but rather a fuller exploration of the pre-learning repertoire. Given this possibility, it
seems incautious to call these "new patterns." (I note that three controls were already performed.
These rule out the following potential sources of the repertoire change: drift over long durations,
preparation for a random force perturbation, and co-contraction. They do not rule out the
possibility that the learning-induced shift is a force axis. This isn’t a fatal concern; whether or not
it corresponds to force changes, it’s still a learning-related change, and discovering it is force
would help us further make sense of it.)

Consistent with the interpretation that the repertoire shift corresponds to a force axis is the fact
that VMR learning, otherwise quite similar, does not show the repertoire shift.

Another way to disambiguate a repertoire shift due to learning from a force axis is to partition the
data into PMd and M1. M1 may show a greater sensitivity to force than does PMd. Is it the case
that the loadings onto the repertoire-shift axis come from the M1 electrodes?

1.5. Is this really a skill? Of course, skill learning is what we want to understand, but it is probably
better to refer only to “adaptation” here. (Arguably, monkey V shows skill, in that he can switch
rapidly among mappings...)

2) Details of the neural activity findings.
2.1. Here the authors combine PMd and M1 recordings. This seems like a strange thing to do.
There could be key differences between the areas, and indeed, identifying those difference is of
high value in the field. A distinction could be made based on anatomical location, or based on the
extent to which preparatory activity is present at the individual channels. Even if no meaningful
differences are seen when this is done, that is worthy of reporting.

The definition of “preparatory activity” is rather narrow, and somewhat non-standard. Given this is
a delayed-reach task, why not consider more of the time prior to the go cue? Of course, there
could be fascinating effects during that period, related to the sensory-motor misalignment that the
animals anticipate experiencing.

2.2. Why is the shift in population space present for all arm movement directions, even unlearned
ones? The shift in the repertoire seems to encode the block that the animal is in. The exciting
possibility is that this is a learning-induced shift in the population space. The duller possibility is
that this is simply explainable as a different “preparatory set” for the two blocks. This could show
up as a difference in the activity of some muscle somewhere or the angle of some joint, or the
force applied to the manipulandum, even for unlearned directions, but maybe not. I am reluctant
to even raise this candidate explanation, because it requires “proving a negative” - that there was
no systemic change in muscle activity anywhere, or even in the animal’s internal preparatory set
(which could occur without any measurable external correlate.) However, readers will wonder it, so
an answer should be offered. And of course, further work will be required to discover the source of
this population shift.

2.3. Fig 4j is confusing in two ways. First, it is described in a single, complex sentence at the very
end of the results section. It deserves some unpacking. Second, and more importantly, the data
are not obviously consistent with previous results on flow fields.
These trajectories appear “tangled”, in a manner that Russo and colleagues recently said does not
happen in motor cortex. Also, this effect seems different from the Shenoy 2012 Nature paper. It
would seem that insofar as linear dynamics explain M1 activity, offsets in trajectories should only
appear if initial conditions are also offset. Here, the four trajectories are bunched together at time move onset-500, but then they appear to diverge. (Or maybe that is just the perspective from which this figure is plotted.) This apparent discrepancy with the literature is one of the strongest reasons to consider separating PMd from M1 activity in the analyses here.

3) Behavior.
I write this fully aware that it is usually difficult to go back and collect additional data from monkeys by the time papers are in review. Given the virus pandemic, this is likely to be all the more true than it usually is. I have considered whether the idiosyncrasies evident in the animals’ behavior have bearing on the paper’s conclusions about neural activity state changes. I think things are safe in that regard, however there are some disconnects between what’s in the text and what’s evident in the figures, and this might cause readers to trip over the discrepancies. Some rewording is suggested to make sure there is a tight alignment between the claims and the evidence.

3.1. On the first page, learning is described as “gradual” for both animals, but fig 1c makes it evident that monkey U learns gradually and does not attain full recovery of performance, whereas monkey V learns rapidly and does achieve full performance.

In fact, digging deeper, one can see that monkey V was always a little off, even during the before-learning block. It is as if he behaves as if the force field is on at all times. Can we even call this “learning”? In a task like this, where the animals probably encounter only a handful of perturbations, should we call this “adaptation” or “task switching”? Arguably, all the conclusions about how M1 stores multiple sensory-motor are just as valid, whether or not the focus is on their initial learning and consolidation, or only on how the animal selects among them, as appropriate for the context. How many sessions did the animals experience with this paradigm? Are all included here? Do they all look largely the same?

If, in light of this, it is important to the authors that this study be about how neural activity changes track learning, then perhaps limit analyses to just the very first sessions when each of the force-field manipulations was introduced.

3.2. Fig 4 depicts relearning, claiming it is faster than initial learning. This is true, but the effect is very fast - seemingly the first trial is by far the most important one. This is fine, but to describe this as “faster relearning” makes one think that the slope of the purple will be larger than the slope of the orange. If the curves are shifted to align on the second trial, it appears this will not be the case.

Minor suggestions:
1. Is there a spatial asymmetry to the generalization? It seems a curl field might impact adjacent targets in the direction of rotation more so than those in the other direction. Fig 1d shows a symmetric fall-off, constructed by averaging over targets. It would be instructive to also flip the sense of rotation (that is, CCW becomes CW) to see if there is in fact a bias in the generalization, with more learning in the “push” direction. It is worth performing this for fig 2e as well.

2. It seems that more could be done with fig 2g. For example, why not color the points that correspond to one session? That would give a sense for the overall variability in the neural shift, which might be interesting to see.

3. Fig 4g is a little busy. How about making one figure for each animal? Also, the gray point gets a little lost at first - how about a line connecting it to the black point? And, why not put arrowheads on all lines, depicting the sequence? And, if you keep with one figure, make it clearer that filled is U and hollow is V.
4. Fig 4j would benefit from some “signposts”. It is easy to misread “Movement onset - 500 ms” as “Movement onset: 500 ms”. How about putting the word “preparation” above the portion of the trajectories to the right of the “movement onset” carets, and “execution” to the left? This will facilitate digesting the figure.

5. If you construct fig 4g without normalizing, are the gray “before learning” and black “after learning” points at roughly the same places in population space?

6. How many curl field sessions were run in total? Were all of them analyzed? Did the first few look any different from the last few?

Editorial remarks:
- Fig 1b: which monkey are these trajectories from?
- Fig 1a: Is it always the case that the target density near the trained target is higher? Can you find a way to indicate all possible test conditions (up, down, right, CW and CCW for each)?
- Line 137: It’s a little odd to use the terms “orthogonal” and “parallel” in such different senses in the same sentence: “a nearly orthogonal dimension” and neural changes that do not “directly parallel behavioral output”.
- Line 225: perhaps “could” should be “would”
- Line 330: “gripped” -> “gripped”
- Line 483: should Fx and Fy depend on condition k? Fx(k) and Fy(k)?
- Typo in reference #26 “Ernandes” -> “Fernandes”

Referee #3 (Remarks to the Author):

General Comments:

This study by Sun et al addresses the fundamental question of how we rapidly learn multiple sets of new motor skills and retain memories of the learning of those skills without the memory of one skill interfering too much with another. Many behavioral studies over the past decades have examined this question but less is known about the underlying neural mechanisms. Single unit recording studies examining the activity of individual neurons in motor cortical regions revealed learning-related changes correlated with behavior but provided conflicting evidence for the retention of a memory of previously learned skills (e.g., Li et al., Neuron, 2001; Arce et al., J Neurosci, 2010; Cherian et al., J Neurophysiol, 2013). More recently, a number of studies have begun to address the mechanisms underlying motor skill learning and memory by using approaches that examine the dynamics of neural populations to reveal strategies not readily apparent at the level of single neurons.

In the current study, the authors use such an approach to address how cortical activity involved in preparing movements (i.e., preparatory activity) helps facilitate skill acquisition. The authors start from the premise that in order to learn new motor skills and retain a memory of that learning without interfering with other learned motor behaviors, there must be ways in which neural population activity can change over the course of learning without directly influencing motor output. To examine the evidence for such changes in neural preparatory activity they used a well-known and extensively-used paradigm for studying learning of new movement kinetics during reaching (curl force field learning task) in which monkeys reach with a haptic device which perturbs their reach trajectories with a force that is proportional to their reach velocity but orthogonal to their reach direction. They recorded the responses of neural populations in the primary motor (M1) and dorsal premotor (PMd) cortices as monkeys adaptively compensated for this perturbation by learning a new association between a given reach target location and the forces required to reach to that target. The authors examined both behavioral responses and changes in neural preparatory activity over the course of learning, how learning in a particular
reach direction generalizes to other directions, the effects of learning, unlearning and relearning to compensate for the same perturbation as well as the impact of sequentially learning two opposing sets of perturbations.

Using sophisticated “neural state analyses” to monitor changes in population-level activity patterns the authors show that, in agreement with previous work (e.g., Jarosiewicz et al., PNAS, 2008; Golub et al., Nat Neurosci, 2018), population preparatory activity exhibits learning-related changes that are closely correlated with changes in behavior and consistent with re-associating existing (before learning) activity patterns with a new behavioral response (reaching to a different target). Importantly, however, they also show that there are neural activity changes that did not directly parallel behavioral output. Specifically, these learning-related “uniform shifts” were present for reach directions that behaviorally show no evidence of learning-related changes and occurred along a roughly orthogonal neural state space dimension to that of force output. The authors further provided evidence that these changes reflect new population activity patterns (i.e., a change in neural repertoire) and that these new activity patterns are different for different tasks (i.e., they reflect the learned task “identity”). In addition, even after “washout” of the newly learned behavior uniform activity shifts persist, consistent with a role in motor memory retention. The authors propose that these “uniform shifts” reflect a mechanism for separating neural preparatory states associated with different learned motor skills and retaining a memory of those learned skills. Importantly, these new activity patterns appear to exist within a portion of neural state space that does not map directly onto motor behavior (“output-null” space) thus facilitating the separation of motor memories that otherwise might interfere with one another.

These are important results which contribute significantly to advancing our understanding of the neural mechanisms underlying the learning and retention of multiple motor skills. In particular, the identification of neurons that maintain persistent changes after behavioral evidence for a recently learned skill is “washed out” (i.e., “memory” neurons; Li et al., Neuron, 2001; Arce et al., J Neurosci, 2010; Richardson et al., J. Neurophysiol, 2012) has long pointed to the retention of motor memory through population-level activity patterns. However, it is only more recently that simultaneous recordings of multiple neurons across motor cortical regions and population-level analyses have started to elucidate the underlying mechanisms. Several studies examining population-level activity have emphasized learning mechanisms which involve re-associating existing neural activity patterns with new behavior (i.e., mechanisms which rely on the same neural repertoire; e.g., Golub et al., Nat. Neurosci, 2018; Sadtler et al., Nature, 2014). However, recent studies have also demonstrated the existence of learning mechanisms that involve the formation of new neural population activity patterns (new neural repertoire; e.g., Oby et al., PNAS, 2019). Most directly related to the current study is that of Perich et al, Neuron, 2018 who identified a signature of learning in the subspace of PMd that does not directly influence M1 activity (i.e., “M1-null” subspace of PMd). It was suggested that this reflects a novel mechanism by which the brain alters planning activity within an “output-null” subspace to set the appropriate initial conditions for subsequent execution of a given adapted motor behavior by M1. The authors of the current study reach a similar conclusion, suggesting that the “uniform shift” they observed in the “output-null” subspace is consistent with the changes previously observed in the “M1-null” subspace by Perich et al., 2018 (i.e., see Discussion lines 277-287). Notably, however, the current study expands on previous work by explicitly showing that these neural activity changes are roughly orthogonal to behavioral output and that they are different for different learned tasks. Perhaps, most importantly, they show that even when the learning is “washed out” shifts within the “output-null” subspace relative to before learning persist suggesting that a memory of the learning is retained. Consequently, while I would not necessarily consider the theoretical ideas presented in the current manuscript to be entirely novel, key aspects of the results are novel and they make a potentially important conceptual advance towards our understanding of the neural mechanisms underlying our capacity to separate and retain motor memories of different motor skills.

Overall, I found that while the results are important and novel there are a number of aspects of
the manuscript that could be improved, particularly from the standpoint of publication in Nature. These include:

1. Sophisticated sets of analyses were performed but the description of those analyses in the Methods section was often hard to follow. This may be partially because I am not an expert in the use of these techniques but then again neither is the general reader of Nature. For that reason, I would strongly recommend revising the methods to make the techniques more accessible to the general reader. Among the most important aspects of this is making sure the motivation for the various analyses comes across clearly. For example, initial analyses used targeted dimensionality reduction (TDR) to relate components of population responses directly related to behavior which makes sense. However, there is then a switch to using PCA as a dimensionality reduction technique that was not well motivated. More importantly, however, the links between the analyses in terms of relationships between the different subspaces is not clear. Based on the text (lines 136-138) which indicates that there were prominent neural changes along a nearly orthogonal dimension that did not parallel behavior and Figs. 2d and f, one gets the impression that there were close relationships between the two sets of subspaces with PC1 and PC2 being closely related to muscle force dimensions TDR1 and TDR2. However, this is never explicitly indicated although it seems quite relevant from the standpoint of establishing the extent to which the uniform shifts (largely apparent along the PC3 dimension) are in fact orthogonal to behavioral (i.e., hand force) changes. Similarly, I felt that the definition of the uniform shift axes in the Methods did not come across clearly. Specifically, according to the text the learning and washout "uniform shift" axes were defined as the axes connecting before- and after-learning centroids and after-learning and washout centroids, respectively. These axes were then orthogonalized against the two TDR axes which I have taken to mean (perhaps incorrectly) that you consider specifically the component of each of these "shift axes" that is orthogonal to the TDR axes. Assuming this is correct, should I then interpret all references to "learning-uniform shift" and "washout uniform shift" in the text as referring to shifts along axes that are orthogonal to TDR1 and TDR2 (i.e., force behavior)? It sounds as if in some analyses this was true and in others it wasn’t. Given the relevance of shifts being orthogonal to behavior (in "output-null" space) for the conclusions of the study whether or not you are explicitly considering shifts orthogonal to TDR1 and TDR2 should come across more clearly not only in the methods but also in the main text. Other areas of potential confusion are outlined below in my specific comments as are a number of aspects of the experimental procedures that were unclear and/or where it seemed to me that important pieces of information were missing.

2. A critical aspect of the conclusion of the study is that the learning-related "uniform shifts" are not directly linked to motor output (i.e., they are within the "output-null" subspace). Consequently, it is particularly important to show that there were no other behavioral changes unrelated to learning the new kinetics of the environment that contributed to changes in neural activity. You consider muscle co-contraction but what about other factors such as speed which is known to influence preparatory activity (e.g., Churchland et al., J Neurophysiol, 2006)? In Extended Data Fig. 6 you show evidence for decreases in speed during the learning block that appear quite modest although I don’t know if that was true for only one or both animals. However, what about during the error clamp block? Was there any evidence for a general progressive increase/decrease in speed that might influence neural activity? It would be useful to plot this as well. Since in many cases you compare activity during error clamp trials to activity in non-error clamp trials was there any evidence that the monkeys could tell in advance whether an upcoming trial would be an error-clamp trial? For example, in the error-clamp block was there any difference in preparatory activity for force-field vs error-clamp trials in the training direction? When performing the TDR analysis did you ever examine models that include variables other than horizontal and vertical hand forces (e.g., speed)? How much of the variance in the data did the force behavioral variables actually account for? In general, the addition of a few more analyses to show that neural activity changes had no correlation with other behavioral changes would be useful to help support the robustness of the conclusions.
3. M1 and PMd neural recordings were lumped together in all analyses. Did you ever examine each region independently? Given the results of the recent study of Perich et al (2018) it would be interesting to know if the uniform shifts you have observed are mainly confined to PMd.

4. Although, as noted above, I found that the Methods sections could use some clarification the main text was generally clear and well-written with appropriate references made to previous work. One comment that applies particularly to the abstract is that it comes across (at least to me) as including quite a lot of field-specific jargon and thus appears to be written for a more specialized neuroscience audience rather than the more general readership of Nature. I would recommend revision of the abstract, introduction and discussion to ensure that the relevance of the study can be easily appreciated by a broad audience. In addition, in terms of presentation it is sometimes unclear whether what is being plotted in a given figure represents the example for an individual case vs an average across sessions, monkeys etc.

Specific Comments:

Fig. 1/Methods: Did monkeys reach in the horizontal plane, vertically or in some intermediate plane?

Fig. 1b: How was early learning vs late learning defined? Similarly how was early washout versus late washout defined? Is late learning at the end of block ii or block iii? It would also be useful to include either in Fig. 1 or in an Extended Data figure some information about how peak speed changed across each of the four blocks for each animal.

Fig. 1c: The time course of learning for monkeys U and V seems to be quite different. Specifically, Monkey U appears to take over 100 trials before reaching a plateau in learning (as one might expect) and even towards the end of learning perpendicular error never reaches zero. In contrast, Monkey V appears to very rapidly (i.e., within ~ 10 trials) reach a perpendicular error of close to zero as if no learning is taking place but the monkey is simply recalling a previously learned behavior that wasn't fully washed out or is employing some sort of behavioral strategy (e.g., slowing his movements) to compensate very rapidly. Can the authors comment on what the difference might be attributed to? For example, was there any evidence that monkey V did employ a strategy of slowing his movements to avoid being perturbed by the field? Was there evidence for more retention of previous learning in monkey V? Was the field strength potentially too weak for monkey V?

Fig. 1d, 2e: Here you plot perpendicular hand force difference (Fig. 1d) or neural angular shift (Fig. 2e) between error-clamp trials and before learning trials as a function of target direction relative to the trained direction to examine generalization. As I understand it you compared the forces generated in error clamp trials in block iii (after learning) to those during “free” unperturbed reaching movements before learning in block i to obtain the “learning” (orange) curve. It is unclear to me, however, how the “no learning” (black) curve was obtained. Did you sometimes perform a block of error clamp trials (i.e., block iii) either following a block ii in which no curl field was applied (i.e., no learning) or immediately following block i?

Lines 86-91 and 410-423: You indicate that you recorded in PMd and M1 (lateral and medial) but it would be valuable to include some further information (possibly even extended data) providing a little more detail about recording sites and the extent to which they overlapped in the two monkeys. For example, you used Utah arrays in monkey U but Plexon V-probes and a Neuropixels probes in monkey V which I assume enabled you to make much deeper recordings in monkey V. In monkey V were you able to record in deeper layers (e.g., caudal M1 recordings in the bank of the central sulcus)? Although you lumped neurons across all areas together in the analyses presented in the manuscript were any analyses performed to examine potential differences across regions (e.g., PMd vs rostral M1 vs caudal M1)?
For the V-probe and Utah-array recordings you indicate that both stable single units and multi-unit isolations were included. Can you provide an estimate of the relative percentage of single-unit vs multi-unit recordings that were used in the analyses? For the 1000 units recorded using the Neuropixels probes was it possible in all cases to isolate single units?

Fig. 2 legend: In part d, are you providing a single session example for learning of a CCW field during upward movements or is this an average across both fields (with sign reversal for CW field), different training directions, monkeys etc?

You indicate here that you also identified prominent unexpected neural changes along a nearly orthogonal dimension that did not directly parallel behavioral output and that this was revealed by applying PCA to the neural population preparatory activity. So far in the paper you have examined neural population activity in a TDR subspace with the goal of relating neural activity to specific movement variables (i.e., hand forces). You then perform a PCA analysis to show that there is a clear separation in before-learning and after-learning repertoire. As noted above, what is less obvious to me is that this is along an orthogonal dimension that did not directly parallel behavioral output. Is that assessment based mainly on the observation that it occurred for directions in which there was no behavioral evidence of learning or is it also true that these shifts were along a dimension orthogonal to force output. Specifically, since most of the “uniform” learning-related change in Fig 2f appears to be along the PC3 dimension this would seem to imply that the PC1/PC2 plane is closely aligned with the TDR1/TDR2 plane. Is that true? More generally it would be helpful to clarify the relationship between PC1, PC2 and TDR1, TDR2. Do the two first principle components largely reflect force? How much of the variance in the data do TDR1 and TDR2 vs PC1 and PC2 account for? How much do the first 3 PCs account for?

On a related note is there any behavioral variable or context that PC3 might be related to (e.g., global differences in speed or perhaps some other variable related to the context of reaching in a block with interspersed error clamp trials)? In your analyses you compare before-learning preparatory activity during “free” reaches to after-learning activity in error clamp trials. I realize this was necessary to be able to characterize a uniform shift across reach directions but it would have been better to be able to compare error clamp trials after-learning to error-clamp trials before learning. Given the existing data, if you simply consider activity for reaches in the training direction and project the preparatory activity during force-field trials (e.g., at the end of the learning block or during the error clamp block ) into PC space does the activity appear at the same location as for after-learning error-clamp trials? Similarly, based on my understanding of what is plotted in Fig. 2e at some stage you must have performed error-clamp trials under “no learning” conditions. Was this ever performed on the same day as a learning experiment such that you could directly compare error-clamp “no learning” population activity to error-clamp “after-learning” activity?

In assessing the ability of random perturbation forces to effect a repertoire change, activity during random-perturbation trials was compared to before learning and after-learning error clamp trials. What happens if you instead compare force-fields trials late in the
learning block or during the error clamp block to random-perturbation trials?

Lines 174-176: You indicate here that there were no obvious signs of co-contraction in late-learning and washout trials. What about during the error-clamp block? Were there any differences in EMG recordings of muscle activation during error-clamp trials in block 3 as compared to before learning trials in block 1 in directions to which you found no behavioral generalization?

Lines 188-206: This section focusses on a geometric analysis of the uniform shifts when comparing learning of multiple force fields by examining the extent of alignment of the "learning uniform shift axes" associated with each of those fields. However, as noted above, the definition of "learning uniform-shift axis" was not so clear. In the text it is defined as the vector pointing from the centroid of the before learning neural repertoire to that of the after learning repertoire. In the methods there is then a further statement about orthogonalizing against the two TDR axes where rotatory neural shifts were observed. Should I take this to mean that after finding the vector connecting before learning and after learning centroids you redefined the learning uniform shift axis to be only the component of the full learning-related shift that was orthogonal to TDR1 and TDR2? More generally, it wasn't completely clear to me whether the shifts observed and plotted in Figure 3 and their geometric relationships reflect changes in neural states that are completely orthogonal to the TDR1 and TDR2 dimensions that reflect force output or whether at least a component of these shifts does incorporate the rotatory shifts observed in the 2D TDR subspace. In other words, to what extent can the geometric relationships between shifts observed in Figure 3 be thought of as shifts within an "output-null" subspace?

Lines 227-228/Fig. 4e,f: As noted earlier, Monkey V appears to both learn and relearn much faster than Monkey U and so quickly that it almost appears as if he has either used some specific behavioral strategy (e.g., slowing movements) or has retained previous learning. Since you compare initial learning to relearning here the latter would appear not to be the case. However, in examining this data I realized that it is unclear from the manuscript whether initial learning means the first time the monkey ever encountered a CW or CCW curl-field in any session and when reaching in any direction or whether it rather refers to the first time the monkey encountered this field in a particular reach direction/session. More specifically, how was the data for the various relearning tasks sessions combined into these plots?

Lines 247-250: Here you show that peri-movement states also shifted away from the before-learning ones but in this case the shift was local to the direction in which there was behavioral evidence of learning and there was no evidence of motor memory retention after washout. I wasn't quite sure how to interpret this. How much of the shift was along PC1 and PC2 axes vs along the PC3 axis? Perhaps, more importantly, how much of this shift was orthogonal to the TDR1 vs TDR2 plane? Although the shifts appear to be local to directions which showed behavioral changes do they nonetheless reflect activity changes that don't directly influence behavior (i.e., "output-null")? I note also that Monkey V did exhibit significant changes for reaching in all directions. What is the authors' interpretation of these results beyond the fact that the shift is less uniform than for preparatory activity?

Lines 284-287: I did not understand what was meant here. Does the word "it" in "it does not" refer to the uniform shift?

Line 330: “griped” should be “gripped”

Line 332: Missing “a” before “point-to-point”

Line 333: “with” should be “within”

Line 352: Either here or elsewhere within the methods the arrangement and spacing of the 12 peripheral targets relative to the trained target should be indicated.
In the error-clamp block (block iii) it is indicated that 70% of the trials were the same as in the learning block and 30% were randomly interleaved error-clamp trials (20 trials per reaching target). If I have understood correctly this implies 240 error clamp trials randomly interspersed among another 560 learning trials in the training direction (i.e., many more than in the learning block). Is this correct? In addition, Fig. 1c shows the force field being turned off after ~200-250 trials followed immediately by washout. This is potentially confusing and it would seem to be worth illustrating what the perpendicular error on learning trials was at the end of the error-clamp block.

How many washout trials were typically performed?

You indicate here that to encourage monkeys to learn or unlearn the curl field you automatically failed a trial if the hand trajectory deviation exceeded a bound. What was that bound? In general, I am surprised to hear that incorporating such a bound was required. Typically when adapting to curl fields as long as a subject corrects towards the end target trajectories naturally become straighter (i.e., via implicit learning) so I wouldn’t have expected monkeys to continue to make curved, inefficient movements. Was there any evidence that incorporating such a bound encouraged monkeys to slow their movements as a means of reducing the perturbing force or was the incorporation of the 700ms reach duration restriction sufficient to prevent that? As a result of these constraints what was the typical success rate during the learning block? The Extended data in Fig.6a show some slowing in late learning. Is this an average for both animals or did each animal slow movements to different extents?

In the relearning experiments you indicate that Monkey U did four sessions and Monkey V did three sessions. What were the reach direction and curl field combinations examined in these sessions? Is it possible that some memory of learning in one session was retained and influenced learning in a subsequent session? Am I correct in assuming that the relearning sessions were similar in structure to the single-field learning sessions except that blocks ii-iv were repeated a second time with the same curl-field?

I wasn’t sure what was meant by “The task had the same temporal duration as in a learning session”. Does this mean that monkeys made center-out reaches for approximately the same time as it normally took to complete blocks i-iv in a learning session? Were error-clamp trials examined in these sessions and if not where does the “no learning” data used in Figs. 1d and 2e come from? I also did not understand what was meant by “we used trials for which the trial IDs matched those in the learning sessions...”. Please clarify.

You indicate here that you pooled V-probe recordings you pooled neurons across multiple sessions for the same curl-field. Does that mean the same curl-field in the same direction?

Here you indicate that Fig. 1c illustrates the mean and s.e.m. of MPE across all sessions. Does that mean that it reflects both learning and relearning trials?

You indicate that different time windows were used in your PCA analyses for both the preparatory and peri-movement epochs depending on whether you were performing neural state analyses vs neural repertoire analyses. What was the motivation for choosing different time windows for these analyses and what kind of impact did this have on the PCs and preparatory states within PC space that were computed? Also unclear is which of the analyses you describe fall into the category of “neural state” vs “neural repertoire” analyses. Both of these issues should be clarified.

In addition, one might expect the time window used for the preparatory epoch to largely focus on activity before and perhaps only very slightly after the go cue. The -50ms to 50ms time window
around the go cue for the neural state analyses seems reasonable. However, the -400ms to -100ms from movement onset window used for the neural repertoire analyses likely starts to overlap with movement-related activity. Again, some clarification should be provided as to why this time window was chosen and what was the impact on the computed PCs and state estimates. If, for the “neural repertoire analyses”, you choose either a window which starts and terminates substantially earlier (i.e., so as to avoid potential overlap with peri-movement neural activity) or the same window as was chosen for the “neural state analyses” what happens?

Lines 476-511: I found this section describing the targeted dimensionality reduction approach you used generally difficult to follow and would recommend revision to make it more accessible to the general reader. I include here only a few specific comments.

Line 483: In this equation should \( \beta_x \) be \( \beta_{i,x} \), \( \beta_y \) be \( \beta_{i,y} \), \( F_x \) be \( F_x(k) \) and \( F_y \) be \( F_y(k) \)? Should \( \beta_{i,j+1} \) be \( \beta_{i,n+1} \)?

Line 491-495: It wasn’t clear to me what you mean by building the 2D hand-force space “without binary indicators (n=0)”. Does this mean that you built this space without taking into account whether a given trial was an error-clamp trial? Was this space built using only before-learning trials? If so that should be clarified here. I was also unclear on how the 3D TDR model was built as it sounds as if this model was built using both before-learning and after-learning trials. In general I did not follow the approach you used here very well.

Line 504: Should the beta coefficient matrix in this equation be transposed? I had trouble following how the dimensions work out here.

Lines 523-532: The definition of the uniform shift axes is quite critical to interpreting the results but, as noted above, I found the description here somewhat confusing and would recommend clarification particularly for a general audience. Of particular relevance is to make clear whether when you quantify shifts along these uniform shift axes you are always talking about shifts that are orthogonal to behavioral force output.

Line 534: When you talk here about orthogonalizing uniform-shift axes are you now referring to orthogonalizing the axes for learning or washout of different fields relative to each other or relative to the force output?

Line 555-566: The description of how you computed the control distributions was difficult to parse and could be clarified.

Lines 574-577: When you refer to neural trajectories here are you referring to the neural trajectory in PC space over the time course of an entire trial (e.g., Fig. 4j)?
Author Rebuttals to Initial Comments:

Introduction
We thank the reviewers for their time and feedback, and we appreciate the insightful comments and suggestions for how to further strengthen the analyses and conclusions presented. Taken together, the reviewers’ comments primarily focus on a few areas which we believe that we have been able to clearly and compellingly clarify. These areas are as follows:

1. Ruling out additional alternative explanations for the uniform shift and neural repertoire changes, including:
   a. Posture changes during learning
   b. Hand speed changes during learning
   c. The use of error clamps after learning
   d. Changes of baseline neural activity (i.e., neural activity before target onset)

2. Providing additional quantification and experimental results throughout the entire manuscript, including:
   a. Analyzing PMd and M1 neural data separately and comparing the results to our findings using data grouped from PMd and M1
   b. Repeating key neural analyses using neural data from an earlier time window during the preparatory period
   c. Expanding the TDR model to 3D with hand speed as an additional regressor, and show that the hand speed axis does not align with the uniform shift axis
   d. Quantifying the relationship between different neural population dimensions (i.e., PC and TDR axes, PC and uniform-shift axes, TDR and uniform-shift axes)
   e. New results from an ‘interference experiment’ to further support the hypothesis that the separation of preparatory states by the uniform shift could reduce interference and promote multi-skill learning

3. More specific comparisons to prior reports in order to clarify the novelty of this work, including:
   a. Comparison to the background shift in Rokni et al. 2007
   b. Connections to changes in the “M1-null” neural subspace in Perich et al. 2019

4. Providing additional details on the methods and clarifying terms, including:
   a. More comprehensive description of methods in order to increase clarity, such as the TDR analysis, the definition of the uniform-shift axis, control experiments and analyses, neural trajectory analyses, etc.
   b. More comprehensive definitions of key terms, such as reassociation vs. re-aiming, motor skill versus motor adaptation, neural population dynamics versus neural population states / activity, etc.
   c. The motivation for using certain quantification methods and for choosing certain time windows in different analyses

5. Increasing the orientation of the abstract and main text to highlight the hypotheses tested in contrast to the more “voyage of discovery” approach presented in the original manuscript.

We have kept these five areas squarely in mind when revising the manuscript as described in the point by point responses below.

Sincerely,
The authors
We wish to thank the reviewers for their many insightful and helpful comments and questions. We believe that we have addressed each point clearly and compellingly. For ease of reading, we have included below all Referee Comments in black text, our replies to the Referees in blue text, and the additions made to the manuscript in red text.

Referee #1 (Remarks to the Author):

This is a review of “Skill-specific changes in cortical preparatory activity during motor learning” by Sun and colleagues. The paper asks an important and long-standing issue in arm motor control and motor learning, specifically the neuronal basis of learning a new dynamical load, in this case the canonical curl-field which has been studied in hundreds of human studies.

The actual experimental approach is straightforward and logical. Two rhesus monkeys were trained to reach while holding a joystick attached to a robot the movement of which veridically controlled the movement of a cursor on a screen that the animal could see. The reach targets were presented to the animal on the same screen. Initially no loads were applied. After some number of baseline reaches, a load was introduced for only one target that pushed the animal’s hand perpendicular to its movement direction with a magnitude proportional to the movement speed (the curl field). This initially displaced the hand but the animals learned to compensate for the load and returned to roughly straight reaches, evidence of learning the applied load. After some amount of learning, the animal started reaching to all 12 potential targets but the other 11 had no curl field. For all these reaches the animal also experienced so-called clamp trials where the robot forced the animal to reach straight through a virtual channel and measured how hard the animal pushed against the channel, providing a direct measure of the forces the animal thought it needed to generate to counter the load. After these trials, the loads were turned off and the animal returned to its baseline behavior. This is essentially the standard approach to this kind of study with the exception of having the curl field in place for only one target location.

In terms of results. First, the animals roughly behave as expected (though see #4 below). They show initial errors after the field is introduced, which is slowly reduced over trials. They generalize this learning to targets nearby but not far away. They show kinematic after-effects (errors in the opposite direction as the load) in the washout epoch and they eventually return to baseline performance again. The core results, and what is to be seen as the major advance here, is essentially a dynamical systems description of the population level neural activity in PMd-M1 (acquired at the single cell level) that is correlated to this behavior. In my view, there are two key findings. First, there exists a neural subspace where shifts in the preparatory state closely reflect the behavior. As expected, therefore, these shifts only apply to those reaches that demonstrate learning. Second, there exists another shift in neural states along a different dimension that applies to all reaching targets uniformly, which implies the formation of some new set of neural activity. When the field is removed and the animal returns to baseline, the uniform shift does not return to baseline rather another uniform shift is introduced. This uniform shift may tag force field environment, and these tags may underlie so-called savings where re-learning a field is faster the second time than the first time.

My general assessment is that this a substantive effort on many fronts and one that usefully drives the field forward. On the other hand, I don’t think the scope of the advance presented here is sufficient given the past literature and the desired venue. There are several critical empirical details missing that could get it there but the amount of work needed is substantial given the animal model being used.

1. Of the key findings, the first one (shifts in neural state that predict behavior) seems a minor advance relative to Bizzi (2001) as well as others (as cited) who demonstrated much of this before. The demonstration of generalization function is nice but rather expected and certainly not a shift in thinking for the field.

Thank you very much for highlighting this important connection to previous work. We agree that the shift of single-neuron tuning curves reported in previous papers (e.g., Li et al., 2001) is an important finding, which uncovered neural changes that can directly predict behavioral changes after learning the force field. Yet it remains unknown from the literature how the preparatory neural state shifts from trial to trial during learning,
leading to a performance asymptote. In the current work, population-level neural analyses allowed us to investigate this gradual process and, importantly, see the systematic and lawful organization of the neural population state through time.

We also agree that the neural generalization curve, on its own, is not surprising given the behavioral generalization pattern and the shift of single-neuron tuning curves. To our knowledge, however, this is the first direct evidence showing a neural population correlate of motor learning generalization, which has been predicted by previous work (Arce et al., 2010). Moreover, the internal model framework for motor learning has been proposed by a rich body of behavioral studies (Lalazar and Vaadia, 2008) while its underlying neural basis remains largely unexplored. Here, our results from the targeted dimensionality reduction (TDR; Mante et al., 2013) analysis indicates that preparatory neural activity is predictive of initial hand forces in the upcoming movement during the learning process. This provides evidence that preparatory neural population activity reflects the update of an internal model for generating new movements.

In a broader sense, by investigating the neural population activity rather than single neuron tuning curves, our work is anchored in a fundamentally different model of how the motor cortex may operate, which centers on neural population dynamics. In the neural population dynamical systems framework, a neural population constitutes a dynamical system that performs computations through the temporal evolution of neural dynamics, for example, to generate and control movement. This framework has helped in our understanding of a new way of thinking about the neural basis for motor control (Shenoy et al., 2013; Vyas et al., 2020a). This framework proposes that preparatory activity (i.e., before the ‘go cue’) sets the initial state from which movement period activity evolves, governed by both the local dynamics of the motor cortex as well as inputs from other brain regions. The preparatory state governs the initial part of a reach, with feedback from the periphery influencing movement period activity after the reach begins, as this is a feedback control system (e.g., Churchland et al., 2012; Kalidindi et al., 2020; Sussillo et al., 2015). Our work here is among the first to investigate the relationship between neural population dynamics, especially the preparatory neural activity, and behavioral output during curl field learning. In particular, the TDR method results reveal the systematic neural population correlates of gradual learning and generalization. This naturally encompasses the previous single-neuron results within this new framework, but provides several additional insights as described above.

The authors recognize this I think and spend most of their time on the second claim about the uniform shift. But is this new? There is a paper by Rokni and colleagues (Neuron, 2007) that not only gets to a similar point but provides a conceptual underpinning for why it may be so – which I note that the authors don’t cite. Now, I don’t think these are identical claims and I think the present paper provides a much stronger and more nuanced analysis empirically. At the very least the authors need to make it clear if and how the present findings relate to this work and what precisely is different and new conceptually.

Thank you for pointing out this important, inadvertent oversight; we have now added the Rokni et al., 2007 citation and an extensive discussion. Importantly, we recognize and appreciate the relevance of this excellent work. We also agree that it is important to highlight the differences between our work and the results presented by Rokni and colleagues, and to clarify what is conceptually new in our work. The previous paper presented changes of neural activity (“background changes” as they refer to it) that happen globally to all reach directions, as did the uniform shift that we found during curl field learning. However, the critical difference between those background changes in Rokni et al., 2007 and this uniform shift is as follows. (1) The background changes (calculated between before-learning and after-washout trials in their learning experiment) are not significantly different from the background drift that they found in the control experiment. That is, they are not specifically related to learning (termed “irrelevant changes” by the authors; page 658, paragraphs 1 and 2). (2) In contrast, the uniform shift in our work was specifically related to learning, though not specific to the trained reach target. It happened gradually during learning but, importantly, it did not happen in control no-learning days (now new Fig. 3c), and it was specific to the learned curl field (now new Fig. 4). Based on the critical difference between the background drift and the uniform shift we report, we believe that we are providing additional important insights and that the effect reported by Rokni and colleagues is different.

The high-dimensional neural population activity can exhibit a wide variety of patterns. The key question we are addressing is: during motor learning, do changes in neural population dynamics relate purely to newly learned
movements, or, alternatively, are there additional neural activity patterns generated that facilitate learning without matching the movement output? Here, through the lens of curl field learning, we found neural population activity patterns in certain dimensions (i.e., the hand force related TDR subspace) that directly relate to movement parameters and likely drive the upcoming movement output, and other neural dimensions (the ‘uniform shift’ axes) that are used for pattern generation or supporting the local dynamics but do not directly link to the movement output. The major conceptually new finding is that when multiple different curl fields were learned sequentially, distinct uniform shifts occurred, each reflecting the identity of the field applied and potentially separating the associated motor memories. The neural geometry of these shifts in preparatory activity could serve to organize skill-specific changes in movement production, facilitating the acquisition and retention of a broad repertoire of motor skills, like an ‘indexing’ function (as suggested by Reviewer #2) that enables different motor memories to be stored and accessed separately. We have updated the abstract and main text with these clarifications ([lines 34-40] and [lines 56-66]).

We believe that the following possibilities reconcile the differences between the Rokni et al. 2007 study and our study:

A. Although the task in Rokni et al. 2007 had a delay period, the neural data analysis used the “-100 to +300 ms around movement onset” time window and so the background drift was found mainly during the peri-movement period. In contrast, we found the uniform shift during the preparatory period (prior to the go cue), and it disappeared during the peri-movement period. Given that for each individual neuron, the tuning properties during preparatory and peri-movement periods are often quite different (Churchland et al., 2010), it is likely that the uniform shift is confined to the preparatory period whereas the background drift of tuning curves is confined to the peri-movement period. It is also likely that the level of background changes depends on brain regions: Rokni et al. 2007 studied M1 and SMA whereas our work studied M1 and PMd. Previous work that reported a lack of background changes in PMd during center-out reaches also pointed out this potential brain region difference (Chestek et al., 2007). The dynamics have been shown to be distinct between SMA and PMd / M1 (Russo et al., 2020), likely reflecting the distinct computational roles in these regions.

B. In contrast with Rokni et al. 2007, Chestek et al. 2007 reported a lack of background changes in their neural recordings when monkeys performed a center-out reaching task. Instead, Chestek and colleagues found considerable stability between neural recordings and behavior over at least 48h. Chestek and colleagues found that if one controls the variability of the behavior, the neural background drift is far lower than previously reported (Carmena, 2005; Rokni et al., 2007). Chestek et al. 2007 also suggested that many of the changes observed by Rokni et al. 2007 might also be attributed to neural noise, in addition to the possible instability associated with synaptic plasticity. Our results may be more robust to this noise of single neurons because of averaging over more trials per condition and by focusing on neural populations.

We have added the text below to the Discussion section to reflect our response to this concern.

[lines 457-459, 469-480] Besides the aforementioned four sets of control experiments and analyses, we want to address a few more alternative, learning-irrelevant explanations for the uniform shift and neural repertoire change. ... Second, previous work reported changes of neural activity that happen globally to all reach directions (“background changes” as they refer to it52), as did the uniform shift that we found during curl field learning. However, the critical difference between those background changes and this uniform shift is as follows. The background changes (calculated between before-learning and after-washout trials in their learning experiment) are not significantly different from the background drift that they found in the control experiment. That is, they are not specifically related to learning. In contrast, the uniform shift in our work was specifically related to learning, though not specific to the trained reach target. It happened gradually during learning but, importantly, it did not happen spontaneously in control no-learning days (Fig. 3h), and it was specific to the learned curl field (Fig. 4).

Therefore, we believe that the background drift reported by Rokni and colleagues and the uniform shift we found are different.

2. Let’s take for granted for the moment that the second key finding about uniform shifts is completely new. Well, I agree with the authors that we are left with a putative mechanism that underlies savings and a mechanism that can underlie the ability to learn multiple force environments. And I agree that’s exciting. Unfortunately, the authors leave it at essentially description and don’t nail the point. They have simply not robustly tested that idea
empirically and not demonstrated its validity beyond the very rudimentary results described at line 224-242. There are interesting ways of getting at it empirically without complex shifts in the neural state directly (though I agree with the authors that would be awesome). I wouldn’t expect this dataset in general but given the desired venue for this work it seems a fair enough ask to empirically address the prediction in a robust manner.

Thank you for raising this important question and, indeed, we do have a concrete conceptualization (theory) in mind and we now have included it in the manuscript ([lines 63-66] and [lines 491-500]) and as explained below. We are grateful for the comment here that this does not come through clearly enough in the original manuscript.

First, we would like to further unpack the major conceptualization in this work: suppose that one wants to learn a CW and a CCW field simultaneously. From previous studies and what we found in the TDR subspace (Fig. 2), we know that there exists a reassociation-like neural change during learning. If this were the only change that existed, one would predict that learning different curl fields would interfere with each other because they share the same neural state repertoire. Instead, we found a uniform shift that separates the before- and after-learning preparatory neural states and perhaps serves as an ‘indexing’ function to store and access different motor skills in the neural state space separately (as suggested by reviewer #2). We think that this is a non-trivial and concrete proposal which could not have been inferred from previous work.

Ideally, we would be able to do spatio-temporally patterned optogenetic perturbations to specifically manipulate neural population activity that largely contributes to the uniform-shift dimension, and test if this impacts the behavioral effect we observed (e.g., memory separation / reduction of interference, memory retention). While this may soon be possible in mice as has been achieved in other brain areas (e.g., Chettih and Harvey, 2019; Marshel et al., 2019; Robinson et al., 2020), it has not yet been demonstrated in rhesus macaques (we hope to achieve spatio-temporal patterning in rhesus macaques in the coming few years with our ongoing collaborations with the Deisseroth and Seidemann Labs). Thus, this is for future work and should be an exciting and critical next step to causally and more directly investigate the role of certain neural population patterns (dimensions) in multi-skill learning and motor memory retention.

Given that this ‘dream experiment’ is not yet technically possible, we conducted an ‘interference experiment’ to further test our hypothesis (the ‘indexing’ hypothesis, see above). The hypothesis is that a uniform shift of preparatory neural states is involved in the separation of motor memories when learning distinct curl fields (as shown in Fig. 3) that would otherwise interfere with each other. We analyzed the neural and behavioral data from the interference experiment and presented the results in [lines 243-319] and Extended Data Figs. 10, 11 of the manuscript (corresponding methods in [lines 584-603] and [lines 829-842]). We have copied the core results below for convenience. Please note that we did only two sessions of the interference experiment with each monkey due to Krishna’s (health-related, where all is ok but Krishna needed to scale back and focus on human clinical trial and computational research) slow ramp down of the monkey lab, as well as the abruptness and severity of the COVID-19 pandemic. Nevertheless, the results were consistent across monkeys and complimentary to findings when monkeys learned multiple fields sequentially (see Fig. 4).

**Main**

[lines 243-319] To further test the indexing hypothesis, we investigated how uniform shifts relate to the simultaneous learning of distinct curl fields and to the interference of learning. The interference of learning is defined by the phenomenon that learning one curl field impedes learning another curl field. The indexing hypothesis predicts that (1) if monkeys are able to learn distinct fields simultaneously, there will be two different field-specific uniform shifts to separate the preparatory neural states, and that (2) this separation will be incomplete if the two fields interfere with each other by partially opposing the shift of preparatory neural states associated with the other field. The bell-shaped generalization curve (Fig. 2d) showed that learning a curl field applied to one reach target partially altered the reach movement to a target 30 degrees away. We thus designed an interference experiment in which opposite curl fields were applied randomly on interleaved trials to two targets separated by 30 degrees (Extended Data Fig. 10a and see Methods). In the interference block, the hand deviation error for each curl field decreased over hundreds of trials (Extended Data Fig. 10b, blue curves), which indicated simultaneous learning of both fields. Nevertheless, more trials were required to learn either field (at least 400 trials per field) than when learning only one field at a time (no more than 200 trials per field, Fig. 5e), which suggests that this simultaneous learning is subject to partial interference between curl fields. In the
To investigate preparatory activity patterns during interference and learning, we first built a 2D subspace related to hand forces using the same TDR approach as described above (see Methods). We found that preparatory neural states in this TDR subspace directly reflected behavioral output. Before learning, preparatory states were spatially aligned corresponding to different reach directions (Extended Data Fig. 10c, color circles). In the interference block, preparatory states gradually rotated in opposite directions for the two curl fields, which is consistent with behavioral simultaneous learning (Extended Data Fig. 10c, left panels). During sequential learning, preparatory states of the two curl fields further separated from each other in opposite directions, which matches the improvement in behavioral performance and corroborates that separation of neural states was incomplete during simultaneous learning (Extended Data Fig. 10c, right panels). Moreover, we observed behavioral and neural generalization of learning both fields in the interference block (Extended Data Fig. 10d).

Given the interference of two fields during simultaneous learning, did preparatory neural states show the uniform-shift patterns as predicted by the indexing hypothesis? We defined two uniform shifts based on the shifts of preparatory states in the sequential-learning block where the learning of two curl fields occurred in isolation (see Methods). Both uniform shifts were orthogonalized against the TDR axes such that they were fully independent of the TDR subspace. We found that these two shifts were 127 degrees (monkey U) and 128 degrees (monkey V) apart. For opposing fields applied to targets separated by 30 degrees, this obtuse separation angle is expected from the geometry of uniform shifts already described, lying in between the orthogonal shifts observed for learning two curl fields applied to two targets 90 degrees apart (see Fig. 4b) and the antiparallel shifts observed for learning two curl fields applied to the same target (see Fig. 4a). The obtuse separation angle also suggests a neural correlate of interference consistent with the indexing hypothesis: in the interference block, the uniform shift of preparatory neural states for one curl field would partially oppose the uniform shift associated with the other field (Extended Data Fig. 10e, small black arrows), thereby only partially separating the preparatory states (Extended Data Fig. 10e, orange and pink circles) and slowing simultaneous learning for both fields. During sequential learning, preparatory states of each curl field (Extended Data Fig. 10e, green and purple circles) moved farther in their specific uniform-shift direction and were further separated from each other. These results provide additional evidence for the indexing hypothesis that distinct uniform shifts separate preparatory neural states for learning different curl fields and reduce interference between them.

In addition, we report here a residual neural shift during interference that was orthogonal to the field-specific uniform shifts and separated error-clamp preparatory states from the before-learning states (see Methods, Extended Data Fig. 10e). This residual shift was not predicted by the indexing hypothesis but does not contradict it: this shift may reflect an attempt to learn or index neural activity patterns for reaching in an unpredictable environment such as the interfering curl fields.

We then performed a second interference experiment with two opposite fields applied to the same reach target (Extended Data Fig. 11a). We found that monkeys were not able to learn both fields simultaneously, as expected, since the curl field identity was not cued and two fields were randomly interleaved, which indicated complete interference (Extended Data Fig. 11b, blue curves). In contrast, monkeys subsequently learned both fields sequentially (Extended Data Fig. 11b, red curves). Here, the indexing hypothesis predicts that, since we expect the uniform shifts associated with the opposing fields to be antiparallel, little net shift would occur along this shared uniform-shift axis in the interference block. Furthermore, we expect that a similar residual interference shift, orthogonal to the field-specific uniform shifts, would be observed as in the previous experiment. Consistent with these predictions, preparatory neural states preceding reaches through these two curl fields remained close to each other during interference (Extended Data Fig. 11c, e). During sequential learning of the two fields, antiparallel uniform shifts separated the preparatory states (Extended Data Fig. 11e), consistent with our findings in Fig. 4a and the indexing hypothesis. Additionally, we found a residual interference shift orthogonal to the field-specific uniform shifts, similar to our observations in the 30-degree interference experiment. Notably, the residual interference shift occurred even though no net learning was observed. We speculate that the residual interference shift likely relates to an attempt to index the neural activity patterns specific to the interference context, which might also facilitate strategies tailored to adapting to an unpredictable environment (e.g., sequential-learning block, learning continued with the hand deviation error further decreasing (Extended Data Fig. 10b, red curves).
impedance control to stabilize the limb\cite{41,42}. We leave it for future work to systematically address the functional roles of the residual neural shift during interference.
Extended Data Fig. 10 | Interference and sequential learning of opposite curl fields applied to two targets 30 degrees apart.

a, Block design of the interference experiment. Monkeys first performed delayed center-out reaches to each of 11 targets with no curl field (block i). Next in block ii, monkeys experienced either a CCW applied to one target or a CW curl field applied to another target in randomly interleaved trials. These two targets were 30 degrees apart. After around 200 trials of each field, curl-field trials were interleaved with error-clamp trials in the error-clamp block (block iii) and curl-field trials accounted for around 80% of all trials. Blocks ii and iii together made up the interference block. At last, in the sequential-learning block (block iv), monkeys learned one field applied to one target for 100 trials and the other field applied to the other target for another 100 trials. b, Behavioral indicators of learning quantified by the hand trajectory deviation error. Hand trajectory error in both curl fields is initially large, decreases in the interference block (blue curves) and further reduces in the sequential-learning block (red curves). One-sided Wilcoxon rank-sum test comparing hand error in the last 20 trials of block iii and block iv: monkey U, \(P_{\text{field1}} = 1.32 \times 10^{-8}\) and \(P_{\text{field2}} = 1.28 \times 10^{-5}\); monkey V, \(P_{\text{field1}} = 2.08 \times 10^{-8}\) and \(P_{\text{field2}} = 1.56 \times 10^{-8}\). c, Preparatory neural states in the force-related 2D TDR subspace. Before-learning states (color circles) radially organize corresponding to 11 reach directions. Error-clamp states (color diamonds) rotate counterclockwise for reach targets near the target that has a CW field and rotate clockwise for targets near the target that has a CCW field. Preparatory states of the two targets that have curl fields (gray and black triangles) rotate opposite their curl field directions in the interference block (left panel) and further separate in the sequential-learning block (right panel). Small black arrows point to the before-learning states of reach targets that later have curl fields. d, Behavioral and neural generalization of simultaneously learning two fields. Behavioral generalization (upper panel) was measured by perpendicular hand force differences between error-clamp trials and before-learning trials, and neural generalization (lower panel) was measured by the rotatory angles from before-learning to error-clamp neural states. Zero degree on the x axis, the middle target between the two targets that had curl fields. Error bars, s.e.m. from resampling. e, 2D plots: preparatory neural states projected into the subspace spanned by the two field-specific uniform shifts. Without orthogonalization, these two uniform shifts are 127 degrees (monkey U) and 128 degrees (monkey V) apart. The uniform shifts were orthonormalized before projection such that: x axis = field 1 uniform shift, y axis = field 2 uniform shift – the projection of field 2 uniform shift on field 1 uniform shift. During interference, preparatory states of each curl field (orange and pink circles) move in its specific uniform-shift direction while also progressing in the other uniform-shift direction, and are significantly separated (Hotelling’s T^2 test: monkey U, \(P = 3.73 \times 10^{-13}\); monkey V, \(P = 2.58 \times 10^{-6}\)). Small black arrows conceptually demonstrate this progression of neural states during interference. During sequential learning, preparatory states of each curl field (green and purple circles) move farther in its specific uniform-shift direction. Distance between preparatory states of these two fields further increases (Hotelling’s T^2 test: monkey U, \(P = 1.51 \times 10^{11}\); monkey V, \(P = 0\)). The shift from the before-learning centroid (gray circle) to the error-clamp centroid (black circle) during interference is in between the uniform shifts of learning each field. 3D plots: a residual interference shift that is orthogonal to the field-specific uniform shifts separates the before-learning and error-clamp neural state centroids. Neural states in the 2D plots are the projection of neural states into the gray plane in the 3D plots. b-e, One session per monkey. Though just one session of this interference experiment was performed with each monkey, the results were consistent across monkeys and complimentary to findings when monkeys learned multiple fields sequentially to support the indexing hypothesis (see Fig. 4).
Extended Data Fig. 11 | Interference and sequential learning of opposite curl fields applied to the same target.

**a,** Block design of the interference experiment. Same as in Extended Data Fig. 10a except that two opposite curl fields were applied to the same reach target. **b,** Behavioral indicators of learning quantified by the hand trajectory deviation error. Hand trajectory error in both curl fields is initially large, only slightly decreases in the interference block (blue curves), and significantly reduces in the sequential-learning block (red curves). One-sided rank-sum test comparing hand error in the last 20 trials of block iii and block iv: monkey U, $P_{field1}$ and $P_{field2} = 1.56 \times 10^{-8}$; monkey V, $P_{field1}$ and $P_{field2} = 1.56 \times 10^{-8}$. **c,** Preparatory neural states in the force-related 2D TDR subspace. Before-learning states (color circles) radially organize corresponding to 11 reach directions. In
the interference block, preparatory states of the two curl fields (gray and black triangles) are mixed together around the before-learning state (left panel). Error-clamp states (color diamonds) of most targets shift from their corresponding before-learning states. These shifts do not show coherent patterns across targets or monkeys and are likely due to noise. In the sequential-learning block, preparatory states of the two curl fields (gray and black triangles) gradually rotate opposite their curl field directions (right panel). The small black arrow points to the before-learning state of the reach target that later has curl fields. **d,** Perpendicular hand force differences between error-clamp and before-learning trials (upper panel), and the rotatory angles from before-learning to error-clamp neural states (lower panel), do not show coherent patterns across targets or monkeys. Zero degree on the x axis, the target that has curl fields. Error bars, s.e.m. from resampling. **e,** Uniform shifts of learning two curl fields and the residual interference shift were defined in the same way as in Extended Data Fig. 10e. The two field-specific uniform shifts are close to antiparallel (monkey U: dot product = -0.79, monkey V: dot product = -0.64), and so we can visualize preparatory neural states in a 2D plane spanned by field 1 uniform shift and the residual interference shift. During interference, preparatory neural states of the two curl fields (pink and red circles) shift away from the before-learning centroid (gray circle) along the residual interference axis, but they remain close to each other (Hotelling’s T² test: monkey U, P = 0.66; monkey V, P = 0.98). During sequential learning, preparatory states of the two curl fields (green and purple circles) are separated by opposite uniform shifts (Hotelling’s T² test: monkey U, P = 2.49 x 10⁻⁸; monkey V, P = 2.90 x 10⁻⁵), similar to Fig. 4a. **b-e,** One session per monkey. Though just one session of this interference experiment was performed with each monkey, the results were consistent across monkeys and complimentary to findings when monkeys learned multiple fields sequentially to support the indexing hypothesis (see Fig. 4).

3. I am worried about subtle changes in behavior and how they were measured and controlled. For example, I would like to understand how the authors control the overall geometry of the animal’s arm and body in their apparatus? From what I can see, it seems to me that a lot about the general state of the animal goes unmeasured or unreported. Can the authors rule out that the key results here (trivially) reflect things like changes in the animal’s posture as a function of trials or conditions?

Thank you very much for this insightful question, which we pay considerable attention to while conducting all experiments, but did not describe here and thus the question noted is most understandable.

To control the overall geometry of the animal’s body and arm, we spent many months training them to sit calmly in a nonhuman primary chair (with side walls, neck plate, and a panel through which they could extend their arm to perform the reaching task) that was customized for them. They sat in the same chair at the same height, with the same position of head fixation, and the chair was locked to the haptic device at the same location. Locations of the display screen and the haptic device were also fixed across all days. Though we did not record and quantitatively measure their posture, we had cameras to monitor their face and body during the experiment. From the cameras we did not see obvious consistent posture changes from the before-learning block to the learning block. This is also consistent with our own observations of each monkey’s behavior, which is part of the standard substantial training process that we have adopted. While it is certainly possible that the monkey could make small changes below the threshold for us to detect, we do not have any reason from observing the animal’s behavior to suspect that this could be responsible for explaining the results presented in this manuscript. Moreover, we note that static offsets in position are responsible for a very small fraction of the total variance of neural population activity (Stavisky et al., 2018). We have added these details about how we controlled the animals’ behaviors to Supplementary Materials [lines 3-19].

We agree that if the monkey changes its sitting posture, it might change the overall neural activity in the motor cortex (Scott and Kalaska, 1997) and so we may expect to observe changes in neural activity throughout a whole trial. This potential confounding mechanism is not consistent with our data, and unlikely to explain our observations for several reasons:

1. The geometric relationships between different uniform shifts reflected the identity of learning different curl fields (Fig. 4). This result supports that the uniform shift is specifically learning-related, unless some unmeasured postural state is closely related to the learned field but is not related to learning.
Given that we did not see a uniform shift during the peri-movement period (Extended Data Fig. 16), the uniform shift that we report during the preparatory period is unlikely to result from posture changes.

As a complementary analysis, we quantified the repertoire change of the baseline neural activity (i.e., when the animal was holding the handle before target onset in each trial). In contrast to the neural repertoire change during the preparatory period (now Fig. 3c), we did not see a significant neural repertoire change of the baseline neural activity (Extended Data Fig. 17 and reproduced below). This further supports the conclusion that the observed uniform shift can not be explained by postural changes. While we cannot rule out behavioral changes that only occurred during the preparatory period (and not during the baseline period or the movement period), we think that this is extremely unlikely. During careful observation of the monkey’s behavior via cameras, we did not observe any unexpected pre-reach movement.

**Extended Data Fig. 17** | There is no significant neural repertoire change of the baseline neural activity (i.e., before target onset in each trial) after learning the curl force field. Black box: control sessions in which monkeys did thousands of center-out reaches without any curl field; red: learning sessions. One-sided Wilcoxon rank-sum test: monkey U, \( P = 0.999 \); monkey V, \( P = 0.595 \).

We have added the text below to the discussion section of the main text to reflect our response to this concern.

**[lines 457-469]** Besides the aforementioned four sets of control experiments and analyses, we want to address a few more alternative, learning-irrelevant explanations for the uniform shift and neural repertoire change. First, changes in the animals’ posture could potentially contribute to the uniform shift. That is, when the body slightly twists, it may change the overall neural activity in the motor cortex and so we may expect a uniform shift or a neural repertoire change throughout a whole trial. Although our animals’ postures were not measured, given that we did not see a uniform shift during the peri-movement period (Extended Data Fig. 16c, d), the uniform shift that we report ought not be merely due to postural changes. Furthermore, as a complementary analysis, we quantified the repertoire change of the baseline neural activity (i.e., when the animal was holding the handle before target onset). In contrast to the neural repertoire change during the preparatory period (Fig. 3c), we did not see a significant neural repertoire change of the baseline neural activity (Extended Data Fig. 17), which further supports that our main results are not substantially due to postural changes.

4. The monkeys show quite disparate behavior, certainly in terms of learning rates. I appreciate that the authors show us both animals for some analyses but they do not do it for all of them. And sometimes unclear how they are lumped together and what effect it has on the results.

We thank the reviewer for highlighting this important point which was not sufficiently clear in the original manuscript. We have added relevant details to the figures, figure captions and methods throughout the revised manuscript.

In an attempt to be maximally helpful, we have also summarized this information of which results came from both monkeys (analyzed separately or lumped together) and which results came from one monkey, as well as our rationale for doing so. This summary has been added to the Supplementary Materials [lines 135-155].
Grouped data: in Fig. 5g, 5h (right panel), 5k and Extended Data Fig. 13f, 13g, we grouped together four sessions of relearning experiment with monkey U and three sessions with monkey V rather than analyze them separately to gain higher statistical power for performing the sign-rank test. Example neuron PSTHs and EMG recordings from both monkeys are grouped together (Extended Data Figs. 1, 5, 6). Note that each panel of the EMG figure is from one specific muscle of one monkey but the 6 total recorded muscles are either from monkey U or monkey V.

Data from only one monkey: we did the random-perturbation experiment only with monkey V (Fig. 3d, e and Extended Data Fig. 18c). We were able to track the same neurons over five sessions only in monkey U (using Utah array implant) to study the uniform shift during multi-session multi-field learning (Fig. 4b, 4c purple distribution, Fig. 4f 8-condition decode, Extended Data Fig. 9c, and Extended Data Fig. 14c 8-condition decode) and the relationship between the uniform shift and learning/relearning rate (Fig. 5i), with the stability of selected neurons is shown in Extended Data Fig. 8.

Data from both monkeys shown separately: all the rest results. Note that plots of example single-trial hand trajectories, visualized neural states and visualized neural trajectories are from one example session in one monkey (Figs. 1b, 2a, 2d, 3a, 3d, 4a, 4d, 5a, 5c, 5j; Extended Data Figs. 4a, 15, 16a, 16e, 18a, 18b, 22) and are consistent in both monkeys: the behavioral and neural quantification results are shown separately for both monkeys unless specified in the “Grouped data” section (Figs. 1c, 1d, 2b, 2e, 3b, 3c, 3e, 4c, 4e, 4f, 5b, 5d, 5e, 5f; Extended Data Figs. 2, 3, 4b, 7, 9a, 9b, 13a-e, 14, 16b-d, 16f, 17); the interference experiment results (Extended Data Figs. 10 and 11) come from two sessions with each monkey.
Referee #2 (Remarks to the Author):

Sun and colleagues examined neural population dynamics in motor and premotor cortex while monkeys learned to adapt their reaches to a force field. This manipulation holds particular interest because it is well-studied in humans, but there is relatively little understanding of the neural basis of how we learn even this simple motor skill. Thus, this behavior is the right place to start in our efforts to understand the neural basis of skill, and in fact, this paper seems likely to stand as one of the first to reveal the neural-population principles of motor learning. As the authors allude to, efforts to tie motor learning to neural changes have in the past largely focused on single neurons, where organized, rational changes were hard to see. To add to the potential impact of the work, it is one of the first to perform high-density recordings (here, neuropixels), and crucially, be able to interpret and make sense of those population signals, rather than simply show them off. The main conceptual advance of this study is to help show how the brain uses task-irrelevant dimensions of neural population activity space to perform useful computations. They put forth an interesting new proposal - that high-dimensional spaces are used to sequester motor memories, to prevent them from interfering. For these reasons, I see this as a high-impact, broad-interest finding, worthy of publication in Nature. However, the paper will improve if the positive aspects of the paper are brought out more impactfully in the writing and analyses. (This review is lengthy, but that’s because the paper is good.)

We thank the reviewer for their very kind comments related to the importance of the problem, and our approach to it. We believe that we have considerably strengthened and streamlined the manuscript as a result of the wonderful, and wonderfully helpful, suggestions offered.

Major Suggestions:
1) Concerns about the text.
   The novel claims of the paper should be emphasized. This paper should add clarity to the terminology in the field. Here are some specific instances where clarity in the presentation could be enhanced, for the betterment of this study and the field.

1.1. As written, the key findings for which the paper seems likely to become known are just below the surface, and need to be brought to the top. The paper as written details a voyage of discovery, but it would be more effective and impactful to present it in a hypothesis-driven manner. That is, “here is our vision, and look! The data support it.” For example, the second-to-last sentence of the abstract says “distinct uniform shifts can separate motor memories” but that would be better as the second sentence! “How does the brain keep track of different motor skills? We hypothesize that motor memories are stored by using uniform shifts in neural population activity space to keep distinct motor memories separated. Our data support this hypothesis.” Maybe the authors would like to give this concept a name? “memory indexing” seems like a good candidate. And, the learning axis also deserves a name.

Naming the concept is a great suggestion, thank you, and we agree that ‘indexing’ is an excellent and well-balanced term for summarizing the neural population patterns that we found in the uniform shift and its potential function (i.e., helping look up the specific motor skill for a given force field and retrieve it). With the reviewer’s permission, which we believe was given, we are very happy to adopt this name and we have now used it throughout in the revised manuscript. We also believe that we understand the overall spirit of this comment, which is that simple names for a few key concepts can be quite helpful. In this spirit, we have also named the learning axis the ‘uniform-shift learning axis’ (or, shortened as the ‘uniform-shift axis’ when not compared to the ‘uniform-shift washout axis’), and used it consistently in the revised manuscript.

Thank you for the helpful suggestions for phrasing the text in a more hypothesis-driven manner. We have rewritten the abstract in this new manner to make our key hypothesis and findings more upfront as suggested:

Abstract
The brain has a remarkable capacity to learn and execute various motor skills, which relies on the variety of patterns that the neural population activity can exhibit. Here we ask what patterns the neural population activity generates to learn a new motor skill. We trained rhesus monkeys to learn a curl force field task\textsuperscript{1} that elicited new arm-movement kinetics for some but not all reaching movements\textsuperscript{2,3}. We hypothesize that new neural activity
patterns are generated in the motor cortex when learning those new movements. We found that in a neural subspace related to hand forces, preparatory activity reassociated with new movements (‘reassociation subspace’). These systematic changes were observed only for learning-altered reaches. We also discovered the formation of new preparatory neural activity patterns along a neural population dimension nearly orthogonal to the reassociation subspace. The new patterns emerged from the shift of preparatory activity along this orthogonal dimension for all reaches including those unaltered by learning (‘uniform-shift learning axis’). During a washout period when the curl field was removed, movement kinetics gradually reverted, but the shift of preparatory neural activity along the uniform-shift learning axis persisted. This persistent shift may retain a motor memory of the learned field and relate to faster relearning of the same curl field. How does the brain keep track of different motor memories when learning multiple different skills? We hypothesize that different motor skills are stored by engaging different uniform-shift learning axes to distinguish motor memories (‘motor memory indexing’). We observed multiple distinct uniform shifts when multiple different curl fields were learned, with each shift reflecting the identity of the curl field applied and potentially separating the associated motor memories. The geometry of these shifts in preparatory activity could serve as an indexing function to organize skill-specific changes in movement production, facilitating the acquisition, retention and retrieval of a broad motor repertoire.

This comes up again in the third paragraph of the Introduction. The phrasing, “Here we asked...” is weaker than the data could support. “Here we show that there are dynamics that occur with learning but do not directly support the new behavior. We propose that these dynamics serve an ‘indexing’ function that enables different motor skills to be stored in a population space. This could provide a mechanism for preventing them from interfering, and rapidly re-accessing them.” This key conceptual advance is again buried in the discussion, only appearing in paragraph four.

Thank you again for the helpful suggestions for phrasing the text in a more hypothesis-driven manner. We agree that this framing helps highlight the concepts and key findings that we indeed have in mind. We have rewritten the introduction part of the main text in this new manner to make our key hypothesis and findings more upfront. We have added the following introduction to the manuscript:

Main

Motor learning encompasses a wide range of phenomena, from relatively low-level mechanisms for calibrating movement parameters, to making high-level cognitive decisions about how to act in a novel environment. Motor adaptation has been a long-standing and widely used paradigm for studying motor learning. Decades of behavioral studies have demonstrated many intriguing phenomena during motor adaptation, such as the error-driven calibration of movements, generalization of learned skills to a new context, savings (faster relearning) or memory retention, and interference between learning multiple skills. Yet their neural mechanisms, in particular the underlying neural population dynamics, remain largely unknown.

In the field of motor control, studying neural population dynamics has provided foundational insight into activity patterns and computational principles not readily apparent at single-neuron resolution. Collectively, these experiments have characterized many different neural activity patterns related to the learning process. However, a key remaining question is: do changes in neural population dynamics relate purely to newly learned movements, or, alternatively, are there additional neural activity patterns generated that facilitate learning without matching the movement output? Here, through the lens of curl field learning, we found changes in neural population activity in certain neural subspaces that directly relate to movement parameters (e.g., movement kinetics and kinematics) and likely guide the movement output. We also found that there are neural population activity patterns in a nearly orthogonal dimension that occur with learning but do not appear to directly support the movement output. Our results suggest that neural patterns in this orthogonal dimension serve an ‘indexing’ function that enables different motor skills to be acquired, stored and accessed separately in the neural population state space. This could provide a mechanism for preventing motor skills from interfering and aiding in rapidly re-accessing them as needed.

I consider figure 2f to be the most significant (sensible and surprising at the same time) finding of the study. As structured, this result sneaks up on the reader, and if you blink, you miss it. Perhaps figure 1 should be
reformulated to show the hypothesized neural outcome. That way, when we arrive at the data that support it, it will stand out more, and be more memorable.

This is an excellent suggestion. We have highlighted the key results by separating Figs. 2f-j (now new Fig. 3a-e) from Fig. 2a-e (now constitutes the entire new Fig. 2), and by using more hypothesis-driven language in the abstract and introduction. In particular, we moved the hypothesis to the front of the introduction in order to help the main results stand out when readers arrive at the data supporting it (the revised introduction is in our response to the last question). We decided to keep plots of the hypothesized neural outcome in Extended Data Fig. 12, because neural results related to the hypothesized neural outcome for multi-field learning in Extended Data Fig. 12 is built upon the neural findings in the single-field learning experiment. We believe that introducing the hypothesized outcome in the main text after the single-field learning results will help readers understand the motivation behind the hypothesis better. This presentation also allows us to lead readers straight to the key neural results of the single-field learning experiment after introducing the task design and behavior.

Perhaps figs 2f-j should be a separate figure from 2a-e? Fig 2d is a nice demonstration of a neural correlate of a known behavioral effect. It is novel, and it is reassuring to see, and a nice validation of the use of the TDR approach here, but it is not the main accomplishment of this study. A simple split into two figures will prevent this material from overshadowing the effect in fig 2f.

This is a great suggestion, which we have followed, thank you. In the revised manuscript, we split the original Fig. 2 into the new Fig. 2 and Fig. 3 to highlight the uniform shift results. We have reproduced the new figures and captions below for convenience:

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**Fig. 2 | Patterns of preparatory activity in the force-related 2D TDR neural subspace.**

**a,** In a 2D neural subspace constructed by TDR capturing the variance due to initial hand forces, preparatory states before-learning (color circles) radially organize as a ‘ring’ corresponding to reach directions (small circles: single-trial states; large circles: trial-averaged states). Top-left inset, color-coded reach targets when the trained target is up. Top-right inset, preparatory states during learning (gray and black circles) gradually rotating from the before-learning state of the trained target (middle) to that of its adjacent reaching target (left or right), in two example learning sessions (a CCW or CW force field applied to up reaches). **b,** Initial hand forces predicted by the 2D TDR preparatory states are correlated with real forces of the upcoming movement; the sign of hand force indicates its direction (monkey U, \( R^2 = 0.77 \) and \( P = 1.63 \times 10^{-13} \); monkey V, \( R^2 = 0.59 \) and \( P = 1.92 \times 10^{-12} \)). Lighter dots, earlier learning trials; darker dots, later learning trials. **c,** Single-trial prediction MSE of initial hand
forces is significantly smaller using original data than shuffled data (one-sided Wilcoxon rank-sum test: monkey U, $P = 0.0003$ in both cases; monkey V, $P = 0.004$ in both cases). For each monkey, left two columns: training-set (before-learning trials) prediction MSE; right two columns: held-out set (learning trials) prediction MSE. Control results (blue) are forces predicted by models built from training sets that have neural and behavior data shuffled. One datapoint per session. d, In the same 2D TDR subspace, preparatory states of error-clamp reaches (color diamonds) consistently rotate in an opposite direction to the curl field direction for targets within 45 degrees from the trained target. Two example monkey U sessions (left panel, learning a CCW field applied to the up target; right panel, learning a CW field applied to the up target), representative of the other learning sessions from both monkeys. e, Changes of preparatory states in the 2D TDR subspace reflect generalization of learning, quantified as the rotatory angles from before-learning to error-clamp neural states. Zero degree on the x axis, the trained target. Error bars, s.e.m. across sessions.

Fig. 3 | A uniform shift of the preparatory neural states along a nearly orthogonal neural population dimension.

a, Preparatory states of the trained target (gray circles) gradually shift away from the before-learning neural repertoire (color circles) and towards a new (significantly different) repertoire in error-clamp trials (color diamonds, the after-learning repertoire). The arrow points from the before-learning neural state to the after-learning state of the trained target. Two-sided Wilcoxon rank-sum test: monkey U, $P = 3.22 \times 10^{-17}$; monkey V, $P = 1.52 \times 10^{-13}$. b, Single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis (see Methods). Normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states. Solid line: linear-log regression. Gray circles and line: across all sessions; blue circles and line: one example session. $P$ and $R^2$ values of corresponding regression curves are also color-coded. c, Preparatory neural state repertoires change similarly for trained and untrained reaches. Black: control sessions in which monkeys did thousands of center-out reaches without any force field; blue (far tgt): far targets more than 45 degrees from the trained target in learning sessions; red (near tgt): near targets within 45 degrees from the trained target in learning sessions (one-sided Wilcoxon rank-sum test: monkey U, $P_{12} = 8.24 \times 10^{-11}$, $P_{13} = 1.01 \times 10^{-9}$, $P_{23} = 0.96$; monkey V, $P_{12} = 5.22 \times 10^{-5}$, $P_{13} = 2.33 \times 10^{-7}$, $P_{23} = 0.059$). d, Preparatory states for before-learning no-perturbation (color circles), before-learning random-perturbation (color triangles) and after-learning (color diamonds) conditions projected to PCs 1-3. e, Larger preparatory neural repertoire changes occur to after-learning states vs. before-learning no-perturbation states (red), and to after-learning states vs. before-
learning random-perturbation states (blue), than to before-learning random-perturbation states vs. no-perturbation states (black). \( P_{12} = 1.83 \times 10^{-5}, P_{13} = 1.83 \times 10^{-5}, P_{23} = 0.37 \).

1.2. Everybody is excited about neural population dynamics, due in large part to the senior author's previous work. However, the term runs the risk of becoming an amorphous catch-all. I suggest using “neural population dynamics” to refer only to short-timescale changes in neural population state - that is, the types of effects captured by jPCA or GPFA, and here in fig 4j. To use the same term to also refer to changes over timescales of minutes only dilutes its meaning and creates some potential for confusion.

Thank you for raising this important point, which is very helpful for keeping straight the different timescales addressed in this study. To help avoid confusion, we have defined our terminology in the methods section and used it consistently throughout this manuscript, which is also copied below for convenience:

**Methods**


1. **Neural population state.** The N-dimensional coordinates (state) of a population of N neurons found by counting the number of action potentials emitted from each neuron within a time bin. The neural population state is then an N-dimensional point, or a lower dimensional point if a dimensionality reduction method is applied to the data. Finally, if multiple trials are averaged together, the average neural population state retains the same definition except that the average number of action potentials emitted from each neuron within a time bin is used.

2. **Initial condition.** The preparatory neural population state at the time of the ‘go cue’ in an instructed-delayed reach task.

3. **Changes in neural population state.** The difference between the neural population states before and after some perturbation, in this case the application of a curl field. In the curl field learning experiments the changes in neural population state can be (i) during learning (i.e., how the preparatory neural population state changes from trial to trial during the application of a curl field or (ii) after complete learning (i.e., after numerous curl field trials such that the after-learning preparatory neural state becomes steady). Changes in initial condition is a specific case of changes in neural population state at the time of the go cue during the preparatory period.

4. **Neural population dynamics.** This typically refers to the time evolution of the neural population state within a trial (i.e., a fairly fast timescale).

5. **Changes in neural population dynamics.** This refers to the changes in the way the neural population state evolves. For example, if we use the standard equation \( \frac{dx(t)}{dt} = A x(t) + B u(t) \) to describe neural population dynamics, changes in neural population dynamics can be represented as the change of matrices \( A \) or \( B \) across trials (i.e., across longer timescales, where \( A \) and \( B \) are assumed to be fixed when studying fast timescale neural population dynamics, but in reality \( A \) and \( B \) are functions of time that come into play across longer timescales and especially likely during learning. While as defined in 4 that the timescale of neural population dynamics itself is typically as short as within a single trial, changes in neural population dynamics typically happen over longer timescales, across multiple trials and minutes or longer.

To summarize, here we use ‘changes in neural population state’ and ‘changes in neural population dynamics’ to mean longer-timescale neural changes across trials, and we use ‘neural population dynamics’ as we have in prior publications when talking about the evolution of neural state over time within a trial.

1.3. Are the effects in fig 2D “reaiming” or “reassociation”? As written, the manuscript seems agnostic on the issue (e.g., end of “reassociation-like changes...” section.) Those terms are used here without a lot of definition or explanation, beyond the relevant citations. Shall we think of reaiming as the neural correlate of a behavioral strategy that can be implemented entirely in the workspace? And, reassociation as a strategy that extends reaiming to include dimensions of neural activity that are not isomorphic with the workspace? Whatever determination the authors make, please don’t pass up the opportunity to define these terms clearly.

Thank you for pointing out both the need, and the opportunity, to define this more clearly. Just to address your question straightaway, before describing how we have addressed it in the manuscript, yes you indeed
understood correctly how we were meaning reassociation and re-aiming. We have copied Fig. 2d here for convenience and so that we can clarify further.

The neural changes in Fig. 2d suggests a re-aiming-like strategy. But, perhaps, preparing for a compensatory force to oppose the curl field would result in similar neural changes (i.e., the rotatory shift of preparatory neural states) even if the monkeys did not explicitly re-aim for a nearby reaching target. So here we use ‘reassocation’ to describe this type of neural shift and ‘re-aiming’ to speculate about the animal’s potential behavioral strategy, which stays valid regardless of whether or not the monkeys actually used the re-aiming strategy.

We have defined these terms in the methods section (copied below for convenience), and use reassocation consistently in the revised manuscript to describe the neural correlates of learning in the 2D TDR subspace.

**Methods**

**Definition of re-aiming and reassocation.** Reassociation⁴ is the neural strategy of learning subject to a fixed, existing repertoire of neural activity patterns. In other words, the neural activity patterns used for a particular behavior change during learning, but remain within the set of neural activity patterns that could be generated by (or empirically observed from) the neural population before learning. Here, the control space is the neural repertoire. Re-aiming is the neural strategy of learning whereby the neural activity patterns used for a given behavior after learning are the same neural activity patterns used for a potentially different behavior before learning. Here, the control space is the behavioral repertoire. Because the neural activity patterns used after learning are part of the existing neural repertoire, re-aiming is an instance of reassocation. Re-aiming has been observed during learning in VMR tasks²⁰,²³,³¹,³², whereby the perturbation applied to the effector is countered by a rotatory shift in neural activity patterns. The rotatory shift of preparatory states we observed in the 2D TDR subspace resembles these re-aiming findings. However, we referred to these types of changes as reassocation-like (as opposed to re-aiming-like) because (1) preparing for a compensatory force to oppose the curl field could result in re-aiming-like rotatory shifts even if the monkeys do not explicitly re-aim for a nearby reaching target, (2) we can directly test for reassocation (based on repertoire change⁴), and (3) all re-aiming-like changes are consistent with reassocation.

1.4. How about the effects in fig 2F? Are those really “new patterns”, or something simpler, like reassocation, or a neural correlate of force? Oby et al (2019) were careful to define “new patterns” as patterns of neural activity that were demonstrably not present during the first day of behavior, prior to learning. That paper made note of the caveat that it was possible that the capacity to exhibit those patterns may have already been present prior to learning, and the animals simply did not express them. Here, that caveat is at least as much of a concern. That is, it seems a viable possibility here that the capacity to exhibit the after-learning repertoire was present prior to learning. Evidence for this is that the repertoire shift illustrated in fig 2f seems to have happened already in the first day. Could it have happened simply by changing the forces the animal exerted? The learning-related changes in behavior probably closely track forces; if the animal can exhibit a shift in neural activity simply by exerting different forces without any learning, then it would be an overstatement to call those “new patterns.” One can envision a control task where the animals simply have to resist a force to keep their hand centered. That seems likely to shift neural activity in a dimension unrelated to kinematics, and we would not call those “new
patterns”, but rather a fuller exploration of the pre-learning repertoire. Given this possibility, it seems incautious to call these “new patterns.” (I note that three controls were already performed. These rule out the following potential sources of the repertoire change: drift over long durations, preparation for a random force perturbation, and co-contraction. They do not rule out the possibility that the learning-induced shift is a force axis. This isn’t a fatal concern; whether or not it corresponds to force changes, it’s still a learning-related change, and discovering it is force would help us further make sense of it.) Consistent with the interpretation that the repertoire shift corresponds to a force axis is the fact that VMR learning, otherwise quite similar, does not show the repertoire shift.

Thank you for bringing up the definition of “new”. As you astutely pointed out, it seems possible that the capacity to exhibit those neural population activity patterns may have already been present prior to learning and the animals simply did not express them, but in our definition, those patterns are still new compared to the neural patterns used in the standard reaching movements before learning the curl field. So here what we mean by “new” is that those neural patterns are specific to moving in a curl field and are not used for a standard set of behaviors such as the center-out reaches or correcting random perturbations. Furthermore, the neural repertoire change did not occur spontaneously in control no-learning days (Fig. 3c). To emphasize this point and help avoid confusion in the revised manuscript, we have clarified the definition of ‘new’ as ‘not used before-learning for standard reaching movements’ and used it consistently. The updated text is copied here:

[lines 170-172] The presence of this uniform shift suggests that a neural repertoire change may occur after learning, that is, the emergence of new preparatory activity patterns that are not used before learning for standard reaching movements.

[lines 444-446] Furthermore, the occurrence of uniform shifts provides evidence for the formation of new activity patterns during short-term motor learning that are not used in standard reaching movements.

Despite this shared potential caveat regarding “new patterns”, there is a disconnect between Oby et al., 2019 and our work here, in that controlling the prosthetic in their work demands specific neural patterns, whereas curl fields in our work require specific behavior. In that sense, the caveat that the brain "could have exhibited those patterns" in the prosthetic case does not necessarily apply as straightforwardly in the curl field case, because the neural patterns specific to moving in a certain curl field do not necessarily exist before learning that field unless the environment demands the generation of field-specific behaviors and thus the generation of behavior-specific neural patterns.

Importantly, we collected control datasets, which verified that these uniform shifts never spontaneously occurred during normal center-out reaches on the no-learning control days. This further supports the idea that those neural patterns we observed are specific to learning the curl field (or, new relative to neural patterns used during the normal reaches).

Moreover, it is almost impossible to exhaust all the existing neural patterns before learning because each animal could only perform a limited number of tasks. Thus, to say that some neural activity pattern is completely novel and has never been expressed before learning is similar to “proving a negative”, as is pointed out by the reviewer in another question below (see 2.2). However, we think that the process of exploring the capacity that is already present, but has never been expressed, and the process of gaining a new capacity de novo, can both be qualified as learning. These two processes could sometimes be indissociable.

Regarding the question of “could it (the repertoire change) have happened simply by changing the forces the animal exerted”: importantly, the uniform shift generalized to all reach directions to the same extent whereas changes in hand forces after learning the curl field showed spatial locality, which supports the idea that it is not a force axis (i.e., the uniform shift is decoupled from the exerted hand forces). Furthermore, the uniform shift persisted after the washout period when forces returned to the before-learning patterns, which provides additional evidence that it is not a force axis. Finally, after we computed the dot product between the uniform-shift axis and the force-related TDR 1 and TDR 2 axes, we found that the uniform-shift axis was nearly orthogonal to the TDR axes (i.e., the dot product was close to zero, Extended Data Fig. 3a in the revised manuscript).
Alternatively, if by “force axis” you mean an axis that is not directly representing the exerted forces but instead indicates whether the animal is in an environment that requires the generation of new forces (in a curl-field-specific manner), we believe that this explanation is the same as the ‘indexing’ function that you helpfully suggested. That is, the uniform shifts index neural population states for the generation of forces associated with specific curl fields (i.e., field-specific motor skills). We think that a future experiment that varies the strength of the curl field and tests if the uniform shift relates to the field strength would be an interesting next step that can further characterize the indexing function of the uniform shift.

Another way to disambiguate a repertoire shift due to learning from a force axis is to partition the data into PMd and M1. M1 may show a greater sensitivity to force than does PMd. Is it the case that the loadings onto the repertoire-shift axis come from the M1 electrodes?

We grouped PMd and M1 data together because we wanted to have a larger total number of neurons to perform our analyses with, and in many ways these neurons on the precentral gyrus respond similarly. We thank the reviewer for the great suggestion of partitioning the data into PMd and M1 populations in order to check if there are differences. In the revised manuscript, we examined PMd and M1 neurons separately to see if PMd and M1 neurons contribute differently to the preparatory neural repertoire shift. The results showed that the PMd population and the M1 population were highly similar. Each on its own had neural repertoire changes after learning the curl field, similar to what we found when we grouped PMd and M1 together. We have added this plot to Extended Data Fig. 19 in the revised manuscript, which is copied below for convenience. We have also added the following text to the Discussion section:

[line 411-416] By investigating PMd and M1 populations separately, we found that a similar uniform shift occurred in each area alone as occurred when the areas were combined (Extended Data Fig. 19). Our results suggest that the uniform shift is not confined to only PMd, but it is confined to the preparatory neural activity in both PMd and M1 and does not occur during the peri-movement period (Extended Data Fig. 16).

![Extended Data Fig. 19](image)

**Extended Data Fig. 19** | ... c, Preparatory neural repertoires change similarly in PMd, M1, or PMd + M1. Black: control sessions in which the monkey did thousands of center-out reaches without any force field (one-sided Wilcoxon rank-sum test: monkey U PMd + M1, $P = 9.14 \times 10^{-10}$; monkey U PMd, $P = 2.08 \times 10^{-17}$; monkey U M1, $P = 4.77 \times 10^{-5}$).

Moreover, monkey V Neuropixels data was obtained via two days of PMd recordings and one day of M1 recording (now new Figs. 2b, 2c, 2e, 3b, 3c, 5b, 5d, 5f, 5g, 5h, 5k). We updated the methods section with this note (see [line 573]). Data from PMd and M1 were analyzed separately and then combined into the same plots. PMd and M1 populations in monkey V also showed similar neural repertoire changes, which provides further support that PMd and M1 neurons contribute similarly to the uniform shift and repertoire change.
1.5. Is this really a skill? Of course, skill learning is what we want to understand, but it is probably better to refer only to “adaptation” here. (Arguably, monkey V shows skill, in that he can switch rapidly among mappings…)

Thank you for raising this important point. To help avoid confusion, we wish to clearly define our terminology and then use it consistently. We will use the terminology as defined in Krakauer et al., 2019. Those definitions are added to the methods section of the revised manuscript and copied below for convenience.

**Methods**

[lines 880-895] Definitions of behavioral terminology

1. **Motor skill.** Motor skill involves precise execution of movements. Typically, motor skill also involves rapid selection of the right action in the right context.

2. **Skill acquisition vs. skill maintenance.** We adopt a two-part operational definition of motor learning: (1) skill acquisition is the process by which an individual acquires the ability to rapidly identify an appropriate movement goal given a particular task context, select the correct action given a sensory stimulus and / or the current state of the body and the world, and execute that action with accuracy and precision; (2) skill maintenance is the ability to maintain performance levels of existing skills under changing conditions. These two aspects of motor learning are each important in their own right, and they likely share overlapping neural circuitry. That said, it is also clear that the brain possesses dedicated mechanisms for skill maintenance (see Adaptation below).

3. **Adaptation.** It is a skill, but more about skill maintenance than acquisition, except for the first time encountering a new environment such as the curl field. The term adaptation is usually used in contrast to *de novo* skill acquisition which involves learning a new motor controller from scratch rather than derived from existing ones.

Given the three definitions above from Krakauer and colleagues, we believe that continuing to use the term skill in the revised manuscript is appropriate.

Moreover, we think that although adaptation and skill acquisition have remarkable differences, there could be cases where they overlap. For instance, adapting to a new force environment could be a skill (but not as complicated as playing tennis) to learn, akin to the difference between walking and snowshoeing, or turning something with your hands versus using a screwdriver.

2) Details of the neural activity findings.

2.1. Here the authors combine PMd and M1 recordings. This seems like a strange thing to do. There could be key differences between the areas, and indeed, identifying those differences is of high value in the field. A distinction could be made based on anatomical location, or based on the extent to which preparatory activity is present at the individual channels. Even if no meaningful differences are seen when this is done, that is worthy of reporting.

Thank you again for this suggestion. As we noted in our response to an earlier question, (1.4), we had previously grouped together PMd and M1 recordings because we wanted to maximize the total number of neurons in our population-based analyses. We agree with the reviewer that it is worthy of investigating whether the neural patterns we observed are specific to PMd or M1. In the revised manuscript, we examined PMd and M1 neurons separately to see if PMd and M1 contribute differently to the TDR subspace and uniform shift axis. We found that population activity in only PMd or only M1 displayed similar rotatory neural shifts in the 2D TDR subspace. We also observed similar neural patterns along the uniform-shift axis that separated the before-learning and after-learning neural states.

In short, when data from the two areas were analyzed separately, the results were quite similar to what we found when we grouped all neurons from PMd and M1 together. Importantly, however, we cannot conclude that there are not important differences in activity between the two areas in this task. We can say, however, that the key analyses and findings already reported in this manuscript did not appear different between populations in PMd and M1.
Therefore, we conclude that the uniform shift was not confined to only PMd or only M1. Instead of being isolated to a specific region, these shifts were mainly confined to the preparatory neural activity in both PMd and M1 (Extended Data Fig. 19). We have included this new figure below for convenience.

Extended Data Fig. 19 | Patterns of preparatory activity in different neural state subspaces with PMd and M1 neural populations analyzed separately.

a, Initial hand forces predicted by the 2D TDR preparatory states are correlated with real forces of the upcoming movement; the sign of hand force indicates its direction (monkey U PMd, $R^2 = 0.41$ and $P = 3.84 \times 10^{-8}$; monkey
U M1, $R^2 = 0.56$ and $P = 7.33 \times 10^{-12}$). Lighter dots, earlier learning trials; darker dots, later learning trials. **b**, Single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis. Solid line: linear-log regression (monkey U PMd, $R^2 = 0.04$ and $P = 6.67 \times 10^{-9}$; monkey U M1, $R^2 = 0.103$ and $P = 5.84 \times 10^{-25}$). **c**, Preparatory neural repertoires change similarly in PMd, M1, or PMd + M1. Black: control sessions in which the monkey did thousands of center-out reaches without any force field (one-sided Wilcoxon rank-sum test: monkey U PMd + M1, $P = 9.14 \times 10^{-10}$; monkey U PMd, $P = 2.08 \times 10^{-17}$; monkey U M1, $P = 4.77 \times 10^{-5}$). **d**, Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period, in both PMd and M1. TO: target onset. GC: go cue. **e**, Tangling scores (Q) of neural trajectories before and after learning during the target-onset period, preparatory period and peri-movement period. Neural trajectories in either PMd or M1 or both have similar tangling patterns over time: they are bunched together around target onset, start to separate during the preparatory period and further diverge during the peri-movement period (left, M1 neurons only; middle, PMd neurons only; right, PMd and M1 neurons pooled). This is consistent with the neural trajectories visualized in **d**.

In addition, we note that monkey V Neuropixels data came from two days of PMd recordings and one day of M1 recording and they were analyzed separately in all related analyses (Figs. 2b, 2c, 2e, 3b, 3c, 5b, 5d, 5f, 5g, 5k in the revised manuscript). We updated the methods section with this note (see [line 572]). Monkey V Neuropixels data also showed that PMd and M1 populations had similar neural changes, which provides further support that PMd and M1 neurons contribute similarly to the TDR subspace and the uniform shift axis.

The definition of "preparatory activity" is rather narrow, and somewhat non-standard. Given this is a delayed-reach task, why not consider more of the time prior to the go cue? Of course, there could be fascinating effects during that period, related to the sensory-motor misalignment that the animals anticipate experiencing.

Thank you for the suggestion on investigating an earlier time window. In the revised manuscript, we have added a plot of PCs 1-3 over time in a representative session (Extended Data Fig. 15, copied below for convenience) to help demonstrate our motivation for choosing the time window -50 to +50 ms from the go cue. This figure shows that the preparatory neural activity maintains stationary during at least -100 to +50 ms from the go cue. More specifically, we chose the time window -50 to +50 ms from the go cue, because it is right before peri-movement activity rises (i.e., near the end of the preparatory period) and shows stronger neural tuning than the early stage of the preparatory period (e.g., 0 to 100 ms after target onset shown in the plot). The -50 to +50 ms time window around the go cue, indicated as the gray shaded area, is within the preparatory period and has strong neural tuning reflected by the first three PCs. We chose this 100 ms duration because if the time window is too wide, the 'condition-independent signal', which usually appears in PC 1, will dominate the variance of the neural dynamics (Kaufman et al., 2016), which is not the major neural pattern we are investigating. We have added the explanations above to the Supplementary Materials [lines 163-182].
Extended Data Fig. 15 | Example PCs 1-3 during time windows -100 to +100 ms from target onset (TO), -100 to +100 ms from go cue (GC), and -200 to +400 ms from movement onset (MOO) in each trial. An example session from monkey U and representative of the other sessions from both monkeys. We applied PCA to trial-averaged neural activity for different reach directions in the before-learning block. Different colors correspond to different reach directions in the before-learning block and color scheme is the same as in Fig. 2a. Neural trajectories in PCs 1-3 are bundled together around the target onset time window and diverge around the go cue time window (i.e., during preparatory period). The time window -50 to +50 ms from go cue (gray shadow) we chose for preparatory neural state analysis is within the preparatory period (near the end of it) and has stronger neural tuning than earlier time windows (e.g., the first 100 ms after target onset).

In addition, we did the same neural state analyses with an earlier time window: -100 to 0 ms from the go cue, which is completely within the delay period. We found that neural changes during this earlier time window were similar to what we found during -50 to +50 ms from the go cue (Extended Data Fig. 13). The new figure is copied here for convenience:
Extended Data Fig. 13 | Patterns of preparatory activity in different neural state subspaces in an earlier preparatory time window (-100 to 0 ms from the go cue).

a, Initial hand forces predicted by the 2D TDR preparatory states are correlated with real forces of the upcoming movement; the sign of hand force indicates its direction (monkey U, $R^2 = 0.80$ and $P = 6.01 \times 10^{-33}$; monkey V, $R^2 = 0.67$ and $P = 1.77 \times 10^{-35}$). Lighter dots, earlier learning trials; darker dots, later learning trials.

b, Single-trial prediction MSE of initial hand forces is significantly smaller using original data than shuffled data (one-sided Wilcoxon rank-sum test: monkey U, $P = 0.03$ in both cases; monkey V, $P = 0.0079$ in both cases). For each monkey, left two columns, training-set (before-learning trials) prediction MSE; right two columns, held-out set (learning trials) prediction MSE. Control results (blue) are forces predicted by models built from training sets that had neural and behavior data shuffled. One datapoint per session.

c, Single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis. Normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states. Solid line: linear-log regression (monkey
U, R² = 0.12 and P = 1.27 x 10⁻²⁶; monkey V, R² = 0.18 and P = 4.08 x 10⁻³³. d, The angular differences between washout states and the before-learning state gradually decrease on a single-trial basis. Solid line: linear regression (monkey U, R² = 0.19, P = 8.4 x 10⁻¹⁰⁰; monkey V, R² = 0.23, P = 5.3 x 10⁻¹⁴¹). e, Distance between washout states and the before-learning state along the uniform-shift learning axis does not show a significant trend of increase or decrease. Solid line: linear regression (monkey U, P = 0.57; monkey V, P = 0.13). Each dot is a single trial. Normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states. f, The uniform shift from the late-washout state to the relearning state is significantly smaller than from the before-learning state to the learning state, along the uniform-shift learning axis (one-sided signed rank test, P = 2.0 x 10⁻⁵, seven sessions from two monkeys combined). g, Centroids (circles) of late-washout and relearning states projected onto the uniform-shift learning and washout axes (seven sessions from two monkeys). All shifts were normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states in each session. Late-washout states are significantly different from the learning state (one-sided signed rank test, P = 0.039 for x < 1 and P = 0.0078 for y > 0). Relearning states are significantly different from late-washout states (one-sided Wilcoxon rank-sum test, P = 0.0078 for Δx > 0 and P = 0.0078 for Δy < 0), but not significantly different from the learning state along the uniform-shift learning axis (two-sided signed rank test, P = 0.22 for comparing x to 1 and P = 0.031 for comparing y to 0). Crosses, the means of washout and relearning neural states.

2.2. Why is the shift in population space present for all arm movement directions, even unlearned ones? The shift in the repertoire seems to encode the block that the animal is in. The exciting possibility is that this is a learning-induced shift in the population space. The duller possibility is that this is simply explainable as a different “preparatory set” for the two blocks. This could show up as a difference in the activity of some muscle somewhere or the angle of some joint, or the force applied to the manipulandum, even for unlearned directions, but maybe not. I am reluctant to even raise this candidate explanation, because it requires “proving a negative” - that there was no systemic change in muscle activity anywhere, or even in the animal’s internal preparatory set (which could occur without any measurable external correlate.) However, readers will wonder about it, so an answer should be offered. And of course, further work will be required to discover the source of this population shift.

Thank you for raising this important question and an interesting alternative explanation. Because the uniform shift unexpectedly happened to the preparatory neural states of all reach targets, the majority of this work was aimed at investigating what computational roles the uniform shift could play during motor learning. As nicely summarized by the reviewer, our results from the multi-field learning and the relearning experiments suggest that the uniform shift may serve an ‘indexing’ function that enables different motor skills to be acquired, stored and accessed in the neural population state space separately. This shift could provide a mechanism for preventing different motor memories from interfering, and rapidly re-accessing them.

Based on our data and analyses, we believe that the alternative explanations raised above are not the major contributor to the uniform shift for the following reasons. (1) Given that we did not observe the uniform shift during the peri-movement period (Extended Data Fig. 16), this shift should not be merely due to changes of postures or the angle of some joint or some muscle activity not measured. (2) We observed the uniform shift persisting after washout (now Fig. 4c, d) and (3) we did not observe the uniform shift during visual-motor rotation (VMR) learning (Extended Data Fig. 4), which suggests that this shift should not be simply due to the switch of blocks (VMR task as described in Vyas et al., 2018, 2020b; but neural data not analyzed in the present way). (4) The geometric relationships between different uniform shifts reflected the identity of learning different curl fields (now Fig. 4). This result supports that the uniform shift is specifically learning-related, here termed indexing as suggested, unless some unmeasured behavior or internal state is closely related to the learned field but is not related to learning.

We have added the reasoning above to the discussion section, which is copied below for convenience.

**Discussion**

[lines 459-469] First, changes in the animals’ posture could potentially contribute to the uniform shift. That is, when the body slightly twists, it may change the overall neural activity in the motor cortex and so we may expect a uniform shift or a neural repertoire change throughout a whole trial. Although our animals’ postures were not measured, given that we did not see a uniform shift during the peri-movement period (Extended Data Fig. 16c,
d), the uniform shift that we report ought not be merely due to postural changes. Furthermore, as a complementary analysis, we quantified the repertoire change of the baseline neural activity (i.e., when the animal was holding the handle before target onset). In contrast to the neural repertoire change during the preparatory period (Fig. 3c), we did not see a significant neural repertoire change of the baseline neural activity (Extended Data Fig. 17), which further supports that our main results are not substantially due to postural changes.

Fourth, we think that this shift should not be simply due to the switch of blocks, because we observed the uniform shift persisting after washout (Fig. 5c, d) and did not observe the uniform shift during VMR learning (Extended Data Fig. 4). Last, the geometric relationships between different uniform shifts reflected the identity of learned curl fields (Fig. 4). This result supports that the uniform shift is specifically learning-related, which we term indexing, unless some unmeasured behavioral or internal brain state is closely related to the learned field but is not related to learning.

We agree with the reviewer that it is effectively impossible to exclude all potential changes in behavior or internal brain states not measured in this work, and they were likely to have changed during learning. It would be really interesting if future work could find some behavior or internal state that is uniform for all reach directions but specific to the learned curl field such that the uniform neural shift we observed could be explained by that behavior or internal state.

2.3. Fig 4j is confusing in two ways. First, it is described in a single, complex sentence at the very end of the results section. It deserves some unpacking.

Thank you for identifying a point which deserves additional clarification. In the revised manuscript, we further unpacked the neural trajectory similarity analysis in the results and methods sections, which is copied below for convenience. Fig. 4 in the original manuscript is now new Fig. 5.

Main
Consistent with this distinction between preparatory and peri-movement states after washout, the late-washout neural trajectory was less similar to the before-learning neural trajectory during movement preparation than during movement execution (Fig. 5j, k). The relative neural trajectory similarity was measured as the distance between washout and after-learning neural trajectories over the distance between washout and before-learning trajectories, and thus a larger value indicated higher similarity to the before-learning neural trajectory (see Methods). Taken together, the uniform shift appears to be a preparatory phenomenon and not prominent in the peri-movement neural activity.

Methods
Measurement of neural trajectory distance (Fig. 5f). We first averaged the neural trajectories across trials for each condition. To estimate the neural trajectory distance between different conditions over time, we performed a Euclidean distance analysis as follows: for the two neural trajectories we were comparing, we quantified the Euclidean distance between pairs of points during the preparatory period (-50 to +50 ms from the go cue) and during the movement period (+100 to +200 ms from movement onset), in the first three PCs. The first three PCs accounted for around 60% - 70% of the variance of the neural trajectory data in all data sets. A smaller distance indicated that the two trajectories were more similar.

Measurement of relative neural trajectory similarity (Fig. 5k). The relative neural trajectory similarity was a metric to compare whether the washout neural trajectory was more similar to the before-learning or the after-learning trajectory. It was quantified as the ratio of neural trajectory distance (see the last section) between washout and after-learning trajectories over that between washout and before-learning trajectories during a certain time window. A larger value indicated higher similarity to the before-learning neural trajectory than to the after-learning neural trajectory. Control analyses measured this metric by splitting all before-learning trials into two random halves, with one half treated as the before-learning trials and the other serving as the sham washout trials. We combined data from two monkeys (seven relearning-experiment sessions including four from monkey U and three from monkey V) to gain a higher statistical power for the one-sided Wilcoxon rank-sum test.
Second, and more importantly, the data are not obviously consistent with previous results on flow fields. These trajectories appear “tangled”, in a manner that Russo and colleagues recently said does not happen in motor cortex. Also, this effect seems different from the Shenoy 2012 Nature paper. It would seem that insofar as linear dynamics explain M1 activity, offsets in trajectories should only appear if initial conditions are also offset. Here, the four trajectories are bunched together at time move onset-500, but then they appear to diverge. (Or maybe that is just the perspective from which this figure is plotted.) This apparent discrepancy with the literature is one of the strongest reasons to consider separating PMd from M1 activity in the analyses here.

We apologize for presenting the neural trajectory results in an inadvertently confusing way. We have added a clearer explanation and we have performed new analyses to address the “tangling” question. (Also, just for the sake of clarity, by Shenoy 2012 Nature paper we believe that this is referring to Churchland*, Cunningham*, …, Shenoy. Nature 2012).

A. Regarding the comment that “these trajectories appear tangled”. (1) Neural trajectories after the go cue only appeared to be tangled, due to the angle of visualization, and were not actually tangled. (2) We quantified the neural tangling for before-learning and after-learning neural trajectories, for the trained reach target, during three time periods: before target onset, during the preparatory period (i.e., after target onset, but before the ‘go cue’) and during the movement period (Extended Data Fig. 19e and copied below for convenience). We found that the neural tangling was significantly smaller during preparatory and movement periods than before target onset, which supports that neural trajectories do not diverge until the target information arrives and is consistent with the literature (Jiang et al., 2020; Russo et al., 2018; Wang et al., 2018). This pattern of tangling remained consistent when we used PMd neurons only, M1 neurons only, as well as PMd and M1 neurons pooled. In summary, Fig. 4j (now Fig. 5j) shows very similar before- and after-learning neural states before target onset which then diverge into different initial conditions during the preparatory period and later generate very different neural trajectories due to the curl field being applied. That is, learning a curl field forms new initial conditions not used before learning, such that they evolve into different neural trajectories. This agrees with the initial-condition hypothesis detailed in previous papers (Afshar et al., 2011; Churchland et al., 2012; Vyas et al., 2018, 2020a).

Extended Data Fig. 19 | ... e, Tangling scores (Q) of neural trajectories before- and after-learning during the target-onset period, preparatory period, and peri-movement period. Neural trajectories in either PMd or M1 or both have similar tangling patterns over time: they are bunched together around target onset, and start to separate during the preparatory period and further diverge during the peri-movement period (Monkey U: left, M1 neurons only; middle, PMd neurons only; right, PMd and M1 neurons pooled). Monkey V results come from three Neuropixels recordings (two in PMd and one in M1) that were analyzed separately and plotted together. The tangling patterns are consistent with the neural trajectories visualized in panel d.

B. Regarding, “the four trajectories are bunched together at time move onset-500 ms, but then they appear to diverge”. At 500 ms before movement onset (i.e., -500 ms), these four trajectories start from the center-hold period which is before the preparatory period begins. As such, they should not be separated by an offset since there was no information about the upcoming trial yet, which supports the idea that the uniform shift does not manifest before movement preparation starts. Neural trajectories then gradually diverged after the go cue, and this is consistent with the ‘initial condition’ theory (e.g., Afshar et al., 2011; Churchland et al., 2006a), because
the initial condition refers to the preparatory neural state after target onset not the center-hold neural state before target onset.

C. To better demonstrate the neural trajectory tangling pattern and its consistency with the literature, we have made a new neural trajectory plot with three alignments: -150 to +150 ms with respect to target onset, -50 to +50 ms with respect to the go cue, and -200 to +400 ms with respect to movement onset (now Fig. 5j). The new trajectories extend time points before the preparatory period, during the preparatory period, and during the entire reach. We have copied the new neural trajectory plot here for convenience.

Fig. 5 | ... j, Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period. TO: target onset. GC: go cue.

We have also made new trajectory plots for PMd and M1 data separately (Extended Data Fig. 19d). Together with the ‘tangling plot’ (Extended Data Fig. 19e), our results show that PMd alone, M1 alone, or PMd and M1 neurons pooled all have a similar neural trajectory structure: (1) before- and after-learning neural trajectories are bunched together around target onset, (2) diverge during movement preparation and execution, and then (3) converge again near the end of the reaches.
Extended Data Fig. 19 | ... d, Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period, in both PMd and M1. TO: target onset. GC: go cue.

D. Conversely, if the before- and after-learning neural population states were not separated during the preparatory period, recent theoretical work could provide an alternative explanation as to why the results would still be consistent with the dynamical systems framework and literature (Linderman et al., 2016).

The initial-condition hypothesis often assumes (for simplicity) that the neural dynamical system in the motor cortex is autonomous and there is only one dynamics matrix \( A \) before and after learning:

\[
dx(t) / dt = A * x(t) + B * u(t) \quad (1)
\]

where \( x(t) \) is the (vector) neural state at time \( t \), \( u(t) \) is the (vector) input to the dynamical system at time \( t \) and \( B \) is the input projection matrix. In this case, the initial-condition hypothesis says that if the initial condition (the preparatory neural state, i.e., \( x(t = 0) \)) is the same, then one expects to see the same neural trajectory, when there are no external inputs \( u(t) \). Finding that neural trajectories cross is what is most often meant by high tangling. In contrast, finding that different initial conditions evolve into different neural trajectories is what is meant by low tangling (technically, trajectories should not come close together yet diverge later in an autonomous system).

However, in reality, the motor cortical dynamics are non-autonomous. Therefore, there could be another scenario during learning where there are two dynamics matrices, \( A_{before-learning} \) and \( A_{after-learning} \) such that:

Before learning, \( \frac{dx(t)}{dt} = A_{before-learning} * x(t) + B * u(t) \) \quad (2)

After learning, \( \frac{dx(t)}{dt} = A_{after-learning} * x(t) + B * u(t) \) \quad (3)

With these two dynamics matrices, even if the neural trajectories before- and after-learning were initiated from the same initial condition (preparatory state), they could evolve separately due to different dynamics matrices and have different neural trajectories. Our results that the initial conditions before and after learning were separated at the time of the go cue cannot tell us whether the dynamics matrix \( A \) changes after learning, and investigating this would be quite interesting for future work to test if learning changes the initial conditions, or the dynamics matrix, or both.

The theoretical framework described above has been added to the Supplementary Materials [lines 47-83].

3) Behavior.
I write this fully aware that it is usually difficult to go back and collect additional data from monkeys by the time papers are in review. Given the virus pandemic, this is likely to be all the more true than it usually is. I have considered whether the idiosyncrasies evident in the animals’ behavior have bearing on the paper’s conclusions about neural activity state changes. I think things are safe in that regard, however there are some disconnects between what’s in the text and what’s evident in the figures, and this might cause readers to trip over the discrepancies. Some re-wording is suggested to make sure there is a tight alignment between the claims and the evidence.

3.1. On the first page, learning is described as “gradual” for both animals, but fig 1c makes it evident that monkey U learns gradually and does not attain full recovery of performance, whereas monkey V learns rapidly and does achieve full performance. In fact, digging deeper, one can see that monkey V was always a little off, even during the before-learning block. It is as if he behaves as if the force field is on at all times. Can we even call this “learning”? In a task like this, where the animals probably encounter only a handful of perturbations, should we call this “adaptation” or “task switching”? Arguably, all the conclusions about how M1 stores multiple sensory-
motor [memories] are just as valid, whether or not the focus is on their initial learning and consolidation, or only on how the animal selects among them, as appropriate for the context.

Thank you for bringing up this very important point, to compare ‘learning’ vs. ‘adaptation’ vs. ‘task switching’. First, we define the following terms to help avoid confusion, which is added to the Supplementary Materials:

Supplementary Materials
[lines 86-101] Additional behavioral terminology. We use the definitions consistent with Krakauer et al. 2019. Motor learning vs. adaptation vs. task switching:

1. Learning (motor). Motor learning encompasses a wide range of behavioral phenomena, from behavioral calibration (such as adaptation), to de novo learning of new skills and to making high-level cognitive decisions about how to act in a novel situation. Consistent with this definition, we think the process of learning new arm forces in the curl field is one type of motor learning, in particular the adaptation of muscle dynamics to the new force environment (Extended Data Figs. 5, 7b).

2. Adaptation. Adaptation is the adjustment of behaviors to a new task condition or environment, which involves task switching (here, switching to the new force environment). Adaptation is considered to be one type of learning aimed at skill maintenance when one encounters changes in the body or environment. It is usually compared to de novo learning, i.e., the acquisition of completely new skills from scratch. Moreover, in our experiment, the animals on average experienced the new force environment for around 1 hour every day and they showed strong aftereffect in the washout block, which demonstrates the process of adaptation.

Second, regarding the comment “monkey V was always a little off”. Although the hand trajectory of monkey V seemed to be a little off in the before-learning block (Fig. 1c), Fig. 1c shows that the hand trajectory error dropped dramatically during learning and Extended Data Fig. 7b shows that the hand force changed greatly after learning in monkey V, which suggests that he learned new arm forces in the curl field rather than purely rely on the slightly-off before-learning behavior.

In the revised manuscript, we recalculated the kinematics error during learning as the average hand deviation error 20 ms around the peak speed, instead of the hand deviation error at the single time point of peak speed (Fig. 1c). This new metric better captured the gradual learning process: it took monkey U at least 100 trials to reach the asymptote of performance during learning, although he still did not attain full recovery of performance; it took monkey V at least 50 trials to reduce the hand lateral deviation back to the before-learning level. Although monkey V learned rapidly, it was not one / two-trial learning, which further supports that it was still a learning process but with a faster improvement in behavioral performance. We have copied the new plot of hand deviation curves here for convenience.

![Fig. 1](image-url)  
**Fig. 1 | c,** Behavioral indicators of learning and washout quantified by the hand trajectory deviation from a straight path (see Methods). Hand trajectory error is initially large, decreases during learning or washout, and then plateaus (close to before-learning level) in late-learning or late-washout trials. Shaded area, s.e.m. across sessions. Early learning is defined as the beginning of the learning block (block ii), late learning is defined as
curl-field trials in the end of the error-clamp block (block iii), early washout is defined as the beginning of the washout block (block iv), and late washout is defined as the end of block iv.

Regardless, we agree with the reviewer that our conclusions about how the neural population patterns we found are related to the curl force field could apply to any of the aforementioned circumstances (gradual learning or task switching, initial learning or consolidation, etc.).

How many sessions did the animals experience with this paradigm? Are all included here? Do they all look largely the same?

In this study, monkey V had a month of behavioral training with a 10 N / (m*s) curl field without neural recording. We then did 40 sessions of V-probe recordings in total, of which 20 sessions were used in the analyses because in those 20 sessions monkey V completed sufficient trials in all blocks to enable our analyses. Out of the 12 Neuropixels sessions with curl fields, our analyses used three sessions of the single-field learning / relearning experiment, one session of the random-perturbation experiment, one session of the opposite-field learning experiment, and two sessions of the interference experiment. Not all sessions with curl field learning were analyzed, and we excluded the following for monkey V: behavioral training sessions (no neural recordings), V-probe sessions without enough trials finished, and Neuropixels sessions without enough active stable units or enough trials finished.

With monkey U, we conducted seven sessions of behavioral training with a 10 N / (m*s) curl field before we started neural recording. We then did 12 sessions of Utah-array recordings (three implanted arrays, each with 96 electrodes) with curl fields, among which we analyzed four sessions of the single-field learning / relearning experiment, three sessions of the opposite-field learning experiment, and two sessions of the interference experiment. Not all sessions with curl field learning were analyzed, and we excluded the following for monkey U: behavioral training sessions (no neural recordings); recording sessions without enough trials finished.

We believe that this is best addressed in the Supplementary Materials, and have thus added it to [lines 102-126] in the Supplementary Materials. We are happy to add more details to the main text if deemed necessary.

Regarding the question “do they all look largely the same”. The behavior of monkey U looked largely the same in behavioral training sessions and neural recording sessions. The neural patterns we investigated in monkey U also looked largely the same in early and later neural recording sessions. Monkey V showed significantly faster learning in late neural recording sessions than in early behavioral training sessions (Supplementary Fig. 2, copied below for convenience). Despite this learning rate difference, other behavioral variables we measured and the neural patterns we investigated in monkey V looked largely the same in both V-probe and Neuropixels recording sessions. Monkey V results in this manuscript were all from the later sessions.

**Supplementary Fig. 2** | The success rates of monkey V in earlier sessions (left panel, behavioral training sessions) and later sessions (right panel, neural recording sessions). It took monkey V around 100 trials to reach performance asymptote in earlier sessions and no more than 30 trials in later sessions. Shaded area, s.e.m. across sessions.
If, in light of this, it is important to the authors that this study be about how neural activity changes track learning, then perhaps limit analyses to just the very first sessions when each of the force-field manipulations was introduced.

(1) Limiting analyses to just the very first sessions appears infeasible to us to do with monkey V data, because monkey V was behaviorally trained on a weaker curl field (10 N / (m*s\(^{-1}\))) applied to up or down targets for a month before we started recording. However, it is possible to do with monkey U data in early learning days of each field, because monkey U was behaviorally trained for only seven days with a weaker curl field (10 N / (m*s\(^{-1}\))) before we started recording, and because each field was repeated no more than 3 times in total. In fact, these relatively earlier earlier monkey U neural recordings were already used for analyses in the original manuscripts, from which we did not observe a significant difference from datasets collected later. The difference in the number of days monkey V and monkey U were exposed to each field could possibly explain why our behavioral analysis showed significantly faster learning in monkey V than in monkey U (Fig. 1c and Fig. 4e in the original manuscript and now Fig. 1c and Fig. 5e).

(2) Here we clarify that this study was not intended to investigate just the first day of encountering curl fields or to track long-term learning across sessions.

3.2. Fig 4 depicts relearning, claiming it is faster than initial learning. This is true, but the effect is very fast - seemingly the first trial is by far the most important one. This is fine, but to describe this as “faster relearning” makes one think that the slope of the purple will be larger than the slope of the orange. If the curves are shifted to align on the second trial, it appears this will not be the case.

We apologize for not being precise with our definition of ‘faster relearning’ based on the hand trajectory error curves in Fig. 4e (now Fig. 5e). We agree with the reviewer that what Fig. 4e depicts is overall not a steeper slope for relearning, but a smaller hand trajectory error from the very early beginning of relearning until getting well-readapted, compared to the initial learning process. Nevertheless, because Fig. 4e shows the rolling average of hand deviation error over 10 trials, we think it was not just the first trial but likely the first 10 trials that were by far the most important to reflect behavioral savings.

In the revised manuscript, we recalculated the kinematics error during learning / relearning as the average hand deviation error 20 ms around the peak speed, instead of the hand deviation error at the single time point of peak speed, which is added to the updated methods section and copied below. **This new metric could better capture the faster relearning process** (now Fig. 5e and copied below). It shows a combination of a smaller initial hand trajectory error and fewer trials needed for hand trajectory error to reach asymptote during relearning. That is the behavioral indicator of ‘savings’.

**Measurement of neural trajectory distance (Fig. 5f).** We first averaged the neural trajectories across trials for each condition. To estimate the neural trajectory distance between different conditions over time, we performed a Euclidean distance analysis as follows: for the two neural trajectories we were comparing, we quantified the Euclidean distance between pairs of points during the preparatory period (-50 to +50 ms from the go cue) and during the movement period (+100 to +200 ms from movement onset), in the first three PCs. The first three PCs accounted for around 60% - 70% of the variance of the neural trajectory data in all data sets. A smaller distance indicated that the two trajectories were more similar.
We found that consistent with the faster behavioral relearning, neural trajectories during relearning approached the well-learned trajectory in fewer trials than during initial learning (now Fig. 5f). We have updated Fig. 4 (now Fig. 5) with the new results, which is copied below for convenience.

**Fig. 5** | ... e, Hand trajectory errors are smaller during relearning than during initial learning (one-sided Wilcoxon rank-sum test: monkey U, \( P = 5.08 \times 10^{-4} \); monkey V, \( P = 0.0015 \)). f, Distance between neural trajectories during learning and the late-learning trajectory decreases over trials (red) but is larger than distance between neural trajectories during relearning and the late-relearning trajectory (blue) until they reach the asymptote. One-sided rank-sum test: monkey U, \( P = 0.0021 \); monkey V, \( P = 6.18 \times 10^{-4} \). e, f, Shaded area, s.e.m. across sessions.

Minor suggestions:
1. Is there a spatial asymmetry to the generalization? It seems a curl field might impact adjacent targets in the direction of rotation more so than those in the other direction. Fig 1d shows a symmetric fall-off, constructed by averaging over targets. It would be instructive to also flip the signs of rotation (that is, CCW becomes CW) to see if there is in fact a bias in the generalization, with more learning in the “push” direction. It is worth performing this for fig 2e as well.

Thank you for the helpful and insightful suggestion. We have added a new figure (Extended Data Fig. 2) to show the behavioral and neural generalization effect with signs of rotation flipped for CCW fields. Indeed, we found a spatial asymmetry to the behavioral and neural generalization in both monkeys, with more learning in the ‘push’ direction (i.e., the direction to oppose the curl field). We have copied the figure below for convenience.
Extended Data Fig. 2 | Behavioral and neural generalization effects are spatially asymmetrical.

We computed the behavioral (a) and neural generalization (b) effects with the sign of the effects flipped for CCW fields to match the effects of CW fields, compared to Fig. 1d and Fig. 2e. We found a spatial asymmetry in the behavioral and neural generalization in monkeys U and V, with more learning in the ‘push’ direction (i.e., the direction to oppose the curl field).

2. It seems that more could be done with fig 2g. For example, why not color the points that correspond to one session? That would give a sense for the overall variability in the neural shift, which might be interesting to see.

Thank you for the suggestion. We made the same scatter plot with points from each session color-coded and fitted into separate curves (copied below), but it is hard to differentiate the colored data points for each session. Therefore, we have made another plot with one example session colored and all the rest remaining gray, which is presented in the revised manuscript (Fig. 3b). We have copied the fully-colored and partially-colored plots below for convenience:

The plot above shows the single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis. Solid line: linear-log regression. Each color represents one session, with the $P$ and $R^2$ values of corresponding regression curves also color-coded.
Fig. 3 | ... b, Single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis (see Methods). Normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states. Solid line: linear-log regression. Gray circles and line: across all sessions; blue circles and line: one example session. \( P \) and \( R^2 \) values of corresponding regression curves are also color-coded.

3. Fig 4g is a little busy. How about making one figure for each animal? Also, the gray point gets a little lost at first - how about a line connecting it to the black point? And, why not put arrowheads on all lines, depicting the sequence? And, if you keep with one figure, make it clearer that filled is U and hollow is V.

Thank you. We grouped together results from monkey U (four relearning sessions) and monkey V (three relearning sessions) to gain more statistical power compared with analyzing each monkey separately, so we decided to keep them grouped. In the revised version, as suggested, we (1) added a line connecting the gray and black points, (2) put arrowheads on all lines depicting the sequence, and (3) added a figure legend showing that filled circles are from monkey U and hollow circles are from monkey V. We have copied the updated plot below for convenience.

Fig. 5 | ... g, Centroids of late-washout and relearning states projected onto the uniform-shift learning and washout axes (seven sessions from two monkeys). All shifts were normalized against the distance between the centroid of before-learning states and the centroid of error-clamp states in each session. Late-washout states are significantly different from the learning state (one-sided signed rank test, \( P = 0.039 \) for \( x < 1 \) and \( P = 0.0078 \) for \( y > 0 \)). Relearning states are significantly different from late-washout states (one-sided rank-sum test, \( P = 0.0055 \) for \( \Delta x > 0 \) and \( P = 0.0012 \) for \( \Delta y < 0 \)), but not significantly different from the learning state (two-sided signed rank test, \( P = 0.22 \) for comparing \( x \) to 1 and \( P = 0.47 \) for comparing \( y \) to 0). Crosses, the means of washout and relearning neural states.

4. Fig 4j would benefit from some “signposts”. It is easy to misread “Movement onset - 500 ms” as “Movement onset: 500 ms”. How about putting the word “preparation” above the portion of the trajectories to the right of the “movement onset” carets, and “execution” to the left? This will facilitate digesting the figure.
Thank you for the great suggestion. The original Fig. 4j trajectories started from the center hold period (i.e., before target onset), not the preparatory period, and so we were not able to add a signpost of “preparation” above all the trajectories before movement onset. In the revised manuscript, we made a new neural trajectory plot with three alignments: -150 to +150 ms from target onset, -50 to +50 ms from the go cue, and -200 to +400 ms from movement onset, with the signposts suggested by the reviewer (now Fig. 5j). The new trajectory plot is copied below for convenience.

![Neural trajectories](image)

**Fig. 5 | ... j.** Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period. TO: target onset. GC: go cue.

5. If you construct fig 4g without normalizing, are the gray “before learning” and black “after learning” points at roughly the same places in population space?

We appreciate the reviewer’s suggestion and agree that it would be very interesting to know the relationship between before-learning states in different sessions and the relationship between after-learning states over multiple sessions.

Unfortunately, we were not able to do this cross-session / cross-monkey comparison without normalizing, primarily because (1) in monkey V, different sessions recorded from different neurons, and (2) in monkey U, the stability of Utah arrays changed over time making it possible to only track some neurons for a limited number of sessions. Due to different neural populations recorded from session to session, the absolute values of the uniform shift varied from day to day and the gray and black points were not around the same places. To make cross-session and cross-monkey results comparable, therefore, we needed to normalize.

6. How many curl field sessions were run in total? Were all of them analyzed? Did the first few look any different from the last few?

In this study, monkey V had a month of behavioral training with a 10 N / (m*s⁻¹) curl field without neural recording. We then did 40 sessions of V-probe recordings in total, of which 20 sessions were used in the analyses because in those 20 sessions monkey V completed sufficient trials in all blocks to enable our analyses. Out of the 12 Neuropixels sessions with curl fields, our analyses used three sessions of the single-field learning / relearning experiment, one session of the random-perturbation experiment, one session of the opposite-field learning experiment, and two sessions of the interference experiment. Not all sessions with curl field learning were analyzed, and we excluded the following for monkey V: behavioral training sessions (no neural recordings), V-probe sessions without enough trials finished, and Neuropixels sessions without enough active stable units or enough trials finished.
With monkey U, we conducted seven sessions of behavioral training with a 10 N / (m*s\(^{-1}\)) curl field before we started neural recording. We then did 12 sessions of Utah-array recordings (three implanted arrays, each with 96 electrodes) with curl fields, among which we analyzed four sessions of the single-field learning / relearning experiment, three sessions of the opposite-field learning experiment, and one session of the interference experiment. Not all sessions with curl field learning were analyzed, and we excluded the following for monkey U: behavioral training sessions (no neural recordings); recording sessions without enough trials finished.

We believe that this is best addressed in the Supplementary Materials, and have thus added it to [lines 102-126] in the Supplementary Materials. We are happy to add more details to the main text if deemed necessary.

Regarding “did the first few look any different from the last few”. The behavior of monkey U looked largely the same in behavioral training sessions and neural recording sessions. The neural patterns we investigated in monkey U also looked largely the same in early and later neural recording sessions. Monkey V showed significantly faster learning in late neural recording sessions than in early behavioral training sessions (Supplementary Fig. 2, copied below for convenience). Despite this learning rate difference, other behavioral variables we measured and the neural patterns we investigated in monkey V looked largely the same in both V-probe and Neuropixels recording sessions. Monkey V results in this manuscript were all from the later sessions.

Supplementary Fig. 2 | The success rates of monkey V in earlier sessions (left panel, behavioral training sessions) and later sessions (right panel, neural recording sessions). It took monkey V around 100 trials to reach performance asymptote in earlier sessions and no more than 30 trials in later sessions. Shaded area, s.e.m. across sessions.

Editorial remarks:
- Fig 1b: which monkey are these trajectories from?
  These trajectories are from monkey U. We have added this detail to the revised figure caption.

- Fig 1a: Is it always the case that the target density near the trained target is higher? Can you find a way to indicate all possible test conditions (up, down, right, CW and CCW for each)?

Yes, as noted by the reviewer, the target density near the trained target was higher to sample more neural states for reaches that were more likely to be altered by learning, for the purpose of studying generalization of learning (e.g., Howard and Franklin, 2015). Ideally, we would like to have equally-spaced reach targets as dense as possible, but because monkeys only perform a limited number of trials each day, a compromise solution we took was to increase the density of targets near the trained reach target.

In the revised manuscript, we have added a figure (Extended Data Fig. 20) to show all the target arrangements when the curl field was applied to different target directions (i.e., the trained target was up, down or right). We have copied the figure below.
Extended Data Fig. 20 | Spatial arrangements of the 12 reaching targets relative to the trained target.
The target density near the trained target (up, down or right) is higher in order to sample more neural states for reaches that are more likely to be altered by learning, for the purpose of studying generalization of learning. Ideally, one would like to have equally-spaced reach targets as dense as possible, but because monkeys could perform a limited number of trials each day, a compromise solution is to increase the density of targets near the trained reach target.

- Line 137: It’s a little odd to use the terms “orthogonal” and “parallel” in such different senses in the same sentence: “a nearly orthogonal dimension” and neural changes that do not “directly parallel behavioral output”. Thank you, we reworded “parallel behavioral output” to now read “reflect the behavioral output” to avoid this potential confusion.

- Line 225: perhaps “could” should be “would”
Thank you, this is now fixed.

- Line 330: “gripped” -> “gripped”
Thank you for pointing out this interesting typo, and it is now fixed.

- Line 483: should Fx and Fy depend on condition k? Fx(k) and Fy(k)?
Thank you, this typo is now fixed.

- Typo in reference #26 “Ernandes” -> “Fernandes”
Thank you, this typo is now fixed.
Referee #3 (Remarks to the Author):

General Comments:

This study by Sun et al addresses the fundamental question of how we rapidly learn multiple sets of new motor skills and retain memories of the learning of those skills without the memory of one skill interfering too much with that of another. Many behavioral studies over the past decades have examined this question but less is known about the underlying neural mechanisms. Single unit recording studies examining the activity of individual neurons in motor cortical regions revealed learning-related changes correlated with behavior but provided conflicting evidence for the retention of a memory of previously learned skills (e.g., Li et al., Neuron, 2001; Arce et al., J Neurosci, 2010; Cherian et al., J Neurophysiol, 2013). More recently, a number of studies have begun to address the mechanisms underlying motor skill learning and memory by using approaches that examine the dynamics of neural populations to reveal strategies not readily apparent at the level of single neurons.

In the current study, the authors use such an approach to address how cortical activity involved in preparing movements (i.e., preparatory activity) helps facilitate skill acquisition. The authors start from the premise that in order to learn new motor skills and retain a memory of that learning without interfering with other learned motor behaviors, there must be ways in which neural population activity can change over the course of learning without directly influencing motor output. To examine the evidence for such changes in neural preparatory activity they used a well-known and extensively-used paradigm for studying learning of new movement kinetics during reaching (curl force field learning task) in which monkeys reach with a haptic device which perturbs their reach trajectories with a force that is proportional to their reach velocity but orthogonal to their reach direction. They recorded the responses of neural populations in the primary motor (M1) and dorsal premotor (PMd) cortices as monkeys adaptively compensated for this perturbation by learning a new association between a given reach target location and the forces required to reach to that target. The authors examined both behavioral responses and changes in neural preparatory activity over the course of learning, how learning in a particular reach direction generalizes to other directions, the effects of learning, unlearning and relearning to compensate for the same perturbation as well as the impact of sequentially learning two opposing sets of perturbations.

Using sophisticated "neural state analyses" to monitor changes in population-level activity patterns the authors show that, in agreement with previous work (e.g., Jarosiewicz et al., PNAS, 2008; Golub et al., Nat Neurosci, 2018), population preparatory activity exhibits learning-related changes that are closely correlated with changes in behavior and consistent with re-associating existing (before learning) activity patterns with a new behavioral response (reaching to a different target). Importantly, however, they also show that there are neural activity changes that did not directly parallel behavioral output. Specifically, these learning-related "uniform shifts" were present for reach directions that behaviorally show no evidence of learning. The authors further provided evidence that these changes reflect new population activity patterns (i.e., a change in neural repertoire) and that these new activity patterns are different for different tasks (i.e., they reflect the learned task "identity"). In addition, even after "washout" of the newly learned behavior uniform activity shifts persist, consistent with a role in motor memory retention. The authors propose that these "uniform shifts" reflect a mechanism for separating neural preparatory states associated with different learned motor skills and retaining a memory of those learned skills. Importantly, these new activity patterns appear to exist within a portion of neural state space that does not map directly onto motor behavior ("output-null" space) thus facilitating the separation of motor memories that otherwise might interfere with one another.

These are important results which contribute significantly to advancing our understanding of the neural mechanisms underlying the learning and retention of multiple motor skills. In particular, the identification of neurons that maintain persistent changes after behavioral evidence for a recently learned skill is "washed out" (i.e., "memory" neurons; Li et al., Neuron, 2001; Arce et al., J Neurosci, 2010; Richardson et al., J. Neurophysiol, 2012) has long pointed to the retention of motor memory through population-level activity patterns. However, it is only more recently that simultaneous recordings of multiple neurons across motor cortical regions and population-level analyses have started to elucidate the underlying mechanisms. Several studies examining population-level activity have emphasized learning mechanisms which involve re-associating existing neural activity patterns with new behavior (i.e., mechanisms which rely on the same neural repertoire; e.g., Golub et al.,
Nat. Neurosci, 2018; Sadtler et al., Nature, 2014). However, recent studies have also demonstrated the existence of learning mechanisms that involve the formation of new neural population activity patterns (new neural repertoire; e.g., Oby et al., PNAS, 2019). Most directly related to the current study is that of Perich et al, Neuron, 2018 who identified a signature of learning in the subspace of PMd that does not directly influence M1 activity (i.e., “M1-null” subspace of PMd). It was suggested that this reflects a novel mechanism by which the brain alters planning activity within an “output-null” subspace to set the appropriate initial conditions for subsequent execution of a given adapted motor behavior by M1. The authors of the current study reach a similar conclusion, suggesting that the “uniform shift” they observed in the “output-null” subspace is consistent with the changes previously observed in the “M1-null” subspace by Perich et al., 2018 (i.e., see Discussion lines 277-287). Notably, however, the current study expands on previous work by explicitly showing that these neural activity changes are roughly orthogonal to behavioral output and that they are different for different learned tasks. Perhaps, most importantly, they show that even when the learning is “washed out” shifts within the “output-null” subspace relative to before learning persist suggesting that a memory of the learning is retained. Consequently, while I would not necessarily consider the theoretical ideas presented in the current manuscript to be entirely novel, key aspects of the results are novel and they make a potentially important conceptual advance towards our understanding of the neural mechanisms underlying our capacity to separate and retain motor memories of different motor skills.

Overall, I found that while the results are important and novel there are a number of aspects of the manuscript that could be improved, particularly from the standpoint of publication in Nature.

Thank you for this accurate and comprehensive summary, and for the kind comments and (as described below) extremely helpful and insightful questions and suggestions. As described below, we have performed numerous new analyses to respond to all questions and comments, and we have created and included several new figures. We believe that these changes have considerably improved the manuscript, and thank the reviewer again.

These include:

1. Sophisticated sets of analyses were performed but the description of those analyses in the Methods section was often hard to follow. This may be partially because I am not an expert in the use of these techniques but then again neither is the general reader of Nature. For that reason, I would strongly recommend revising the methods to make the techniques more accessible to the general reader. Among the most important aspects of this is making sure the motivation for the various analyses comes across clearly. For example, initial analyses used targeted dimensionality reduction (TDR) to relate components of population responses directly related to behavior which makes sense. However, there is then a switch to using PCA as a dimensionality reduction technique that was not well motivated.

Thank you, we agree that there are many opportunities to help the reader understand the relatively complex analyses. We do appreciate that we are asking rather a lot of the reader despite our best attempts to be clear and concise, and we have now redoubled our efforts.

In particular, as the reviewer noted, there are specific reasons for using targeted dimensionality reduction (TDR; which we introduced in Mante et al., 2013 and principal components analysis (PCA; which is a decades old ‘classic’ technique familiar to many), which we have worked hard to clarify. More specifically, TDR is a supervised method that includes behavioral data in addition to neural data, which enables us to look for neural patterns that directly reflect the behavioral variables (e.g., movement kinetics) that we included in the regression. In contrast, PCA is an unsupervised method that includes only neural data, and is useful when we explore neural activity patterns that might not be directly related to behavior variables (e.g., the uniform shift). We first discovered the uniform shift using PCA, which we might have missed had we used TDR or similar methods. This discovery was surprising, but was also why we looked with a ‘wide lens’, as we were not specifically searching for such a neural pattern.

After finding the uniform shift with PCA, we defined the uniform-shift learning axis as the vector connecting the centroid of before-learning states to the centroid of after-learning states. It turned out that this shift was nearly orthogonal to TDR dimensions 1 and 2 (see the plot of dot products between TDR and PC axes in our answer to the next question). We then orthogonalized the uniform-shift axis against TDR1 and TDR 2, before we
quantified the amount of neural changes along the uniform-shift axis during learning / relearning and before we quantified the relationship between different uniform-shift axes. We did this because we wanted to investigate the role of the uniform shift independent of TDR dimensions 1 and 2.

We revised the main text to reflect the description above and further explain to the reader our motivation for using TDR and PCA, which is reproduced below for convenience:

**[lines 99-109]** We first asked what signatures of curl field learning could be identified from the neural population activity, in particular, during movement preparation. Recent human behavioral studies have suggested the importance of movement preparation for curl field learning, but the underlying neural mechanism remains unknown. Investigating the preparatory neural activity (defined in Methods) allows us to observe how the motor cortex may update movement preparation to generate the newly learned behavior. To estimate neural subspaces predictive of specific movement variables, we used targeted dimensionality reduction (TDR, see Methods), a supervised dimensionality reduction method that operates on neural data and behavioral data. Because precise control of hand forces is critical for learning curl field dynamics, we first focused on a low-dimensional subspace that captured across-trial neural variance related to horizontal and vertical hand forces.

**[lines 146-169]** Having found preparatory neural patterns in the reassociation space that directly correlated with the upcoming movements, we next asked if there are preparatory patterns that facilitate learning but are not tightly linked to the motor output. Without knowing what specific neural activity patterns to look for to address this question, we used principal component analysis (PCA), an unsupervised dimensionality reduction method that operates on neural data alone. By applying PCA to the neural population preparatory activity, we identified prominent and unexpected neural changes along PC 3, while PC 1 and 2 dimensions largely overlapped with the reassociation space (see Methods; Extended Data Fig. 3a). We found that during curl field learning, preparatory states gradually shifted away from the before-learning repertoire (the set of neural states for reaching to all targets before learning), as visualized in the subspace of PCs 1-3 (Fig. 3a gray circles). The after-learning repertoire, which is the set of preparatory states following learning and measured with error-clamp trials, was significantly separated from the before-learning repertoire (Fig. 3a).

Strikingly, this shift of preparatory states was observed preceding reaches to both trained and untrained targets, and therefore was a 'uniform shift' related to learning but independent of the specifically trained target. In order to determine the axis that best captures this uniform shift, as this need not align precisely with PC 3, we defined the 'uniform-shift learning axis' as the vector pointing from the centroid of the before-learning neural repertoire to the centroid of the after-learning neural repertoire in the full dimensional neural space (see Methods). This uniform-shift axis was nearly orthogonal to the reassociation space (Extended Data Fig. 3a). To be sure that the uniform-shift learning axis was fully independent of the reassociation space, we orthogonalized it against the TDR 1 and 2 axes. Finally, we projected full-dimensional neural data onto the uniform-shift axis and quantified the shift of the preparatory neural states along this axis (see Methods; Fig. 3b), which reflects the gradual learning process on a single-trial basis.

More importantly, however, the links between the analyses in terms of relationships between the different subspaces is not clear. Based on the text (lines 136-138) which indicates that there were prominent neural changes along a nearly orthogonal dimension that did not parallel behavior and Figs. 2d and f, one gets the impression that there were close relationships between the two sets of subspaces with PC1 and PC2 being closely related to muscle force dimensions TDR1 and TDR2. However, this is never explicitly indicated although it seems quite relevant from the standpoint of establishing the extent to which the uniform shifts (largely apparent along the PC3 dimension) are in fact orthogonal to behavioral (i.e., hand force) changes. Similarly, I felt that the definition of the uniform shift axes in the Methods did not come across clearly. Specifically, according to the text the learning and washout “uniform shift” axes were defined as the axes connecting before- and after-learning centroids and after-learning and washout centroids, respectively. These axes were then orthogonalized against the two TDR axes which I have taken to mean (perhaps incorrectly) that you consider specifically the component of each of these “shift axes” that is orthogonal to the TDR axes. Assuming this is correct, should I then interpret all references to “learning-uniform shift” and “washout uniform shift” in the text as referring to shifts along axes that are orthogonal to TDR1 and TDR2 (i.e., force behavior)? It sounds as if in some analyses this was true and in others it wasn’t. Given the relevance of shifts being orthogonal to behavior (in “output-null” space) for the
conclusions of the study whether or not you are explicitly considering shifts orthogonal to TDR1 and TDR2 should come across more clearly not only in the methods but also in the main text. Other areas of potential confusion are outlined below in my specific comments as are a number of aspects of the experimental procedures that were unclear and/or where it seemed to me that important pieces of information were missing.

Thank you for pointing out that, despite our initial best attempts to be clear, we inadvertently were not clear. All of the points raised are fully understandable and, we believe, quite addressable as described below and as is now in the text. The reviewer is correct that all references to “learning uniform shift” and “washout uniform shift” in the text are shifts along axes that are orthogonalized against the TDR axes. Below we clarify our process of analyzing the uniform shift.

When we first defined the uniform-shift axis by connecting before-learning and after-learning neural state centroids (or connecting after-learning and washout centroids), we did not assume that it was orthogonal to TDR 1 and TDR 2. To examine the relationship between the TDR axes, the PC axes and the uniform-shift axis, we calculated dot products between each pair of them. A dot product around 0 indicates orthogonality between axes whereas a dot product near 1 indicates large overlapping between axes. After we took the dot product between the uniform-shift axis and TDR 1 or TDR 2, we found that the uniform-shift axis was nearly orthogonal to the TDR axes (i.e., the dot product was close to zero, see Extended Data Fig. 3a in the revised manuscript). Moreover, TDR 1 and TDR 2 largely overlapped with PC 1 and PC 2, and the uniform-shift axis largely overlapped with PC 3. We have copied the plot of dot products below for convenience:

![Extended Data Fig. 3](image)

**Extended Data Fig. 3 | Relationships between neural population dimensions and total neural variance different dimensions explained.**

a, Relationships between different neural population dimensions (defined by TDR axes, uniform-shift axis and PCs), measured by their pairwise dot products (see Methods). Values close to 1 indicate that two dimensions are closely aligned whereas values close to 0 indicate that two dimensions are nearly orthogonal. In each session, we calculated the dot product of TDR 1 and PC 1 and the dot product of TDR 1 and PC 2, and then took the larger value of the two dot products (TDR 1 vs. PC 1 / 2). We also calculated the dot product of TDR 2 and the PC axis not used for multiplying with TDR 1 (TDR 2 vs. PC 1 / 2). The PC 1 / 2 plane largely overlaps with the TDR 1 / 2 plane (black boxes). The TDR 1 axis, TDR 2 axis and the TDR axis related to hand speed are all nearly orthogonal to the uniform-shift learning axis (blue and red boxes). PC 3 largely overlaps with the uniform-shift learning axis (yellow boxes). Monkey U: n = 4 sessions; monkey V: n = 3 sessions.

We then orthogonalized each uniform-shift axis against TDR 1 and TDR 2 axes, before we quantified the amount of uniform shift during learning / relearning (Figs. 2g, 4g, 4h in the original manuscript and now Figs. 3b, 5g, 5h) and before we quantified the relationship between different uniform shift axes (Fig. 3 in the original manuscript and now Fig. 4). We did this because we wanted to investigate the role of the uniform shift fully independent of
TDR axes, and more generally, to investigate neural population patterns related to learning but not directly reflecting movement kinetics / kinematics.

We have updated the methods section and the main text with these clarifications, which is reproduced below for convenience:

Main
[lines 150-153, 161-167] By applying PCA to the neural population preparatory activity, we identified prominent and unexpected neural changes along PC 3, while PC 1 and 2 dimensions largely overlap with the reassociation space (see Methods; Extended Data Fig. 3a). In order to determine the axis that best captures this uniform shift, as this need not align precisely with PC 3, we defined the ‘uniform-shift learning axis’ as the vector pointing from the centroid of the before-learning neural repertoire to the centroid of the after-learning neural repertoire in the full dimensional neural space (see Methods). This uniform-shift axis was nearly orthogonal to the reassociation space (Extended Data Fig. 3a). To be sure that the uniform-shift learning axis was fully independent of the reassociation space, we orthogonalized it against the TDR 1 and 2 axes.

Methods
[lines 771-774] We orthogonalized each uniform-shift axis against the two TDR axes where we found rotatory neural shifts, before we did any quantification analysis of the uniform shift; the uniform-shift axes were also always orthogonalized relative to each other before we projected and plotted preparatory neural states on them (see Figs. 4a, b, d, and 5g).

Last, we would like to clarify the definition of preparatory dimensions and ‘output-null / output-potent’ dimensions of neural population activity. We apologize for not defining these concepts clearly and have added them to the Supplementary Materials (copied below for convenience).

Supplementary Materials
[lines 183-193] ‘Output-null’ and ‘output-potent’ dimensions in neural population neural activity. Neural population activity during movement preparation (preparatory neural activity) is always output-null because there is no explicit movement output during the preparatory period, regardless of whether or not the neural activity is correlated with certain behavior variables in the upcoming movement. In this work, both the TDR components and the uniform shift of the preparatory activity are in output-null dimensions, with the former more directly related to movement output variables. Output-potent dimensions are defined by components of neural population activity during movement execution that directly relate to certain behavior variables of the movement output (especially muscle activity as defined in Kaufman et al. 2014). Neural population activity during movement execution contains both output-null and output-potent components (e.g., Kaufman et al. 2014; Stavisky et al. 2017).

2. A critical aspect of the conclusion of the study is that the learning-related “uniform shifts” are not directly linked to motor output (i.e., they are within the “output-null” subspace). Consequently, it is particularly important to show that there were no other behavioral changes unrelated to learning the new kinetics of the environment that contributed to changes in neural activity. You consider muscle co-contraction but what about other factors such as speed which is known to influence preparatory activity (e.g., Churchland et al., J Neurophysiol, 2006)? In Extended Data Fig. 6 you show evidence for decreases in speed during the learning block that appear quite modest although I don’t know if that was true for only one or both animals. However, what about during the error clamp block? Was there any evidence for a general progressive increase/decrease in speed that might influence neural activity? It would be useful to plot this as well.

Thank you for raising this important alternative explanation, and we apologize for being unclear about whether the plots came from one or both animals. Extended Data Fig. 6 in the original manuscript came from one example session of monkey V. In the revised manuscript, we added a new plot showing the hand speed in before-learning, late-learning, error-clamp and late-washout trials for monkeys U and V (Extended Data Fig. 7a) averaged over multiple sessions. We have reproduced the plot below.
Overall (averaged over all single-field learning sessions for each monkey), the peak hand speed of monkey U was around 20 mm/s (5% of the peak speed) slower in late-learning / error-clamp / late-washout trials than in before-learning trials while monkey V maintained the before-learning peak hand speed in learning and washout trials, but slowed down by around 40 mm/s (10% of the peak speed) in error-clamp trials. In individual sessions, changes of the peak hand speed across all blocks varied from day to day and we did not observe a consistent pattern: for instance, sometimes the peak hand speed was slower than before-learning only in late-learning trials but not in error-clamp or late-washout trials; sometimes the peak speed was slower than before-learning in error-clamp and late-washout trials but not in late-learning trials; sometimes the peak speed was even faster than before-learning in all the other blocks, etc. (individual-session data not shown). In contrast to this variability of hand speed changes in different sessions, we observed the uniform shift of preparatory states consistently in each individual session and in both monkeys, which supports the idea that change of hand speed is not a major contributing factor to the learning-related uniform shift.

To provide additional evidence, we also expanded the TDR model by adding the hand speed to the regressors. We found that the hand force axes (TDR 1 and TDR 2) explained 40% to 50% of the total variance of the preparatory neural activity, the hand speed TDR axis explained 10% to 20% of the total variance of the preparatory neural activity, and the uniform-shift axis explained 10% to 20% of the total variance (Extended Data Fig. 3b and copied below in our answer to the reviewer’s question “when performing the TDR analysis did you ever examine models that include variables other than horizontal and vertical hand forces (e.g., speed)?”). We also found that the hand speed TDR axis was nearly orthogonal to the uniform-shift axis (Extended Data Fig. 3a and copied above in our answer to the reviewer’s first question). These results suggest that the hand speed axis does influence the preparatory activity, as pointed out by the reviewer and shown in previous work (e.g., Churchland et al., 2006b; Even-Chen et al., 2019, but importantly it barely overlaps with the uniform-shift axis.

All the results presented here have been added to the main text [lines 200-206].

Since in many cases you compare activity during error clamp trials to activity in non-error clamp trials was there any evidence that the monkeys could tell in advance whether an upcoming trial would be an error-clamp trial? For example, in the error-clamp block was there any difference in preparatory activity for force-field vs error-clamp trials in the training direction?

Thank you very much for bringing up the potential concern about the error clamp contributing to the uniform shift. For the trained target, monkeys could not tell whether it was a curl field learning trial or an error-clamp trial. However, because all the other targets only had error-clamp trials, the monkey might be able to tell in advance that trials with these targets had an error clamp. To address this question directly, in the revised manuscript, we compared the preparatory neural states of the trained target in late-learning and error-clamp trials. We found that late-learning and error-clamp preparatory neural states were not significantly different (Extended Data Fig. 18b). We used Hotelling’s T² test (for multivariate data) to compare the late-learning preparatory state and the error-clamp preparatory state, which showed that they were not significantly different in all single-field learning
sessions from two monkeys ($P > 0.05$ in each of four monkey U sessions, $P > 0.1$ in each of 20 monkey V V-probe sessions and three monkey V Neuropixels sessions). We have copied the figure below (arrows point to the before-learning and error-clamp neural states of the trained target):

Extended Data Fig. 18 | ... b, In the error-clamp block (block iii) of learning sessions, the late-learning preparatory state (black circle) and error-clamp state of the trained target (purple diamond next to the arrow) are not significantly different (Hotelling’s $T^2$ test: $P > 0.05$ for all learning sessions of monkey U and $P > 0.1$ for all learning sessions of monkey V). Arrows point to the before-learning state (purple circle) and error-clamp state (purple diamond) of the trained target. The plot is from one example session of monkey U.

When performing the TDR analysis did you ever examine models that include variables other than horizontal and vertical hand forces (e.g., speed)? How much of the variance in the data did the force behavioral variables actually account for? In general, the addition of a few more analyses to show that neural activity changes had no correlation with other behavioral changes would be useful to help support the robustness of the conclusions.

Thank you for this very helpful suggestion. In the revised manuscript, we expanded the TDR model by adding the hand speed to the regressors. We found that the hand force axes (TDR 1 and TDR 2) explained around 40% to 50% of the total variance of the preparatory neural activity, the hand speed TDR axis explained approximately 10% to 20% of the total variance of the preparatory neural activity, and the uniform-shift axis explained around 10% to 20% of the total variance (Extended Data Fig. 3b). We also found that the hand speed axis was nearly orthogonal to the uniform-shift axis (Extended Data Fig. 3a). These results provide evidence that the hand speed axis does influence the preparatory activity, as pointed out by the reviewer, but importantly it barely overlaps with the uniform-shift axis. The figure of variance explained is copied below for convenience. All the results presented here about the hand speed axis have been added to the main text [lines 200-206].
Extended Data Fig. 3 | Relationships between neural population dimensions and total neural variance
different dimensions explained.

a, Relationships between different neural population dimensions (defined by TDR axes, uniform-shift axis and PCs), measured by their pairwise dot products (see Methods). Values close to 1 indicate that two dimensions are closely aligned whereas values close to 0 indicate that two dimensions are nearly orthogonal. In each session, we calculated the dot product of TDR 1 and PC 1 and the dot product of TDR 1 and PC 2, and then took the larger value of the two dot products (TDR 1 vs. PC 1 / 2). We also calculated the dot product of TDR 2 and the PC axis not used for multiplying with TDR 1 (TDR 2 vs. PC 1 / 2). The PC 1 / 2 plane largely overlaps with the TDR 1 / 2 plane (black boxes). The TDR 1 axis, TDR 2 axis and the TDR axis related to hand speed are all nearly orthogonal to the uniform-shift learning axis (blue and red boxes). PC 3 largely overlaps with the uniform-shift learning axis (yellow boxes). Monkey U: n = 4 sessions; monkey V: n = 3 sessions.

b, c, The portion of total neural activity variance explained by the TDR 1 and TDR 2 (hand force related) axes, TDR 3 (hand speed related) axis, uniform-shift axis and PCs 1-3. TDR 1 and TDR 2 account for approximately 40%-50% of total variance; the uniform-shift learning axis accounts for around 10%-20% of total variance; PC 1 and PC 2 account for approximately 50%-60% of total variance, and the first three PCs account for around 70%-80% of total variance.

3. M1 and PMd neural recordings were lumped together in all analyses. Did you ever examine each region independently? Given the results of the recent study of Perich et al (2018) it would be interesting to know if the uniform shifts you have observed are mainly confined to PMd.

Thank you for the great question. We grouped PMd and M1 recordings together because we wanted to have a larger total number of neurons with which to perform our analyses. In this revision, we examined PMd and M1 neurons separately to see if PMd and M1 neurons contribute differently to the TDR subspace and uniform shift axis. The results show that PMd and M1 populations alone showed neural shifts in the 2D TDR subspace and along the uniform-shift axis similar to what we found when we grouped PMd and M1 together. The uniform shift...
was not confined to only PMd, but it was mainly confined to the preparatory neural activity in both PMd and M1 (Extended Data Fig. 19). We discuss the connection between our work and Perich et al., 2018 in the revised manuscript, which is copied below.

[lines 408-422] Perich and colleagues recently reported neural changes in the ‘M1-null’ subspace of PMd for rapid learning, where the M1-null subspace represents the component of the PMd population activity that does not directly influence M1 population activity\textsuperscript{22}. Is the uniform shift in our work confined to the M1-null subspace of PMd? By investigating PMd and M1 populations separately, we found that a similar uniform shift occurred in each area alone as occurred when the areas were combined (Extended Data Fig. 19). Our results suggest that the uniform shift is not confined to only PMd, but it is confined to the preparatory neural activity in both PMd and M1 and does not occur during the peri-movement period (Extended Data Fig. 16). Perhaps the uniform shift we found here is a different type of learning-related change: it lives in the ‘muscle-null’ subspaces of PMd and M1 and is not necessarily confined to the M1-null subspace of PMd. Alternatively, it is also possible that most M1 neurons in \textsuperscript{22} did not have significant preparatory activity and consequently the neural changes they found in the preparatory period were only confined to PMd. While there is no inconsistency between our findings and those by \textsuperscript{22}, further work is needed to precisely relate these studies.

The new figure analyzing PMd and M1 separately is reproduced below for convenience:
Extended Data Fig. 19  | Patterns of preparatory activity in different neural state subspaces with PMd and M1 neural populations analyzed separately.

a, Initial hand forces predicted by the 2D TDR preparatory states are correlated with real forces of the upcoming movement; the sign of hand force indicates its direction (monkey U PMd, $R^2 = 0.41$ and $P = 3.84 \times 10^{-8}$; monkey U M1, $R^2 = 0.56$ and $P = 7.33 \times 10^{-12}$). Lighter dots, earlier learning trials; darker dots, later learning trials.

b, Single-trial neural shift during learning from the before-learning state along the uniform-shift learning axis. Solid line: linear-log regression (monkey U PMd, $R^2 = 0.04$ and $P = 6.67 \times 10^{-9}$; monkey U M1, $R^2 = 0.103$ and $P = 5.84 \times 10^{-25}$).

c, Preparatory neural repertoires change similarly in PMd, M1, or PMd + M1. Black: control sessions in
which the monkey did thousands of center-out reaches without any force field (one-sided Wilcoxon rank-sum test: monkey U PMd + M1, \( P = 9.14 \times 10^{-10} \); monkey U PMd, \( P = 2.08 \times 10^{-12} \); monkey U M1, \( P = 4.77 \times 10^{-5} \)).

d. Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period, in both PMd and M1. TO: target onset. GC: go cue.

e. Tangling scores (Q) of neural trajectories before and after learning during the target-onset period, preparatory period and peri-movement period. Neural trajectories in either PMd or M1 or both have similar tangling patterns over time: they are bunched together around target onset, start to separate during the preparatory period and further diverge during the peri-movement period (left, M1 neurons only; middle, PMd neurons only; right, PMd and M1 neurons pooled). This is consistent with the neural trajectories visualized in d.

In addition, we note that monkey V Neuropixels data came from two days of PMd recordings and one day of M1 recording and they were analyzed separately in all related analyses (Figs. 2b, 2c, 2e, 3b, 3c, 5b, 5d, 5f, 5g, 5h, 5k in the revised manuscript). We updated the methods section with this note (see [line 573]). Monkey V Neuropixels data also showed that PMd and M1 populations had similar neural changes, which provides further support that PMd and M1 neurons contribute similarly to the TDR subspace and the uniform shift axis.

4. Although, as noted above, I found that the Methods sections could use some clarification the main text was generally clear and well-written with appropriate references made to previous work. One comment that applies particularly to the abstract is that it comes across (at least to me) as including quite a lot of field-specific jargon and thus appears to be written for a more specialized neuroscience audience rather than the more general readership of Nature. I would recommend revision of the abstract, introduction and discussion to ensure that the relevance of the study can be easily appreciated by a broad audience.

Thank you, we agree that there are many opportunities to better help the reader understand the overall study, including the methods and analyses while using less (unintentional) field-specific jargon. We appreciate that we are asking rather a lot of the readers despite our best attempts to be clear and concise, and we have now revised the abstract ([lines 18-40]), introduction ([lines 42-66]), discussion ([lines 397-508]) and methods ([lines 510-961]) to make them more accessible to the general readership.

In addition, in terms of presentation it is sometimes unclear whether what is being plotted in a given figure represents the example for an individual case vs an average across sessions, monkeys etc.

Thank you for highlighting this important point which was not sufficiently clear in the original manuscript. We have added relevant details to the figures and figure captions throughout the revised manuscript.

In an attempt to be maximally helpful, we have also summarized this information of which results came from both monkeys (analyzed separately or lumped together) and which results came from one monkey, as well as our rationale for doing so. This summary has been added to the Supplementary Materials [lines 135-155].

Grouped data: in Fig. 5g, 5h (right panel), 5k and Extended Data Fig. 13f, 13g, we grouped together four sessions of relearning experiment with monkey U and three sessions with monkey V rather than analyze them separately to gain higher statistical power for performing the sign-rank test. Example neuron PSTHs and EMG recordings from both monkeys are grouped together (Extended Data Figs. 1, 5, 6). Note that each panel of the EMG figure is from one specific muscle of one monkey, but the 6 total recorded muscles are either from monkey U or monkey V.

Data from only one monkey: we did the random-perturbation experiment only with monkey V (Fig. 3d, e and Extended Data Fig. 18c). We were able to track the same neurons over five sessions only in monkey U (using Utah array implant) to study the uniform shift during multi-session multi-field learning (Fig. 4b, 4c purple distribution, Fig. 4f 8-condition decode, Extended Data Fig. 9c, and Extended Data Fig. 14c 8-condition decode) and the relationship between the uniform shift and learning / relearning rate (Fig. 5i), with the stability of selected neurons is shown in Extended Data Fig. 8.
Data from both monkeys shown separately: all the rest results. Note that plots of example single-trial hand trajectories, visualized neural states and visualized neural trajectories are from one example session in one monkey (Figs. 1b, 2a, 2d, 3a, 3d, 4a, 4d, 5a, 5c, 5j; Extended Data Figs. 4a, 15, 16a, 16e, 18a, 18b, 22) and are consistent in both monkeys; the behavioral and neural quantification results are shown separately for both monkeys unless specified in the “Grouped data” section (Figs. 1c, 1d, 2b, 2c, 2e, 3b, 3c, 3e, 4c, 4e, 4f, 5b, 5d, 5e, 5f; Extended Data Figs. 2, 3, 4b, 7, 9a, 9b, 13a-e, 14, 16b-d, 16f, 17); the interference experiment results (Extended Data Figs. 10 and 11) come from two sessions with each monkey.

We have also added to the methods section more details about the number of sessions used in each analysis. We copy the relevant portions of the methods section here for convenience:

[lines 644-651] In this study, monkey U results included 12 recording sessions (four sessions of the relearning experiment, three sessions of the opposite-field experiment, two sessions of the interference experiment, and three sessions of the center-out control experiment); monkey V results included nine Neuropixels recording sessions (three sessions of the relearning experiment, one session of the opposite-field experiment, two sessions of the interference experiment, two sessions of the center-out control experiment, and one session of the random-perturbation experiment) and two sets of V-probe recordings for learning two different curl fields (20 sessions in total).

Specific Comments:

Fig. 1/Methods: Did monkeys reach in the horizontal plane, vertically or in some intermediate plane? The plane within which the monkeys used the haptic device to move their hands was 45 degrees inclined. [lines 518-519] in the methods section.

Fig. 1b: How was early learning vs late learning defined? Similarly how was early washout versus late washout defined? Is late learning at the end of block ii or block iii?

(1) Early learning was defined as the beginning of the learning block (block ii), late learning was defined as the end of block ii in the original manuscript and as the end of the error-clamp block (block iii) in the new manuscript (consistent for all neural, EMG and behavioral analyses). [lines 566-569] in the methods section.

(2) Early washout was defined as the beginning of the washout block (block iv), and late washout is defined as the end of block iv (consistent for all neural, EMG and behavioral analyses). [lines 566-569] in the methods section.

(3) In the revised manuscript, we plotted a new version of Fig. 1c with late learning defined by curl field trials at the end of the error-clamp block (block iii) instead of the learning block (block ii). This new plot is copied below in our answer to the next question about Fig. 1c.

It would also be useful to include either in Fig. 1 or in an Extended Data figure some information about how peak speed changed across each of the four blocks for each animal.

We have made a new figure comparing the hand speed across before-learning, late-learning, error-clamp, and late-washout trials for both monkeys (Extended Data Fig. 7a). We have copied the new hand speed plot here (time 0 is movement onset):
Extended Data Fig. 7  |  a, Hand speed in before-learning (black), late-learning (red), error-clamp (purple), and late-washout (blue) trials for monkey U and monkey V averaged over multiple sessions. Shaded area, s.e.m. across sessions. Time zero, movement onset.

Overall (averaged over all single-field learning sessions for each monkey), the peak hand speed of monkey U was around 20 mm s\(^{-1}\) slower in late-learning / error-clamp / late-washout trials than in before-learning trials while monkey V maintained the before-learning peak hand speed in learning and washout trials, but slowed down by around 40 mm s\(^{-1}\) (10% of the peak speed) in error-clamp trials. In individual sessions, changes of the peak hand speed across all blocks varied from day to day and we did not observe a consistent pattern: for instance, sometimes the peak hand speed was slower than before-learning only in late-learning trials but not in error-clamp or late-washout trials; sometimes the peak speed was slower than before-learning in error-clamp and late-washout trials but not in late-learning trials; sometimes the peak speed was even faster than before-learning in all the other blocks, etc. (individual-session data not shown). In contrast to this variability of hand speed changes in different sessions, we observed the uniform shift of preparatory states consistently in each individual session and in both monkeys, which supported that the change of hand speed should not be the major contributing factor to the learning-related uniform shift.

Fig. 1c: The time course of learning for monkeys U and V seems to be quite different. Specifically, monkey U appears to take over 100 trials before reaching a plateau in learning (as one might expect) and even towards the end of learning perpendicular error never reaches zero. In contrast, monkey V appears to very rapidly (i.e., within ~ 10 trials) reach a perpendicular error of close to zero as if no learning is taking place but the monkey is simply recalling a previously learned behavior that wasn't fully washed out or is employing some sort of behavioral strategy (e.g., slowing his movements) to compensate very rapidly. Can the authors comment on what the difference might be attributed to? For example, was there any evidence that monkey V did employ a strategy of slowing his movements to avoid being perturbed by the field? Was there evidence for more retention of previous learning in monkey V? Was the field strength potentially too weak for monkey V?

Thank you for raising these interesting questions, which we have addressed individually below.

Our explanation for "why monkey V learned much faster than monkey U": in V-probe recording days, in order to have more than 100 neurons collectively, monkey V learned each field at least five times. We made sure that days which had the same curl field were at least seven days apart to minimize the effect of overtraining. We also trained monkey V on a weaker curl field (10 N / (mm s\(^{-1}\))) one month before we started neural recording to make sure that his behavior was consistent enough for long-term neural recordings. We chose this weaker curl field for behavioral training sessions with the intent to minimize the chance of overtraining monkey V before we started any neural recording. In contrast, monkey U was exposed to the weaker curl field (10 N / (mm s\(^{-1}\))) for only seven behavioral-training days before we started the Utah array recordings, and each field was repeated no more than three times in total. We have added these details to the Supplementary Materials [lines 102-118].

We also updated the way we quantified hand deviation error: instead of using the maximum hand deviation error at a single time point, we averaged the lateral hand trajectory deviation over a 20 ms time window around the
peak hand speed, which could reflect the gradual learning process better ([lines 678-685] in the methods section). We plotted a new version of Fig. 1c using this method, which is copied below for convenience:

**Fig. 1 | ... c,** Behavioral indicators of learning and washout quantified by the hand trajectory deviation from a straight path (see Methods). Hand trajectory error is initially large, decreases during learning or washout, and then plateaus (close to before-learning level) in late-learning or late-washout trials. Shaded area, s.e.m. across sessions. Early learning is defined as the beginning of the learning block (block ii), late learning is defined as curl-field trials in the end of the error-clamp block (block iii), early washout is defined as the beginning of the washout block (block iv), and late washout is defined as the end of block iv.

To show that monkey V did not employ a strategy of slowing his movements: we made a new plot comparing hand speed across before-learning, late-learning, error-clamp and late-washout trials for both monkeys (Extended Data Fig. 7a, copied above in our answer to the last question). Overall (averaged across all sessions for each monkey), the peak hand speed of monkey U was around 20 mm*s⁻¹ (5% of the peak speed) slower in late-learning/error-clamp/late-washout trials than in before-learning trials while monkey V maintained the before-learning peak hand speed in learning and washout trials, but slowed down by around 40 mm*s⁻¹ (10% of the peak speed) in error-clamp trials. This comparison supports that monkey V did not employ a strategy of significantly slowing down his movements to avoid being perturbed by the field, and that slowing down was not the main reason why monkey V learned faster than monkey U.

Behaviorally monkey V did adapt much faster in late recording sessions compared to the early training sessions. It took monkey V more than 50 trials to adapt to the curl field with a success rate above 80% in earlier sessions (Supplementary Fig. 2 and copied below for convenience). However, we were not able to collect neural evidence of memory retention across sessions in monkey V due to the nature of V-probe and Neuropixels recordings: we sampled different neural populations in different sessions and so could not track memory in the same neural population.
Supplementary Fig. 2 | The success rates of monkey V in earlier sessions (left panel, behavioral training sessions) and later sessions (right panel, neural recording sessions). It took monkey V around 100 trials to reach performance asymptote in earlier sessions and no more than 30 trials in later sessions. Shaded area, s.e.m. across sessions.

We believe that the field strength 14N / (m*s⁻¹) was sufficiently strong given that in earlier behavioral training sessions it took monkey V at least 50 trials to adapt to a weaker curl field. We also know that in the neural recording sessions, monkey V generated significantly larger hand forces after learning the curl field (Extended Data Fig. 7b), and he showed large aftereffect due to learning in early-washout trials (Fig. 1c green curve). These observations are consistent with the learning pattern that we observed in monkey U although at a faster time scale. The faster learning rate could potentially be due to transfer learning, i.e., knowledge or skills gained while solving one problem applied to a different but related problem (Seidler, 2010).

Fig. 1d, 2e: Here you plot perpendicular hand force difference (Fig. 1d) or neural angular shift (Fig. 2e) between error-clamp trials and before learning trials as a function of target direction relative to the trained direction to examine generalization. As I understand it you compared the forces generated in error clamp trials in block iii (after learning) to those during “free” unperturbed reaching movements before learning in block i to obtain the “learning” (orange) curve. It is unclear to me, however, how the “no learning” (black) curve was obtained. Did you sometimes perform a block of error clamp trials (i.e., block iii) either following a block ii in which no curl field was applied (i.e., no learning) or immediately following block i?

We thank the reviewer for pointing out where we could have been clearer regarding the control experiment. We clarify the experimental design below to address this question: as noted by the reviewer, in those no-learning sessions, we performed an error-clamp block (block iii) in which center-out reaches with and without the error clamp were randomly mixed together following a block ii in which no curl field was applied. The no-learning black curve was obtained by calculating the hand force difference between the error-clamp trials in block iii and no-clamp trials in block i in the no-learning control sessions.

To help avoid confusion with the no-learning datasets, we have updated the center-out control experiment part of the methods section with a summary of trials and blocks in the center-out control experiment that were used in the analyses, which is copied below for convenience:

[lines 604-615] Center-out reach control experiment. This control experiment was conducted to verify that the changes in neural population activity patterns were learning-related, not merely due to the instability of recording. In control sessions (monkey U, n = 3; monkey V, n = 2), monkeys U and V made thousands of delayed center-out reaches to one of 12 targets without any curl force field. This control experiment had the same number of blocks as the principal learning task (see above), and each block had approximately the same number of trials to match their corresponding blocks in learning sessions (e.g., if trial 200 in a learning session is in block ii, then trial 200 in a control session is also in block ii except that the control session does not have a curl field and only has center-out reaches). The non-learning control results in Figs. 1d, 2e, 3c and Extended Data Fig. 2 came from comparing behavioral and neural data in error-clamp trials of block iii and no-clamp trials of block i, to be consistent with trials used in analyses of the learning data. Block ii in control sessions did not have a curl field and so there was no learning.

Lines 86-91 and 410-423: You indicate that you recorded in PMd and M1 (lateral and medial) but it would be valuable to include some further information (possibly even extended data) providing a little more detail about recording sites and the extent to which they overlapped in the two monkeys. For example, you used Utah arrays in monkey U but Plexon V-probes and a Neuropixels probes in monkey V which I assume enabled you to make much deeper recordings in monkey V. In monkey V were you able to record in deeper layers (e.g., caudal M1 recordings in the bank of the central sulcus)?

Thank you for the helpful suggestion. In the revised manuscript, we have added a figure (Extended Data Fig. 21, left panel) to show locations of the three Utah arrays implanted in monkey U, one in PMd, one in medial M1 (M1M) which we define to mean medial gyral M1 and one in lateral M1 (M1L) which we define to mean lateral gyral M1.
Monkey V had an acrylic head implant with a recording chamber over left PMd and M1 (coordinates A16, L15; Extended Data Fig. 21, right panel). In this recording chamber (19 mm diameter), a series of small burr holes (3 mm diameter) were drilled sequentially as needed across the entire recording period through the acrylic implant and skull to access dura and brain. In monkey V, Neuropixels and V-probes could record all six layers of the cortex, but were not long enough to reach deep within caudal M1.

The figure of recording sites is reproduced below for convenience.

**Extended Data Fig. 21 | Utah-array implant locations in monkey U and recording sites in monkey V.**

Recordings were performed in PMd and M1 in the hemisphere contralateral to the reaching arm. Left panel: an intraoperative photo of three Utah-array implants in monkey U. Right panel: a schematic map illustrating the approximate locations of recording sites in monkey V based on stereotactic coordinates; data in this work include units recorded from multi-electrode V-probes and Neuropixels probes. Histology has not yet been done on either monkey. Using the cortical landmarks, we estimate that the recording sites in monkey V largely overlap with the lateral half of the area covered by the three Utah arrays in monkey U. AS: Spur of arcuate. CS central sulcus. PCD: precentral dimple.

Although you lumped neurons across all areas together in the analyses presented in the manuscript were any analyses performed to examine potential differences across regions (e.g., PMd vs rostral M1 vs caudal M1)?

**It is a great suggestion to examine PMd and M1 neurons separately.** In the original manuscript, we grouped PMd and M1 (we recorded rostral M1 only) data together because we wanted to have a larger total number of neurons to perform our population-based analyses with. In this revision, we examined PMd and M1 neurons separately to see if PMd and M1 neurons contribute differently to the TDR subspace and uniform shift axis. The results showed that PMd and M1 populations alone showed neural shifts in the 2D TDR (hand-force related) subspace and along the uniform-shift axis similar to what we found with PMd and M1 grouped together (Extended Data Fig. 19). We have copied the new figure analyzing PMd or M1 separately in our response to an earlier question from the reviewer (general question #3, page 49 in this document).

In addition, we note that monkey V Neuropixels data came from two days of PMd recordings and one day of M1 recording and they were analyzed separately in all related analyses (Figs. 2b, 2c, 2e, 3b, 3c, 3b, 5d, 5f, 5g, 5h, 5k in the revised manuscript). We updated the methods section with this note (see [line 573]). Monkey V Neuropixels data also showed that PMd and M1 populations had similar neural changes, which provides further support that PMd and M1 neurons contribute similarly to the TDR subspace and the uniform-shift axis.

**Lines 424-439: For the V-probe and Utah-array recordings you indicate that both stable single units and multi-unit isolations were included.** Can you provide an estimate of the relative percentage of single-unit vs multi-unit recordings that were used in the analyses? For the 1000 units recorded using the Neuropixels probes was it possible in all cases to isolate single units?
We estimated the proportion of stable single units for Utah-array, V-probe and Neuropixels recordings as follows, which has been added to the Supplementary Materials.

**Supplementary Materials**

[lines 156-162] The V-probe and Utah array recordings usually include both single and multi-unit isolations, and after spike sorting, we kept stable single units and multi-units. Approximately 40% of all sorted Utah-array units were single units while for V-probe recordings the percentage was around 50%. Neuropixels probes could record single units with the currently highest quality to our knowledge. Among all recorded units, the portion of stable single units was usually between 40% and 70%. After spike sorting, at least 80% of all sorted Neuropixels units were single units.

Below, we also provide more details describing our spike sorting process.

(1) We used MKsort (written by Prof. Matthew Kaufman when he was in the group) to sort Utah-array and V-probe data (we have added a screenshot below of the MKsort GUI to show the waveform and the ISI distribution of an example well-isolated single unit). Using MKsort, units were sorted with a hoop-sorting algorithm, with the ISI distributions displayed for the sorted units. Potential spikes were first identified when the filtered voltage dropped below a user-defined threshold (here we used -3.5 x RMS). These spikes were sorted by placing a lower and upper voltage threshold (the ‘hoop’ as noted in the plot below) at specific times relative to the initial threshold crossing. Users can view spike waveforms across all trials throughout the whole recording session (the horizontal scroll bar as noted in the plot below), and adjust the hoops if the amplitude slightly changes over time. The MKsort GUI allowed us to select units that had (i) relatively constant waveforms throughout each recording session and (ii) fewer ISI violations, and thus were more likely to be single units.

![MKsort GUI screenshot](image)

(2) Neuropixels probes allow for high confidence in the spike sorting, since virtually all recorded neurons are visible by multiple recording sites along the probe. Units were spike sorted using Kilosort (Pachitariu et al., 2016) and Phy to provide the automated and manual cluster curation steps, respectively, to ensure single-unit isolations and neuron stability over the course of a recording session (the Supplementary Fig. 3 in Trautmann et al., 2019 shows our criteria for manual curation of single units using the Phy Template-GUI). In the manual curation step, individual neurons were inspected to ensure that the waveform template across multiple channels was consistent with a single neuron, that there was a low incidence of refractory period violations, and that the template amplitude was relatively constant throughout the full duration of the recording period. Clusters which violated any of these properties, suggesting either the inclusion of spikes from multiple neurons or a lack of temporal
stability in firing properties (e.g., if the unit was only visible for a portion of the experimental session) were excluded from all analyses presented here. In practice, approximately 300 neurons were recorded during a typical experimental session using a single Neuropixels probe. For a given session, roughly 100 - 200 (40% - 70%) of these units were isolatable as single units and stable throughout the duration of the session, which were the units we manually curated and used for analyses.

In addition, Trautmann et al. 2019 shows that neural population dynamics and scientific conclusions are quite similar using multi-unit threshold crossings rather than sorted neurons, at least in four different studies on movement control. Therefore, we speculate that our findings are likely to remain similar whether the neural recordings only have single units or also include multi-units.

Fig. 2 legend: In part d, are you providing a single session example for learning of a CCW field during upward movements or is this an average across both fields (with sign reversal for CW field), different training directions, monkeys etc?

This is a single example session (monkey U) for learning a CCW field applied to up reaches, and the preparatory neural states of the trained target rotate clockwise. For learning a CW field, the preparatory states rotate counterclockwise.

In Fig. 2d of the revised manuscript (copied below for convenience), we have added another example of neural changes in the 2D TDR subspace for learning a CW field applied to up reaches, which happened in the opposite direction compared to changes of neural states for learning a CCW curl field. We have also noted in the figure caption that the plots were generated from two example sessions from monkey U and representative of the other single-field learning sessions from both monkeys.

When learning a CW or a CCW curl field applied to other target directions, neural states in the TDR plane showed patterns of changes similar to these examples: the largest neural changes opposing the curl field were around the trained target and neural changes degraded when the probed reach targets were far from the trained target. Monkey V showed similar neural changes to monkey U in this 2D TDR subspace. The visualization of projected 2D neural states in other monkey U and monkey V sessions are not shown, but quantified neural changes for learning different curl fields across learning sessions in both monkeys are shown in Fig. 2e.

**Fig. 2 | d.** In the same 2D TDR subspace, preparatory states of error-clamp reaches (color diamonds) consistently rotate in an opposite direction to the curl field direction for targets within 45 degrees from the trained target. Two example monkey U sessions (left panel, learning a CCW field applied to the up target; right panel, learning a CW field applied to the up target), representative of the other learning sessions from both monkeys.

Lines 136-139: You indicate here that you also identified prominent unexpected neural changes along a nearly orthogonal dimension that did not directly parallel behavioral output and that this was revealed by applying PCA to the neural population preparatory activity. So far in the paper you have examined neural population activity in a TDR subspace with the goal of relating neural activity to specific movement variables (i.e., hand forces). You
then perform a PCA analysis to show that there is a clear separation in before-learning and after-learning repertoire. As noted above, what is less obvious to me is that this is along an orthogonal dimension that did not directly parallel behavioral output. Is that assessment based mainly on the observation that it occurred for directions in which there was no behavioral evidence of learning or is it also true that these shifts were along a dimension orthogonal to force output. Specifically, since most of the “uniform” learning-related change in Fig 2f appears to be along the PC3 dimension this would seem to imply that the PC1/PC2 plane is closely aligned with the TDR1/TDR2 plane. Is that true? More generally it would be helpful to clarify the relationship between PC1, PC2 and TDR1, TDR2. Do the two first principal components largely reflect force? How much of the variance in the data do TDR1 and TDR2 vs PC1 and PC2 account for? How much do the first 3 PCs account for?

As correctly noted by the reviewer, the PC 1 and PC 2 plane was closely aligned with the TDR 1 and TDR 2 plane, which suggests that they largely reflect hand force. In the revised manuscript, we have added a new plot (Extended Data Fig. 3a) of the dot products between PC 1 / PC 2 axes and TDR 1 / TDR 2 axes, to show that these PC-defined and TDR-defined planes largely overlapped. This new plot also includes the dot products between the uniform-shift axis and TDR 1 and 2 axes to show that the uniform-shift axis was nearly orthogonal to TDR axes, and between the uniform-shift axis and PC 3 to show that they largely overlapped (dot products close to 0 indicates orthogonality and dot products around 1 indicates a substantial overlap). Therefore, our assessment of this orthogonality between the uniform shift and the force output is based on the quantification result that the uniform-shift axis was nearly orthogonal to the force-related TDR dimensions.

We have also added a plot of variance explained by TDR 1, TDR 2, PC 1, PC 2, PC 3 and the uniform-shift learning axis (Extended Data Fig. 3b, c). TDR 1 and TDR 2 accounted for 40% - 50% of total variance; the uniform-shift learning axis accounted for around 10% - 20% of total variance; PC 1 and PC 2 accounted for 50% - 60% of total variance, and the first three PCs accounted for 70% - 80% of total variance. We copy the figure below for convenience.
Extended Data Fig. 3 | Relationships between neural population dimensions and total neural variance
different dimensions explained.

a, Relationships between different neural population dimensions (defined by TDR axes, uniform-shift axis and
PCs), measured by their pairwise dot products (see Methods). Values close to 1 indicate that two dimensions
are closely aligned whereas values close to 0 indicate that two dimensions are nearly orthogonal. In each
session, we calculated the dot product of TDR 1 and PC 1 and the dot product of TDR 1 and PC 2, and then
took the larger value of the two dot products (TDR 1 vs. PC 1 / 2). We also calculated the dot product of TDR 2
and the PC axis not used for multiplying with TDR 1 (TDR 2 vs. PC 1 / 2). The PC 1 / 2 plane largely overlaps
with the TDR 1 / 2 plane (black boxes). The TDR 1 axis, TDR 2 axis and the TDR axis related to hand speed are
all nearly orthogonal to the uniform-shift learning axis (blue and red boxes). PC 3 largely overlaps with the
uniform-shift learning axis (yellow boxes). Monkey U: n = 4 sessions; monkey V: n = 3 sessions. b, c, The portion
of total neural activity variance explained by the TDR 1 and TDR 2 (hand force related) axes, TDR 3 (hand speed
related) axis, uniform-shift axis and PCs 1-3. TDR 1 and TDR 2 account for approximately 40% - 50% of total
variance; the uniform-shift learning axis accounts for around 10% - 20% of total variance; PC 1 and PC 2 account
for approximately 50% - 60% of total variance, and the first three PCs account for around 70% - 80% of total
variance.

On a related note is there any behavioral variable or context that PC3 might be related to (e.g., global differences
in speed or perhaps some other variable related to the context of reaching in a block with interspersed error
clamp trials)? In your analyses you compare before-learning preparatory activity during “free” reaches to after-
learning activity in error clamp trials. I realize this was necessary to be able to characterize a uniform shift across
reach directions but it would have been better to be able to compare error clamp trials after-learning to error-
clamp trials before learning. Given the existing data, if you simply consider activity for reaches in the training
direction and project the preparatory activity during force-field trials (e.g., at the end of the learning block or
during the error clamp block ) into PC space does the activity appear at the same location as for after-learning
error-clamp trials? Similarly, based on my understanding of what is plotted in Fig. 2e at some stage you must
have performed error-clamp trials under “no learning” conditions. Was this ever performed on the same day as
a learning experiment such that you could directly compare error-clamp “no learning” population activity to error-
clamp “after-learning” activity?

Thank you for bringing up the potential concern about the error clamp contributing to the uniform shift. In the
revised manuscript, we compared the preparatory neural states for the trained target in late-learning (block iii)
and error-clamp trials. We found that late-learning and error-clamp preparatory neural states of the trained target
were not significantly different (Extended Data Fig. 18b, an example and representative session from monkey
U). We used Hotelling’s $T^2$ test to compare the late-learning preparatory state and the error-clamp preparatory
state, which showed that they were not significantly different in all single-field learning sessions from two
monkeys ($P > 0.05$ for four monkey U sessions, $P > 0.1$ for 20 monkey V V-probe sessions and three monkey V
Neuropixels sessions). The plot is copied below for convenience (arrows point to the before-learning and error-
clamp neural states of the trained target).

Extended Data Fig. 18 | ... b, In the error-clamp block (block iii) of learning sessions, the late-learning
preparatory state (black circle) and error-clamp state of the trained target (purple diamond next to the arrow) are
not significantly different (Hotelling’s $T^2$ test: $P > 0.05$ for all learning sessions of monkey U and $P > 0.1$ for all
learning sessions of monkey V). Arrows point to the before-learning state (purple circle) and error-clamp state
(purple diamond) of the trained target. The plot is from one example session of monkey U.
In control sessions (i.e., when monkeys did thousands of center-out reaches without any curl field), we performed no-clamp and error-clamp trials under ‘no learning’ conditions. Because the no-clamp and error-clamp trials were randomly interleaved, the monkey did not know that he was in an error-clamp trial until after he began reaching. Therefore, we do not expect to see any difference in the preparatory neural activity between no-clamp and error-clamp trials. As a complementary analysis, we compared the ‘error-clamp, no-learning’ neural state to the ‘no-clamp, no-learning’ state and found that they were not significantly different (Extended Data Fig. 18a, an example and representative session from monkey U; Hotelling’s $T^2$ test, $P > 0.5$ for all control sessions from both monkeys). We have copied the plot below for convenience.

![Plot showing no-learning session with error-clamp and no-clamp trials](image)

**Extended Data Fig. 18** | The uniform shift is not due to the error clamp.  
**a,** Preparatory states in error-clamp (color diamond) and no-clamp (color circle) trials are not significantly different in no-learning control sessions (Hotelling’s $T^2$ test: $P > 0.5$ for all control sessions of monkeys U and V). The plot is from one example session of monkey U.

Unfortunately, we do not have ‘error-clamp, no learning’ trials in a learning session to directly compare them to the ‘error-clamp, after learning’ trials. But given that in control experiments the ‘error-clamp, no-learning’ neural state and the ‘no-clamp, no learning’ state are not significantly different, we think that our current results should remain similar if we could use the ‘error-clamp, no-learning’ trials.

Lines 163-166: To rule out recording instabilities you compared repertoire change values in learning sessions to those in “non-learning” sessions. Did you also perform an error-clamp block in these non-learning sessions and is this where the “no learning” data for Fig. 1d and Fig. 2e comes from? This was not at all clear to me from the methods. I have understood that in Fig. 2h the repertoire change for trained reaches was obtained by quantifying the distance between before learning and after-learning error clamp trials. What was quantified in the control/no-learning case? Was it the distance between activity at the beginning of practise (i.e., before-learning block) and that during error-clamp trials performed in the equivalent of block 3 but after thousands of center-out movements? Alternatively was it simply activity during control reaches before and after thousands of trials that was compared?

Yes, the reviewer is correct that we performed an error-clamp block in these non-learning sessions in which center-out reaches with or without the curl field were randomly mixed together.

The repertoire change in the non-learning case was the distance between neural activity at the beginning of the center-out reaching task (i.e., equivalent to the before-learning block / block i in learning sessions) and that during error-clamp trials performed in the equivalent of block iii but after thousands of center-out movements.

To help avoid confusion with the no-learning datasets, we have updated the center-out control experiment part of the methods section with a summary of trials and blocks in the center-out control experiment that were used in the analyses, which is copied below for convenience:

**[lines 604-615] Center-out reach control experiment.** This control experiment was conducted to verify that the changes in neural population activity patterns were learning-related, not merely due to the instability of recording. In control sessions (monkey U, $n = 3$; monkey V, $n = 2$), monkeys U and V made thousands of delayed
center-out reaches to one of 12 targets without any curl force field. This control experiment had the same number of blocks as the principal learning task (see above), and each block had approximately the same number of trials to match their corresponding blocks in learning sessions (e.g., if trial 200 in a learning session is in block ii, then trial 200 in a control session is also in block ii except that the control session does not have a curl field and only has center-out reaches). The non-learning control results in Figs. 1d, 2e, 3c and Extended Data Fig. 2 came from comparing behavioral and neural data in error-clamp trials of block iii and no-clamp trials of block i, to be consistent with trials used in analyses of the learning data. Block ii in control sessions did not have a curl field and so there was no learning.

Lines 169-173: In assessing the ability of random perturbation forces to effect a repertoire change, activity during random-perturbation trials was compared to before-learning and after-learning error clamp trials. What happens if you instead compare force-fields trials late in the learning block or during the error clamp block to random-perturbation trials?

We thank the reviewer for the suggestion. In the revised manuscript, we performed repertoire analyses using late-learning preparatory activity (from the last 100 learning trials in the error-clamp block) and found significant repertoire change of the preparatory states after learning compared to before-learning random-perturbation states (Extended Data Fig. 18c). We once again found significant neural repertoire changes when comparing force-field trials to random-perturbation or no-perturbation trials. We copy the plot here for convenience:

![Extended Data Fig. 18](attachment:Extended Data Fig. 18.png)

Extended Data Fig. 18 | ... c, Larger preparatory repertoire changes occur to late-learning force-field states vs. before-learning no-perturbation states (red), and to late-learning force-field states vs. before-learning random-perturbation states (blue), than to before-learning random-perturbation vs. no-perturbation states (black). $P_{12} = 1.83 \times 10^{-5}$, $P_{13} = 1.83 \times 10^{-5}$, $P_{23} = 0.99$. The results are similar to Fig. 3e where we used after-learning error-clamp trials to compute the repertoire changes.

Lines 174-176: You indicate here that there were no obvious signs of co-contraction in late-learning and washout trials. What about during the error-clamp block?

We made a new figure comparing EMG recordings of muscle activity across before-learning, late-learning, error-clamp, and late-washout trials (Extended Data Fig. 5 and copied below). EMG signals looked quite similar in the late-learning and error-clamp trials (block iii) for the trained target.

Were there any differences in EMG recordings of muscle activation during error-clamp trials in block 3 as compared to before learning trials in block 1 in directions to which you found no behavioral generalization?
We also compared EMG signals in error-clamp trials (block iii) and before-learning trials (block i) for 135 degree targets (i.e., far targets that consistently showed almost no behavioral generalization across sessions and monkeys, see Fig. 1d), and their muscle activity curves looked similar. This comparison is added to Extended Data Fig. 6 and copied below for convenience:

Extended Data Fig. 5 | EMG signals of 6 upper limb muscles (bicep, radialis flexor, radialis extensor, pectoralis, posterior deltoid, lateral deltoid) in before-learning, learning, error-clamp, and washout blocks.

Time zero, movement onset. One example condition: CW curl field applied to down reaches. EMG signals do not show signs of muscle co-contraction during learning (red). Muscle activity during the preparatory period remains flat and around the same level across all blocks (two-sided rank-sum test: $P > 0.3$ for all pairs of comparison except for $P < 0.0001$ when comparing late-learning / error-clamp bicep activity with before-learning / late-washout bicep activity). Muscle activity patterns in before-learning (black) and late-washout trials (blue) are very similar. Muscle activity patterns in late-learning (red) and error-clamp trials (purple) are very similar. Shaded area, s.e.m across trials. EMG activity shows similar temporal patterns to previous intramuscular recordings$^{54,66}$. 
Extended Data Fig. 6 | EMG signals of 6 upper limb muscles (bicep, radialis flexor, radialis extensor, pectoralis, posterior deltoid, lateral deltoid) in before-learning and error-clamp blocks for the 135-degree reach target.

Time zero, movement onset. One example condition: CW curl field applied to down reaches. Muscle activity of reaching to the target 135 degrees away from the trained target (i.e., far targets with almost no behavioral generalization, see Fig. 1d) in before-learning (black) and error-clamp trials (purple) show similar temporal patterns. Shaded area, s.e.m across trials.

Lines 188-206: This section focuses on a geometric analysis of the uniform shifts when comparing learning of multiple force fields by examining the extent of alignment of the “learning uniform shift axes” associated with each of those fields. However, as noted above, the definition of “learning uniform-shift axis” was not so clear. In the text it is defined as the vector pointing from the centroid of the before learning neural repertoire to that of the after learning repertoire. In the methods there is then a further statement about orthogonalizing against the two TDR axes where rotatory neural shifts were observed. Should I take this to mean that after finding the vector
connecting before learning and after learning centroids you redefined the learning uniform shift axis to be only the component of the full learning-related shift that was orthogonal to TDR1 and TDR2? More generally, it wasn’t completely clear to me whether the shifts observed and plotted in Figure 3 and their geometric relationships reflect changes in neural states that are completely orthogonal to the TDR1 and TDR2 dimensions that reflect force output or whether at least a component of these shifts does incorporate the rotatory shifts observed in the 2D TDR subspace. In other words, to what extent can the geometric relationships between shifts observed in Figure 3 be thought of as shifts within an "output-null" subspace?

As correctly pointed out by the reviewer, the uniform-shift axis did not incorporate the dimensions where rotatory shifts were observed (which reflected the force output). To help clear up potential confusion, here is how we defined the ‘uniform-shift learning axis’: after finding the vector connecting before-learning and after learning centroids, we redefined the uniform-shift learning axis to be only the component of the full learning-related shift that was orthogonal to TDR 1 and TDR 2, that is, this uniform shift did not incorporate the rotatory shifts in the 2D TDR subspace.

We clarify that everything in the preparatory activity is ‘output-null’, and we apologize for not defining ‘output-null’ clearly enough. Our understanding is that your question is asking about a ‘force-unrelated’ subspace in the preparatory activity. In Fig. 3 (now Fig. 4), we orthogonalized all uniform-shift axes against TDR axes before analyzing the geometric relationships between those uniform-shift axes. Therefore, the uniform shifts were always within a force-unrelated subspace.

We have updated the main text and the methods section with the clarifications about the uniform-shift axis in [lines 165-167] and [lines 763-774]. We have added the clarification about the output-null subspace to Supplementary Materials [lines 183-193].

Lines 227-228/Fig. 4e,f: As noted earlier, monkey V appears to both learn and relearn much faster than monkey U and so quickly that it almost appears as if he has either used some specific behavioral strategy (e.g., slowing movements) or has retained previous learning. Since you compare initial learning to relearning here the latter would appear not to be the case. However, in examining this data I realized that it is unclear from the manuscript whether initial learning means the first time the monkey ever encountered a CW or CCW curl-field in any session and when reaching in any direction or whether it rather refers to the first time the monkey encountered this field in a particular reach direction / session. More specifically, how was the data for the various relearning tasks sessions combined into these plots?

Thank you very much for highlighting this important detail which was not sufficiently clear in the original text. First, in response to "...it almost appears as if he has either used some specific behavioral strategy (e.g., slowing movements)", we made a new figure comparing hand speed across before-learning, late-learning, error-clamp and late-washout trials for both monkeys (Extended Data Fig. 7a). Overall (averaged across all sessions for each monkey), the peak hand speed of monkey U was around 20 mm·s⁻¹ (5% of the peak speed) slower in late-learning / error-clamp / late-washout trials than in before-learning trials while monkey V maintained the before-learning peak hand speed in learning and washout trials, but slowed down by around 40 mm·s⁻¹ (10% of the peak speed) in error-clamp trials. This comparison supports that during learning, monkey V did not employ a strategy of significantly slowing down his movements to avoid being perturbed by the field, and that slowing down was not likely the main reason why monkey V learned faster than monkey U. We copy the hand speed plots below for convenience:
Extended Data Fig. 7 | ... a, Hand speed in before-learning (black), late-learning (red), error-clamp (purple), and late-washout (blue) trials for monkey U and monkey V averaged over multiple sessions. Shaded area, s.e.m. across sessions. Time zero, movement onset.

Second, ‘initial learning’ refers to the first time the monkey encountered a specific field applied to a particular reach direction in a particular session, and ‘relearning’ refers to the second time adapting to the same field in the same session.

In Fig. 4e,f (now Fig. 5e,f), for each monkey separately, we did behavioral and neural comparisons between the initial learning and relearning blocks within each relearning experiment session (n = 4 for monkey U, and n = 3 for monkey V). We then combined results from these individual sessions into those plots, that is, the shaded area in Fig. 4e,f comes from s.e.m across sessions for each monkey separately. We have updated the figure captions with this note.

Lines 247-250: Here you show that peri-movement states also shifted away from the before-learning ones but in this case the shift was local to the direction in which there was behavioral evidence of learning and there was no evidence of motor memory retention after washout. I wasn’t quite sure how to interpret this. How much of the shift was along PC1 and PC2 axes vs along the PC3 axis? Perhaps, more importantly, how much of this shift was orthogonal to the TDR1 vs TDR2 plane?

We first clarify how we interpret “no evidence of motor memory retention after washout” in the peri-movement activity: the retention of the neural changes in preparatory states but not in peri-movement states suggests that it is a specific feature of the preparatory neural population activity. Because memory is not always manifest in behavior output, we think it is more likely to be retained in preparatory neural activity (always output-null) than peri-movement neural activity (usually directly guides the movement output). Previous work also shows that the dominant PCs in the preparatory activity are orthogonal to dominant PCs in the peri-movement activity (Elsayed et al., 2016), which further supports that there are neural activity patterns purely in the preparatory neural subspace, such as the uniform shift here.

Below we compare the TDR axes, PCs, and the shift from before-learning to after-learning states of the trained target (termed ‘peri-movement local shift’), using the peri-movement neural data. We took the dot product between the local shift and TDR 1 and 2 axes during the peri-movement period, and found that this local shift axis significantly overlapped with the TDR axes (i.e., the dot product was much greater than zero, $P = 9.77 \times 10^{-4}$, signed rank test with monkey U and monkey V data combined). We also found that this shift significantly overlapped with PCs 1-3 ($P = 0.03$, signed-rank test compared to 0, with monkey U and monkey V data combined). Plots of the pairwise dot products between those axes during the peri-movement period are added to the Supplementary Materials (copied below for convenience). The corresponding text is added to [lines 22-35] in Supplementary Materials.
Supplementary Fig. 1 | The relationship between peri-movement neural dimensions.
Pairwise dot products between PCs 1-3 and the ‘peri-movement local shift’ (i.e., the shift from the before-learning to the after-learning peri-movement neural states of the trained target), and pairwise dot products between TDR 1 and 2 axes and the peri-movement local shift. During the peri-movement period, PCs 1-3 significantly overlap with the local shift ($P = 0.03$, signed rank test compared to 0, with monkey U and monkey V data combined). TDR 1 and 2 axes also significantly overlap with the local shift ($P = 9.77 \times 10^{-4}$, signed rank test compared to 0, with monkey U and monkey V data combined).

Although the shifts appear to be local to directions which showed behavioral changes do they nonetheless reflect activity changes that don’t directly influence behavior (i.e., “output-null”)?

Based on the results shown above, here is our sense of what is happening:

1. Given that TDR axes significantly overlap but are not completely aligned with this peri-movement shift, it is possible that this shift also reflects neural changes that do not directly influence behavior.

2. Previous work (e.g., Kaufman et al., 2014; Stavisky et al., 2017) shows that peri-movement activity contains both output-potent and output-null components.

I note also that monkey V did exhibit significant changes for reaching in all directions. What is the authors’ interpretation of these results beyond the fact that the shift is less uniform than for preparatory activity?

Thank you for pointing out this smaller yet significant repertoire change of peri-movement neural states for reaching to far targets in monkey V (Extended Data Fig. 16d). In a few (but not all) learning sessions, we found that monkey V showed hand force changes after learning for targets that were far from the trained target, but to a lesser extent than hand force changes for nearby targets. That is, hand force changes in those sessions still followed the bell-shaped generalization curve despite including a weak global change for all reach directions. We think that this behavioral phenomenon was reflected in the peri-movement neural shift that was not uniform in general although it contained a weak shift for all reach directions. We think that these details are fairly far from the focus of the paper (preparatory neural activity), but we are open to include them in the manuscript if deemed necessary.

Lines 284-287: I did not understand what was meant here. Does the word “it” in “it does not” refer to the uniform shift?

Yes, “it” refers to the uniform shift, or the neural dimension in which the uniform shift occurs. We have updated the entire paragraph to discuss how our work connects to the ‘M1-null’ neural subspace in Perich et al., 2018 (copied below for convenience).

[lines 402-422] Previous work showed that the preparatory neural activity mainly occupies the ‘output-null’ subspace of neural population activity in PMd and M1\textsuperscript{44}, but it remained unknown if all output-null activity is tuned
for movement output parameters. In this work, the uniform shift of preparatory neural states occurs to reach directions where the movement is unaltered during learning, which indicates that uniform shifts and the resulting new activity patterns not only live in the output-null subspace, but also reflect components of the output-null activity that do not directly relate to the movement output. Perich and colleagues recently reported neural changes in the 'M1-null' subspace of PMd for rapid learning, where the M1-null subspace represents the component of the PMd population activity that does not directly influence M1 population activity. Is the uniform shift in our work confined to the M1-null subspace of PMd? By investigating PMd and M1 populations separately, we found that a similar uniform shift occurred in each area alone as occurred when the areas were combined (Extended Data Fig. 19). Our results suggest that the uniform shift is not confined to only PMd, but it is confined to the preparatory neural activity in both PMd and M1 and does not occur during the peri-movement period. Perhaps the uniform shift we found here is a different type of learning-related change: it lives in the 'muscle-null' subspaces of PMd and M1 and is not necessarily confined to the M1-null subspace of PMd. Alternatively, it is also possible that most M1 neurons did not have significant preparatory activity and consequently the neural changes they found in the preparatory period were only confined to PMd. While there is no inconsistency between our findings and those by, further work is needed to precisely relate these studies.

Line 330: “giped” should be “gripped”
Thank you for pointing out this embarrassing typo, and it is now fixed.

Line 332: Missing “a” before “point-to-point”
Thank you, this typo is now fixed.

Line 333: “with” should be “within”
Thank you, this typo is now fixed.

Line 352: Either here or elsewhere within the methods the arrangement and spacing of the 12 peripheral targets relative to the trained target should be indicated.
Thank you for this suggestion. We have added a figure (Extended Data Fig. 20) to the methods section (see lines 532-534) to show three spatial arrangements of the 12 reaching targets relative to the trained target (up, down, or right). We copy the figure here for convenience:
Extended Data Fig. 20 | Spatial arrangements of the 12 reaching targets relative to the trained target.
The target density near the trained target (up, down or right) is higher in order to sample more neural states for reaches that are more likely to be altered by learning, for the purpose of studying generalization of learning. Ideally, one would like to have equally-spaced reach targets as dense as possible, but because monkeys could perform a limited number of trials each day, a compromise solution is to increase the density of targets near the trained reach target.

Line 356-357: In the error-clamp block (block iii) it is indicated that 70% of the trials were the same as in the learning block and 30% were randomly interleaved error-clamp trials (20 trials per reaching target). If I have understood correctly this implies 240 error clamp trials randomly interspersed among another 560 learning trials in the training direction (i.e., many more than in the learning block). Is this correct?
Yes, this is correct. The error clamp block had many more learning trials than the learning block to keep the adapted behavior. We have added this description to the methods section [lines 548-549].

In addition, Fig. 1c shows the force field being turned off after ~200-250 trials followed immediately by washout. This is potentially confusing and it would seem to be worth illustrating what the perpendicular error on learning trials was at the end of the error-clamp block.
In the revised manuscript, we have made a new Fig.1c showing the perpendicular hand error in the last 100 learning trials in the error clamp block (block iii) instead of the last 20 trials in block ii. Instead of using the max hand deviation error at a single time point, we averaged the lateral hand trajectory deviation over a 20 ms time window around the peak hand speed. To avoid the potentially confusing ‘cumulative trial count’ in the older plot, we instead marked along the x axis the number of trials included in the hand curve of each condition (the updated Fig.1c was copied in our response to an earlier question from the reviewer, on page 53).

Line 359-360: How many washout trials were typically performed?
For both monkeys, at least 500 (ranging between 539 and 666) washout trials were typically performed. We have added this description to the methods section [lines 573-575].
You indicate here that to encourage monkeys to learn or unlearn the curl field you automatically failed a trial if the hand trajectory deviation exceeded a bound. What was that bound?
The bound was 20 mm lateral to the straight line between the center and the reach target on both sides. We have added this description to the methods section [lines 561-563].

In general, I am surprised to hear that incorporating such a bound was required. Typically when adapting to curl fields as long as a subject corrects towards the end target trajectories naturally become straighter (i.e., via implicit learning) so I wouldn’t have expected monkeys to continue to make curved, inefficient movements. Was there any evidence that incorporating such a bound encouraged monkeys to slow their movements as a means of reducing the perturbing force or was the incorporation of the 700 ms reach duration restriction sufficient to prevent that?

We appreciate the reviewer’s perspective and think it is important to determine if the behavioral bound is sufficient to encourage learning and prevent slowing down in the meantime. To really address this question, we need to do control experiments without the spatial bound or the temporal constraint. Unfortunately, we did not do such control experiments and so we are not able to directly address whether the incorporation of the 700 ms duration and the lateral deviation punishment could promote learning and prevent slowing down. Therefore, we have added the following text to the methods section to clarify this point.

[lines 558-565] In learning and washout blocks, we introduced a path efficiency check, where we automatically failed a trial if the hand trajectory deviation (perpendicular to the straight-line trajectory to the reach target) exceeded a bound, with the intent to encourage monkeys to learn or wash out the curl field (rather than accept making highly curved and inefficient movements to targets). This bound was 20 mm lateral to the straight line between the center and the reach target on both sides. In all trials throughout all sessions, monkeys were required to finish each reach movement within 700 ms or the trial would be counted as a failure. However, we did not have experimental data to sufficiently show that the spatial bound and time limit significantly promoted learning.

As a result of these constraints what was the typical success rate during the learning block?
The success rate is an important detail to report. In the learning block, the typical success rate dropped to around 40% at the beginning, and after reaching asymptote, it was around 70% - 80% for monkey U, and 80% to 90% for monkey V. We have added this description to the methods section [lines 543-545].

The Extended data in Fig.6a show some slowing in late learning. Is this an average for both animals or did each animal slow movements to different extents?

Extended Data Fig.6a is from one animal (monkey V). In this revised version, we have made a new figure comparing hand speed across before-learning, late-learning, error-clamp, and late-washout trials for both monkeys (Extended Data Fig. 7). Overall (averaged across all sessions for each monkey), the peak hand speed of monkey U was around 20 mm*s⁻¹ (5% of the peak speed) slower in late-learning, error-clamp, and late-washout trials than in before-learning trials while monkey V maintained the before-learning peak hand speed in learning and washout trials, but slowed down by around 40 mm*s⁻¹ (10% of the peak speed) in error-clamp trials. In individual sessions, changes of the peak hand speed across all blocks varied from day to day and we did not observe a consistent pattern: for instance, sometimes the peak hand speed was slower than before-learning only in late-learning trials but not in error-clamp or late-washout trials; sometimes the peak speed was slower than before-learning in error-clamp and late-washout trials but not in late-learning trials; sometimes the peak speed was even faster than before-learning in all the other blocks, etc. (single-session data not shown).

In summary, this comparison did not show evidence of monkeys slowing down their movements as a means of reducing the perturbation force during learning. We copy the new plots of hand speed comparisons below (they are also presented in our response to earlier questions from the reviewer):
Extended Data Fig. 7 | ... a, Hand speed in before-learning (black), late-learning (red), error-clamp (purple) and late-washout (blue) trials for monkey U and monkey V averaged over multiple sessions. Shaded area, s.e.m. across sessions. Time zero, movement onset.

Lines 372-376: In the relearning experiments you indicate that monkey U did four sessions and monkey V did three sessions. What were the reach direction and curl field combinations examined in these sessions? Is it possible that some memory of learning in one session was retained and influenced learning in a subsequent session? Am I correct in assuming that the relearning sessions were similar in structure to the single-field learning sessions except that blocks ii-iv were repeated a second time with the same curl-field?

Thank you very much for these important questions, which we have addressed individually below.

(1) Reach direction and curl field combinations in these single-field learning sessions are as follows:
   Monkey U - Down CW, Down CCW, Up CW, and Up CCW.
   Monkey V - Down CW, Down CCW, and Right CW.

We have added curl field configurations to the methods section [lines 532-534] with a diagram in Extended Data Fig. 20 (copied above in our response to an earlier question from the reviewer, on page 68).

(2) The comparison of behavioral performance of monkey V in early and late sessions supports that there was some memory retained across sessions (Supplementary Fig. 2), as noted by the reviewer. However, we always tried to minimize the frequency of learning the same curl field by applying different curl fields over at least five successive sessions. Therefore, we think that some memory of learning in a previous session would not influence learning in a subsequent session but might influence a much later session when we applied the same field again. Unfortunately, we were not able to collect direct neural evidence of memory retention across sessions in monkey V due to the nature of V-probe and Neuropixels recordings: we sampled different neural populations in different sessions and so could not track memory in the same neural population. We have added a plot comparing success rates of monkey V in the earlier and later sessions to the Supplementary Materials, which is copied below for convenience.
Supplementary Fig. 2 | The success rates of monkey V in earlier sessions (left panel, behavioral training sessions) and later sessions (right panel, neural recording sessions). It took monkey V around 100 trials to reach performance asymptote in earlier sessions and no more than 30 trials in later sessions. Shaded area, s.e.m. across sessions.

(3) Yes, it is correct that “relearning sessions were similar in structure to the single-field learning sessions except that blocks ii-iv were repeated a second time with the same curl field”. We have added this description to the methods section [lines 575-577].

Lines 387-389: I wasn’t sure what was meant by “The task had the same temporal duration as in a learning session”. Does this mean that monkeys made center-out reaches for approximately the same time as it normally took to complete blocks i-iv in a learning session? Were error-clamp trials examined in these sessions and if not where does the “no learning” data used in Figs. 1d and 2e come from? I also did not understand what was meant by “we used trials for which the trial IDs matched those in the learning sessions….”. Please clarify.

Thank you for pointing out where we could have been clearer regarding the control experiment. “The same temporal duration” means in control sessions, we designed each block to have approximately the same number of trials to match their corresponding blocks in learning sessions (e.g., if trials #200-300 in a learning session are in block ii, then trials around #200-300 in a control session are also in block ii except that the control trial does not have a curl field). This also explains what was meant by “we used trials for which the trial IDs matched those in the learning sessions”. For instance, if we analyzed trials #200-300 in learning sessions, we would also use trials with IDs around #200-300 in control sessions to perform the control analysis.

In control sessions, ‘no-learning’ results came from comparing behavioral and neural data in error-clamp trials of block iii and no-clamp trials of block i, to be consistent with trials used in analyses of the learning data. Block ii in control sessions did not have a curl field and so there was no learning.

We have updated the center-out control experiment part of the methods section in the revised manuscript to reflect the clarifications, which is copied below for convenience:

[lines 604-615] Center-out reach control experiment. This control experiment was conducted to verify that the changes in neural population activity patterns were learning-related, not merely due to the instability of recording. In control sessions (monkey U, n = 3; monkey V, n = 2), monkeys U and V made thousands of delayed center-out reaches to one of 12 targets without any curl force field. This control experiment had the same number of blocks as the principal learning task (see above), and each block had approximately the same number of trials to match their corresponding blocks in learning sessions (e.g., if trial 200 in a learning session is in block ii, then trial 200 in a control session is also in block ii except that the control session does not have a curl field and only has center-out reaches). The non-learning control results in Figs. 1d, 2e, 3c and Extended Data Fig. 2 came from comparing behavioral and neural data in error-clamp trials of block iii and no-clamp trials of block i, to be consistent with trials used in analyses of the learning data. Block ii in control sessions did not have a curl field and so there was no learning.

Lines 436-438: You indicate here that you pooled V-probe recordings you pooled neurons across multiple sessions for the same curl-field. Does that mean the same curl-field in the same direction?
Yes, this is correct. We pooled V-probe recordings together for the same curl field applied to the same direction (i.e., sessions with the same curl field configuration). We have added this description to the methods section [lines 664-666].

Lines 456-457: Here you indicate that Fig. 1c illustrates the mean and s.e.m. of MPE across all sessions. Does that mean that it reflects both learning and relearning trials?
We apologize for being unclear about which trials are used in the figure. Fig. 1c only reflects the behavior during learning but not relearning trials across all sessions. Relevant details have been added to the figure caption of Fig. 1c.
You indicate that different time windows were used in your PCA analyses for both the preparatory and peri-movement epochs depending on whether you were performing neural state analyses vs neural repertoire analyses. What was the motivation for choosing different time windows for these analyses and what kind of impact did this have on the PCs and preparatory states within PC space that were computed? Also unclear is which of the analyses you describe fall into the category of “neural state” vs “neural repertoire” analyses. Both of these issues should be clarified.

Thank you very much for the very helpful suggestions. We have addressed them individually below.

(1) Among all neural analyses, Fig. 3c, 3e, Extended Data Fig. 4b, Extended Data Fig. 16d, Extended Data Fig. 17, Extended Data Fig. 18c, Extended Data Fig. 19c are from the ‘neural repertoire’ analysis; Fig. 5f, 5j-k, Extended Data Fig. 19d, 19e are from the ‘neural trajectory’ analysis, and the rest are from ‘neural state’ analyses.

(2) We have added relevant details about choosing certain time windows to the Supplementary Materials [lines 163-182]. In an attempt to be maximally helpful, we have also summarized this information of which time windows were chosen for which analysis, as well as our rationale for doing so.

For the neural state analysis, we chose time windows -50 to +50 ms from the go cue and 0 to +100 ms from movement onset, because the former is right before peri-movement activity rises (i.e., near the end of the preparatory period, with stronger neural tuning than the earliest stage of the delay period) and the latter is in the early stage of the peri-movement period before sensory signal comes to the motor cortex (i.e., mainly motor signal).

For the neural trajectory analysis, we chose time windows -150 to +150 ms from target onset, -50 to +50 ms from the go cue and -200 to +400 ms from movement onset to cover the time periods before movement preparation, during movement preparation and during movement execution.

For the neural repertoire analysis, we chose the time windows -400 to -100 ms and 0 to 600 ms from movement onset and averaged the neural activity every 100 ms, because we wanted to denoise the data (by averaging over time) and meanwhile generate multiple before- and after-learning states for each reach direction (by choosing a larger time window) to more thoroughly represent the before- and after-learning neural pattern repertoires.

In the revised manuscript, we added a plot of neural trajectories in PCs 1-3 (Extended Data Fig. 15, and copied below for convenience) to help demonstrate our motivation for choosing those time windows:

1. The -50 to +50 ms time window around the go cue, indicated as the gray area, is within the preparatory period and has strong neural tuning reflected by the first three PCs.
2. The 0 to +600 ms and 0 to +100 ms time windows from movement onset chosen for peri-movement neural repertoire and neural state analyses do have strong peri-movement activity.
3. Peri-movement activity starts to rise around 150 ms before movement onset. So the -400 to -100 ms time window from movement onset for the preparatory neural repertoire analysis is not precise because it partially overlaps with the peri-movement period, as pointed out by the reviewer. In the revised manuscript, we chose the -500 to -200 ms time window from movement onset for the preparatory neural repertoire analysis (see our response to the next question).

Despite using new time windows, our key results remained the same. We thus think that the time windows we chose did not have a significant impact on the neural effect we observed.
Extended Data Fig. 15 | Example PCs 1-3 during time windows -100 to +100 ms from target onset (TO), -100 to +100 ms from go cue (GC), and -200 to +400 ms from movement onset (MOO) in each trial.

An example session from monkey U and representative of the other sessions from both monkeys. We applied PCA to trial-averaged neural activity for different reach directions in the before-learning block. Different colors correspond to different reach directions in the before-learning block and color scheme is the same as in Fig. 2a. Neural trajectories in PCs 1-3 are bundled together around the target onset time window and diverge around the go cue time window (i.e., during preparatory period). The time window -50 to +50 ms from go cue (gray shadow) we chose for preparatory neural state analysis is within the preparatory period (near the end of it) and has stronger neural tuning than earlier time windows (e.g., the first 100 ms after target onset).

In addition, one might expect the time window used for the preparatory epoch to largely focus on activity before and perhaps only very slightly after the go cue. The -50ms to 50ms time window around the go cue for the neural state analyses seems reasonable. However, the -400ms to -100ms from movement onset window used for the neural repertoire analyses likely starts to overlap with movement-related activity. Again, some clarification should be provided as to why this time window was chosen and what was the impact on the computed PCs and state estimates. If, for the “neural repertoire analyses”, you choose either a window which starts and terminates substantially earlier (i.e., so as to avoid potential overlap with peri-movement neural activity) or the same window as was chosen for the “neural state analyses” what happens?

Thank you for alerting us that this needs further clarification. As described above, for the preparatory neural repertoire analysis, we chose the wider time window -400 to -100 ms with respect to movement onset, because we wanted to denoise the data (by averaging over time) and meanwhile generate multiple before- and after-learning states for each reach direction (by choosing a larger time window) to more thoroughly represent the before- and after-learning neural pattern repertoires.

However, the neural trajectories in PCs 1-3 (see above and Extended Data Fig. 15) show that peri-movement activity starts to rise around 150 ms before movement onset. Therefore, we agree with the reviewer that the -400 to -100 ms time window from movement onset for the preparatory neural repertoire analysis was not precise because it partially overlapped with the peri-movement period. In the revised manuscript, to avoid the peri-movement activity, we chose the -500 to -200 ms time window from movement onset for the preparatory neural repertoire analysis. We found significant neural repertoire changes after learning the curl field (now new Fig. 3c),...
which was similar to our findings using the -400 to -100 ms time window from movement onset. The new plots of the preparatory neural repertoire change are used in the revised manuscript and copied below.

Despite using new time windows, our key results remained the same. We thus think that the time windows we chose did not have a significant impact on the neural effect we observed.

Fig. 3 | ... c. Preparatory neural state repertoires change similarly for trained and untrained reaches. Black: control sessions in which monkeys did thousands of center-out reaches without any force field; blue (far tgt): far targets more than 45 degrees from the trained target in learning sessions; red (near tgt): near targets within 45 degrees from the trained target in learning sessions (one-sided Wilcoxon rank-sum test: monkey U, $P_{12} = 8.24 \times 10^{-11}$, $P_{13} = 1.01 \times 10^{-9}$, $P_{23} = 0.96$; monkey V, $P_{12} = 5.22 \times 10^{-5}$, $P_{13} = 2.33 \times 10^{-7}$, $P_{23} = 0.059$).

Lines 476-511: I found this section describing the targeted dimensionality reduction approach you used generally difficult to follow and would recommend revision to make it more accessible to the general reader. I include here only a few specific comments.

Thank you. We revised the TDR part in the methods section to make it more accessible to the general reader (see lines [707-751]), and in addition incorporated the next three specific comments.

Line 483: In this equation should $\beta_x$ be $\beta_{i,x}$, $\beta_y$ be $\beta_{i,y}$, $F_x$ be $F_x(k)$and $F_y$ be $F_y(k)$? Should $\beta_{i,j+1}$ be $\beta_{i,n+1}$?

Thank you, yes, this typo is now fixed.

Line 491-495: It wasn’t clear to me what you mean by building the 2D hand-force space “without binary indicators (n=0)”. Does this mean that you built this space without taking into account whether a given trial was an error-clamp trial? Was this space built using only before-learning trials? If so that should be clarified here. I was also unclear on how the 3D TDR model was built as it sounds as if this model was built using both before-learning and after-learning trials. In general I did not follow the approach you used here very well.

Thank you for pointing this out to us. Yes, this 2D hand-force space was built using only before-learning trials, and therefore we did not include the indicator variable of whether or not a given trial was an error-clamp trial or a before-learning trial. We have added this clarification to the Targeted dimensionality reduction (TDR) part of the methods section (see [lines 722-725]).

On the other hand, the 3D TDR model was built with both before-learning and error-clamp trials, using hand forces and a binary indicator of trials types as regressors: an indicator 0 represents before-learning trials, and 1 represents error-clamp trials. Before-learning and error-clamp neural states projected onto this indicator axis were separated uniformly for all reach targets. We have added more details on the 3D TDR model to the methods section (see [lines 732-735]). To help demonstrate the results of the 3D TDR model, here we present plots of (1) rotatory neural shift in the hand force 2D TDR plane (left panel), and (2) the uniform shift of error-clamp preparatory neural states from before-learning states along this indicator axis (right panel) in an example session (up target was the trained target). These plots have been added to the revised manuscript as Extended Data Fig. 22.
Extended Data Fig. 22 | Neural preparatory states in the expanded 3D TDR subspace.
The 3D subspace was constructed by TDR capturing the variance due to initial hand forces and a binary indicator of the trial condition (before-learning vs. after-learning error clamp trials). a, In the 2D hand-force subspace (defined by TDR 1 and TDR 2 axes), preparatory states before-learning (color circles) radially organize as a ‘ring’ corresponding to reach targets; preparatory states of error-clamp reaches (color diamonds) consistently rotate in an opposite direction to the curl field direction for targets within 45 degrees from the trained target. One example and representative session (monkey U). These rotatory neural shifts are very similar to what we observed in Fig. 2d. b, Along the TDR 3 axis, this 3D model revealed a uniform shift during learning similar to what we observed along PC 3 in the PCA subspace (Fig. 3a). One example and representative session (monkey U).

Line 504: Should the beta coefficient matrix in this equation be transposed? I had trouble following how the dimensions work out here.
Thank you for catching this mistake. The beta coefficient matrix should be transposed, which is now fixed.

Lines 523-532: The definition of the uniform shift axes is quite critical to interpreting the results but, as noted above, I found the description here somewhat confusing and would recommend clarification particularly for a general audience. Of particular relevance is to make clear whether when you quantify shifts along these uniform shift axes you are always talking about shifts that are orthogonal to behavioral force output.

We apologize for not defining the uniform-shift axes clearly. These uniform-shift axes for learning or washout of different fields were all orthogonalized against the force-related subspace (defined by TDR 1 and 2 axes) before we did all quantification analyses; they were also orthogonalized relative to each other before we projected and plotted preparatory states along those uniform-shift axes. We did this because we wanted to investigate the role of the uniform shift fully independently of TDR axes, and more generally, to investigate neural population patterns related to learning but not directly reflecting movement kinetics / kinematics.

In the methods section of the revised manuscript, we have made it clear that the uniform-shift axes were always orthogonalized against TDR axes before we quantified them (see [lines 771-772]).

Line 534: When you talk here about orthogonalizing uniform-shift axes are you now referring to orthogonalizing the axes for learning or washout of different fields relative to each other or relative to the force output?

Thank you for this important question. As described in our answer to the last question, these uniform-shift axes for learning or washout of different fields were all orthogonalized against the force-related TDR subspace (defined by TDR axes 1 and 2) before we did all quantification analyses. They were also orthogonalized relative to each other before we projected and plotted preparatory states along those uniform-shift axes.

We have updated the methods section [lines 772-774] with the clarifications described above.
The description of how you computed the control distributions was difficult to parse and could be clarified.

We apologize for not explaining the control distributions clearly. In the methods section of the revised manuscript, we further unpacked and reworded how we generated the control distributions for parallel, antiparallel and orthogonal uniform-shift axes. We have copied below the updated methods for the control distributions for convenience:

[lines 795-813] To quantitatively test for these geometric relationships, we compared the observed uniform shifts to control distributions generated for each relationship. Each control distribution was defined by a set of before- and after-learning preparatory states resampled from the observed data, and computed in the following ways to reflect the corresponding geometric relationship: (1) Parallel / antiparallel case: for each learned curl field, we resampled its before-learning and after-learning trials and measured the dot products between all the resampled uniform-shift axes. Without noise intrinsic to the data, these resampled uniform-shift axes for the same curl field should be truly parallel and the dot product should be 1. The antiparallel distributions were constructed from taking the inverse of the parallel distributions but with a separate independent set of resampling. (2) Orthogonal two-field case: we first used all trials of learning two different curl fields applied to two different reach targets to define their trial-averaged uniform-shift axes, as described in the last section. We orthogonalized these two trial-averaged uniform-shift axes, denoted as \( \vec{v}_1 \) and \( \vec{v}_2 \). We then resampled trials of learning these two fields: in the \( i \)th round of resampling, we defined and orthogonalized the resampled uniform-shift axes for the two fields, denoted as \( \vec{v}_{1i} \) and \( \vec{v}_{2i} \) \( (i = 1, 2, \ldots, n) \). We measured the dot products between \( \vec{v}_1 \) and \( \vec{v}_{2i} \), as well as between \( \vec{v}_2 \) and \( \vec{v}_{1i} \), because without noise intrinsic to the data, they should be truly orthogonal and the dot product should be 0. (3) Orthogonal washout case: we applied the same procedure as in the orthogonal two-field case, except that we used learning and washout trials of one single field rather than learning trials of two different fields.

Lines 574-577: When you refer to neural trajectories here are you referring to the neural trajectory in PC space over the time course of an entire trial (e.g., Fig. 4)\)?

The neural trajectories were plotted over the time course of -500 to +500 ms from movement onset in the PCs 1-3 subspace. This time window covered the end of the center-hold period (before target onset), the preparatory period, and the peri-movement period. Although it did not cover the time course of an entire trial, the left-out time points were either within the early center-hold period (i.e., several hundred milliseconds before the target onset and so all neural trajectories were bunched together) or after the reach target was acquired (so all neural trajectories joined together again).

In the revised manuscript, we made a new neural trajectory plot with three alignments: -150 to +150 ms with respect to target onset, -50 to +50 ms with respect to the go cue, and -200 to +400 ms with respect to movement onset (now Fig. 5). The new trajectories extend time points before the preparatory period, during the preparatory period, and during the entire reach. We have copied the new neural trajectory plot here for convenience.
Fig. 5 | Neural trajectories of before-learning, late-learning, late-washout and late-relearning conditions (-150 to +150 ms from target onset, covered by the gray circle; -50 to +50 ms from the go cue, covered by the gray ellipse; and -200 to +400 ms from movement onset). The movement preparation and execution periods are noted on the trajectories. The late-washout trajectory (green) is less similar to the before-learning trajectory (black) during the preparatory than the movement-execution period. TO: target onset. GC: go cue.

References


Reviewer Reports on the First Revision:

Referee #1 (Remarks to the Author):

This is a resubmitted version of a paper I previously reviewed. The authors have done a very thorough job responding to my comments and those of the other reviewers, with substantive additions to the analyses and presentation. I believe now that the results are robust and represent a substantial advance in our understanding of the neural basis of force field adaptation and motor learning more generally. I do have some further questions, mostly suggestions, which are detailed below more or less in order of how they come up in the revised manuscript.

1. I find the title unclear -- more generally, I am confused about the relationship between skill and motor learning. Are skills the things that develop during the kind of motor learning paradigm being studied here? I am not sure that would be the general take. I would probably go away from skill in this experimental context which would change the title and text throughout. If the authors want to stretch their neck out with skill then may as well go all the way and drop the "during motor learning" and re-crafting the sentence around skill much like they have it in the Abstract. Such a title would be more accessible to non-experts.

2. In the Abstract, the authors introduce the goal ("Here we ask...") then talk about what the monkey did (We trained...) and then the hypothesis ("We hypothesize"). The back and forth flips in present and past tense is a bit confusing. I would either switch the hypothesis to past tense (in which case also switch the one lower down, line 34) or change the order to put the hypothesis in present tense after the "here we ask" sentence.

3. I find lines 53-55 problematic. First, it takes a bit long to get into on line 53, I would delete the sentence starting with "collectively" and go straight to the sentence with starting with "However,..." but deleting the However and making it sharper. I worry that the more general reader will not understand what this is all about and what the possibilities are and they will need to if they are going to keep reading.

4. Line 177. I think this VMR finding is too easily missed. The "furthermore" and it being mid-paragraph does not help accentuate it. I would suggest saying a bit more here to make sure the reader gets this point now rather than in the discussion.

5. As a general point, there are a lot of "see Methods" throughout the Results. In the Methods the authors do a great job of pointing out for which figures that part of the Methods is related. However, coming from the Results to the Methods it's a hard link to make and that makes the paper hard to digest given how analytically loaded it is. I would suggest that the authors point to a specific subheading in the Methods in these calls (e.g. see Relearning Experiments in Methods). Some new headings may be required.

6. Lines 478-480. The authors have done a nice job summarizing the Rokni paper and how it differs from their own. But then they conclude that background drift is simply not the same thing as uniform shift. That may be true but it seems like a bit too much of a shrug. When I suggested this paper, I was hoping/expecting the authors to respond by contrasting the analytical/experimental approaches and the lever that it gives the present paper. If the authors think they can do this fairly to the Rokni work, I think it would be useful in terms of highlighting the novelty and impact of this work.

Andrew Pruszynski
Referee #2 (Remarks to the Author):

Sun and colleagues have written a paper that is easy to read, thorough and rigorous in its approach, and most of all, teaches us something important that we didn’t already know about motor cortex. To conduct this re-review, I first read the paper from scratch. Then I read carefully the team’s response to my first review. I then perused the other two reviewers’ comments, and Sun’s responses. In all, I judge that the authors have improved the paper in light of the reviewers’ comments. Even in its current form, the paper now resembles those that rise to the level of Nature. Below are suggestions that the author may wish to consider if they would provide for further improvements. To start, I comment on why I think this paper is likely to be influential.

Some of the strong suits:
- Figure 3 shows a quite remarkable result. It didn’t have to turn out this way. And, having seen this, the interpretation is straightforward and powerful. It’s this result, in my opinion, that’s going to really get people thinking. It’s especially cool that this uniform shift occurs even for untrained targets. It’s really like this is a context signal coming into M1 from somewhere else, probably via somatosensory cortex, as soon as the animals “feel” the curl field.
- Likewise, figure 4 did not need to show as clean and straightforward a result as it did.
- Clarity about nomenclature is an additional nice touch, and more clear statements about terminology like this are needed in the field. Overall, the methods section itself is a contribution to the literature.

Potential improvements:
- Could figure 2 have turned out any other way? While it’s nice that the neural implementation of “re-aiming” is so straightforward and direct (and, impressive that their methods could reveal it), I wonder, can the authors somehow elevate the impact of this, the paper’s first figure showing neural data? Is there an argument to be made that something different could have happened here? Or, another way to elevate the significance of figure 2 might be to explore, what are the larger consequences of this finding? The authors mention that the same “re-association” is seen during VMR learning and BCI learning. Can we claim from this result that even in curl field adaptation, there is a VMR-like re-aiming component? Do these results indicate that we can safely equate “re-aiming” with “re-association”?
- Regarding figure 3, is it interesting that this shift is “nearly orthogonal”? That is, on one hand, the shift better not have a strong projection onto the TDR space of the targets, since it would interfere with movement to the targets. So, it makes good functional sense that the shift would be orthogonal. On the other hand, most arbitrary lines are going to be nearly orthogonal to an arbitrary plane in 244-D space.
- Are these really “new” neural activity patterns? One might argue that any patterns seen within a couple hundred trials are nothing but discovering new motifs of an existing circuit, or exploring the pre-existing repertoire. Is “new” simply anything that’s outside the repertoire observed during a baseline task? This would be like, “new to the experimenter” but probably not “new to the monkey”. It might be helpful to distinguish three senses of “new patterns”: first, those that lead directly to learning-related behavioral improvement. Second, those that are specific to the behavior, but do not directly cause it. Third, those that accompany learning, but are unrelated to the new abilities. I would say that the authors’ findings belong in the second category. (And incidentally, most studies of learning might actually belong in only the third category.) The language on line 450 conflates the first two categories, and it might be worthwhile to clarify - part of what’s implied by the “indexing” function is that the new neural activity patterns that implement the indexing, by construction, cannot directly drive behavior, so “new” in the sense of the first
category isn't an option here. If the authors agree this is a valuable distinction, then providing a definition after line 896 would benefit the field.

- Along the lines of parsing nomenclature, it might help to have one place where the authors’ claims are laid out very directly. Shorthand words obviously can't do justice to the whole concept, but that caveat aside, it seems the key claim here is “reassociation occurs in the TDR target subspace and new activity patterns appear orthogonal to it.” It might be worth it to be explicit about this somewhere. (Forgive me if I overlooked an explicit statement to that effect.) Along these lines, if I’ve kept track of all the nuances, TDR reassociation occurs during VMR learning, but new patterns don’t show up during VMR learning. All this is worth summarizing somewhere prominent.

- Is adaptation a skill? If so, what should we call something like throwing a baseball or playing piano? That is, shouldn’t we reserve that term for behaviors that bear some resemblance to things most people think of when they think of skills? By the authors’ terms, they are studying “skill maintenance” here. And thus perhaps the title over-promises what is actually delivered, since readers will probably assume “skill acquisition” is what’s referred to in the title.

- Can we really rule out postural shifts just by looking at baseline responses? What if posture has a gain effect (as has often been reported, going back to the 1980s) rather than an additive effect? Would you be able to see it in the baseline?

- Overall, in the paragraph beginning on line 457, the most important point is the very last one. Namely, the clean and sensible (at least in hindsight) geometrical relationship observed here demands an explanation. The one the authors offer is clean and elegant, and for those who do not wish to accept it, the burden of proof is on them to come up with a compelling alternative. Without getting as argumentative as that sounds, it might be a “best foot forward” construction for this paragraph to to say, "It’s hard to find an alternative explanation than the “indexing” we propose. However, before we can draw this conclusion, we must first consider a few learning-irrelevant explanations.”

Suggestions:
- Line 150: Since you’re already defining PCA, it’s worth it to explain that the dimensions are ranked by variance accounted-for. That will help when you next refer to PCs 1, 2, and 3.

- Line 164: It’s a little jarring to read (paraphrasing) that “the uniform-shift axis was nearly orthogonal to the reassociation space” and then “we made it orthogonal”. It will help to explain why this was done, and why it’s okay to do (e.g. it doesn’t “bake in” any subsequent results.)

- Fig 5b caption - make it clear what each point is. (Presumably one point per a trial; all sessions shown.)

- Fig 5j is probably going to look nicer if the grid lines are more spread-out and/or a lighter gray.

- In the very final sentences, the cerebellum and basal ganglia are mentioned. That’s fine, but it leaves out much. In light of the fact that the learning here seems to be mostly preparing to counteract the push of a curl field, it seems prudent to mention haptic areas (somatosensory cortex, thalamus, and dorsal spinal cord) as among those that might be responsible for learning in this task.

- Extended figure 3 title seems to be missing the word “in” - “neural variance *in* different dimensions”. Also, that figure has some conspicuous whitespace. Why not sketch in a little geometrical definition of the dot product and variance explained metrics?

- Extended figure 15 would benefit from an inset showing the target location - to - color
relationship.

Journal Review Criteria:

Summary of the key results
Provided above.
Originality and significance: if not novel, please include reference
Findings are original and significant.
Data & methodology: validity of approach, quality of data, quality of presentation
Methods are valid, data is of top quality, presentation is dense but appropriate to justify the claims.
Appropriate use of statistics and treatment of uncertainties
Statistics appropriately deployed; uncertain is dealt with well by the use of models.
Conclusions: robustness, validity, reliability
Conclusions are robust and justified.
Suggested improvements: experiments, data for possible revision
Mild text revisions would improve things. New experiments and data are NOT necessary to support the claims.
References: appropriate credit to previous work?
Yes.
Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Yes, lucid and clear.

Referee #3 (Remarks to the Author):

General Comments:

Overall the revised manuscript is considerably improved in terms of presentation and clarity. The authors have made substantial revisions to write parts of the manuscript in a more hypothesis-driven manner which brings out the key motivation for the work and emphasizes why it is of broad relevance. They have also clarified many aspects of the methodology which were difficult to follow or where information was missing. Thus, they have addressed many of my concerns with respect to this issue (but see a few comments below). In addition, they have made exceptional efforts to provide additional quantification of the experimental results as well as to include new results (even during COVID) to help support their conclusions. Many of my previous concerns have been very well addressed and the efforts of the authors to clarify my many questions are most appreciated. However, after careful rereading of the manuscript and consideration of the extensive additional material the authors have provided I find that while aspects of this work are certainly novel and important, I am not yet convinced of the conclusion. As outlined below, my strong feeling is that the relevance and importance of this study can best be appreciated in a more expanded format (i.e., slightly more specialized journal) that allows sufficient space to more fully address the results and their implications in the context of previous work as well as the limitations in interpretation.

In particular, I am enthusiastic about the notion that the uniform shifts in preparatory activity that the authors have observed along neural population dimensions orthogonal to force/motor output could reflect a potential mechanism for keeping track of and retaining memories of different motor skills while minimizing interference between them (i.e., that these shifts in activity reflect a sort of "indexing function"). However, despite my enthusiasm about the idea, after re-reviewing the manuscript I still don't feel completely convinced of aspects of the authors' conclusions (much as I would like to be) for a couple of reasons:

1. The first is that while I am convinced that the uniform shifts observed over the course of learning indeed reflect a change in repertoire relative to before learning and that it is task-specific,
I am not yet convinced that the shifts are not closely linked to the motor output. As outlined below, I have a number of remaining questions with regards to this. Perhaps one could argue that the relationship between the shifts and motor output is not the most important issue, but after careful consideration I do think this is an essential issue in terms of level of novelty. If the uniform shift is not in fact decoupled from the motor output, it may still reflect a mechanism for keeping track of and retaining distinct memories of different motor skills. The results would still be novel in several respects and make an important contribution to furthering our understanding of skill learning at the neural population level. However, if the shift does have a direct relationship to the motor output I also think that the novelty of the study would be considerably reduced, as other studies have previously addressed aspects of such results and/or their conceptual underpinnings (e.g., Li et al., 2001; Rockni et al., 2007; Perich et al., 2018). It is the idea that preparatory activity acts as an “indexing function” largely independent from motor output that I think is most novel here and so I do feel that convincing the reader of this is important when it comes to publishing this study in Nature.

Admittedly I am not an expert on the analysis techniques that are being used here (whereas the authors certainly are) so I think it is highly likely that with some additional clarifications and analyses the authors could indeed resolve my remaining questions/confusion regarding the relationship between the observed shift and motor output. However, even so, there remains the question of whether Nature is the best venue for this manuscript. My feeling is that it is likely that a substantial proportion of readers would have many of the same questions and concerns that I and the other reviewers have raised. For the most part, I think the authors have done an excellent job of addressing them but this has required providing substantial additional information largely in the form of extended data figures (of which there are now 22). In view of this, my conclusion is that the relevance and importance of this study can best be appreciated by presenting the results in a slightly longer format that allows sufficient space to fully discuss a broader range of results, their implications, and their novelty in a more comprehensive fashion (i.e., in a more specialized journal than Nature).

2. Assuming that one can be completely convinced that the uniform shift is truly taking place along a dimension orthogonal to motor output, a second issue (also previously raised by reviewer 1) is that while the results would be highly suggestive of a putative mechanisms for savings and the ability to learn and retain multiple motor skills, actually proving this is much more difficult. The authors have done an admirable job, under the circumstances, of attempting to provide some more concrete evidence for this through their new interference tasks results. Despite this, I am not convinced that these new results add much beyond what was already illustrated in Figures 4 and 5, although I do think they provide a potentially interesting population-level illustration of how preparatory states shift uniformly during interference (i.e., the interference shift). However, approximately 1.5 pages in the main text (lines 243-319) are dedicated to the description of these results which appear only in Extended data figures and in my opinion this was detrimental to the flow of the article. Again, my impression is that this work can better be appreciated if the results are presented in a slightly longer format in a more specialized journal where there is more space to address their relevance and implications.

Major Comments:

1. As noted above, despite many clarifications regarding the methodology that the authors have provided I still have a few questions about the relationship between the uniform shift and motor output. In particular, since my first reading of this manuscript I am now questioning whether it makes sense to only use before-learning trials to build the 2D TDR subspace. It seems to me that in a dynamic learning task it would not be surprising to find that this subspace changes. More specifically, the idea that similar neural activity patterns are activated but “reassigned” to a new reach direction makes sense in the context of a visuomotor learning task, but for learning a dynamic skill we might expect there to be at least some new associations between neural activity
patterns and force. In that case, could it not be that the TDR1 and TDR2 axes defining the 2-D hand-force subspace change over the course of learning in a way that could potentially include a component orthogonal to the orientation of that subspace before learning? My concern is that, if this is the case, the observed uniform shift while clearly reflecting a new repertoire, might not in fact reflect a repertoire completely orthogonal to force output.

I think that this is really the same or a similar issue to that raised by Reviewer 2 who suggested that the learning-induced shift axis could be a force axis. The authors have already responded to this but it seems to me that an important assumption underlying their response is that the 2-D hand-force subspace is not changing over the course of learning (i.e., or at least not changing in an orthogonal direction relative to before learning). Has this actually been verified? I do realize that some attempt to address this has been made by using the TDR space built based on before learning trials to predict forces after learning, but I have some concerns about the difference between a strong correlation vs an accurate prediction (see Minor comments below).

In general, while much of the analysis is based on the 2D TDR space built using only before learning trials, other analyses combined before learning and error clamp trials and overall it is not clear to me how well the TDR axes and/or the planes they span were aligned with each other across these different analyses. Perhaps, most importantly, what I would really like to know is if the TDR hand-force subspace is instead built based only on error-clamp data after learning what is the orientation of that subspace relative to the 2-D hand force subspace built based on before learning trials? Do the TDR1/TDR2 planes remain aligned?

2. I remain somewhat confused about what PC3 might represent. Specifically, while the uniform shift appears predominantly along the PC3 axis I can see from Extended Data Figure 15 that PC3 activity appears substantially tuned to movement direction. One could almost say it seems to reflect preferred directional tuning along an axis somewhere between the 45o and 90o target directions (although maybe that’s a stretch). However, I was somewhat surprised by this as I would not have expected a PC dimension that is completely unrelated to behavioral output to reflect so much directional tuning. Could the authors comment on this and provide an explanation/interpretation?

In addition, I think it would be useful to also see: 1) What PC4 looks like and how much of the variance it accounts for; 2) What all the top 4 PCs look like both before learning as well as after learning in error clamp trials. Does an uneven sampling of movement directions have any impact on these analyses?

3. Ruling out all possible “trivial” explanations for the uniform shift across movement directions, including ones to which learning did not generalize, is admittedly an almost impossible task. The authors have already made substantial efforts to rule out, to the extent possible, a number of issues such as postural changes, muscle co-contraction, the "error-clamp state", hand speed, etc. However, one thing I noted when examining Extended Data Figures 5 and 6 is that the reduction in flexor activity (i.e. observed in the Bicep and Pectoralis muscles) associated with learning to compensate for a CW curl-field during down reaches (Ext. Data Fig. 5) remains apparent, albeit to less of an extent, when reaching to a “far” target 135 deg away (Ext. Data Fig. 6). Specifically, Biceps and Pectoralis activity during error clamp trials appears to be reduced by ~30% which is not negligible. Is this something that is seen uniformly across directions? Similarly, extensor activity (Lateral/Posterior deltoid) seems slightly increased. Given these observations I think it makes sense to more thoroughly investigate changes in individual muscle activities across all directions (but separately for learning different fields in different directions) to see what evidence there may be for a component of muscle activity change that is uniform across directions.
Minor Comments:

Fig 1. legend: It would be useful here to clarify in part (d) that the no learning data comes from comparing error-clamp and null-field trials (from the 1st block?) in the "center-out reach control" experiment.

Fig 2 legend: It would be useful to indicate in part (a) which monkey(s) the data comes from.

Lines 121-124: Here you indicate that projecting after learning trials into the 2D TDR hand-force subspace built using before learning trials yielded predictions of the new hand forces which were strongly correlated with the observed ones and exhibited high accuracy. I agree that the real hand force is indeed well correlated with the prediction but when it comes to accuracy it seems that we should know something about the slope of a regression between actual and predicted values and how close the slope is to 1 (intercept is to 0) to know how accurate the predictions made based on the before learning 2D TDR subspace are.

Lines 243-319: As noted above, this section describing the results of interference experiments provides a reasonably detailed description of results which only appear in Extended data figures and I felt this substantially detracted from the flow of the article. These results are potentially interesting but in my opinion should be presented in a format in which the data figures can be integrated with the main text.

Extended Figures 10c and 11c: These figures are very crowded and I found it difficult to see much. For example it wasn’t clear to me whether there was any shift at all in the top panels of Figure 10c and my impression was that the little triangles for monkey V on the bottom right were not pointing in the correct direction. I could easily be wrong but the point is that I think the presentation here could be improved perhaps by focusing on the targets of interest and removing the others.

Lines 295-298 and Figures 10e,11e: It seems that the most prominent (and new) observation here is that there is a considerable uniform shift associated with the interference environment (i.e., attempting to simultaneously learn opposing curl fields). Although the authors suggest that this shift may reflect an "attempt to learn or index neural activity patterns for reaching in an unpredictable environment" it seems to me that most likely explanation for this shift is that it reflects the preparatory activity associated with muscle co-contraction (changing limb impedance). Is that what the authors meant by their statement? Did they look at muscle activity?

Lines 480-484 and Extended Data Fig. 18a: Here you provide evidence that preparatory states in error-clamp and no-clamp trials are not significantly different in the no-learning control sessions. Overall (i.e., across all reach directions) that does seem to be the case but it also looks as if for certain individual reach directions there might be substantial differences between error-clamp and no-clamp states. One problem is that it’s hard to see this clearly (and this could easily be an illusion) since preparatory states are plotted here with a different perspective as compared to most other plots (e.g., Fig 18b, 3a, 5c). Can the authors comment and show the plot in Fig 18a with a similar perspective to that in Fig. 18b?

Line 532: In the end, how much learning data was collected for a right reaching target as compared to up/down reaching targets? It sounds to me as if there was much more up/down reaching data collected and this makes me wonder if this could have biased the analyses/results in any fashion.

Line 604: In block ii of the control experiment (i.e., replacing the learning block in learning task experiments) were null-field trials made to a single target as in learning experiments or were reaches more or less evenly distributed across the 12 targets? Similarly, in block iii were many null-field trials made to 1 target with only error-clamp trials made to the other targets (i.e., similar to a learning experiment) or were both null-field and error-clamp trials distributed across
directions more or less evenly? What was the relative percentage of error-clamp vs null-field trials in the error clamp block?

Line 759: I’m still not entirely sure what ”using center-out reach trials matching the trial IDs in a learning session” means. Were error-clamp trials with a particular trial ID in block iii of the learning task also error-clamp trials in block iii of the control task such that when you looked at changes in neural repertoire in the control task you were also comparing null-field to error clamp trials?

Lines 831-832: For the interference experiment data analyses you now used both before-learning and error-clamp trials to build the TDR model. Why not only use only before-learning trials as in the previous analyses? Does it make any difference whether you use only before-learning trials, only error-clamp trials or both in these analyses?

Typos:

Abstract, line 36: Should be “shifts”. 
We wish to sincerely thank the reviewers for their many insightful and helpful comments and questions throughout the review process, including the current comments and suggestions. We believe that we have comprehensively addressed the questions raised through additional analyses, clarification in the text and overall streamlining of the figures and text. More specifically on this last point, we reorganized many figure panels, particularly in the Extended Data, and streamlined the text to further increase its accessibility.

In particular, the central question raised by the reviewers regards possible alternative explanations for the uniform shift which may not relate to motor learning. While we had already carefully studied these alternative explanations internally, and included some discussion of these in the manuscript in various places, the reviewers’ comments indicate a clear request and opportunity for us to better convince the readers that these alternatives can be empirically rejected. Consequently, in our revision, we have collected and organized a comprehensive discussion of the various alternative explanations for the uniform shift into a detailed writeup (Supplemental Note 2), and within it, we have provided extensive methodological, analytical, and empirical evidence that summarizes quantitatively how much of the uniform shift we observe could possibly relate to any of these potential confounding variables, including all those enumerated by reviewers (i.e., the “background changes” in the Rokni et al. work [Reviewer 1], the postural gain effect [Reviewer 2], changes in EMG activity [Reviewer 3], and changes in hand forces [Reviewer 3]). Our results reveal that these potential variables account for only a very small amount of the uniform shifts. We include a detailed description of these new analyses performed and the reasoning for each analysis along with a clear description of how these variables could influence preparatory activity alongside the results of each analysis and our interpretation of each result. This is now all clearly organized in Supplementary Note 2 and Supplemental Table 1. By treating all of these variables in the same analytical (regression) framework, we believe that this straightforward evidence should assuage the questions / concerns, as well as provide a single, linear reference for the reader to consult.

We have also adopted the extremely helpful suggestions from Reviewer 3, and used this opportunity to considerably reorganize and simplify the manuscript. We have rearranged many figure panels (including main figures and extended data figures) as well as the corresponding text, and moved redundant panels to extended data. This allowed us to integrate the important interference experiment results within the main figure flow as Figure 4, as recommended by Reviewer 3. The new manuscript contains 5 main figures with substantially reduced size (roughly 50% reduction) and 10 thoughtfully reorganized extended data figures (compared to 22 in the previous version), which is consistent with Nature’s format. We believe that these changes help
continue to ensure that we report all of the findings thoroughly while also focusing on the key major results in the main text and main figures.

Sincerely,
Xulu, Dan, Krishna and the additional authors
Author Rebuttals to First Revision:

Point-by-point reply to Reviewers

For ease of reading, we have included below all reviewers’ comments in blue text, our replies to the reviewers in black text and the new modifications made to the manuscript are italicized.

Reviewer #1:

This is a resubmitted version of a paper I previously reviewed. The authors have done a very thorough job responding to my comments and those of the other reviewers, with substantive additions to the analyses and presentation. I believe now that the results are robust and represent a substantial advance in our understanding of the neural basis of force field adaptation and motor learning more generally. I do have some further questions, mostly suggestions, which are detailed below more or less in order of how they come up in the revised manuscript.

Thank you very much for your kind words. We are pleased and gratified that our extensive revisions in response to your and the other Reviewers’ suggestions were viewed as having done a “very thorough job” such that the “results are robust and represent a substantial advance in our understanding”. We deeply appreciate your time and extremely insightful suggestions.

1. I find the title unclear -- more generally, I am confused about the relationship between skill and motor learning. Are skills the things that develop during the kind of motor learning paradigm being studied here? I am not sure that would be the general take. I would probably go away from skill in this experimental context which would change the title and text throughout. If the authors want to stretch their neck out with skill then may as well go all the way and drop the “during motor learning” and re-crafting the sentence around skill much like they have it in the Abstract. Such a title would be more accessible to non-experts.

Thank you for pointing out this potential concern regarding using the term “skills” in the title and main text. We agree that upon further reflection, as we were urged to do here, that this title is potentially confusing, and therefore we have updated the title to hopefully avoid this confusion:

Cortical preparatory activity indexes learned motor memories

Here, we provide a bit of additional context regarding our rationale for this new title. Previously in the Methods section of our manuscript (copied below for convenience), we defined the behavioral terminology regarding skills according to Krakauer et al. 2019 and used those definitions consistently in the manuscript:
1. **Motor skill.** Motor skill involves precise execution of movements. Typically, motor skill also involves rapid selection of the right action in the right context.

2. **Skill acquisition vs. skill maintenance.** We adopt a two-part operational definition of motor learning: (1) skill acquisition is the process by which an individual acquires the ability to rapidly identify an appropriate movement goal given a particular task context, select the correct action given a sensory stimulus and/or the current state of the body and the world, and execute that action with accuracy and precision; (2) skill maintenance is the ability to maintain performance levels of existing skills under changing conditions. These two aspects of motor learning are each important in their own right, and they likely share overlapping neural circuitry. That said, it is also clear that the brain possesses dedicated mechanisms for skill maintenance (see Adaptation below).

3. **Adaptation.** It is a skill, but more about skill maintenance than acquisition, except for the first time encountering a new environment such as the curl field. The term adaptation is usually used in contrast to de novo skill acquisition which involves learning a new motor controller from scratch rather than derived from existing ones.

Based on these definitions by John Krakauer and colleagues, in this study we focus more on skill maintenance than skill acquisition by studying curl field learning, although new movements are generated during the learning process. However, as Reviewers 1 and 2 point out, when seeing “skills” in the title, readers are likely to assume de novo skill acquisition rather than the motor learning paradigm in this study. Therefore, we have decided to move away from “skills” in the manuscript.

As mentioned earlier, we now use a new title without “skills” and add in the term “indexing”, which, as recognized by all reviewers, is the major novel finding. *We have also removed “skills” from the main text and adjusted related sentences accordingly.*

In summary, we are grateful to have this brought to our attention as we are indeed more confident that this will not inadvertently mislead readers, perhaps especially casual readers.

2. In the Abstract, the authors introduce the goal (“Here we ask...”) then talk about what the monkey did (We trained...) and then the hypothesis (“We hypothesize”). The back and forth flips in present and past tense is a bit confusing. I would either switch the hypothesis to past tense (in which case also switch the one lower down, line 34) or change the order to put the hypothesis in present tense after the “here we ask” sentence.

Thank you for the suggestion. *We have modified the Abstract to keep the tense consistent.*

3. I find lines 53-55 problematic. First, it takes a bit long to get into on line 53, I would delete the sentence starting with “collectively” and go straight to the sentence with starting with “However,...” but deleting the However and making it sharper. I worry that the more general
reader will not understand what this is all about and what the possibilities are and they will need to if they are going to keep reading.

Thank you for kindly pointing this out, and we now see the problem. We believe that we have fixed this problem by doing exactly what was suggested: we deleted the “collectively, ...” sentence and “However” in the manuscript.

4. Line 177. I think this VMR finding is too easily missed. The “furthermore” and it being mid-paragraph does not help accentuate it. I would suggest saying a bit more here to make sure the reader gets this point now rather than in the discussion.

Thank you, we certainly agree that this point deserves additional emphasis and we see now how we inadvertently failed to highlight it sufficiently. We have now further elaborated on the VMR finding:

In contrast, we did not observe a uniform shift or repertoire change during VMR learning (Extended Data Fig. 2g). This result agrees with the re-aiming strategy reported in other VMR studies, which is a subset of reassociation (Vyas et al. 2018, 2020).

5. As a general point, there are a lot of “see Methods” throughout the Results. In the Methods the authors do a great job of pointing out for which figures that part of the Methods is related. However, coming from the Results to the Methods it’s a hard link to make and that makes the paper hard to digest given how analytically loaded it is. I would suggest that the authors point to a specific subheading in the Methods in these calls (e.g. see Relearning Experiments in Methods). Some new headings may be required.

This is a great suggestion, and we agree it should really help readers navigate more efficiently, thank you. We have added specific subheadings to the revised manuscript’s main text when pointing readers to the corresponding Methods section to assure consistency.

6. Lines 478-480. The authors have done a nice job summarizing the Rokni paper and how it differs from their own. But then they conclude that background drift is simply not the same thing as uniform shift. That may be true but it seems like a bit too much of a shrug. When I suggested this paper, I was hoping/expecting the authors to respond by contrasting the analytical/experimental approaches and the lever that it gives the present paper. If the authors think they can do this fairly to the Rokni work, I think it would be useful in terms of highlighting the novelty and impact of this work.
Our apologies for inadvertently appearing to have 'shrugged' this off in any way -- we would like to assure you that this most certainly was not our intent. We also thank you for this great suggestion to include a more detailed and methodologically-oriented comparison of the Rokni et al. 2007 paper and our study to help further highlight the novelty and impact of this work. In this revision, we have added a detailed analytical and quantitative comparison, together with a summary of the key findings in the Rokni et al. 2007 paper and our study, now colocated with a collection of other considerations regarding the uniform shift in Supplementary Note 2.

As in their paper, we partition the discussion of the background changes reported in Rokni et al. 2007 into two components - a drift in firing rate offset and drifts in preferred directions. Regarding the drifts in offset, Supplementary Note 2 now includes the following:

Rokni et al. (2007) report background changes of neural tuning curves that occur slowly over many trials during normal reaches in a familiar environment. These background changes occur in tandem with and on top of learning related changes accompanying curl field adaptation. Rokni et al. parcellate these changes into two sources: changes in the tuning curve offset and changes in the cosine-tuning. Here, we discuss the background changes in offset, and discuss the changes in cosine-tuning in the following section. Background changes in offset affect the firing rates observed for all reach directions, as did the uniform shift that we found during curl field learning. Because these changes in offset would use the same functional form as the true-uniform shift in Eqn. 5, it is possible for these changes in offset to create a uniform shift of neural states along an orthogonal axis in neural state space.

However, there are several important differences between our learning-related uniform shift results and the results reported in Rokni et al.. First, we note that Rokni et al. are reporting changes in the neural tuning during movement and do not study preparatory activity. We are not aware of any reports demonstrating similar background drifts in neural tuning curve offsets during motor preparation, and in particular no reports of drifts in offset that appear specifically during preparation and not during movement, which would be required to match the preparation-specific uniform shift we report here.

Second, and more critically, Rokni et al. find that learning-related changes in neural tuning (calculated between before-learning and after-washout trials in their learning experiment) are not significantly different from the background changes that they observe in the control experiment with no learning. That is, these background changes are not specifically related to learning. In contrast, the uniform shift we observed occurred only during learning. No uniform shift was observed either in control no-learning sessions of equivalent duration
(which matches the non-learning experiments of Rokni et al, Fig. 2h, Ext. Data Fig. 2e) or in control experiments with randomly delivered mechanical perturbations (which required generation of larger muscle forces in an unfamiliar environment, Fig. 2j). Furthermore, the background changes reported by Rokni et al. are considered to be the result of drift due to noise that is passively tolerated due to the inherent redundancy of a motor cortex with many neurons and few readout dimensions for behavior (i.e. a very large output null space). In our results, the uniform shift was specific to parameters of the learned curl field, both in terms of curl field direction and the directional target to which the curl field was trained (Fig. 3). For these reasons, we believe that the continuously occurring, non-specific background drift reported by Rokni and colleagues is fundamentally different from the learning-related uniform shift we observe during curl field adaptation, which we argue serves to index distinct motor memories. Furthermore, because no results are presented for motor preparatory activity in Rokni et al. (2007), we are unable to perform further direct, quantitative comparisons.

Regarding the changes in tuning direction, we explicitly assess whether changes in neuron tuning direction and the uniform shift we found. As discussed in the subsequent section of Supplementary Note 2, we have performed a regression analysis which directly quantified how much of the uniform shifts could be explained by changes in neuron tuning direction (see extensive Supplementary Note 2). The cross-validated $R^2$ values (0.18±0.04 for monkey U and 0.16±0.06 for monkey V) showed that changes in preferred tuning direction accounted for only a small fraction of the variance of uniform shifts (see Supplementary Table 1 which is copied below for convenience). Based on the quantitative results, we believe that the background drift reported by Rokni and colleagues and the uniform shift we found are different.

<table>
<thead>
<tr>
<th>Model formulation</th>
<th>Equation</th>
<th>CV $R^2$ ± s.e.m.</th>
<th>Monkey U</th>
<th>Monkey V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in endpoint forces</td>
<td>Eqn. 6</td>
<td>0.06±0.02</td>
<td>0.11±0.06</td>
<td></td>
</tr>
<tr>
<td>Changes in direction tuning</td>
<td>Eqn. 10</td>
<td>0.18±0.04</td>
<td>0.16±0.06</td>
<td></td>
</tr>
<tr>
<td>Changes in endpoint force tuning</td>
<td>Eqn. 11</td>
<td>0.21±0.07</td>
<td>0.22±0.05</td>
<td></td>
</tr>
<tr>
<td>Change in muscle forces (time-averaged)</td>
<td>Eqn. 12 with $m_{tavg}$</td>
<td>-0.09±0.01</td>
<td>-0.08±0.01</td>
<td></td>
</tr>
<tr>
<td>Change in muscle forces (PCA)</td>
<td>Eqn. 12 with $m_{pca}$</td>
<td>0.04±0.01</td>
<td>0.14±0.01</td>
<td></td>
</tr>
<tr>
<td>Postural gain changes</td>
<td>Eqn. 13</td>
<td>0.21±0.07</td>
<td>0.18±0.05</td>
<td></td>
</tr>
<tr>
<td>True uniform shift</td>
<td>Eqn. 5</td>
<td>0.90±0.03</td>
<td>0.93±0.02</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 1.** Explained variance of the observed uniform shift in preparatory activity by multiple alternative models, in addition to the preferred learning-related uniform shift model. Variance explained is reported as the five-fold cross-validated $R^2$ computed as $1 - \text{sum squared error/variance}$.

Taking a step back, we recognize the Rokni, Richardson, Bizzi (a true pioneer and luminary in the field, and far ahead of his time in many ways of course) & Seung work (Neuron, 2007) most
certainly provided some of the very first tantalizing insights into this extremely important and interesting question regarding the existence of neural changes that do not necessarily relate to behavior during motor learning. Our endeavor to investigate neural activity patterns that may not directly relate to movement output and the major findings (“uniform shifts” and the “indexing functionality”) are inspired by their investigations and findings. Now when it comes to the actual experimental / analytical approaches, the Rokni work performed nearly 15 years ago simply did not have the tools to simultaneously record large neural populations or the analytical methods which we now have to more directly address this question at a neural population level (e.g., electrode array recordings, dynamical systems framework style analyses, etc.).

Additionally, a comparison of our methodological approach is as follows. (1) Rokni and colleagues used a curl field learning task that applied curl forces to all reach targets which altered the movement kinetics of all reaches. Importantly, our task applied curl forces to only one reach target which altered the movement kinetics for a subset of reaches while retaining the ability to generate original movements for other reaches, due to the natural limits of generalization. This specifically-designed task allowed us to directly dissociate behavioral changes (i.e., “local generalization”) from the neural changes (i.e., “uniform shifts” present for all reaches). (2) Rokni and colleagues found the “background changes” by analyzing single-neuron tuning curves, and a comparison between learning and control datasets revealed that those background changes are irrelevant to learning. In contrast, our work applied population-based analytical methods and discovered that uniform shifts are specific to the learned curl field and do not spontaneously occur in control days (i.e., thus uniform shifts are learning-relevant), although they are not directly related to the movement output. The uniform shifts and their orthogonal relationship with the reassociation subspace are inherently a population-activity feature and thus were presumably visible if Rokni and colleagues happened to look for population features, as opposed to just single-neuron responses and tuning curves as discussed in their 2007 Neuron paper.

In summary, while we certainly were inspired by their pioneering study, we did not build our experimental or analytical approaches directly on Rokni and colleagues’ work. We believe that our population-based experimental and analytical methods, and consequently our findings of the “indexing functionality”, are the major novelty of this work.

Andrew Pruszynski

Andrew, as you kindly signed your excellent review, we believe that it is appropriate to thank you directly here for your extremely helpful and insightful comments and suggestions. Thank you! Krishna (and Xulu, Dan & all authors)
Reviewer #2:

Sun and colleagues have written a paper that is easy to read, thorough and rigorous in its approach, and most of all, teaches us something important that we didn’t already know about motor cortex.

Thank you very much, we are so pleased to read the above (“easy to read”, “thorough and rigorous”, “teaching us something new”), and we do not take it for granted that this is exceptionally special to hear expressed so clearly. Frankly, at an ‘emotional level’ this is so wonderful and uplifting, especially as we all have endured the most harrowing year of most of our lives with COVID. Thank you!

To conduct this re-review, I first read the paper from scratch. Then I read carefully the team’s response to my first review. I then perused the other two reviewers’ comments, and Sun’s responses.

Wow, we are deeply appreciative of such a thorough and rigorous re-review. We of course wish that doing so did not consume so much of your time, but we know that it undoubtedly did. Simply, thank you.

In all, I judge that the authors have improved the paper in light of the reviewers’ comments. Even in its current form, the paper now resembles those that rise to the level of Nature.

Thank you.

Below are suggestions that the author may wish to consider if they would provide for further improvements.

Certainly, we really respect and value your / reviewers’ insights and suggestions, as part of the seminally important peer review process.

To start, I comment on why I think this paper is likely to be influential.

Some of the strong suits:

- Figure 3 shows a quite remarkable result. It didn’t have to turn out this way. And, having seen this, the interpretation is straightforward and powerful. It’s this result, in my opinion, that’s going to really get people thinking. It’s especially cool that this uniform shift occurs even for untrained
targets. It’s really like this is a context signal coming into M1 from somewhere else, probably via somatosensory cortex, as soon as the animals “feel” the curl field.

Thank you, we fully agree, and indeed we hope that it helps the field by opening up quite new ways of conceptualizing neural population activity and motor learning and memory. While we always want to be extremely conservative and not overreach, to the extent that this does influence in a helpful way, well that would be wonderful. Indeed, let’s all advance science step by step.

- Likewise, figure 4 did not need to show as clean and straightforward a result as it did.

Agreed, the results in Figures 3 and 4 surprised us as well and strike us as one of those “once you see it, one can’t really ‘unsee’ it as it all just fits together rather simply and, presumably, correctly.”

- Clarity about nomenclature is an additional nice touch, and more clear statements about terminology like this are needed in the field. Overall, the methods section itself is a contribution to the literature.

Thank you, we are grateful to hear that our focused and highly intentional attempts to be clear on the nomenclature (else it could be very burdensome and confusing) and in the methods (so that readers can as readily as possible replicate / use these analyses) are recognized. Wonderful!

Potential improvements:

- Could figure 2 have turned out any other way? While it’s nice that the neural implementation of “re-aiming” is so straightforward and direct (and, impressive that their methods could reveal it), I wonder, can the authors somehow elevate the impact of this, the paper’s first figure showing neural data? Is there an argument to be made that something different could have happened here? Or, another way to elevate the significance of figure 2 might be to explore, what are the larger consequences of this finding? The authors mention that the same “re-association” is seen during VMR learning and BCI learning. Can we claim from this result that even in curl field adaptation, there is a VMR-like re-aiming component? Do these results indicate that we can safely equate “re-aiming” with “re-association”?

Thank you for these important questions to help us highlight the significance of the reassociation results presented in Fig. 2. Based on changes in single-neuron tuning curves reported in previous work (e.g., Li, Padoa-Schioppa, and Bizzi 2001; Padoa-Schioppa, Li, and
Bizzi 2002), we predicted the rotatory neural shifts in the 2D TDR subspace and did not expect to find alternative neural activity patterns there.

We think a broader significance of finding rotatory shifts in the 2D TDR subspace during curl field learning, as summarized in the manuscript, is as follows:

*This rotatory shift is consistent with the reassociation strategy observed during short-term brain-computer interface (BCI) learning or during VMR learning, and we thus label the 2D TDR subspace as a ‘reassociation space’. Neural states in the reassociation space suggest that reusing existing activity patterns may be a common strategy at least partially benefiting different learning contexts*[^4[^20][31][36][38]].

The observation of reassociation in different motor learning contexts suggest that motor cortical neurons may try reassociating existing neural patterns first when learning new behaviors, and if reassociation alone is not sufficient to complete learning, new neural activity patterns may be generated (e.g. uniform shifts in the case of learning new curl forces).

We have added these additional clarifications to the main text to help highlight the broader significance of the reassociation findings ([lines 464-471]), which is copied here for convenience:

*To adapt to the new force environment when learning a curl force field, subjects need to acquire new movement kinetics (Extended Data Fig. 1d). This task demand is a major feature differentiating curl field learning from other short-term learning contexts in which reassociation of existing neural activity patterns is sufficient to support behavioral learning[^4[^20][31][32][37][38]]. The observation of reassociation in different motor learning contexts suggests that motor cortical neurons may try reassociating existing neural patterns first when learning new behaviors, and if reassociation alone is not sufficient to complete learning, the motor system may engage different learning processes and new neural activity patterns.*

Although the rotatory neural shifts during curl field learning resembles the re-aiming strategy during VMR, we do not think we can conclude that curl field learning has a VMR-like component in it (see the definitions below). More generally, we do not think that we can equate “re-aiming” with “reassociation”. Instead, we believe that “re-aiming” is one specific type of “reassociation” and “reassociation” encompasses a wider range of phenomena. Thus, we used reassociation to describe the rotatory neural shifts throughout the manuscript. The definition of these two terms is copied below from the Methods section of the manuscript:

[^4]: Sun, O’Shea, et al. Response to Reviewers
**Definition of re-aiming and reassociation.** Reassociation[^4] is the neural strategy of learning subject to a fixed, existing repertoire of neural activity patterns. In other words, the neural activity patterns used for a particular behavior change during learning, but remain within the set of neural activity patterns that could be generated by (or empirically observed from) the neural population before learning. Here, the control space is the neural repertoire. Re-aiming is the neural strategy of learning whereby the neural activity patterns used for a given behavior after learning are the same neural activity patterns used for a potentially different behavior before learning. Here, the control space is the behavioral repertoire. Because the neural activity patterns used after learning are part of the existing neural repertoire, re-aiming is a subset of reassociation. Re-aiming has been observed during learning in VMR tasks[^60], whereby the perturbation applied to the effector is countered by a rotatory shift in neural activity patterns. The rotatory shift of preparatory states we observed in the 2D TDR subspace resembles these re-aiming findings. However, we referred to these types of changes as reassociation-like (as opposed to re-aiming-like) because (1) preparing for a compensatory force to oppose the curl field could result in re-aiming-like rotatory shifts even if the motor system does not explicitly re-aim for a nearby reaching target, (2) curl field learning likely demands more than purely re-aiming which is sufficient in VMR learning, (3) we can directly test for reassociation (based on repertoire change[^52,60]), and (4) all re-aiming-like changes are consistent with reassociation.

- Regarding figure 3, is it interesting that this shift is “nearly orthogonal”? That is, on one hand, the shift better not have a strong projection onto the TDR space of the targets, since it would interfere with movement to the targets. So, it makes good functional sense that the shift would be orthogonal. On the other hand, most arbitrary lines are going to be nearly orthogonal to an arbitrary plane in 244-D space.

Thank you for raising this important point which we certainly wish to be clear about. In addition to the functional benefits of being orthogonal pointed out by the reviewer, we think that the orthogonality is interesting because it suggests that the after-learning TDR plane is a separate plane from the before-learning TDR plane rather than a translation of neural states in the same plane, which demonstrates a specific type of neural repertoire change. The orthogonality supports the idea that the motor cortical neural activity has the flexibility to explore a different part of the neural state space rather than remain constrained in a given region of space during short-term motor learning. It could enable the neural population activity to explore a richer repertoire of patterns and consequently generate a wider range of behaviors. This is readily envisioned if one considers that different regions of a neural state space can have quite different dynamics (Remington et al. 2018; Vyas, Golub, et al. 2020).

We agree with the reviewer that “most arbitrary lines are going to be nearly orthogonal to an arbitrary plane in 244-D space”. Indeed, many orthogonal neural dimensions are available given the high-dimensional neural population activity. Yet we believe that not all of those arbitrary
lines show geometrical relationships associated with specific curl fields. The interesting aspect of these uniform shifts is not in the inherent difficulty of finding an orthogonal dimension along which to shift. Instead, it is in how the motor system uses multiple orthogonal dimensions to index different learned fields. The uniform-shift axis is not one of those random orthogonal axes but instead related to learning a specific curl field. We have added these clarifications to the manuscript ([lines 245-250] and [lines 479-484]), which is copied below for convenience:

The existence of different uniform shifts specific to learning different curl fields and their geometric relationships provide evidence that the uniform shift is not an arbitrary change in neural population activity but relevant to learning. These results suggest that these uniform shifts may serve an indexing function that enables distinct motor memories to be acquired, stored, and accessed by exploiting different neural population state subspaces...

...Although many orthogonal neural dimensions are available given the high-dimensional neural population activity, not all of them show geometrical relationships associated with learning specific curl fields. The interesting aspect of these uniform shifts is not in the inherent difficulty of finding an orthogonal dimension along which to shift. Instead, it is in how the motor cortex uses these readily available orthogonal dimensions to index distinct learned fields.

- Are these really “new” neural activity patterns? One might argue that any patterns seen within a couple hundred trials are nothing but discovering new motifs of an existing circuit, or exploring the pre-existing repertoire. Is “new” simply anything that’s outside the repertoire observed during a baseline task? This would be like, “new to the experimenter” but probably not “new to the monkey”. It might be helpful to distinguish three senses of “new patterns”: first, those that lead directly to learning-related behavioral improvement. Second, those that are specific to the behavior, but do not directly cause it. Third, those that accompany learning, but are unrelated to the new abilities. I would say that the authors’ findings belong in the second category. (And incidentally, most studies of learning might actually belong in only the third category.) The language on line 450 conflates the first two categories, and it might be worthwhile to clarify - part of what’s implied by the “indexing” function is that the new neural activity patterns that implement the indexing, by construction, cannot directly drive behavior, so “new” in the sense of the first category isn’t an option here. If the authors agree this is a valuable distinction, then providing a definition after line 896 would benefit the field.

We agree that it is important to distinguish different senses of “new patterns” and thank the reviewer for this helpful enumeration. We apologize for being unclear about the uniform shifts, new neural activity patterns as a result of the uniform shifts, and their relationships with the
movement output. Our findings showed that the uniform shifts and the associated new activity patterns implemented an indexing function specific to a learned curl field but did not directly correlate with the movement kinetics output. Based on these findings, we think that the uniform shifts and the associated new activity patterns do not directly lead to the movement kinetics and likely belong to the second category listed by the reviewer. We do, however, wish to be extremely careful with using causal terminology. There may be an indirect yet causal role in learning yet to be established, but the results demonstrated here are ultimately correlative.

We also agree that a clear definition of “new patterns” is very valuable, and as the reviewer astutely points out, it seems possible that the capacity to exhibit those neural population activity patterns may have already been present prior to learning and the animals did not express them in a simpler baseline task. The reviewer is correct that what we mean by “new” is that those neural patterns are specific to moving in a curl field and are not used for a standard set of behaviors such as the center-out reaches or correcting random perturbations. So in our definition, those patterns are still new compared to the neural patterns used in the standard reaching movements before learning the curl field, and we thus agree that we could be discovering new motifs of an existing circuit related to the new behaviors demanded by the force environment that we would otherwise not have observed. Nevertheless, our results suggest that the motor cortex has the flexibility to explore different neural state subspaces during short-term motor learning when needed to generate environment-specific new behaviors.

To emphasize this point and help avoid confusion in the revised manuscript, we have added our definition of “new patterns” and the clarification described in the first paragraph to the Definitions of neural terminology section in Methods ([lines 922-929]).

Along the lines of parsing nomenclature, it might help to have one place where the authors’ claims are laid out very directly. Shorthand words obviously can’t do justice to the whole concept, but that caveat aside, it seems the key claim here is “reassociation occurs in the TDR target subspace and new activity patterns appear orthogonal to it.” It might be worth it to be explicit about this somewhere. (Forgive me if I overlooked an explicit statement to that effect.) Along these lines, if I’ve kept track of all the nuances, TDR reassociation occurs during VMR learning, but new patterns don’t show up during VMR learning. All this is worth summarizing somewhere prominent.

This is a great suggestion on how we could lay out the major findings more explicitly. As you accurately summarized, we found that (1) during curl field learning, reassociation occurred in the 2D TDR subspace while uniform shifts and the associated new activity patterns appeared
orthogonal to it, and (2) during VMR learning, reassociation occurred without new activity patterns generated and no neural repertoire changes observed.

In the original manuscript, we summarized the curl field learning results in the abstract as “we found that in a neural subspace related to hand forces, preparatory activity reassociated existing activity patterns with new movements (‘reassociation subspace’). These systematic changes were observed only for learning-altered reaches. We also discovered the formation of new preparatory neural activity patterns along a neural population dimension nearly orthogonal to the reassociation subspace. The new patterns emerged from the shift of preparatory activity along this orthogonal dimension for all reaches including those unaltered by learning (‘uniform-shift learning axis’).” We described the VMR results in the main text as “this rotatory shift is consistent with the reassociation strategy observed during short-term brain-computer interface learning or during VMR learning, and we thus name the 2D TDR subspace as a ‘reassociation space’,” and as “furthermore, we did not observe a uniform shift or repertoire change during VMR learning (Extended Data Fig. 4).” We briefly summarized all these findings in Discussion as “in a 2D TDR subspace, we found reassociation-like changes of preparatory neural states similar to those reported across multiple learning contexts. We also discovered a surprising uniform shift of preparatory neural states that may be specific to the context of learning curl force fields.” However, we agree that the manuscript lacked a more prominent summary to overview and compare curl field learning and VMR results.

In the revised manuscript, we have added to the first paragraph of Discussion a summary of neural changes regarding reassociation and uniform shifts during curl field learning and VMR, which is copied here for convenience:

**In a 2D TDR subspace (reassociation subspace), we found reassociation-like changes of preparatory neural states similar to those reported across multiple learning contexts such as VMR learning and short-term BCI learning. We also discovered a uniform shift of preparatory neural states that was orthogonal to the reassociation subspace and may be specific to the context of learning curl force fields.**

- Is adaptation a skill? If so, what should we call something like throwing a baseball or playing piano? That is, shouldn’t we reserve that term for behaviors that bear some resemblance to things most people think of when they think of skills? By the authors’ terms, they are studying “skill maintenance” here. And thus perhaps the title over-promises what is actually delivered, since readers will probably assume “skill acquisition” is what’s referred to in the title.
Thank you for pointing out the potential concern regarding “skills” in the title. Previously in the Methods section of our manuscript, we defined the behavioral terminology regarding skills according to Krakauer et al. 2019 and used those definitions consistently in the manuscript. Based on those definitions, here by studying curl field learning, we focus more on “skill maintenance” than “skill acquisition”, although new movements are generated during the learning process. However, as the reviewer points out, when seeing “skills” in the title, readers are likely to assume de novo skill acquisition rather than the motor learning paradigm in this study. Therefore, and also as Reviewer 1 had a quite similar suggestion, we have decided to move away from using the term “skills” in the manuscript.

We have updated the title by removing “skills” and adding in the term “indexing”, which, as recognized by all reviewers, is the major novel finding. The new title is:

*Cortical preparatory activity indexes learned motor memories*

- Can we really rule out postural shifts just by looking at baseline responses? What if posture has a gain effect (as has often been reported, going back to the 1980s) rather than an additive effect? Would you be able to see it in the baseline?

Thank you for bringing up the possible relationship between the postural gain effect and the uniform shift. Because the baseline activity of most neurons is significantly higher than zero, we think that postural shifts, if any, should be reflected in the baseline neural activity. Given that we did not observe uniform shifts or neural repertoire changes in the baseline neural activity, we believe that uniform shifts and neural repertoire changes are not related to postural shifts.

Nevertheless, in case focusing on baseline neural activity can not rule out this possibility and additional analyses are needed, as we noted at the beginning of our response, we have collected and expanded our discussion of several alternative explanations for the uniform shift, including the influence of posture and the possibility of gain related changes in Supplemental Note 2. Concretely, we have performed a regression-based analysis with a multiplicative gain term on top of the additive behavioral variables to directly address how much of the uniform shifts could be explained by postural gain changes. The cross-validated $R^2$ values (0.21±0.07 for monkey U and 0.18±0.05 for monkey V) showed that changes in preferred tuning direction accounted for only a small fraction of the variance of uniform shifts (see Supplementary Table 1 which is copied below for convenience). Based on the quantitative results, we believe that the posture-related gain changes do not explain the uniform shift.
- Overall, in the paragraph beginning on line 457, the most important point is the very last one. Namely, the clean and sensible (at least in hindsight) geometrical relationship observed here demands an explanation. The one the authors offer is clean and elegant, and for those who do not wish to accept it, the burden of proof is on them to come up with a compelling alternative. Without getting as argumentative as that sounds, it might be a “best foot forward” construction for this paragraph to say, “It’s hard to find an alternative explanation than the “indexing” we propose. However, before we can draw this conclusion, we must first consider a few learning-irrelevant explanations.”

Thank you for the very helpful suggestion on phrasing. We have rephrased the sentence that leads the discussion of control results as follows:

> Finally, we addressed analytically and empirically an additional set of alternative explanations for the uniform shift, including learning-irrelevant changes in single neuron activity, hand forces, muscle activity and posture, as well as the application of error clamps (discussed in Supplementary Note 2, Extended Data Fig. 5).

In order to streamline the entire manuscript, we have also moved the discussion of all the relevant control experiments, analyses, and analytical derivations, including new work to address questions raised in this round of reviews, to Supplementary Note 2 in Supplementary Materials).

Suggestions:

- Line 150: Since you’re already defining PCA, it’s worth it to explain that the dimensions are ranked by variance accounted-for. That will help when you next refer to PCs 1, 2, and 3.
Thank you for bringing up how we could explain PCA more clearly. In the revised manuscript, we modified the sentence around line 150 as follows:

... we used principal component analysis (PCA), an unsupervised dimensionality reduction method that operates on neural data alone and extracts neural dimensions explaining the largest amount of variance.

We also added the following sentences to the PCA section in Methods:

The dimension that captures the greatest amount of neural variance is principal component dimension 1 (PC1), the second most is component 2, and so forth. Importantly, the PCs are orthogonal to each other.

- Line 164: It’s a little jarring to read (paraphrasing) that “the uniform shift axis was nearly orthogonal to the reassociation space” and then “we made it orthogonal”. It will help to explain why this was done, and why it’s okay to do (e.g. it doesn’t “bake in” any subsequent results.)

Thank you for raising this important point that deserves further explanation. The purpose of orthogonalizing the uniform-shift axis against the reassociation space was to make sure that the uniform-shift learning axis was fully independent of the reassociation space. Consequently, the reassociation subspace would not ‘bake in’ ( spuriously result in) any subsequent investigations of the uniform shift axis, as pointed out by the reviewer.

We believe that it is okay to orthogonalize the uniform-shift axis for the following reasons. (1) The uniform-shift axis was intrinsically nearly orthogonal to the reassociation space before we performed any further investigation of it and thus our orthogonalization process would not substantially change this axis. (2) The uniform-shift axis still explained approximately 10% - 20% of total neural variance after orthogonalization, similar to the amount of variance explained before orthogonalization. (3) Orthogonalization was a necessary process allowing us to examine a neural dimension that did not involve the force-related neural dimensions (i.e. TDR 1 and 2 axes) which we already investigated.

We have added these additional explanations to Methods ([lines 769-772]) in the revised manuscript.

- Fig 5b caption - make it clear what each point is. (Presumably one point per a trial; all sessions shown.)
Thank you for the suggestion. We have added to Fig. 5b caption that each point corresponds to one trial and that single-trial data points from all sessions are shown.

- Fig 5j is probably going to look nicer if the grid lines are more spread-out and/or a lighter gray.

This is a great suggestion. We have made the grid lines in Fig. 5j (now Extended Data Fig. 8g) more spread out and in lighter gray. And thus they are far less prominent, yet still there as a subtle visual guide.

- In the very final sentences, the cerebellum and basal ganglia are mentioned. That’s fine, but it leaves out much. In light of the fact that the learning here seems to be mostly preparing to counteract the push of a curl field, it seems prudent to mention haptic areas (somatosensory cortex, thalamus, and dorsal spinal cord) as among those that might be responsible for learning in this task.

Thank you for pointing out this important, inadvertent oversight. We agree with the reviewer that haptic-related areas such as the somatosensory cortex, thalamus, brainstem, and dorsal spinal cord play critical roles in curl field learning.

We have now added those brain regions to Discussion ([lines 489-491]).

- Extended figure 3 title seems to be missing the word “in” - “neural variance *in* different dimensions”. Also, that figure has some conspicuous whitespace. Why not sketch in a little geometrical definition of the dot product and variance explained metrics?

We apologize for the confusing language in the figure title “Relationships between neural population dimensions and total neural variance different dimensions explained”. We agree with the reviewer that the title could have been clearer, and have modified the figure title as “Relationships between neural population dimensions and total neural variance explained by different dimensions”.

A geometrical definition of the dot product metric is a great idea, and we have added that to Extended Data Fig. 3a in the revised manuscript, which is copied below for convenience.
Extended Data Fig. 3 | Relationships between neural population dimensions and total neural variance explained by different dimensions.

**a,** Relationships between different neural population dimensions (defined by TDR axes, uniform-shift axis and PCs), measured by their pairwise dot products (see Measurement of relationships between neural population dimensions in Methods). Values close to 1 indicate that two dimensions are closely aligned whereas values close to 0 indicate that two dimensions are nearly orthogonal. In each session, we calculated the dot product of TDR 1 and PC 1 and the dot product of TDR 1 and PC 2, and then took the larger value of the two dot products (TDR 1 vs. PC 1 / 2). We also calculated the dot product of TDR 2 and the PC axis not used for multiplying with TDR 1 (TDR 2 vs. PC 1 / 2). The PC 1 / 2 plane largely overlaps with the TDR 1 / 2 plane (black boxes). The TDR 1 axis, TDR 2 axis and the TDR axis related to hand speed are all nearly orthogonal to the uniform-shift learning axis (blue and red boxes). PC 3 largely overlaps with the uniform-shift learning axis (yellow boxes). Monkey U: 4 sessions; monkey V: 3 sessions. Right panel: a schematic illustration of projecting data points from axis α to axis β and the corresponding dot product.

Extended figure 15 would benefit from an inset showing the target location - to - color relationship.

Again thank you for the great suggestion.

*In our revision, we have made a new plot of PCs 1-4 over time (as suggested by Reviewer 3) and added an inset showing the color-coded target locations, which is copied below for convenience.*
**Extended Data Fig. 9 | ... a,** Example PCs 1-4 during time windows -100 to +100 ms from target onset (TO), -100 to +100 ms from go cue (GC), and -200 to +400 ms from movement onset (MOO) in each trial. An example session from monkey U and representative of the other sessions from both monkeys. We applied PCA to trial-averaged neural activity for different reach directions in the before-learning and error-clamp blocks. Different colors correspond to different reach directions. Across all learning sessions in both monkeys, PC 1 explained for 30 - 40% of the total variance, PC 2 explained for approximately 10 - 20% of the total variance, PC 3 explained for 8 - 10% of the total variance, and PC 4 accounted for 6 - 8% of the total variance in all sessions across both monkeys. Neural trajectories in PCs 1-4 are bundled together around the target onset time window and diverge around the go cue time window (i.e., during preparatory period). Error-clamp neural trajectories all shifted from their corresponding before-learning trajectories in PC 3. We chose the time window of -50 to +50 ms relative to the go cue (gray shadow) for preparatory neural state analysis. This is within the preparatory period (near the end of it) and has stronger neural tuning than at earlier times (e.g., the first 100 ms after target onset). Upper left inset: color-coded reach directions.
Reviewer #3:

General Comments:

Overall the revised manuscript is considerably improved in terms of presentation and clarity. The authors have made substantial revisions to write parts of the manuscript in a more hypothesis-driven manner which brings out the key motivation for the work and emphasizes why it is of broad relevance. They have also clarified many aspects of the methodology which were difficult to follow or where information was missing. Thus, they have addressed many of my concerns with respect to this issue (but see a few comments below).

Thank you, we deeply appreciate your time, energy and dedication -- especially in this extremely challenging time of COVID -- to not only review the original manuscript but to review the revised manuscript. We are grateful to read the above regarding “considerably improved ... presentation and clarity”, “more hypothesis-driven... brings out key motivation ... and broad relevance”, and “addressed many of my concerns.”

In addition, they have made exceptional efforts to provide additional quantification of the experimental results as well as to include new results (even during COVID) to help support their conclusions.

Thank you, we sincerely appreciate the kind recognition that this has been a time of COVID making literally everything more difficult. Bringing new data and results to bear on the insightful questions you asked was a challenge, but we appreciate being urged to do so as we certainly believe that it has strengthened the manuscript considerably. We feel the same for the extensive additional quantifications that you previously asked for. Simply, thank you.

Many of my previous concerns have been very well addressed and the efforts of the authors to clarify my many questions are most appreciated.

Thank you, we believe that this has indeed sharpened the presentation and strengthened the data in support of our hypotheses. We believe that this should substantially help the reader understand the concepts, analyses and conclusions.

However, after careful rereading of the manuscript and consideration of the extensive additional material the authors have provided I find that while aspects of this work are certainly novel and important, I am not yet convinced of the conclusion.
Thank you for sharing this, and allowing us the opportunity to address this. We do believe that the conclusions are well supported, so it is our responsibility to present our data, analyses, and conclusions in an accurate and compelling way. After an “around the clock” effort by all authors in the past few weeks we believe that we have addressed your questions with more analytical and empirical evidence (please see below).

As outlined below, my strong feeling is that the relevance and importance of this study can best be appreciated in a more expanded format (i.e., slightly more specialized journal) that allows sufficient space to more fully address the results and their implications in the context of previous work as well as the limitations in interpretation.

We certainly respect this view, and we respectfully believe that it is possible to share this research in an accessible way even in a short format. We believe that this line of fundamental neuroscience, including how the brain is able to learn and retain memories about new movements as well as reduce the interference between memories, is both quite novel and of wide interest. We would hope that being able to share with the widest possible scientific audience (i.e., the Nature readership) this fundamental neuroscience investigation is possible. We have continued to do our very best to address the remaining questions and suggestions that you kindly shared. We were more than happy to do everything that we could to make the science as rigorous, and presentation as streamlined, as possible.

As we note below, we have reorganized the figure flow and accompanying text considerably to better fit the succinct format of Nature. More concretely, we have (1) combined current Figs. 2 and 3 into one figure as a new Fig. 2, (2) reduced the size of main figure panels by roughly 50% and reduced the number of extended data figures from 22 to 10, (3) presented the interference plots in a more simplified format that focuses on the major findings, (4) moved the major interference results to main figures (as Fig. 4), and (5) moved the discussion of alternative explanations for the uniform shift to Supplementary Materials that we believe could help shorten the main text and meanwhile maintain the comprehensiveness of the entire manuscript. We expand on these changes below in our response.

In particular, I am enthusiastic about the notion that the uniform shifts in preparatory activity that the authors have observed along neural population dimensions orthogonal to force/motor output could reflect a potential mechanism for keeping track of and retaining memories of different motor skills while minimizing interference between them (i.e., that these shifts in activity reflect a sort of “indexing function”).
Thank you, yes that is indeed the heart of this investigation and discovery, and we too are extremely enthusiastic about how it can lead to a new way of thinking and can generate a new set of experiments in the field.

However, despite my enthusiasm about the idea, after re-reviewing the manuscript I still don’t feel completely convinced of aspects of the authors’ conclusions (much as I would like to be) for a couple of reasons:

1. The first is that while I am convinced that the uniform shifts observed over the course of learning indeed reflect a change in repertoire relative to before learning and that it is task-specific, I am not yet convinced that the shifts are not closely linked to the motor output. As outlined below, I have a number of remaining questions with regards to this. Perhaps one could argue that the relationship between the shifts and motor output is not the most important issue, but after careful consideration I do think this is an essential issue in terms of level of novelty.

Thank you, we believe that we understand your overarching question. We took this concern very seriously and have addressed each question below.

If the uniform shift is not in fact decoupled from the motor output, it may still reflect a mechanism for keeping track of and retaining distinct memories of different motor skills. The results would still be novel in several respects and make an important contribution to furthering our understanding of skill learning at the neural population level.

Yes, we agree. But as we discuss below, to the limits of the field's current abilities -- save for a new technology that we are unaware of being brought to bear on this question -- we do not find a movement correlate.

The central question raised by the reviewer here regards possible alternative explanations for the uniform shift, including changes in hand forces and changes in EMG activity. Consequently, in our revision, we have collected and organized a comprehensive discussion of the various alternative explanations for the uniform shift into a detailed writeup (Supplemental Note 2), and within it, we have provided extensive methodological, analytical, and empirical evidence that summarizes quantitatively how much of the uniform shift we observe could possibly relate to any of these potential confounding variables. Our results reveal that these potential variables account for only a very small amount of the uniform shifts. We include a detailed description of these new analyses performed and the reasoning for each analysis along with a clear description of how these variables could influence preparatory activity alongside the results of each analysis.
and our interpretation of each result. This is now all clearly organized in Supplementary Note 2 and Supplemental Table 1. By treating all of these variables in the same analytical (regression) framework, we believe that this straightforward evidence should assuage the questions / concerns, as well as provide a single, linear reference for the reader to consult.

As described below, we have also performed new analyses and have added clarifications to resolve the reviewer’s remaining questions, in particular the major question about TDR planes before- and after-learning as well as the question about EMG activity before- and after-learning of all recorded muscles across all reaching targets. We believe that we are able to address each point clearly and comprehensively.

However, if the shift does have a direct relationship to the motor output I also think that the novelty of the study would be considerably reduced, as other studies have previously addressed aspects of such results and/or their conceptual underpinnings (e.g., Li et al., 2001; Rockni et al., 2007; Perich et al., 2018). It is the idea that preparatory activity acts as an “indexing function” largely independent from motor output that I think is most novel here and so I do feel that convincing the reader of this is important when it comes to publishing this study in Nature.

Thank you, understood. We agree that if the shift were simply related to changes in motor output, this would in some sense “trivialize” the uniform shift, and ruling out this explanation was a central concern in our experimental design and analysis. We have expanded on this possibility and considered it in detail in Supplemental Note 2 in various forms in a central, organized way. Additionally, please see the summary above and the point by point responses below where we have done and included considerable additional analyses to support our conclusions.

Admittedly I am not an expert on the analysis techniques that are being used here (whereas the authors certainly are) so I think it is highly likely that with some additional clarifications and analyses the authors could indeed resolve my remaining questions/confusion regarding the relationship between the observed shift and motor output.

Thank you for noting that this may well be addressable, and we fully agree that it is, and we have included a comprehensive additional set of analyses to answer this as definitively as (to our knowledge) the field can at this point in time.

Said more plainly perhaps, we are confident in the results (1) having spent multiple years cross checking every aspect that we can think of, (2) having discussed these detailed analyses and results with colleagues, and included already in the manuscript many analyses that colleagues
were curious about, and (3) having addressed questions, including quantitative analyses, that have naturally arisen (as is always the case; no particular concern with this work or anything) as part of many conference presentations (e.g., SFN, MLMC SFN-pre meeting, Neural Control of Movement (NCM), COSYNE). In all cases the findings hold up very well and quite simply, without requiring any ‘gymnastics’. It is actually a very ‘clean story’ in our experience and the dynamical systems framework required to conduct these analyses is relatively straightforward all things considered (e.g., Shenoy, Sahani, and Churchland 2013; Vyas, Golub, et al. 2020; Vyas, O’Shea, et al. 2020; Vyas et al. 2018; Mante et al. 2013; Peixoto et al. 2021; etc.).

However, even so, there remains the question of whether Nature is the best venue for this manuscript. My feeling is that it is likely that a substantial proportion of readers would have many of the same questions and concerns that I and the other reviewers have raised. For the most part, I think the authors have done an excellent job of addressing them but this has required providing substantial additional information largely in the form of extended data figures (of which there are now 22). In view of this, my conclusion is that the relevance and importance of this study can best be appreciated by presenting the results in a slightly longer format that allows sufficient space to fully discuss a broader range of results, their implications, and their novelty in a more comprehensive fashion (i.e., in a more specialized journal than Nature).

Thank you for sharing your extremely helpful perspective, and we can appreciate that an important discovery must be conveyed in a readily accessible manner. We respectfully submit, however, that we believe that the current manuscript does achieve this (as Reviewer 1 and Reviewer 2 appear to be conveying). But we always wish to improve and so we have tried in a very concerted effort to streamline it even further here, as described throughout this Reply to Reviewers document. We believe that after streamlining, the number and complexity of extended data figures, and panels in the main figures, are consistent with what is found in current Nature papers (having again surveyed many recent Nature papers across fields). Again, we believe that the interest, broad importance and the way that this discovery will hopefully help many research groups structure new experiments merit publication in a non-specialty journal.

Pragmatically, we have considerably streamlined the presentation of the manuscript, by reorganizing the figure flow to focus the main figures on the essential findings and creating ample room for the interference experiment as a main figure. We have also reorganized the extended data figures from 22 (which we agree needed to be better organized so as to not burden the careful reader) down to a more manageable 10, each containing more logically related, thematically consistent content. Moreover, in the Discussion, we had interspersed the
core discussion with various thoughts on alternative explanations for the uniform shift. This both disrupted the flow of thought and was insufficient to be a comprehensive, convincing treatment of these concerns, a point which each of the reviewers has raised in various ways. We have replaced this treatment with Supplemental Note 2. This format gave us ample space to explore the alternatives thoroughly, bringing to bear an analytical treatment of some of the alternatives (working out the math to argue which explanations could and could not create a uniform shift in theory), a set of regression-based analyses to quantitatively assess the suggested alternatives, and enumerate the related threads of evidence from our methodology and various control experiments. We feel that this revised organization better serves the two competing goals for the manuscript—achieving a clear, succinct, and convincing presentation of the core results, and convincing the careful reader that the uniform shift is truly related to learning and exhibits the requisite characteristics to function as an index of motor memories.

2. Assuming that one can be completely convinced that the uniform shift is truly taking place along a dimension orthogonal to motor output, a second issue (also previously raised by reviewer 1) is that while the results would be highly suggestive of a putative mechanisms for savings and the ability to learn and retain multiple motor skills, actually proving this is much more difficult. The authors have done an admirable job, under the circumstances, of attempting to provide some more concrete evidence for this through their new interference tasks results. Despite this, I am not convinced that these new results add much beyond what was already illustrated in Figures 4 and 5, although I do think they provide a potentially interesting population-level illustration of how preparatory states shift uniformly during interference (i.e., the interference shift). However, approximately 1.5 pages in the main text (lines 243-319) are dedicated to the description of these results which appear only in Extended data figures and in my opinion this was detrimental to the flow of the article.

Thank you for the very helpful and insightful questions and suggestions, which we absolutely take to heart and have again worked to balance: the balancing being between streamlining the main manuscript and asking readers to trust our summary (but always backing our summary up with extended data and supplementary notes of course) versus adding more text to the main manuscript to explain our rationale as we believe was requested in the previous round. First, importantly, we re-balanced the interference experiment section so as to further streamline the manuscript. We have rearranged many figure panels (including main figures and extended data figures) as well as the corresponding text, and moved redundant panels to extended data. This allowed us to integrate the important interference experiment results within the main figure flow as Fig. 4. We believe that its inclusion in the main figure flow makes the manuscript stronger.
In our view, the interference results serve as a counterpoint to the multi-field learning experiments. In the multiple field results, the organization of the uniform shifts along orthogonal dimensions is consistent with successful indexing of all three within the neural state space. In contrast, in the interference experiment, we leverage our understanding of the geometry of these uniform shifts (the angles between fields as a function of their spatial location) to design a learning situation where the uniform shifts end up being not fully orthogonal, forming an obtuse angle where progress along one uniform shift partially undoes progress along the other. Consistent with a role for the uniform shift in organizing and indexing motor memories, the monkeys learn both fields more slowly due to interference between the closely-spaced, opposite fields.

Of course, we cannot demonstrate causally that this geometry is causally related to the difficulty (or facility) of learning the fields without more precise causal manipulation tools beyond the scope of this manuscript. Furthermore, we do not claim to have proven that the uniform shift indexing function we describe is the sole locus for retaining and recalling motor memories, but given the central involvement of M1 and PMd in motor control and motor learning, we do feel that our results clearly argue that this indexing function is a key component of the holistic neural mechanisms used by the brain-wide motor system. Furthermore, we feel that this close link between behavioral learning and the organization of changes of neural preparatory activity along different dimensions (i.e. the systematic and evidently predictable structure of the angles between the uniform shifts) is compelling, and opens many exciting avenues for future research.

Again, my impression is that this work can better be appreciated if the results are presented in a slightly longer format in a more specialized journal where there is more space to address their relevance and implications.

Following our extensive reorganization, the new manuscript contains 5 main figures with substantially reduced size (roughly 50% reduction) and 10 thoughtfully reorganized extended data figures (compared to 22 in the previous version), which is consistent with Nature’s format. We believe that these changes help continue to ensure that we report all of the findings thoroughly while also focusing on the key major results in the main text and main figures.

Major Comments:

1. As noted above, despite many clarifications regarding the methodology that the authors have provided I still have a few questions about the relationship between the uniform shift and motor output.
In particular, since my first reading of this manuscript I am now questioning whether it makes sense to only use before-learning trials to build the 2D TDR subspace. It seems to me that in a dynamic learning task it would not be surprising to find that this subspace changes. More specifically, the idea that similar neural activity patterns are activated but “reassigned” to a new reach direction makes sense in the context of a visuomotor learning task, but for learning a dynamic skill we might expect there to be at least some new associations between neural activity patterns and force. In that case, could it not be that the TDR1 and TDR2 axes defining the 2-D hand-force subspace change over the course of learning in a way that could potentially include a component orthogonal to the orientation of that subspace before learning? My concern is that, if this is the case, the observed uniform shift while clearly reflecting a new repertoire, might not in fact reflect a repertoire completely orthogonal to force output.

I think that this is really the same or a similar issue to that raised by Reviewer 2 who suggested that the learning-induced shift axis could be a force axis. The authors have already responded to this but it seems to me that an important assumption underlying their response is that the 2-D hand-force subspace is not changing over the course of learning (i.e., or at least not changing in an orthogonal direction relative to before learning). Has this actually been verified? I do realize that some attempt to address this has been made by using the TDR space built based on before learning trials to predict forces after learning, but I have some concerns about the difference between a strong correlation vs an accurate prediction (see Minor comments below).

Thank you for this question, and again please see below for how we believe we have been able to directly address this. We have assembled responses to these questions below in order to hopefully be as clear as possible, and as efficient with your time as possible. Specifically, we have explored the possibility of changing a TDR space (changes in the relationship of neural activity to the endpoint forces) in Supplemental Note 2, both analytically and empirically, and have been able to show that allowing for this possibility does not explain the uniform shift.

In general, while much of the analysis is based on the 2D TDR space built using only before learning trials, other analyses combined before learning and error clamp trials and overall it is not clear to me how well the TDR axes and/or the planes they span were aligned with each other across these different analyses. Perhaps, most importantly, what I would really like to know is if the TDR hand-force subspace is instead built based only on error-clamp data after learning what is the orientation of that subspace relative to the 2-D hand force subspace built based on before learning trials? Do the TDR1/TDR2 planes remain aligned?

Thank you for this very important point, to compare the TDR 1 and 2 axes before- and after-learning. We performed the same TDR regression using only error-clamp trials to build the 2D
TDR subspace, and took the dot products of before-learning and error-clamp TDR axes. The dot product plot has been added to the manuscript as Extended Data Fig. 5d and copied below for convenience. Our results show that TDR 1 and 2 axes after learning remain aligned with the before-learning TDR axes.

Moreover, even if error-clamp TDR axes are not completely aligned with the before-learning TDR axes, as long as the error-clamp TDR axes are also orthogonal to the uniform-shift axis, this result still supports our conclusion that the uniform-shift axis is not a force axis. Indeed, we found that like the before-learning TDR axes, the error-clamp TDR 1 and TDR 2 axes were also orthogonal to the uniform-shift axis. A detailed discussion of these results has been added to the Error Clamp section in Supplementary Note 2.

Extended Data Fig. 5d. TDR axes using only error-clamp trials (TDR-EC) and TDR axes using only before-learning trials (TDR-BL) are largely aligned. The dot products between error-clamp TDR 1 axes and before-learning TDR 1 axes are close to 1 (i.e., highly aligned), and same for TDR 2 axes (black boxes). The error-clamp TDR axes are nearly orthogonal (i.e., dot product around 0) to the uniform-shift axis (red boxes), similar to the before-learning TDR axes shown in Extended Data Fig. 3a. One-sided Wilcoxon rank-sum test: monkey U, \( P = 0.029 \); monkey V, \( P = 7.9 \times 10^{-3} \).

In addition, we would like to further explain why we think it is not a force axis based on the results we previously presented in the original manuscript. Importantly, all the quantitative analyses investigating the uniform shift were performed after we orthogonalized the uniform-shift axes against the force-related TDR axes. Consequently, we believe that the major findings related to the uniform shift (Figs. 2-5) did not reflect the force output. Furthermore, the uniform shift generalized to all reach directions to the same extent whereas changes in hand forces after learning the curl field showed spatial locality, which supports the idea that it is not a force axis (i.e., the uniform shift is decoupled from the exerted hand forces). The uniform shift also
persisted after the washout period when forces returned to the before-learning patterns, which provides additional evidence that it is not a force axis. This mismatch between the hand force changes and the uniform shift is not consistent with what one would expect from a force axis. This mismatch also does not depend on the assumption brought up by the reviewer that “the 2D TDR subspace did not change over the course of learning”.

Lastly, we would like to address the concern that “the uniform shift could be a force axis” as thoroughly as possible with different analytical methods. In the previous revision, we provided our reasoning and performed the dot-product analyses to address this question. In this new revision, we have used an additional approach to directly analyze the relationship between the uniform shift and hand forces. Specifically, we performed regression-based analyses to study how much of the uniform shift could be explained by changes in hand forces (see Supplementary Note 2). The cross-validated $R^2$ values (0.06±0.02 for monkey U and 0.11±0.06 for monkey V) showed that changes in hand forces accounted for only a small fraction of total variance of uniform shifts. These new regression results further support the idea that the uniform-shift axis is not a force axis (see Supplementary Table 1 which is copied below for convenience).

<table>
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<td>Changes in endpoint force tuning</td>
<td>Eqn. 11</td>
<td>0.21±0.07</td>
</tr>
<tr>
<td>Change in muscle forces (time-averaged)</td>
<td>Eqn. 12 with $m_{\text{tavg}}$</td>
<td>−0.09±0.01</td>
</tr>
<tr>
<td>Change in muscle forces (PCA)</td>
<td>Eqn. 12 with $m_{\text{pca}}$</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Postural gain changes</td>
<td>Eqn. 13</td>
<td>0.21±0.07</td>
</tr>
<tr>
<td>True uniform shift</td>
<td>Eqn. 5</td>
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Supplementary Table 1. Explained variance of the observed uniform shift in preparatory activity by multiple alternative models, in addition to the preferred learning-related uniform shift model. Variance explained is reported as the five-fold cross-validated $R^2$ computed as 1 – sum squared error/variance.

2. I remain somewhat confused about what PC3 might represent. Specifically, while the uniform shift appears predominantly along the PC3 axis I can see from Extended Data Figure 15 that PC3 activity appears substantially tuned to movement direction. One could almost say it seems to reflect preferred directional tuning along an axis somewhere between the 45o and 90o target directions (although maybe that’s a stretch). However, I was somewhat surprised by this as I would not have expected a PC dimension that is completely unrelated to behavioral output to reflect so much directional tuning. Could the authors comment on this and provide an explanation/interpretation?
Thank you for identifying a point which deserves additional explanation. Our interpretation of why PC 3 was tuned to movement directions is as follows: Extended Data Fig. 15 (now Extended Data Fig. 9a) shows PCs of neural activity over time in a trial rather than time-averaged neural states. When we analyze neural data over time (i.e., they inform us about neural trajectories), PCs 1-3 are usually different from PCs 1-3 of time-averaged neural-state data (e.g., Kaufman et al. 2016 shows PC 1 as the condition-independent component of the neural-trajectory data in contrast to the directional tuning component of the time-averaged neural-state data). This reflects additional variation exhibited over time in the trajectories, which PCA seeks to capture. In this study, we identified and studied the uniform shift using the neural-state data averaged over a preparatory time window. We found that the uniform-shift axis largely overlapped with the PC 3 axis and was almost orthogonal to the behavior-related TDR axes. However, this finding does not imply that the PC 3 of the neural-trajectory data should show the same patterns. Our results show that PC 3 of the neural-trajectory data has strong directional tuning while PC 3 of the neural-state data does not.

Furthermore, Extended Data Fig. 3a (the dot product between neural axes) shows that PC 3 of the neural-state data is not completely aligned with the uniform-shift axis. Thus, we think it is likely for PC 3 to be partially related to behavioral output. Additionally, the old plots of PCs over time only used before-learning trials. As shown below in a new plot of PCs over time (in our response to the next question), once we include error-clamp trials, PC 3 variance is dominated by the uniform shift rather than the directional tuning.

Lastly, because this figure shows PCs over time in a trial, the variance of neural activity is usually dominated by the peri-movement activity, and so the PCs generally primarily explain peri-movement related variance. Consequently, we see more directional tuning in the top PCs especially during the peri-movement period than when we only look at the preparatory neural data.

In addition, I think it would be useful to also see: 1) What PC4 looks like and how much of the variance it accounts for; 2) What all the top 4 PCs look like both before learning as well as after learning in error clamp trials. Does an uneven sampling of movement directions have any impact on these analyses?

Thank you for raising these interesting questions. We have replaced Extended Data Fig. 15 (now Extended Data Fig. 9a) with a new plot showing PCs 1-4 over time in before-learning (solid lines) and error-clamp (dashed lines) trials from an example session, which is copied below for convenience. PC 1 explained for 30 - 40 % of the total variance, PC 2 explained for approximately
10 - 20% of the total variance, PC 3 explained for 8 - 10% of the total variance, and PC 4 accounted for 6 - 8% of the total variance in all sessions across both monkeys.

Extended Data Fig. 9 | ... a, Example PCs 1-4 during time windows -100 to +100 ms from target onset (TO), -100 to +100 ms from go cue (GC), and -200 to +400 ms from movement onset (MOO) in each trial. An example session from monkey U and representative of the other sessions from both monkeys. We applied PCA to trial-averaged neural activity for different reach directions in the before-learning and error-clamp blocks. Different colors correspond to different reach directions. Across all learning sessions in both monkeys, PC 1 explained for 30 - 40% of the total variance, PC 2 explained for approximately 10 - 20% of the total variance, PC 3 explained for 8 - 10% of the total variance, and PC 4 accounted for 6 - 8% of the total variance in all sessions across both monkeys. Neural trajectories in PCs 1-4 are bundled together around the target onset time window and diverge around the go cue time window (i.e., during preparatory period). Error-clamp neural trajectories all shifted from their corresponding before-learning trajectories in PC 3. We chose the time window of -50 to +50 ms relative to the go cue (gray shadow) for preparatory neural state analysis. This is within the preparatory period (near the end of it) and has stronger neural tuning than at earlier times (e.g., the first 100 ms after target onset). Upper left inset: color-coded reach directions.

We also subsampled the neural data for eight out of the 12 reaching directions and performed PCA on the subsampled data. We made a plot of PCs 1-4 for those evenly-sampled movement directions (copied below), which shows that the uneven distribution of reaching directions did
not impact the PCs. We think that this plot might not rise to the level of importance of including it in the manuscript -- but we are happy to include it if deemed necessary.

3. Ruling out all possible “trivial” explanations for the uniform shift across movement directions, including ones to which learning did not generalize, is admittedly an almost impossible task. The authors have already made substantial efforts to rule out, to the extent possible, a number of issues such as postural changes, muscle co-contraction, the “error-clamp state”, hand speed, etc.

Thank you for noting that we have made every attempt to rule out these potential factors, including performing the requested additional analyses last round.

However, one thing I noted when examining Extended Data Figures 5 and 6 is that the reduction in flexor activity (i.e. observed in the Bicep and Pectoralis muscles) associated with learning to compensate for a CW curl-field during down reaches (Ext. Data Fig. 5) remains apparent, albeit to less of an extent, when reaching to a “far” target 135 deg away (Ext. Data Fig. 6). Specifically, Biceps and Pectoralis activity during error clamp trials appears to be reduced by ~30% which is not negligible. Is this something that is seen uniformly across directions?
Similarly, extensor activity (Lateral/Posterior deltoid) seems slightly increased. Given these observations I think it makes sense to more thoroughly investigate changes in individual muscle activities across all directions (but separately for learning different fields in different directions) to see what evidence there may be for a component of muscle activity change that is uniform across directions.

Thank you for highlighting the control experiments and analyses that we performed to test for these alternative explanations, and thank you for bringing up the important question about muscle activity across all reaching directions. We compared before-learning and error-clamp muscle activity for all reaching directions and did not observe any muscle activity change that was uniform across directions. We have added all the EMG plots to Extended Data Fig. 4 (copied below for convenience). These plots provide a summary of the EMG activity in before-learning and error-clamp trials for six recorded muscles in 12 reaching directions.
Extended Data Fig. 4 | EMG signals of 6 upper limb muscles (bicep, radialis flexor, radialis extensor, pectoralis, posterior deltoid, lateral deltoid)... b, EMG signals in before-learning (black) and error-clamp (purple) blocks do not show a uniform shift across all 12 reaching targets. For all six muscles, EMG activity increases in some directions and decreases in other directions. Muscle activity of reaching to the target 135 degrees away from the trained target (i.e., far targets with almost no behavioral generalization, see Fig. 1d) in before-learning and error-clamp trials show similar temporal patterns. Shaded area, s.e.m across trials. Time zero, movement onset. One example condition: CW curl field applied to down reaches.

In order to make monkeys feel as comfortable with the EMG sensors as possible, we only recorded one or two muscles per session and collectively a set of six muscles when learning a CW curl field applied to down reaches. We did not collect EMG data for learning other different curl fields. Nevertheless, we believe that the EMG results shown here support the idea that the muscle activity did not change uniformly across all reaching directions after learning, in contrast to the uniform shift of preparatory neural activity.

Lastly, we analyzed the relationship between the uniform shift and muscle activity to directly address the concern that “the uniform shift could be related to changes in EMG” using another quantitative approach. Specifically, we have performed regression-based analyses to assess how much of the uniform shift can be explained by changes in the EMG activity (similar to the regression analysis described in our response to your major comment 1). The cross-validated R² values (-0.09±0.01 for monkey U and -0.08±0.01 for monkey V, see Supplementary Table 1 which is copied below for convenience) showed that changes in EMG activity accounted for only a small fraction of total variance of uniform shifts. These new regression results helped rule out the contribution of changes in EMG activity to the neural uniform shift. These results (alongside the analytical formulation that would explain to a reader the essence of the very concern you have raised) is in Supplementary Note 2.

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Supplementary Table 1. Explained variance of the observed uniform shift in preparatory activity by multiple alternative models, in addition to the preferred learning-related uniform shift model. Variance explained is reported as the five-fold cross-validated \( R^2 \) computed as 1 − sum squared error/variance.

Minor Comments:
Fig 1. legend: It would be useful here to clarify in part (d) that the no learning data comes from comparing error-clamp and null-field trials (from the 1st block?) in the “center-out reach control” experiment.

Thank you for the helpful suggestion. We have added to Fig. 1d caption that “the no-learning control results come from comparing hand forces in error-clamp trials of block iii and no-clamp trials of block i in control experiment sessions”.

Fig 2 legend: It would be useful to indicate in part (a) which monkey(s) the data comes from.

Thank you again. We have added to Fig. 2a caption which monkey (monkey U) the data comes from.

Lines 121-124: Here you indicate that projecting after learning trials into the 2D TDR hand-force subspace built using before learning trials yielded predictions of the new hand forces which were strongly correlated with the observed ones and exhibited high accuracy. I agree that the real hand force is indeed well correlated with the prediction but when it comes to accuracy it seems that we should know something about the slope of a regression between actual and predicted values and how close the slope is to 1 (intercept is to 0) to know how accurate the predictions made based on the before learning 2D TDR subspace are.

Thank you for pointing out the importance of presenting the slope and intercept of this regression conducted for this purpose. For the regression lines in Fig. 2b, the slope is 0.6388 for monkey U and 0.409 for monkey V; the intercept is 0.1074 for monkey U and 0.0234 for monkey V. We have added the slopes and intercepts to the figure caption. Fig. 2c of the original manuscript presented the MSE of the TDR regression, which was aimed at reporting the TDR prediction accuracy quantitatively.

Lines 243-319: As noted above, this section describing the results of interference experiments provides a reasonably detailed description of results which only appear in Extended data figures and I felt this substantially detracted from the flow of the article. These results are potentially interesting but in my opinion should be presented in a format in which the data figures can be integrated with the main text.

Thank you, we agree that presenting the interference experiment in the main figures could help highlight the main results. In order to integrate the interference results with the main figures while also keeping the main text succinct, we have (1) combined current Figs. 2 and 3 into one
figure as a new Fig. 2, (2) reduced the size of main figure panels by roughly 50%, (3) presented the interference plots in a more simplified format that focuses on the major findings (see our response to the next question), (4) moved the major interference results to main figures (as Fig. 4), and (5) moved the discussion of alternative explanations for the uniform shift to Supplementary Materials that we believe could help shorten the main text and meanwhile maintain the comprehensiveness of the entire manuscript.

Extended Figures 10c and 11c: These figures are very crowded and I found it difficult to see much. For example it wasn’t clear to me whether there was any shift at all in the top panels of Figure 10c and my impression was that the little triangles for monkey V on the bottom right were not pointing in the correct direction. I could easily be wrong but the point is that I think the presentation here could be improved perhaps by focusing on the targets of interest and removing the others.

Thank you for pointing out how we could present the interference results more clearly. After double-checking, we believe that the little triangles are pointing to the correct direction. We agree with the reviewer that these two figures are very dense and could be challenging for readers to parse. In this new revision, we have removed neural states of the farthest four targets from all TDR plots and focus on neural states of the seven reach directions influenced by the curl fields. The modified plots are copied below for convenience.

Fig. 4 | Interference and sequential learning of opposite curl fields applied to two targets 30 degrees apart... c, Preparatory neural states in the force-related 2D TDR subspace. Before-learning states
(color circles) spatially organize corresponding to reach directions. Error-clamp states (color diamonds) rotate counterclockwise for reach targets near the target that has a CW field and rotate clockwise for targets near the target that has a CCW field. Preparatory states of the two targets that have curl fields (gray and black triangles) rotate opposite their curl field directions in the interference block (left panel) and further separate in the sequential-learning block (right panel). Small arrows point to the before-learning states of reach targets that later have curl fields (trained targets). Neural states of seven (out of 11) targets are visualized in c, and quantification of neural state changes for all 11 targets is shown in d.

Extended Data Fig. 7 | Interference and sequential learning of opposite curl fields applied to the same target... c, Preparatory neural states in the force-related 2D TDR subspace. Before-learning states (color circles) spatially organize corresponding to reach directions. In the interference block, preparatory states of the two curl fields (gray and black triangles) are mixed together around the before-learning state (left panel). Error-clamp states (color diamonds) of most targets shift from their corresponding before-learning states. These shifts do not show coherent patterns across targets or monkeys and are likely due to noise. In the sequential-learning block, preparatory states of the two curl fields (gray and black triangles) gradually rotate opposite their curl field directions (right panel). The small arrow points to the before-learning state of the reach target that later has curl fields (trained target). Neural states of seven (out of 11) targets are visualized in c, and quantification of neural state changes for all 11 targets is shown in d.

Lines 295-298 and Figures 10e,11e: It seems that the most prominent (and new) observation here is that there is a considerable uniform shift associated with the interference environment (i.e., attempting to simultaneously learn opposing curl fields). Although the authors suggest that this shift may reflect an “attempt to learn or index neural activity patterns for reaching in an unpredictable environment” it seems to me that most likely explanation for this shift is that it reflects the preparatory activity associated with muscle co-contraction (changing limb impedance). Is that what the authors meant by their statement? Did they look at muscle activity?
Thank you for raising this important potential explanation for the “residual interference uniform shift”. As the reviewer astutely points out, muscle co-contraction or impedance control could be a likely strategy in this situation (Franklin et al. 2003; Heald, Franklin, and Wolpert 2018). And by “an attempt to learn or index neural activity patterns for reaching in an unpredictable environment”, we indeed meant that “the residual interference shift likely relates to an attempt to index the neural activity patterns specific to the interference context, which might also facilitate strategies tailored to adapting to an unpredictable environment (e.g., impedance control via co-contraction to stabilize the limb)”. We have edited the manuscript with these clarifications ([lines 323-327]). We also note that due to the impact of COVID and the slow, deliberate and systematic ramp down of the nonhuman primate lab (due to Krishna Shenoy’s need to carefully tend to his health and not try to do ‘too much’, following my pancreatic cancer diagnosis years back), we did not record muscle activity in the interference experiment to directly test whether or not monkeys increased muscle co-contraction during interference.

This residual uniform shift was a new finding in the interference experiment that we did not expect but wanted to touch upon and leave our speculative explanations in the manuscript. We did not perform experiments to further investigate this shift systematically and we believe that it is beyond the scope of this work. But we believe it would be interesting for future work to address.

Lines 480-484 and Extended Data Fig. 18a: Here you provide evidence that preparatory states in error-clamp and no-clamp trials are not significantly different in the no-learning control sessions. Overall (i.e., across all reach directions) that does seem to be the case but it also looks as if for certain individual reach directions there might be substantial differences between error-clamp and no-clamp states. One problem is that it’s hard to see this clearly (and this could easily be an illusion) since preparatory states are plotted here with a different perspective as compared to most other plots (e.g., Fig 18b, 3a, 5c). Can the authors comment and show the plot in Fig 18a with a similar perspective to that in Fig. 18b?

This is a great suggestion. In the revision, we plotted Extended Data Fig. 18a (now Extended Data Fig. 5a) with a perspective similar to the perspectives in Fig. 3a (now Fig. 2f) and Extended Data Fig. 18b (now Extended Data Fig. 5b), which is copied below for convenience. This new plot shows that in control no-learning sessions, error-clamp and no-clamp preparatory states are mixed together without a uniform shift.
Extended Data Fig. 5 | The uniform shift is not due to the error clamp.

a, Preparatory states in error-clamp (color diamond) and no-clamp (color circle) trials are not significantly different in no-learning control sessions (Hotelling’s T2 test: P > 0.5 for all control sessions of monkeys U and V). The plot is from one example session of monkey U...

Hotelling’s $T^2$ test showed that preparatory neural states in error-clamp and no-clamp trials were not significantly different in the no-learning control sessions across all reach directions. Importantly, we also did not observe a uniform shift between error-clamp (block iii) states and no-clamp (block i) states in all those control sessions. Together with other results shown in the new Extended Data Fig. 5, these findings support the idea that the uniform shift of preparatory states is not due to the error clamp.

Line 532: In the end, how much learning data was collected for a right reaching target as compared to up/down reaching targets? It sounds to me as if there was much more up/down reaching data collected and this makes me wonder if this could have biased the analyses/results in any fashion.

Thank you for bringing up the concern about a potential target-dependent bias. Out of the 13 learning datasets (monkeys U and V), 3 were collected for a right reaching target with curl fields and 10 for up / down reaching targets with curl fields. We have added this detail to Methods in the manuscript (lines 642-645). The reviewer is correct that more datasets had a curl field applied to the up / down targets than to the right target. However, we believe that this did not bias the results in a target-dependent fashion for the following reason: in all analyses, we investigated each dataset separately before grouping them together for plotting or statistical tests. When comparing results from different datasets, we did not find differences in the main results in a target-dependent fashion except that neural states visualized in the TDR subspace were spatially organized according to the reaching target arrangement of a specific session. Lastly, we want to note here that before neural data collection, our monkeys had performed center-out reaches to evenly-distributed targets in many early training sessions so they were certainly experienced in reaching to balanced targets. Thus, we believe that the main results
across all learning datasets were consistent and do not expect any bias due to more data being collected for up / down targets.

Line 604: In block ii of the control experiment (i.e., replacing the learning block in learning task experiments) were null-field trials made to a single target as in learning experiments or were reaches more or less evenly distributed across the 12 targets? Similarly, in block iii were many null-field trials made to 1 target with only error-clamp trials made to the other targets (i.e., similar to a learning experiment) or were both null-field and error-clamp trials distributed across directions more or less evenly? What was the relative percentage of error-clamp vs null-field trials in the error clamp block?

In blocks ii and iii of the control experiment, null-field trials were evenly distributed across the 12 targets. In block iii, 70% of trials were null-field trials and 30% were error-clamp trials (same ratio as curl-field trials vs. error-clamp trials in the learning experiment). We have added this detail to the Center-out reach control experiment part in Methods ([line 594]).

Line 759: I’m still not entirely sure what “using center-out reach trials matching the trial IDs in a learning session” means. Were error-clamp trials with a particular trial ID in block iii of the learning task also error-clamp trials in block iii of the control task such that when you looked at changes in neural repertoire in the control task you were also comparing null-field to error clamp trials?

Thank you for identifying a point which deserves additional clarification. By “matching the trial IDs”, we meant that the trial indices (within a session) for trials in a certain block of the control task are within the same range as trials in the same block of the learning task (for instance, all error-clamp trials could have trial indices between 500 and 1000 in both learning and control tasks). In other words, this is how we assure that we are matching the average time each day across the different blocks. But the particular trial index of a certain trial type in the learning task was not necessarily exactly the same as in the control task. As the reviewer noted, when we looked at neural repertoire changes in the control task, we always compared the null-field trials in block i to error-clamp trials in block iii, same as what we did when computing neural repertoire changes in the learning task. We have adjusted the language under subheadings Center-out reach control experiment and Measurement of changes to the neural repertoire in Methods accordingly to further clarify the control experiment design ([lines 597-602] and [lines 755-760]).

Lines 831-832: For the interference experiment data analyses you now used both before-learning and error-clamp trials to build the TDR model. Why not only use only before-learning trials as in the previous analyses? Does it make any difference whether you use only before-learning trials, only error-clamp trials or both in these analyses?
Thank you for identifying the distinction between the interference experiment analyses and the learning experiment analysis, which deserves additional clarification.

First, we would like to clarify our reason for using both before-learning trials (in the before-learning block) and error-clamp trials (in the interference block) in the TDR analysis of the interference experiment data. In all analyses, we wanted to investigate how learning shifted preparatory neural states. However, there was a major difference between the interference experiment and the learning experiment: in the interference experiment, the actual learning (i.e., sequential learning of two curl fields without interference) happened after the interference block; in contrast, in the learning experiment, learning happened right after the before-learning block. Thus, for the interference experiment data, building the TDR subspace with both before-learning and error-clamp trials was a slightly cleaner way to isolate only the effect of sequential learning on neural states. Nevertheless, we have tried both ways of building the TDR subspace, as suggested by the reviewer, and the results remained similar (see plots below).

Moreover, as discussed in our response to the reviewer’s major comment 1, TDR 1 axes before-and after-learning were largely aligned in the learning experiment; so were TDR 2 axes. Here in the interference experiment, we compared TDR 1 and 2 axes using only before-learning (or, only error-clamp) trials to TDR 1 and 2 axes constructed with both before-learning and error-clamp trials. We found that TDR 1 axes in all cases were largely aligned, and same for TDR 2 axes. The alignment of TDR axes was measured by their pairwise dot products close to 1. The plot of dot products has been added to Extended Data Fig. 5 and is copied below.

Extended Data Fig. 5 | ... e, The 2D TDR planes constructed from only before-learning trials, only error-clamp trials, and both are aligned. Dot products between TDR axes using only before-learning (BL) trials, only error-clamp (EC) trials, and both (BL+EC). Left column: TDR axes using only before-learning trials were largely aligned with their corresponding TDR axes using both before-learning and error-clamp trials.
Right column: TDR axes using only error-clamp trials were largely aligned with their corresponding TDR axes using both before-learning and error-clamp trials. One-sided sign-rank test compared to 0.8: monkey U + monkey V, $P = 3.9 \times 10^{-3}$...

Consistent with the dot product relationships, the TDR results using only before-learning trials or only error-clamp trials remained similar to our original results. The plot of TDR neural states in one representative session has been added to Extended Data Fig. 5 (copied below for convenience) and we are happy to show plots from all sessions if deemed helpful and/or necessary (again we are attempting to not inflate the manuscript’s length). Therefore, we believe that it did not make a fundamental difference whether we used before-learning trials, error-clamp trials, or both to build the 2D TDR subspace.

Extended Data Fig. 5 | ... f, Preparatory neural states show similar patterns in the force-related 2D TDR subspace built from only before-learning (BL) trials, only error-clamp (EC) trials, or both (BL+EC). One example and representative session from monkey V. Small black arrows point to the before-learning states of reach targets that later have curl fields (trained targets). Lighter gray triangles: earlier learning trials; darker gray triangles: later learning trials.

Typos:

Abstract, line 36: Should be “shifts”.
Thank you for pointing out this typo; it is now fixed.

References
Franklin, David W., Rieko Osu, Etienne Burdet, Mitsuo Kawato, and Theodore E. Milner. 2003. “Adaptation to Stable and Unstable Dynamics Achieved by Combined Impedance Control


Reviewer Reports on the Second Revision:

Referee #3 (Remarks to the Author):

General Comments:

The authors have done an excellent job of addressing my comments. The clarifications and additional analyses were most appreciated and have alleviated my remaining concerns regarding potential confounds in the interpretation of the results. The authors have gone to great lengths to convince the reader that the observed uniform shift indeed reflects an indexing of motor memories as opposed to other potentially confounding variables and have organized all this information into a comprehensive presentation and discussion of the related analyses in Supplemental Note 2. I recognize that this was an enormous amount of work but I do believe this has considerably strengthened the manuscript as readers will certainly be convinced that every effort has been made to rule out potential alternatives and that the proposed interpretation of the uniform shift is indeed appropriate.

The authors have also done an excellent job of revising and reorganizing the manuscript such that the key figures, including those associated with the interference experiments, now appear within the main text, with more redundant panels appearing in the extended data figures. The article reads very well, all the key results are readily accessible within the main text and despite a reduction in the size and number of figures the results are very completely presented. After streamlining, the manuscript is far more accessible to a broad audience and, I believe, entirely appropriate for publication in Nature.

Most importantly, the results substantially advance our understanding of the neural basis for learning and retaining motor memories of multiple learned skills/force environments and the way to think about this capacity at the level of neural populations.