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Binding during sequence learning does not alter cortical representations of individual actions

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Authors:
Patrick Beukema[1,2], Jörn Diedrichsen[3] and Timothy Verstynen[4,2]
[1]Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260
[2]Center for the Neural Basis of Cognition, University of Pittsburgh & Carnegie Mellon University, Pittsburgh, Pennsylvania, 15213
[3]Brain and Mind Institute, Departments of Statistics & Computer Science, University of Western Ontario
[4]Departments of Psychology & Biomedical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania, 15213

Corresponding author:
Timothy Verstynen
Department of Psychology
342C Baker Hall
Carnegie Mellon University
Pittsburgh, Pennsylvania, 15213
email: timothyv@andrew.cmu.edu

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ABSTRACT

As a sequence of movements is learned, serially ordered actions get bound together into sets in order to reduce computational complexity during planning and execution. Here we examined how actions become naturally bound over the course of learning and how this learning impacts cortical representations of individual actions. Across five weeks of practice, neurologically healthy human subjects learned either a complex 32-item sequence of finger movements (Trained group, N=9; 3 female) or randomly ordered actions (Control group, N=9; 3 female). Over the course of practice, responses during sequence production in the Trained group became temporally correlated, consistent with responses being bound together under a common command. These behavioral changes, however, did not coincide with plasticity in the multivariate representations of individual finger movements, assessed using fMRI, at any level of the cortical motor hierarchy. This suggests that the representations of individual actions remain stable, even as the execution of those same actions become bound together in the context of producing a well learned sequence.

SIGNIFICANCE STATEMENT

Extended practice on motor sequences results in highly stereotyped movement patterns that bind successive movements together. This binding is critical for skilled motor performance – yet it is not currently understood how it is achieved in the brain. We examined how binding altered the patterns of activity associated with individual movements which make up the sequence. We found that fine finger control during sequence production involved correlated activity throughout multiple motor regions; however, we found no evidence for plasticity of the representations of elementary movements. This suggests that binding is associated with plasticity at a more abstract level of the motor hierarchy.

INTRODUCTION

Being able to combine simple movements into coordinated sets of actions is critical to many everyday skills, such as typing on the computer or driving a manual transmission car (Lashley, 1951). Over the course of evolution the brain has solved this sequencing problem multiple times, resulting in many interacting algorithms that facilitate the consolidation of complex skills (for review see Beukema and Verstynen 2018). One of these algorithms is the process of set building, also called chunking or binding (Verwey 1996). Binding serial actions into sets improves computational efficiency during the production of complex actions by representing multiple movements under a single selection command (Ramkumar et. al, 2016).

To illustrate this process consider the graphical model presented in Figure 1. On each trial, the manual response to a visual cue occurs through a hierarchical system of perception, selection (e.g., key), and motor planning (e.g., finger movement), that are all represented as latent states with their own independent sources of noise. In this example, the serial order of cues across trials follows a deterministic sequential order. Prior to training (Figure 1A), each response is selected and planned independently of the other responses. Once the order of cues is learned (Figure 1B), the brain can consolidate the selection process such that a set of motor plans is represented under a single selection state. This selection state is triggered by the presentation of the first stimulus in the series, after which subsequent motor commands are cued by the internal state, rather than by the visual cues. This produces faster responses to items within a set, as well as a correlation in responses within bound sets due to their shared upstream command (Figure 1C; Verstynen et al., 2012; Acuna et al., 2014; Lynch et al., 2017).

<INSERT FIGURE 1 HERE>

***Figure 1**: The process of response binding **A**. One each trial, (t), a visual stimulus (s) triggers an appropriate finger response (y), in this case reflecting a response time (RT). In the case of unbound actions, the visual perception (u), selection (w), and motor planning (x) processes are all represented as latent states that operate independently across trials. **B**. With training, the intermediary process of selection binds multiple motor plans together as a set. Each set of actions, τ, is triggered by the visual stimulus of the first item in the set. Subsequent actions are then internally triggered, rather than relying on external visual cues. This example shows two bound sets, a three item set followed by a two item set. **C**. The autocorrelation function of response times for bound actions (dashed line) should exhibit a significant correlation across trials, while unbound actions (solid line) should not exhibit a temporal autocorrelation. **D**. A schematic of four hypothetical voxels in cortical sensory motor networks during the execution of either the index or middling finger, with darker colors reflecting stronger movement-evoked responses. Before training, each finger representation is associated with a unique neural activation pattern. After training, the representations of bound finger movements share more activation and the neural activation patterns are more similar.*

Some forms of non-sequential motor learning rely on the reorganization of movement representations in motor networks (Nudo et. al. 1996), suggesting that action binding during sequence learning could alter internal motor representations of individual movements themselves; however, this effect has been largely unexplored. By examining neural representational patterns, previous work has shown that the structure of individual fingers in primary motor cortex is organized according to their co-articulation during natural hand movements (Ejaz et. al., 2015), suggesting a degree of plasticity of the cortical representations of individual digits (Merzenich et al. 1984). Indeed, artificial manipulations of pairwise finger correlations alters the distance between finger representations in primary somatosensory cortex (Kolasinski et. al. 2016), although representations of individual fingers can persist in the cortex even decades after amputation (Kikkert et al. 2016), suggesting some degree of rigidity in sensory areas (Makin & Bensmaia 2017). Thus it remains unclear whether elementary sensory or motor representations are plastic and subject to changes over time.

If individual actions are bound under a common motor command, then the internal representations of those actions, at some level of the motor hierarchy, should change with learning. One possibility is that if two movements are executed repeatedly in a sequence, then the activation of one finger movement may pre-activate the following movement. In the extreme, this model makes the prediction that two fingers that are regularly paired together will become enslaved together over time, thereby reducing behavioral flexibility (Lashley, 1951). This, however, is not typically observed. It is therefore more likely that the process of binding alters the representation of contextually cued actions in upstream regions linked to more abstract response selection (Diedrichsen and Kornysheva, 2015), which would predict observing altered representations in higher premotor areas (e.g., premotor and parietal regions). Wherever this binding process happens, the multivariate activity pattern for the two bound movements should become more similar in that region (Figure 1D).

Here we tested this hypothesis using a combination of behavioral analysis and event-related fMRI. Binding was measured behaviorally by looking at the naturalistic emergence of correlations between successive behavioral responses after training on a unimanual 32-item sequence. Population-level representations of visually-cued single finger movements in the cortex were measured using multivariate analysis of fMRI data both before and after five weeks of training on the complex sequence. If the simple binding hypothesis is correct, then cortical representations for individual actions that are bound should be reduced following prolonged practice at the motor sequence task.

MATERIALS AND METHODS

Participants

Eighteen right-handed participants (6 female, mean age: 26 years) were recruited locally from Carnegie Mellon University (CMU) and the University of Pittsburgh. Two authors (PB and TV) were included in the sample. All participants provided informed consent and were financially compensated for their time. All experimental protocols were approved by the Institutional review board at CMU.

Experimental Design and Statistical Analysis

Participants were trained for 25 nonconsecutive days on a variant of the serial reaction time task (Nissen and Bullemer, 1987). Participants were instructed to train for at least 5 days a week, but could chose to take time off (no more than 2 days) at their discretion, and not in the days leading up to the scan. All experimental procedures were performed on a laptop running Ubuntu 14.04. At the beginning of each training session, participants were instructed to place their right hand over the ”h” (index),”j” (middle), ”k” (ring), and ”l” (pinky) key. Each trial consisted of a presentation of one of four unique fractal cues appearing on a black background. Each cue was uniquely mapped to one of four keys on the keyboard (Figure 2A). The trial ended either when the participant executed a response or once a maximum response window expired, depending on which event happened first. A description of the adaptive response window is presented in the next paragraph. After a trial termination, the next cue was presented after a 250 ms inter-trial interval. Each trial block consisted of 256 trials and was followed by a rest period where the mean response time (RT) and accuracy for that block was provided to the participant. On each training day, participants completed 1792 trials, separated into 7 trial blocks. RT was calculated as the delay between stimulus presentation and a key press. Stimulus presentation and recording

was controlled with custom written software in Python using the open source Psychopy package (Peirce, 2007). The software used for training is available on GitHub (CoAxLab, n.d.).

Prior to the first session, subjects were assigned to either a Trained group (n=9; 3 female) or a Control group (n=9; 3 female). For participants in the Trained group, trial blocks were separated into two types: blocks of pseudo randomly ordered cues (Random; blocks 1,2,6) or blocks of deterministically ordered cues following an embedded 32-element sequence (Sequence; blocks 3,4,5,7). Figure 2B shows the blockwise structure for a single subject in the Trained group. Trials during the Random blocks were constrained such that repeated presentations of the same cue were excluded. This was done so that Random trial blocks would appear more similar to the Sequence trial blocks. The 32 element sequence presented on Sequence blocks consisted of the following key presses: 3-4-2-3-1-4-2-1-3-4-3-4-1-3-4-2-1-2-4-2-3-1-2-1-2-4-3-1-3-1-2-4 using the mapping (1-index finger, 2-middle finger, 3-ring finger, 4-little finger). Each Sequence block began in a random position of the sequence. For the first 2 blocks, the response threshold for each trial was set to 1000 ms. To encourage faster responses, the response window of blocks 3-5 was adaptively controlled such that the response window on one trial block was the mean plus one standard deviation of the RTs from the previous trial block. If that value fell below 200 ms or if the accuracy on the preceding block was less than 75%, the threshold was reset to 1000 ms. The threshold was removed for the final probe blocks (6 and 7) so that participants could move as quickly as they chose. For the Control group, the procedure was nearly identical to the Trained group, with the exception that all 7 blocks consisted of pseudorandomly ordered trials, i.e. there was no exposure to Sequence blocks.

Analysis of training data

Data analysis was conducted with custom python code which is available on GitHub (https://github.com/CoAxLab/binding_manuscript) along with source data to generate all manuscript figures. All behavioral analysis during training focused on responses during the last two trial blocks (probe blocks) when no adaptive response window was applied: Random and Sequence conditions for the Trained group, Random and Random conditions for the Control group. Differences in response time (RT) and accuracy (percent correct responses) were measured as the difference in the means between the last two blocks, normalized by the standard deviation of values in trial block 6, i.e., z-scored difference in performance (Verstynen et al., 2012). In the Trained group this reflects the sequence specific change in performance on each day. Since 3 subjects completed 24/25 days of training, average group visualizations are presented for day 24 so as to evaluate the same state of learning for all subjects.

Binding was measured by computing the autocorrelation of the series of RTs within each probe trial block. The first 32 trials were excluded to remove the exponential decay as it distorts the autocorrelation analysis (Verstynen et al., 2012). The linear trend was then removed by regression and the residuals were used to calculate the autocorrelation function for lags 1 through 31, following the same procedure as described in (Verstynen et al., 2012; Lynch et al., 2017).

Positive autocorrelations could be confounded by the fact that the Trained group executed faster responses than the Control Group. Therefore, we also examined the correlation as a function of the inter-press interval using linear regression. The IPI was computed as the time between successive key presses, and the correlation was computed as before. For every subject we computed the slope of the linear regression line between IPI and correlation (Figure 3D).

Since the autocorrelation function measures general associations across all sequential lags, it is not sensitive to specific associations between individual elements, and therefore cannot

be used to measure binding between specific finger pairs. Therefore, we conducted a secondary analysis on the same data but examined pairwise correlations between each distinct element (1-32) in the sequence across cycles. Average correlations, ordered by sequence element, are shown in Figure 4A-B. Binding between successive elements is reflected by increases in correlations before compared to after training.

To measure how much the correlation between finger responses matches the statistical structure of the trained sequence, we collapsed the elementwise correlation matrices by finger identity (index, middle, ring, pinky), forming 4x4 observed correlation matrices. To measure the similarity of the observed binding structure to the expected binding structure, we computed the mean squared error between the finger pairing frequencies of the sequence and observed correlations. This gives a normalized similarity measure for how well the pattern of correlations in the behavioral responses matches the pairwise similarities of the trained sequence.

Imaging acquisition

Participants were scanned twice, the day before training started (pre-training) and within 2 days of training completion (post-training). All participants were scanned at the Scientific and Brain Research Center at Carnegie Mellon University on a Siemens Verio 3T magnet fitted with a 32-channel head coil. High-resolution T1-weighted anatomical images were collected for visualization and surface reconstruction (MPRAGE, 1 mm isotropic, 176 slices). A fieldmap with dual echo-time images (TR: 746 ms, TE1: 5.00 ms, TE2: 7.46 ms, 66 slices, 2 mm isotropic) was acquired to correct for fieldmap inhomogeneities. For the functional imaging sessions, we acquired 241 T2* weighted echo-planar imaging volumes (2 mm isotropic, TR: 2000ms, TE: 30.3 ms, MB factor: 3, 66 slices, A >> P, FoV: 192 mm, interleaved ascending

order, flip angle: 79 deg, matrix size: 96x96x66, slice thickness: 2.00 mm). For the finger mapping task, we collected a total of 6 runs resulting in 1446 volumes. Functional images were oriented so as to maximize coverage of the entire cortex and cerebellum. All imaging data is openly available at OpenNeuro: https://openneuro.org/datasets/ds001233/versions/00003.

Neuroimaging tasks

We collected a set of finger mapping runs to estimate the activation patterns evoked by performing each distinct cue-response pair in isolation (i.e. not embedded within a sequence). Prior to the first scan, subject learned the mapping of cue to effector. The same stimuli from the behavioral experiments were projected on an MR-compatible LCD screen mounted at the rear of the scanner. Participants could see this screen through a mirror mounted on the head coil. Responses were recorded on a five-key MR compatible response glove (PST Inc.) placed under the right hand. Each effector (e.g., individual cue-response pairing) was presented in isolation on each trial with no structured order between trials. Thus, the paradigm only measured responses to individual cued movements, not the sequence itself. Each trial type was repeated 12 times per run totaling 72 trials per session. Subjects were instructed to press the cued key several times following stimulus presentation until the cue disappeared from the screen (1 second). The inter-trial interval was sampled according to an exponential distribution ranging from 6-18 seconds. Between runs, subjects were given the option to take several minutes of rest.

Imaging Analysis

Functional imaging data were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) and custom Matlab and Python functions. Raw functional EPI images were realigned to the first

volume. No slice time correction was applied due to the fast TR. These realigned images were then corrected for field distortions using the field maps. All analyses were performed in native functional space. Structural T1 images were used to reconstruct the pial and white surfaces using Freesurfer (Fischl, 2012). All custom code is publicly available (CoAxLab, n.d.).

All analyses of task-related responses were performed using a region of interest (ROI) approach. Anatomical ROIs were defined separately for each subject, using the surface based Brodmann areas extracted from Freesurfer (Fischl et al., 2008) following similar conventions as described in (Wiestler and Diedrichsen, 2013). The hand voxels of the primary motor cortex (M1) were defined as the surface nodes with the highest probability of belonging to Brodmann area (BA) 4, 1 cm above and below the hand knob (Yousry et al., 1997). S1 was defined as the nodes in BA1 BA2, BA3a, or BA3b, 1 cm above and below the hand knob. Premotor cortex was defined as the nodes belonging to BA6 medial (PMv) or lateral (PMd) to the medial frontal gyrus. Supplementary motor area (SMA) was defined as the voxels in BA6 along the medial wall. The Freesurfer atlas was used to define the superior parietal gyrus, as well as the putamen and caudate as these regions are not defined by Brodmann areaa. As a control ROI, we extracted the voxels belonging to primary auditory cortex as this region would not be expected to exhibit any significant decoding of the visually-cued finger patterns. Each surface based ROI was projected back into native functional space.

Analysis for effector representations was performed using representational similarity analysis (RSA, Kriegeskorte et al., 2008) using the crossnobis estimator (Nili et al., 2014, Walther et al., 2015). A GLM with regressors for each effector was fit for each mapping run, along with the six head motion regressors (x, y, z, pitch, yaw, roll). Omissions and incorrect key presses were regressed out of the model. Raw time series were orthogonalized by eigenvector

decomposition and projected into the principal component space to minimize model bias in the decoding. To estimate the differences between finger patterns, we used a cross-validated estimate of the Mahalanobis distance between activity patterns for each effector (Diedrichsen et. al. 2016). The "crossnobis" distance has the advantage over other distance measures in that it is unbiased, since noise is orthogonalized across runs, resulting in an expected distance of 0 if a voxel or region does not reliably distinguish two finger patterns (Ejaz et al., 2015). The estimated distance ($\hat{d}_{i,j}$) between the patterns (u) of two fingers (i,j) was averaged across every pair (m,l) of runs (M) resulting in (6 choose 2) = 15 folds using the following equation:

$$\hat{d}_{i,j} = \sum_{l,m;l\neq m}^{M} \frac{(u_i^m - u_j^m)^T (u_i^l - u_j^l)}{M(M-1)}$$

Equation 1

Unlike correlation distances, Mahalanobis distances can exceed the value of 1. Furthermore the cross-validated nature of the crossnobis estimate also allows *d* to become negative. The pairwise distances between each of the fingers are summarized in a representational dissimilarity matrix. To test for encoding and plasticity within each voxel or ROI, we extracted the average distance between each pair of fingers pattern (K=4) using the following equation:

$$H = \sum_{i\neq j}^{K} \frac{\hat{d}_{i,j}}{K^2 - K}$$

Equation 2

To examine the extent of finger representations across all of cortex, we conducted a surface-based searchlight (Oosterhof et al., 2011), assigning every surface node an H value based on the local (p=160) patterns surrounding an approximately 10 mm radius. Values for the number of voxels (p) and radius were chosen based on previous studies (Yokoi et. al. 2017). This

searchlight approach enabled us to examine the entire H distribution across all voxels in each of the ROIs to confirm that each region reliably discriminated individual effectors. Due to the observed positive skew, we extracted the median H for all regions across all subjects and conducted a one sample t-test against 0, in order to establish whether a region reliably decoded the single finger movement representations.

Changes in representational distances were estimated by calculating the difference in H values, for each ROI, between the post-training and pre-training imaging sessions (i.e., H_{post}-H_{pre}). For each ROI we calculated both pre-trainig and post-training H values using the responses from all voxels in the region mask. To estimate group-level training effects, the average difference in H from these voxels was calculated for each subject and each ROI. The change in H values was determined by looking for consistent patterns across subjects, within each ROI. Along with the group level effects, we also calculated the significance of changes in H at the single subject level.

In addition to the standard null hypothesis tests, a repeated measures ANOVA was used to examine the influence of training on distances in each ROI. Bayesian repeated measures ANOVA with a JZS prior over all models was used to determine the inclusion Bayes Factor to measure the extent to which the data supported inclusion of the interaction effect (JASP Team, 2017, jasp-stats.org). The guidelines in (Kass and Raftery, 1998) were used to interpret the weight of the evidence in support of the null hypothesis.

RESULTS

Learning-related changes in behavior

To assess how training impacted performance, we compared the evolution of response times and accuracy across days for the Trained and Control groups. Figure 2B illustrates all trial-wise responses during a single day for a subject in the Trained group. While responses during random trial blocks (black dots) remained relatively constant, the response times during sequence trial blocks (green dots) get steadily faster with training. The last two trial blocks were used to probe learning across time. On average both the Control (dashed line, Figure 2C) and Trained subjects (dashed line, Figure 2D) exhibited a general improvement in response speeds during the final random trial block (block 6). This general across-session speeding of responses during a trial block with random sequences likely reflects the improved learning of the cue-response mapping across days. During the final sequence block (block 7), however, sequence-specific responses in the Trained group also decreased rapidly across training days. Repeated measures ANOVA indicated a significant block x time effect: $F(23,368) = 15.37$, $p = 7.93 \times 10^{-41}$, with average response times dropping just below 200ms at the end of training (solid line, Figure 2D). As expected, this effect was not observed in the Control group, $F(23,368) = 0.77$, $p = 0.76$, where the final trial block did not contain an embedded sequence (solid line, Figure 2C). In order to capture sequence-specific changes in response speed, we normalized the mean response time for the final trial block (sequence in Trained group, random in Control group) by the mean and variance of response times during trial block 6 (random in both groups; see Methods). This analysis depicts a steady improvement in sequence specific response times across the 5 weeks for the Trained group, with sequence block responses approximately 4 standard deviations faster than the random trial blocks at the end of training (Figure 2E). Repeated measures ANOVA indicated a significant group by time effect, $F(23,368) = 12.79$, $p = 1.67 \times 10^{-34}$. Unlike response speed, average accuracy during the final trial block gradually rose at a steady rate for both

groups, saturating at around 90% for the Trained group and 85% for the Control group, with no significant between group differences, $F(1, 368) = 0.36$, $p = 0.99$ (Figure 2F).

<INSERT FIGURE 2 HERE>

Figure 2: *Task design and behavioral performance.* ***A****. Participants practiced a serial reaction time task in which each finger movement was prompted by a unique cue.* ***B****. Representative reaction time plot from Day 12. Each dot represents the response time on one trial.* ***C****. Reaction times for the Control group for random trials on blocks 6 and 7.* ***D****. Reaction times for the Trained group for the random trials (block 6) and sequence trials (block 7).* ***E****. Mean z-scored reaction times as a function of day for the Control group (blue) and Trained group (peach).* ***F****. Mean accuracy (correct trials/total trials) in the final trial block, as a function of day, for the Control group (blue) and Trained (peach) group. Shaded regions in panels C-F show standard error.*

There are several ways that responses could get faster during the sequence blocks (see Beukema and Verstynen, 2018). The binding hypothesis (Figure 1B), however, makes the specific prediction that serially successive actions that are bound under a shared motor plan should exhibit a correlation in their responses over time, as a consequence of arising from a common, high-level motor plan (Figure 1C). For an index of binding, we used the autocorrelation of RTs during the last trial block for both groups (Verstynen et. al. 2012). Figure 3 shows the autocorrelation functions for early (Day 1), middle (Day 12), and late (Day 24) stages of practice for the Control (Figure 3A) and Trained (Figure 3B) groups separately. While participants in the Control group did not show reliable autocorrelation structure in RTs with training, we did see evidence of an emergent structure in the Trained group. Specifically, participants in the Trained group showed no evidence of an autocorrelation in their RTs at Day 1; however, by the middle of training a pronounced autocorrelation of temporally adjacent responses emerged. This correlation increased throughout the training period, tapering off at approximately the middle of training (Day 12) (Figure 3B inset).

To exclude the possibility that the observed increases in RT autocorrelations are simply the result of executing faster responses, we also examined the correlations in consecutive inter-trial RTs as a function of inter-press interval (IPI). If the increased correlation in temporally adjacent RTs was simply the result of faster responses, then a negative relationship should exist between the observed autocorrelation and the inter press interval, with higher correlations for faster responses, and little or no correlation for slower responses. A representative example of the relationship between the IPI and the RT correlation is shown in Figure 3C reveals no clear association. Across all subjects, the slope of the regression line between the two variables was not significantly different from zero (Figure 3D). This result suggests that the observed increases in correlation are due to executing responses under a shard motor command and not the result of speed increases alone.

We next set out to examine the structure of the associations across movements by examining the pairwise correlations between items in the sequence. For this analysis we organized the data into a matrix of 32 responses by cycles. We then looked at the correlations between different sequence elements across cycles of sequence production. Before practice, this 32x32 correlation matrix does not show much structure, with all items approximately equally correlated (Figure 4A). After training, a clear structure in the correlations emerged, with local clusters of correlated responses found along the diagonal of the matrix (Figure 4B).

If these clusters of correlated responses in the sequence reflected the inter-finger transition frequency (Figure 4C), then the pairing frequency of individual fingers should determine the degree of similarity between finger responses. Thus we repeated our inter-item correlation analysis, except rather than mapping response to each item in the sequence, we mapped it to the finger that executed the response. This was done by creating a new matrix of

single trial response times with each column representing a finger and each row representing a cycle through the sequence and then calculating the 4x4 correlation matrix of inter-finger responses. The similarity between the observed correlations and expected correlations based on the pairwise frequencies (Figure 4D) was computed using the mean squared error (MSE). The mean observed correlation matrix across all subjects on the final day of training is shown in Figure 4E. There was increased similarity between the observed and expected correlations across days (Figure 4F) in the Trained group $F(23,184)=0.0026$, but the structure in the Control group remained unchanged $F(23,184)=0.41$, resulting in a significant group by time interaction, $F(368,23) = 1.90$, $p = 0.0079$. These results indicate that binding occurs in a principled way that originates at least in part in the statistical structure of the sequence.

<INSERT FIGURE 3 HERE>

Figure 3: *Binding in behavioral responses.* ***A,B*** *Mean autocorrelation function for lags 1-31 during early (day 1, purple), middle (day 12, cyan) and late training (day 24, black) for the Control group (A) and Trained group (B). The asterisks indicates the significant lags, at a cut-off ($p<0.05$), for the final training day. Inset in B shows the lag 1 correlation as a function of day for the Trained group. Shaded regions show standard error of the mean.* ***C****. Representative correlations as a function of the inter press interval showing that the correlation does not appear to be a function of executing faster responses.* ***D****. Boxplots showing the slopes of the linear regression lines from the correlation by IPI relationship depicted in C for each of the Trained subjects on the sequence trials (seq) and the random trials (ran)*

<INSERT FIGURE 4 HERE>

Figure 4: A,B*. Average correlation between each element in the sequence during the final trial block for the Trained group, during Day 1 (A) and Day 24 (B).* ***C****. The 32 element sequence showing frequency of each finger transition (i-index, m-middle, r-ring, l-little)* ***F****. Pairwise frequencies between each finger* ***D****. Average observed correlations between fingers at the end of training collapsed across subjects.* ***E****. The MSE between the pairwise frequencies (panel F) and observed correlation matrix computed separately for each subject. Smaller numbers indicate increased similarity to the expected pairwise frequencies (F). Shaded regions show standard error.*

Stable motor representations after training.

In order to directly measure multivariate cortical representations of the individual cued movements, we used a rapid-event-related fMRI design consisting of presentations of each cued finger press followed by a period of fixation (Figure 5A). An ROI analysis was performed on the cortical motor network including primary motor cortex, M1; primary somatosensory cortex, S1; dorsal premotor cortex, PMd; ventral premotor cortex, PMv; supplementary motor area, SMA; and the superior parietal lobule, SPL. These regions were anatomically localized using Brodmann areas extracted from Freesurfer (see Materials and Methods). These regions are shown on the group average surface (Figure 5C). In each of the cortical motor ROIs, we quantified the activity pattern related to each cued finger movement and then calculated a cross-validated Mahalanobis (crossnobis) distance between the activity patterns for each cued finger pair (Figure 5B). If two cued fingers generate the same cortical activity patterns, then the corresponding distance between them will be 0. However, if two finger movements consistently generate dissimilar finger patterns, then the corresponding distance will be positive. Cross-validation allows us to test the value of the distance estimates directly against zero (Diedrichsen and Kriegeskorte 2017, Walther et. al. 2016, Diedrichsen et. al. 2016). The distances between every possible pair of fingers is summarized in a representational dissimilarity matrix (RDM) for each ROI (Figure 5D).

While the magnitude of the representational distances is slightly smaller than distances reported in previous studies (Ejaz et. al. 2015), likely due to the use of an event-related design in our study, the relative representational patterns that we observed in primary motor and primary somatosensory cortex qualitatively matches previous reports. Specifically the index finger is furthest from the little finger, while the middle and ring fingers are close together. This pattern of

representational distances is also similar to what is observed in the other cortical motor regions, although the overall between effector distances are smaller in these premotor regions (Figure 5D). To confirm that each region has reliably different representations for the fingers, we computed the average cross-validated pairwise distance between all finger movements (Figure 5B see Materials and Methods). Average distance (H) greater than 0 indicate above-chance encoding (Diedrichsen and Kriegeskorte 2017). In order to estimate the reliability of this encoding across subjects, we extracted the median distance across voxels within each searchlight for each subject and ROI. The median was chosen in order to account for the fact that the distribution of H values within a region is highly skewed. A one-sample t-test on those median values (one median per subject), after adjusting for multiple comparisons using a Bonferonni correction, found significant separation of cued finger representations (i.e., positive average distances) in the cortical sensorimotor areas, but not the A1 control region nor the putamen (Table 1). A follow up paired samples t-test (within subject) showed that H was greater in M1, S1, PMd, PMv, and SPL, but not in SMA, when compared against A1 (Table 1).

Along with the cortical regions, we also examined the distances between finger representations within the caudate and the putamen (inset of Figure 4E). Overall the distances within the striatum were significantly separable within the caudate but not the putamen. However, the magnitude of the representational distances was very weak in these subcortical regions, with distances several orders of magnitude smaller than in any cortical regions.

Overall, the analysis of cortical representations of individual fingers is consistent with previous studies (Ejaz et. al. 2015), confirming that the patterns of activity in the motor network can reliably discriminate individual effectors. This effect is substantially weaker in subcortical regions, likely having to do with the lower signal-to-noise of the BOLD signal in the striatum

and other regions of the basal ganglia. Therefore, these regions of interest were excluded from further analysis.

<INSERT FIGURE 5 HERE>

Figure 5: *Multivariate activity patterns during cued finger movements.* ***A****. fMRI task schematic. Participants executed single finger movements on the button glove following a variable period of fixation. The cue-finger mapping was identical to that used during the training.* ***B****. Example of a representational dissimilarity matrix showing similar finger patterns that result in small distances and dissimilar finger patterns that result in large distances. The average crossnobis similarity (i.e., H) was used as a test statistic for assessing decoding in each ROI and for assessing representational plasticity.* ***C****. Regions of interest masks overlaid in blue on the group average surface.* ***D****. Average representational dissimilarity matrices for each region. Each colored square within the RDM indicates the distance between those two fingers (i=index, m=middle, r=ring, l=little)* ***E,F****. Violin plots show the distributions of median H values in cortical motor areas (E) and the striatum (F) across subjects. Black circles inside plots show individual data. Asterisks indicate significance at* $\alpha = 0.05$ *after correcting for multiple comparisons (Bonferonni). Primary motor cortex (M1), primary somatosensory cortex (S1), premotor dorsal cortex (PMd), premotor ventral cortex (PMv), superior parietal lobule (SPL), supplementary motor area (SMA), and primary auditory cortex (A1), caudate (Cau), putamen (Put).*

To determine whether the emergence of binding in the behavioral responses coincides with alterations of these representational distances of individual cued actions, we measured how average distances changed for each cortical motor ROI before and after training. The simple form of the binding hypothesis is that the representations of frequently paired actions will become more similar (Figure 1D) after training, predicting that the distances between frequently paired movements will decrease after practice only in the Trained group. When looking at all pairwise distances (Figure 6A) we were unable to find a reliable influence of sequence training on the average pattern distances in any cortical motor region. In most areas, the distances decreased only marginally for both Trained and Control groups together, but the finger patterns remained largely separable, with patterns exhibiting a high degree of stability. Across all regions, we failed to detect a reliable interaction between group and time that would be indicative of a

training effect in representational distances (all p>0.26, full statistics reported in Table 2). In order to evaluate the evidence in support of the null hypothesis that the interaction is not present, we conducted a JZS Bayes Factor (BF) ANOVA with uniform prior across all models and found evidence in support of the null model that training does not influence distances. The BF's ranged from 0.099-0.658 (Table 2), which can be considered positive anecdotal evidence in support of the Null hypothesis (Kass & Raftery, 1995).

<INSERT FIGURE 6 HERE>

Figure 6: *Stable representational distances after training.* ***A.*** *Pairwise finger distances included in overall distance analysis.* ***B****. Bar plots show mean ROI H values in the pre- and post-training scans separately for each group. Error bars show standard error. Gray circles are individual data points.* ***C****. Finger pair frequencies were asymmetrically distributed in the trained sequence (see Figure* ***4D****). Some finger pairs, e.g. index and little were infrequently paired, whereas other finger pairs e.g. index and middle were frequently paired.* ***D,E****. Bar plots show mean H for frequent pairs B (D) and infrequent pairs (E) in the pre- and post-training scans separately for each group. Error bars show standard error. Circles are individual data points. No comparison was found to be statistically significant at* α*=0.05.*

Of course, looking at changes in overall representational distances may not be sensitive enough to pick up changes in the representational distances of only a few finger pairs. The simple plasticity model we proposed in the Introduction predicts that the greatest plasticity should be observed in the finger pairs most often executed together in the sequence. If the distances decreased for the more frequently paired effectors, but increased for the less frequently paired effectors this may result in a net change for the overall average distance near 0. To explore this possibility, we re-analyzed the distance changes by looking at the frequently and infrequently occurring finger pairs in the sequence structure itself (Figure 4C). Based on the pairing frequencies, we identified four frequently used finger pairs (index-middle, index-ring, middle-little, ring-little) and two infrequently used pairs (Figure 6C) (middle-ring and index-

little). Qualitatively, the pattern of distances for each pair type appeared to match what was observed in the overall distance patterns, with higher distances in M1 and S1, and lower distances in the premotor and parietal regions. Thus, much like the overall distance patterns, we were unable to resolve focal changes in representational distances in either of the most frequently (Figure 6D) or infrequently (Figure 6E) paired effectors. Across all regions, two-way repeated measures ANOVA indicated no significant group-by-time interaction for either frequently paired (all $p > 0.26$, full statistics provided in Table 3) or infrequently paired fingers (all $p > 0.13$, full statistics provided in Table 4). The Bayesian ANOVA revealed anecdotal evidence in favor of the null hypothesis for both the frequently (BFs: 0.108-0.631, Table 3) and infrequently (BFs: 0.108-0.391, Table 4) paired fingers.

DISCUSSION

Here we examined whether the binding of serial actions during long-term sequence learning alters the cortical representations of individual cue-response pairings. We found that during sequence production, temporally adjacent responses develop a high degree of correlation in their response speeds, consistent with participants binding multiple responses together under a unified command so as to reduce computational complexity (see also Verstynen et. al. 2012, Ramkumar et. al. 2016, Lynch et. al. 2017). Using a multivariate pattern analysis approach, based on the cross-validated Mahalanobis estimator, we also replicated previous studies showing that cortical motor areas reliably distinguish between activation patterns of individually cued finger responses (Ejaz et. al. 2015). We were, however, unable to find evidence for learning-related changes in this representational structure of cued finger responses in any of the cortical regions tested. Taken together, these findings show that the process of binding actions into chunked sets during

long-term skill learning does not impact the representation of individual cued actions, suggesting that binding relies on changing more complex levels of representation beyond individual movements.

At first glance, the absence of plasticity in population level representations of individual actions that we observed appears to be incompatible with previous reports of plasticity in sensorimotor cortex. Kolansinki and colleagues (2016) found that the representational distances of individual fingers shifted in S1 after physically yoking two fingers together for a period of 24 hours. In their study, the sensory representations of the two yoked fingers remained spatially and temporally identical, however the unyoked fingers altered their distances, suggesting a possible compensatory effect in the sensory representations themselves. In contrast to this observation, other papers have shown that finger representations in S1 are still robust and distinct even decades after amputation (Kikkert et al., 2016), suggesting that the sensory representations of digits have some a degree of robustness. In contrast to these sensory representation studies, our task here relied on training associations between temporally independent movements in a specific context. It is possible that, had we trained on chord-like movements, where multiple fingers are simultaneously engaged (Verstynen et. al. 2005), for a longer period of time, we might have observed similar changes in cortical sensorimotor representations, a hypothesis that is left open to future studies.

Alternatively, there is a strong rationale for why single effector representations would remain stable in cortical sensorimotor networks, particularly motor execution areas like M1, after long-term sequence learning. First, binding responses at the execution level may be a maladaptive strategy for maintaining a flexible movement repertoire (Lashley, 1951). For example, if index finger movements were consistently bound with middle finger movements

because a single daily task required them to work together in sequential fashion, then they might exhibit a prepotent response in inappropriate contexts. In order to maximize flexibility, it would be beneficial for the movements to be bound at a more abstract motor planning stage, upstream from execution processes. Second, practice may involve refining the control of execution-level representations without necessarily impacting the representations themselves. This would suggest that the process of binding during the consolidation of complex movement sequences is dependent on plasticity mechanisms at hierarchically higher level of processing (Wong et. al. 2015).

Of course, it is possible that there is plasticity in the representations of individual sensorimotor effectors during long-term sequence learning, but limitations in our experimental design may preclude identifying those changes. First, while the duration of training we used was longer than many classic sequence learning experiments in humans, five weeks may still not be enough time to lead to measurable representational changes in primary motor cortex. This concern is tempered by the fact that we were able to show strong evidence of action binding in the behavioral responses. A second methodological limitation is the lack of power to observe what is likely a relatively modest effect size. Previous studies of sensory representational plasticity provide a reasonable measure of the true effect size, suggesting we are reasonably powered (Kolasinski, 2016). While, it is true that the number of samples was comparatively low for a typical univariate functional imaging study (at 9 participants per group), several design choices alleviate this concern. We collected a substantial amount of data per subject. Each subject was scanned for approximately 2 hours before training, and 2 hours after training, with 6 identical and independent imaging sessions per run. This relatively large volume of data per subject enabled us to obtain robust estimates of the population patterns of interest. Thus, while

605 the number of subjects was modest, we do not believe that our results are simply the result of
606 insufficient power.

607 Despite these limitations, our experiment clearly shows that five weeks of training on a
608 complex unimanual sequence task does not alter the sensorimotor representations of individual
609 effectors despite clear evidence of binding in the motoric actions. This suggests that execution
610 level representations remain stable during learning and that proficiency is likely controlled by a
611 higher level within the motor hierarchy.
612

Region	Mean	t(17) H>0	p-value H>0	95% CI (low, high)	t(17) $H>H^{A1}$	p-value $H>H^{A1}$
M1	4.92	7.91	*2.11×10^{-7}	3.61, 6.23	7.10	*8.89×10^{-7}
S1	5.23	10.13	*6.42×10^{-9}	4.14, 6.32	9.40	*1.90×10^{-8}
PMd	1.07	5.30	*2.91×10^{-5}	0.64, 1.49	3.02	*0.0037
PMv	1.66	12.06	*4.60×10^{-10}	1.37, 1.95	4.80	*8.29×10^{-5}
SMA	0.57	4.08	*3.87×10^{-4}	0.28, 0.87	-0.006	0.49
SPL	1.57	8.44	*8.71×10^{-4}	1.18, 1.97	5.13	*4.13×10^{-5}
CAU	0.005	3.46	0.0014	0.002, 0.008	-2.75	0.0067
PUT	0.003	1.98	0.031	-0.0002, 0.007	-2.77	0.0065
A1	0.57	2.78	6.45×10^{-3}	0.14, 1.01	n.a.	n.a.

Table 1: T-statistics, and associated p-values testing whether H is significantly greater than 0 (H>0) and whether H is significantly greater than in the control region ($H>H^{A1}$). * indicates significance based on a Bonferroni corrected threshold (0.05/9) in order to control the family wise error rate.

Region	F(1,16)	p-value	Inclusion BF
M1	1.820	0.214	0.161
S1	0.069	0.800	0.141
PMd	0.492	0.503	0.099
PMv	1.673	0.232	0.658
SMA	5.092	0.054	0.182
SPL	0.004	0.950	0.145

Table 2: F-statistics and p-values for testing significance of interaction effect (group x time) from repeated measures ANOVA for mean distances. Inclusion Bayes Factor (BF) is the ratio of the posterior over the prior probability of the model including the interaction term.

624

Region	F(1,16)	p-value	Inclusion BF
M1	1.585	0.243	0.196
S1	0.030	0.867	0.208
PMd	0.089	0.773	0.108
PMv	1.914	0.204	0.631
SMA	6.440	0.035	0.309
SPL	0.001	0.971	0.125

625 ***Table 3****: F statistics and p-values for testing significance of interaction effect (group x time)*
626 *from repeated measures ANOVA for frequently paired fingers. Inclusion Bayes Factor (BF) is*
627 *the ratio of the posterior over the prior probability of the model including the interaction term.*

628
629

Region	F(1,16)	p-value	Inclusion BF
M1	1.744	0.223	0.135
S1	1.265	0.293	0.218
PMd	1.718	0.226	0.108
PMv	0.708	0.425	0.391
SMA	0.309	0.593	0.149
SPL	0.036	0.854	0.183

Table 4: F statistics and p-values for testing significance of interaction effect (group x time) from repeated measures ANOVA for the infrequently paired fingers. Inclusion Bayes Factor (BF) is the ratio of the posterior over the prior probability of the model including the interaction term.

638 **References**

639 Acuna D.E., Wymbs N.F., Reynolds C.A., Picard N, Turner R.S., Strick P.L., Grafton S.T.,
640 Kording K (2014) Multi-faceted aspects of chunking enable robust algorithms. *Journal of*
641 *neurophysiology*, 112(8), 1849–1856.

642 Beukema P, Verstynen T. (2018). Predicting and Binding: interacting algorithms supporting the
643 consolidation of sequential motor skills. *Current Opinion in Behavioral Sciences* 20:98-103

644 CoAxLab (n.d.) CoAxLab/binding_manuscript: source data and code for stable representations
645 manuscript. Available at: https://github.com/CoAxLab/binding_manuscript

646 Diedrichsen, J., & Kriegeskorte, N. (2017). Representational models: A common framework for
647 understanding encoding, pattern-component, and representational-similarity analysis. *PLoS*
648 *computational biology*, *13*(4), e1005508.
649
650 Diedrichsen, J., & Kornysheva, K. (2015). Motor skill learning between selection and execution.
651 *Trends in cognitive sciences*, *19*(4), 227-233.
652
653 Diedrichsen, J., Provost, S., & Zareamoghaddam, H. (2016). On the distribution of cross-
654 validated Mahalanobis distances. arXiv preprint arXiv:1607.01371.
655
656 Ejaz N., Hamada M., & Diedrichsen J. (2015) Hand use predicts the structure of representations
657 in sensorimotor cortex. *Nature Neuroscience* 18(7), 1034-1040.

658 Fischl B. (2012). FreeSurfer. *Neuroimage* 62:774–781.

659 Fischl B., Rajendran N., Busa E., Augustinack J., Hinds O., Yeo B.T., Mohlberg H., Amunts K.,
660 & Zilles K (2008) Cortical folding patterns and predicting cytoarchitecture. *Cerebral Cortex*
661 18(8), 1973–1980.

662 JASP Team (2017). JASP (Version 0.8.4). Retrieved from https://jasp-stats.org/
663
664 Jin, X., Tecuapetla, F., & Costa, R. M. (2014). Basal ganglia subcircuits distinctively encode the
665 parsing and concatenation of action sequences. *Nature neuroscience*, *17*(3), 423-430.
666
667 Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the american statistical*
668 *association*, *90*(430), 773-795.
669
670 Kikkert, S., Kolasinski, J., Jbabdi, S., Tracey, I., Beckmann, C. F., Johansen-Berg, H., & Makin,
671 T. R. (2016). Revealing the neural fingerprints of a missing hand. *Elife*, *5*, e15292.
672
673 Kolasinski, J., Makin, T. R., Logan, J. P., Jbabdi, S., Clare, S., Stagg, C. J., & Johansen-Berg, H.
674 (2016). Perceptually relevant remapping of human somatotopy in 24 hours. Elife, 5, e17280.
675

676 Kriegeskorte N., Mur M., Bandettini P. (2008). Representational similarity analysis - connecting
677 the branches of systems neuroscience. *Frontiers in systems neuroscience* 2.

678 Lashley, K.S. (1951). The problem of serial order in behavior. In: *Cerebral mechanisms in*
679 *behavior*, pp 112–136.

680 Lynch B., Beukema P., Verstynen T. (2017). Differentiating Visual from Response Sequencing
681 During Long-term Skill Learning. *Journal of cognitive neuroscience* 29(1), 125–136.

682 Makin, T. R., & Bensmaia, S. J. (2017). Stability of sensory topographies in adult cortex. *Trends*
683 *in cognitive sciences*, *21*(3), 195-204.

684 Merzenich, M. M., Nelson, R. J., Stryker, M. P., Cynader, M. S., Schoppmann, A., & Zook, J.
685 M. (1984). Somatosensory cortical map changes following digit amputation in adult
686 monkeys. *Journal of comparative Neurology*, *224*(4), 591-605.

687 Nili H., Wingfield C., Walther A., Su L., Marslen-Wilson W., Kriegeskorte N. (2014). A toolbox
688 for representational similarity analysis. *PLoS computational biology* 10(4):e1003553.

689 Nissen M.J., Bullemer P. (1987). Attentional requirements of learning: Evidence from
690 performance measures. *Cognitive psychology,* 19(1), 1–32.

691 Nudo R.J., Milliken G.W., Jenkins W.M., Merzenich M.M. (1996). Use-dependent alterations of
692 movement representations in primary motor cortex of adult squirrel monkeys. *Journal of*
693 *Neuroscience,* 16(2), 785–807.

694 Oosterhof N.N., Wiestler T., Downing P.E., Diedrichsen J. (2011). A comparison of volume-
695 based and surface-based multi-voxel pattern analysis. *Neuroimage*, 56(2), 593–600.

696 Peirce J.W. (2007). PsychoPy-Psychophysics software in Python. *Journal of neuroscience*
697 *methods,* 162(1), 8–13.

698 Ramkumar P., Acuna D.E., Berniker M., Grafton S.T., Turner R.S., Kording K.P. (2016)
699 Chunking as the result of an efficiency computation trade-off. *Nature communications,*
700 7:12176.

701 Verstynen T., Diedrichsen J., Albert N., Aparicio P., Ivry R.B. (2005). Ipsilateral motor cortex
702 activity during unimanual hand movements relates to task complexity. *Journal of*
703 *neurophysiology,* 93(3), 1209–1222.

704 Verstynen T.D., Phillips J., Braun E., Workman B., Schunn C., Schneider W. (2012) Dynamic
705 Sensorimotor Planning during Long-Term Sequence Learning : The Role of Variability,
706 Response Chunking and Planning Errors. *PLoS One* 7:e47336.

707 Verwey W.B. (1996). Buffer loading and chunking in sequential keypressing. *Journal of*
708 *Experimental Psychology: Human Perception and Performance*, 22(3):544–562.

709 Walther A., Nili H., Ejaz N., Alink A., Kriegeskorte N., Diedrichsen J. (2015). Reliability of
710 dissimilarity measures for multi-voxel pattern analysis. *Neuroimage,* 137, 188-200.

711 Wiestler T., Diedrichsen J. (2013). Skill learning strengthens cortical representations of motor
712 sequences. *eLife*, 2013:1–20.

713 Wong, A. L., Lindquist, M. A., Haith, A. M., & Krakauer, J. W. (2015). Explicit knowledge
714 enhances motor vigor and performance: motivation versus practice in sequence tasks.
715 *Journal of Neurophysiology, 114*(1), 219-232.

716 Wymbs N.F., Bassett D.S., Mucha P.J., Porter M.A., Grafton S.T. (2012). Differential
717 recruitment of the sensorimotor putamen and frontoparietal cortex during motor chunking in
718 humans. *Neuron*, 74(5), 936–946.

719 Yokoi A, Diedrichsen J. 2017. Does human primary motor cortex represent sequences of finger
720 movements? bioRxiv, 157438
721
722 Yousry T.A.., Schmid U.D., Alkadhi H., Schmidt D., Peraud A., Buettner A., Winkler P. (1997)
723 Localization of the motor hand area to a knob on the precentral gyrus. A new landmark.
724 *Brain: a journal of neurology*, 120(1), 141–157.