

Sequence action representations contextualize during rapid skill learning

Reviewed Preprint

v2 • March 17, 2025

Revised by authors

Reviewed Preprint

v1 • November 11, 2024

Debadatta Dash, Fumiaki Iwane, William Hayward, Roberto Salamanca-Giron, Marlene Bonstrup, Ethan Buch , Leonardo G Cohen 

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States • Department of Neurology, University of Leipzig Medical Center, Leipzig, Germany

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

eLife Assessment

This **valuable** study investigates how the neural representation of individual finger movements changes during the early period of sequence learning. By combining a new method for extracting features from human magnetoencephalography data and decoding analyses, the authors provide **incomplete** evidence of an early, swift change in the brain regions correlated with sequence learning, including a set of previously unreported frontal cortical regions. The addition of more control analyses to rule out that head movement artefacts influence the findings, and to further explain the proposal of offline contextualization during short rest periods as the basis for improvement performance would strengthen the manuscript.

<https://doi.org/10.7554/eLife.102475.2.sa4>

Abstract

Activities of daily living rely on our ability to acquire new motor skills composed of precise action sequences. Early learning of a new sequential skill is characterized by steep performance improvements that develop predominantly during rest intervals interspersed with practice, a form of rapid consolidation. Here, we ask if the millisecond level neural representation of an action performed at different locations within a skill sequence contextually differentiates or remains stable as learning evolves. Optimization of machine learning decoders to classify sequence-embedded finger movements from MEG activity reached approximately 94% accuracy. The representation manifolds of the same action performed in different sequence contexts progressively differentiated during rest periods of early learning, predicting skill gains. We conclude that sequence action representations contextually differentiate during early skill learning, an issue relevant to brain-computer interface applications in neurorehabilitation.

Introduction

Motor learning is required to perform a wide array of basic daily living activities, intricate athletic endeavors, and professional skills. Whether it's learning to type more quickly on a keyboard¹, improve one's tennis game², or play a piece of music on the piano³ – all of these skills require the ability to execute sequences of actions with precise temporal coordination. Action sequences thus form the building blocks of fine motor skills⁴. Initial exposure to acquisition of a new skill results in rapid performance improvements during the initial practice session (i.e. – early learning), which is up to four times larger in magnitude compared to offline performance improvements reported following overnight sleep^{1,5}.

Recent work advanced our understanding of the mechanisms supporting early skill learning. The density of fast hippocampo-neocortical skill memory replay events increases approximately three-fold during rest periods of early learning with their density explaining differences in the magnitude of micro-offline gains across subjects⁶. Jacobacci et al. (2020)⁷ reported BOLD fMRI changes in the hippocampus and precuneus (regions also identified using MEG)⁶ linked to micro-offline gains. These functional changes were coupled with rapid alterations in brain microstructure in the order of minutes, suggesting that the same network that operates during rest periods of early learning undergoes structural plasticity over several minutes following practice⁸. Chen et al. (2024)⁹ and Sjøgård et al (2024)¹⁰ provided crucial direct evidence from intracranial EEG in humans linking sharp-wave ripple density during rest periods (known markers for neural replay)¹¹ in the human hippocampus (80-120 Hz) to micro-offline gains during early learning. Thus, converging evidence now links hippocampal activity, neural replay dynamics, and micro-offline performance gains as mechanisms supporting rapid consolidation during early learning.

The neural representation of a sequential skill binds discrete individual actions (e.g. - single piano keypress) into complex, temporally and spatially precise sequence representations (e.g. - a refrain from a piece of music)^{12,13}. The neural representation or manifold¹⁴ of sequential skills has been characterized in humans^{3,15,16}. Previous fMRI reports showed that the representation of individual motor sequence actions may remain relatively stable over days or weeks of practice, after a memory is formed¹⁷. However, it is not known if individual sequence action representations differentiate or remain stable during early skill learning, when most prominent performance improvements occur, and the memory is not yet fully formed¹. Furthermore, it is not known if the neural representations for identical movements occurring at different positions of a sequential skill differentiate (or contextualize) as a function of learning, an issue crucial to the robustness of BCI applications.

Investigating the contextualization of discrete action representations at a millisecond level is challenging since both the individual actions and the skill sequence they are embedded within are concurrently represented in changing neural activity dynamics during learning^{16,18}. To address this problem, we constructed a series of decoders aimed at predicting keypress actions from magnetoencephalographic (MEG) neural activity, dependent upon the learning state and the local sequence context (ordinal position) the keypress action is performed within. Implementing this novel approach allowed us to determine that individual sequence actions are indeed contextualized during early skill learning resulting in differentiation of their neural representations, and that the degree of this differentiation predicts skill gains. This representational contextualization was also observed to predominantly develop during rest rather than during practice intervals in parallel with rapid consolidation of skill.

Results

Participants engaged in a well characterized sequential skill learning task^{1,5,6} that involved repetitive typing of a sequence (4-1-3-2-4) performed with their (non-dominant) left hand over 36 trials with alternating periods of 10s *practice* and 10s *rest* (*inter-practice rest*; *Day 1 Training*; **Figure 1A**). Individual keypress times and finger keypress identities were recorded and used to quantify skill as the correct sequence speed (keypresses/s)¹.

Participants reached 95% of maximal skill (i.e. - Early Learning) within the initial 11 practice trials (**Figure 1B**), with improvements developing over inter-practice rest periods (micro-offline gains) accounting for almost all of total learning across participants (**Figure 1B inset**).¹ In addition to the reduction in sequence duration during early learning, the relative duration of component keypress transitions displayed increased temporal regularity across sequence iterations (**Figure 1C**). On average across subjects, $2.32\% \pm 1.48\%$ (mean $\pm$ SD) of all keypresses performed were errors, which were evenly distributed across the four possible keypress responses. While errors increased progressively over practice trials, they did so in proportion to the increase in correct keypresses, so that the overall ratio of correct-to-incorrect keypresses remained stable over the training session.

On the following day, participants were retested on performance of the same sequence (4-1-3-2-4) over 9 trials (*Day 2 Retest*), as well as single-trial performance on 9 different untrained sequences (*Day 2 Controls*: 2-1-3-4-2, 4-2-4-3-1, 3-4-2-3-1, 1-4-3-4-2, 3-2-4-3-1, 1-4-2-3-1, 3-2-4-2-1, 3-2-1-4-2, and 4-2-3-1-4). As expected, an upward shift in performance of the trained sequence ($0.68 \pm \text{SD } 0.56$ keypresses/s; $t = 7.21$, $p < 0.001$) was observed during *Day 2 Retest*, indicative of an overnight skill consolidation effect for the trained sequence (**Figure 1 - figure supplement 1A**).

Keypress actions are represented in multi-scale hybrid-space manifolds

To investigate the possibility of contextualization of neural representations, we constructed a set of decoders to predict keypress actions from MEG activity as a function of both the learning state and the ordinal position of the keypress within the sequence. We first characterized the spectral and spatial features of keypress state representations by comparing performance of decoders constructed around broadband (1-100Hz) or narrowband [δ - (1-3Hz), θ - (4-7Hz), α - (8-14 Hz), β - (15-24 Hz), γ - (25-50Hz) and high γ -band (51-100Hz)] MEG oscillatory activity. We found that decoders trained on broadband data consistently outperformed those trained on narrowband activity, and that whole-brain parcel- (148 brain regions or parcels) or voxel-space (15684 voxels)¹⁹ decoders exhibited greater accuracy (parcel: $t = 1.89$, $p = 0.035$, $df = 25$, Cohen's $d = 0.17$; voxel: $t = 7.18$, $p < 0.001$, $df = 25$, Cohen's $d = 0.76$) than individual voxel-space intra-parcel decoders (**Figure 2**). Specifically, while the highest performing intra-parcel decoders predicted keypresses with up to 68.77% ($\pm$ SD 7.6%) accuracy, whole-brain inter-parcel decoder accuracy reached 74.51% ($\pm$ SD 7.34%) (**Figure 2**, also see **Figure 2 - figure supplement 1**).

Next, we asked if the combination of intra- and inter-parcel activity patterns, constituting a multi-scale hybrid space, improve further keypress predictions. We constructed hybrid space decoders ($N = 1295 \pm 20$) combining all inter-parcel activity with top-ranked intra-parcel features (covering 8 regions, see **Methods**, **Figure 3A**). Decoder accuracy was higher for hybrid ($78.15\% \pm \text{SD } 7.03\%$ accuracy and a weighted mean F1 score of $0.78 \pm \text{SD } 0.07$) than for voxel- ($74.51\% \pm \text{SD } 7.34\%$; paired t -test: $t = 6.30$, $p < 0.001$, $df = 25$, Cohen's $d = 0.39$) and parcel- ($70.11\% \pm \text{SD } 7.48\%$; paired t -

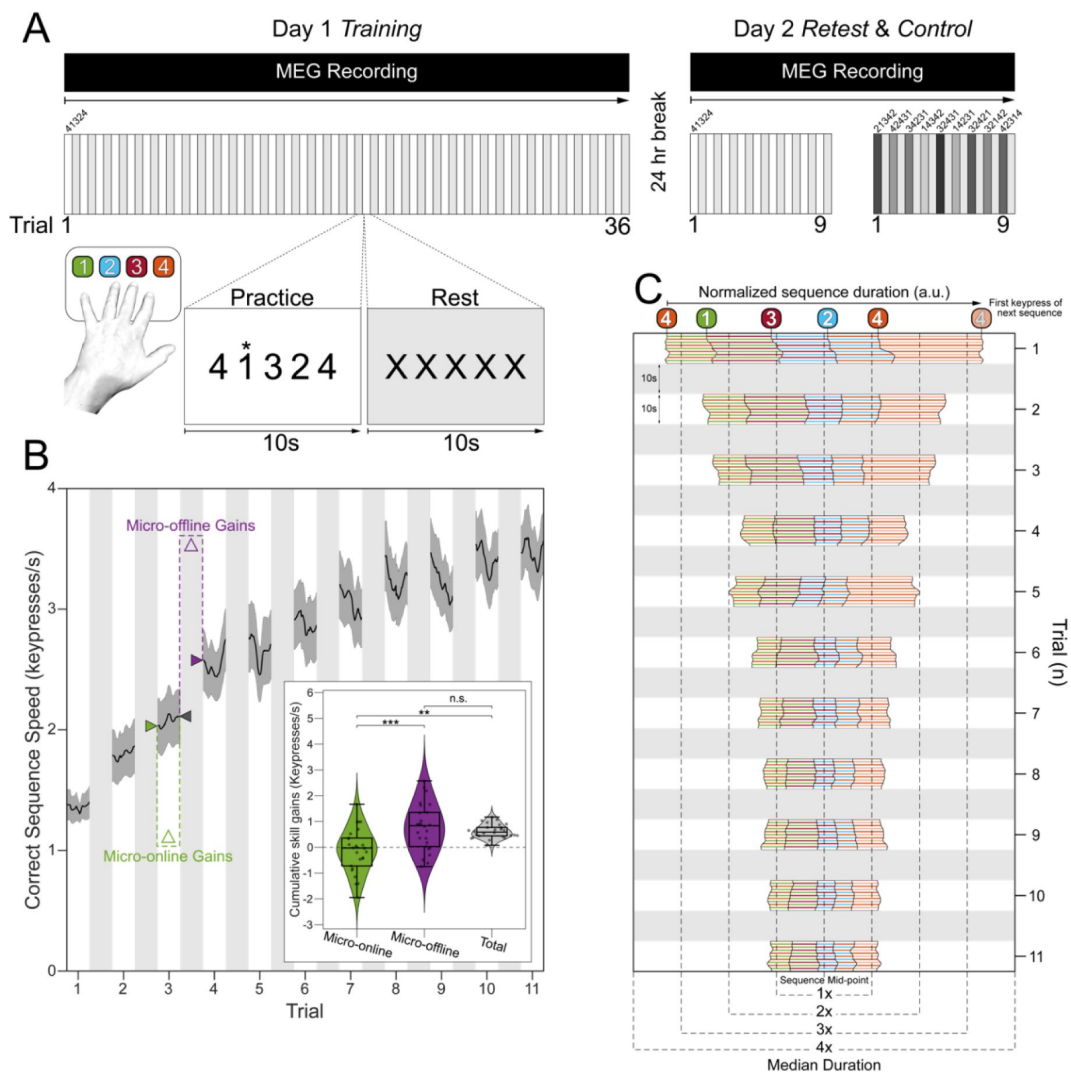


Figure 1.

Experimental design and behavioral performance.

A. Skill learning task. Participants engaged in a procedural motor skill learning task, which required them to repeatedly type a keypress sequence, “4 – 1 – 3 – 2 – 4” (1 = little finger, 2 = ring finger, 3 = middle finger, and 4 = index finger) with their non-dominant, left hand. The *Day 1 Training* session included 36 trials, with each trial consisting of alternating 10s practice and rest intervals. After a 24-hour break, participants were retested on performance of the same sequence (4-1-3-2-4) for 9 trials (*Day 2 Retest*) as well as single-trial performance on 9 different sequences (*Day 2 Control*; 2-1-3-4-2, 4-2-4-3-1, 3-4-2-3-1, 1-4-3-4-2, 3-2-4-3-1, 1-4-2-3-1, 3-2-4-2-1, 3-2-1-4-2, and 4-2-3-1-4). MEG was recorded during both Day 1 and Day 2 sessions with a 275-channel CTF magnetoencephalography (MEG) system (CTF Systems, Inc., Canada). **B. Skill Learning.** As reported previously^{1,2}, participants on average reached 95% of peak performance by trial 11 of the *Day 1 Training* session (see **Figure 1 - figure supplement 1A** for results over all *Day 1 Training* and *Day 2 Retest* trials). At the group level, total early learning was exclusively accounted for by micro-offline gains during inter-practice rest intervals (**Figure 1B** inset). **C. Keypress transition time (KTT) variability.** Distribution of KTTs normalized to the median correct sequence time for each participant and centered on the mid-point for each full sequence iteration during early learning (see **Figure 1**—figure supplement 1B for results over all *Day 1 Training* and *Day 2 Retest* trials). Note the initial variability of the relative KTT composition of the sequence (i.e. – 4-1, 1-3, 3-2, 2-4, 4-4), before it stabilizes by trial 6 in the early learning period.

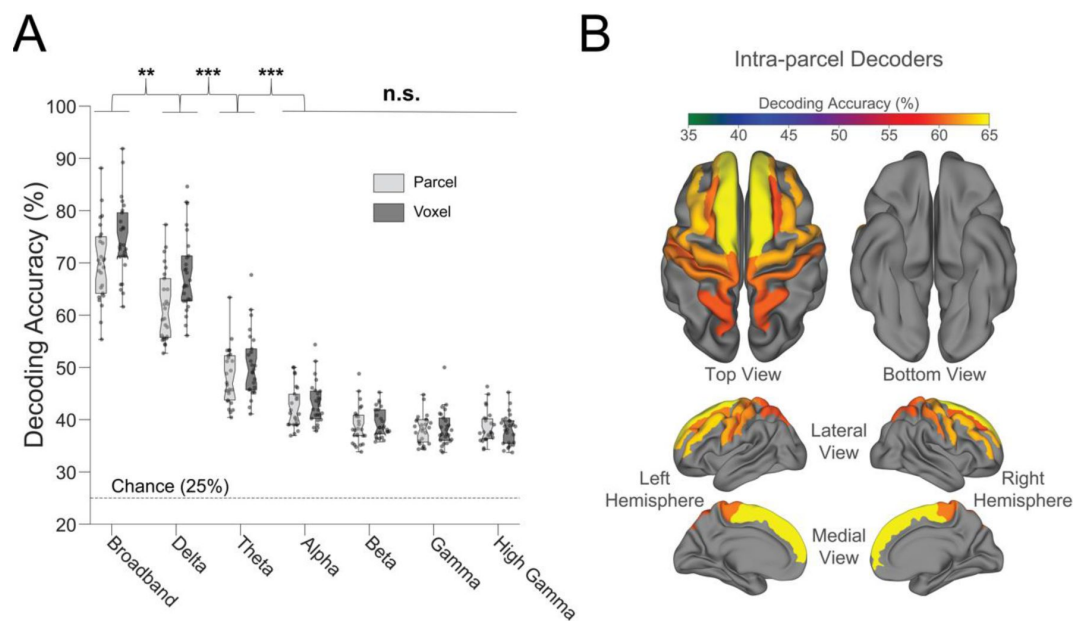


Figure 2.

Spatial and oscillatory contributions to neural decoding of finger identities

A. Contribution of whole brain oscillatory frequencies to decoding. When trained on broadband activity relative to narrow frequency band features, decoding accuracy (i.e. - test sample performance) was highest for whole-brain voxel- ($74.51\% \pm \text{SD } 7.34\%$, $t = 8.08$, $p < 0.001$) and parcel-space ($70.11\% \pm \text{SD } 7.11\%$, $t = 13.22$, $p < 0.001$) MEG activity. Thus, decoders trained on whole-brain broadband data consistently outperformed those trained on narrowband activity. Dots depict decoding accuracy for each participant. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns.: not significant. **B. Contribution of intra-parcel brain oscillatory frequencies to decoding.** Performance of the top performing decile of intra-parcel decoders (see **Methods**, [Figure 2](#)—figure supplement 1) over different frequency bands (top-panel; decoding accuracy is color coded and listed for each parcel and frequency band assessed). Broadband activity resulted in best accuracy closely followed by delta band activity. Broadband intra-parcel decoding accuracy mapped to a standard brain surface (FreeSurfer *fsaverage* brain) and color-coded by accuracy (bottom panel). Note that bilateral superior frontal cortex (yellow, 69%) contributed the most to decoding accuracy followed by middle frontal, pre- and post-central regions ($> 60\%$).

test = 12.08, $p < 0.001$, $df = 25$, Cohen's $d = 0.98$) spaces (**Figure 3B**, **Figure 3 – figure supplement 1**). Thus, a multi-scale hybrid-space representation best characterizes keypress action manifolds.

We implemented several dimensionality reduction or manifold extraction strategies including principal component analysis (PCA), multi-dimensional scaling (MDS), minimum redundant maximum relevance (MRMR), and linear discriminant analysis (LDA)²⁰ to map the input feature (parcel, voxel, or hybrid) space to a low-dimensional latent space¹⁴. LDA-based manifold extraction led to the greatest classifier performance gains, improving keypress decoding accuracy to $90.47\% \pm \text{SD } 4.84\%$ (**Figure 3B**; weighted mean F1 score = $0.91 \pm \text{SD } 0.05$). In comparison, LDA-based dimensionality reduction also improved parcel-space decoder performance ($82.95\% \pm \text{SD } 5.48\%$) but reduced voxel-space decoder accuracy ($40.38\% \pm \text{SD } 6.78\%$; also see **Figure 3 – figure supplement 2**). Notably, decoding of index finger keypresses (executed at two different ordinal positions in the sequence) exhibited the highest false negative (0.116 per keypress) and false positive (0.043 per keypress) misclassification rates compared with all other digits (false negative rate range = $[0.067 \text{ } 0.114]$; false positive rate range = $[0.020 \text{ } 0.037]$; **Figure 3C**), raising the hypothesis that the same action could be differentially represented when executed within different contexts (i.e. - different learning states or sequence locations).

We assessed the robustness of this hybrid strategy for decoding individual actions during skill learning over multiple sessions by applying it to data collected on the following day during the *Day 2 Retest* (9-trial retest of the same trained sequence) and *Day 2 Control* (single-trial performance of 9 different untrained sequences) blocks. The decoding accuracy for *Day 2* MEG data remained high ($87.11\% \pm \text{SD } 8.54\%$ for the trained sequence during *Retest*, and $79.44\% \pm \text{SD } 5.54\%$ for the untrained *Control* sequences; see confusion matrices in **Figure 3 – figure supplement 3**). This indicates that the hybrid decoding strategy is particularly reliable for decoding keypress actions during skill learning and the neural representations of the learned sequence remain stable the following day. Although the keypress state decoding performance was lower for untrained *Control* sequences relative to the trained sequence, greater accuracy of the hybrid approach over purely parcel- or voxel-space input features was still observed. Thus, this hybrid approach allows robust sequential finger movement decoding across multiple days and sequences.

Inclusion of keypress sequence context location optimized decoding performance

Next, we tracked the trial-by-trial evolution of keypress action manifolds as training progressed. Keypress neural representations were observed to progressively differentiate during early learning (**Figure 4**). A representative example in **Figure 4A** (top row) depicts progressive representational clustering of four-digit representations from trials 1, 11, and 36. The spatial representation of these clusters changed over the course of training, from predominant involvement of pre-central areas in trial 1 to post-central, superior, and middle frontal cortex contributions in later trials 11 and 36 (**Figure 4A**, bottom row). Thus, a shift in activity from pre-central areas to post-central, superior, and middle frontal cortex paralleled improvements in decoding performance (also see **Figure 4 – figure supplement 1** for trial-by-trial quantitative feature importance score changes during skill learning).

Correct performance of the training sequence required pressing the index finger twice (4-1-3-2-4) at two contextually different ordinal positions (sequence positions 1 and 5). Inclusion of sequence location information (i.e. – sequence context) for each keypress action (five sequence elements with the one keypress represented twice at two different locations) improved decoding accuracy ($t = 7.09$, $p < 0.001$, $df = 25$, Cohen's $d = 0.86$, **Figure 4B**) from $90.47\% (\pm \text{SD } 3.44\%)$ to $94.15\% (\pm \text{SD } 4.84\%)$; weighted mean F1 score: 0.94), and reduced overall misclassifications by 54.3% (from 219 to 119; **Figure 3C** and **4B**). This is supported by greater differentiation in neural representations of the two different index finger keypresses embedded at different points within the sequence

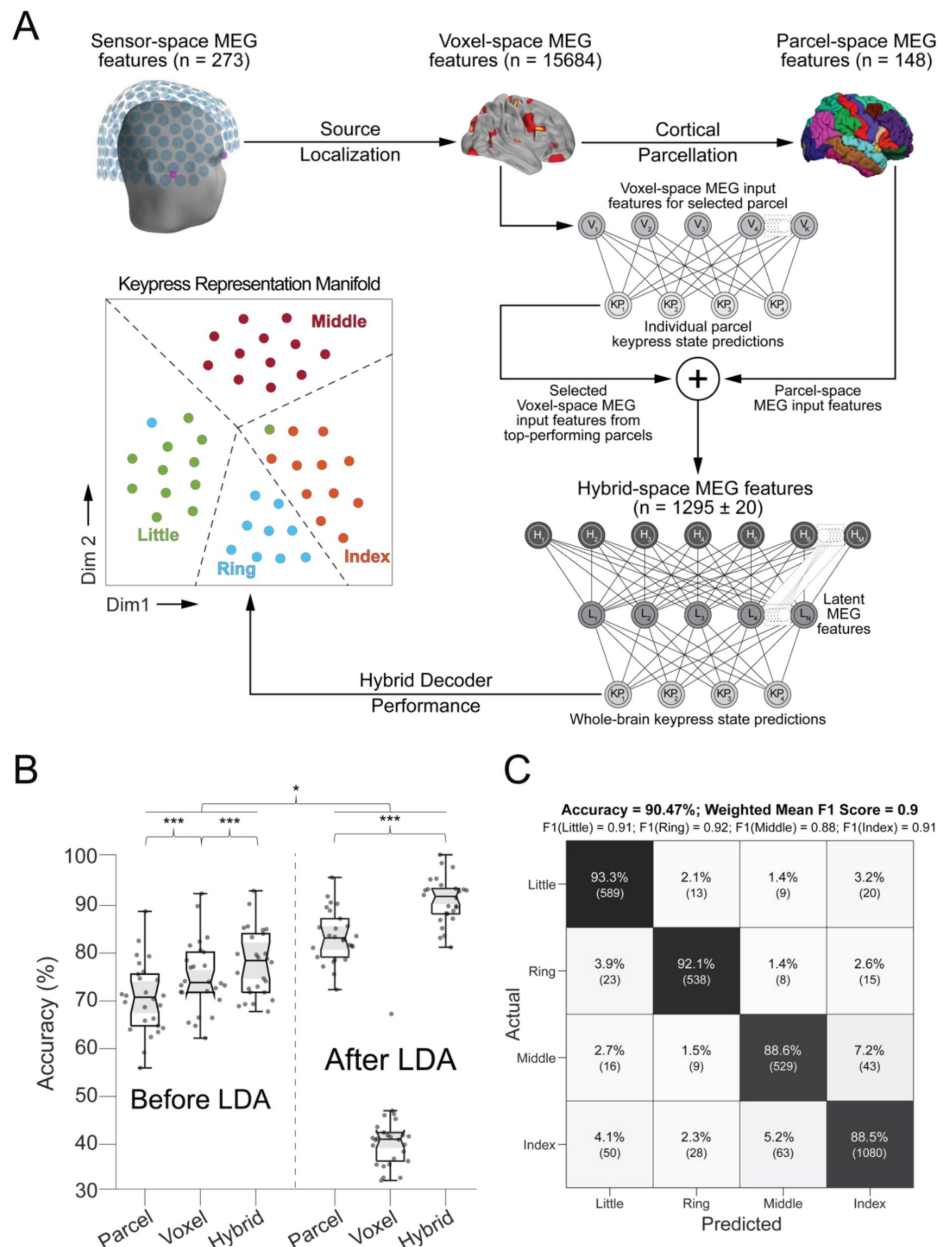


Figure 3.

Hybrid spatial approach for neural decoding during skill learning

A. Pipeline. Sensor-space MEG data ($N = 272$ channels) were source-localized (voxel-space features; $N = 15684$ voxels), and then parcellated (parcel-space features; $N = 148$) by averaging the activity of all voxels located within an individual region defined in a standard template space (Desikan-Killiany Atlas). Individual voxel-space regional decoders (intra-parcel decoders) were then constructed and ranked. The final hybrid-space keypress state (i.e. – 4-class) decoder was constructed using all parcel-spaces and top-ranked intra-parcel voxel input features (see Methods). **B. Decoding performance across parcel, voxel, and hybrid spaces.** Note that decoding performance was highest for the hybrid space approach compared to performance obtained for whole-brain voxel- and parcel spaces. Addition of linear discriminant analysis (LDA)-based dimensionality reduction further improved decoding performance for both parcel- and hybrid-space approaches. Each dot represents accuracy for a single participant and method. “***” indicates $p < 0.001$ and “*” indicates $p < 0.05$. **C. Confusion matrix of individual finger identity decoding for hybrid-space manifold features.** True predictions are located on the main diagonal. Off-diagonal elements in each row depict false-negative predictions for each finger, while off-diagonal elements in each column indicate false-positive predictions. Please note that the index finger keypress had the highest misclassifications ($n = 141$ or 47.5% of all prediction errors).

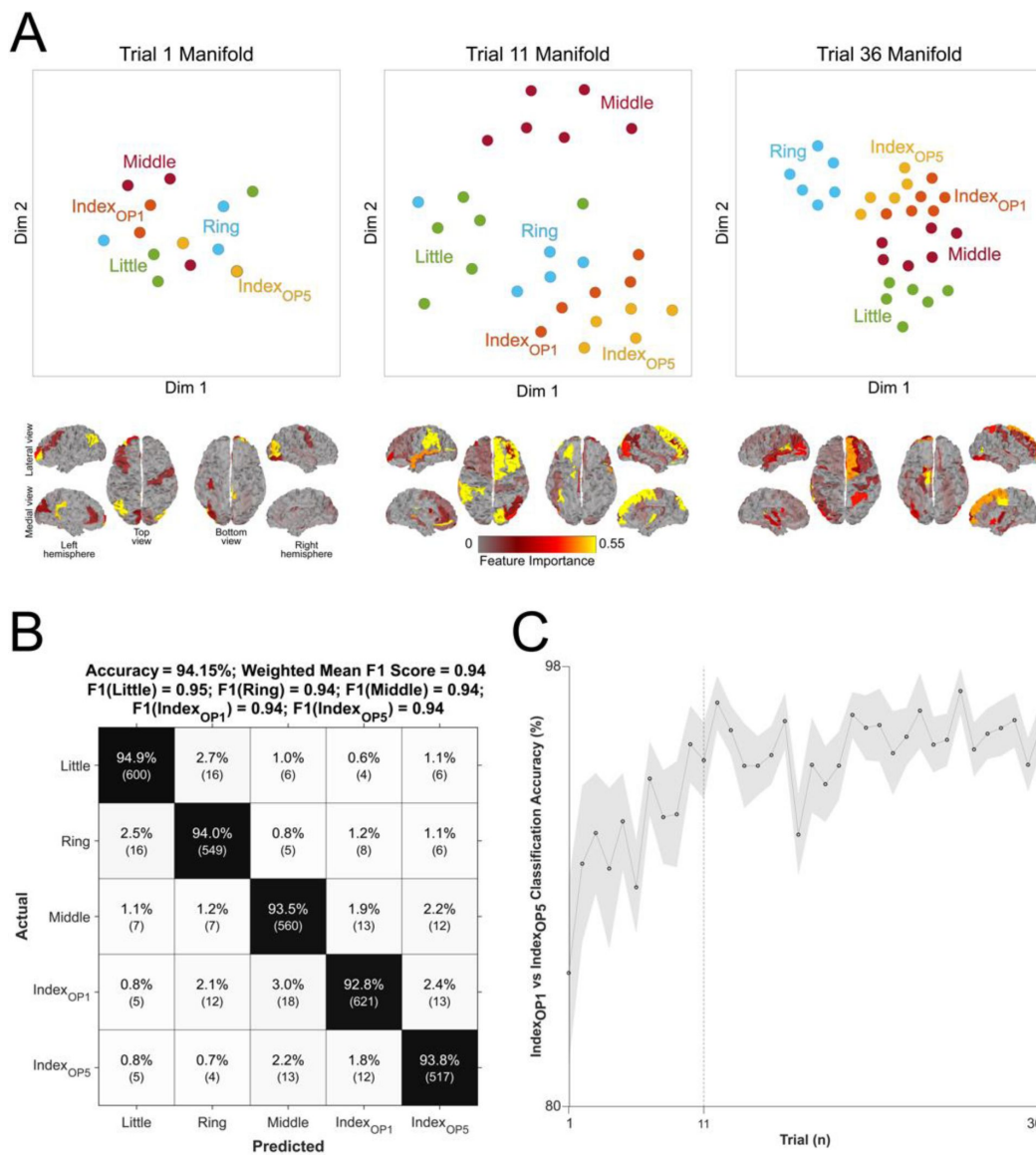


Figure 4.

Evolution of keypress neural representations with skill learning

A. Keypress neural representations differentiate during early learning. t-SNE distribution of neural representation of each keypress (top scatter plots) is shown for trial 1 (start of training; top-left), 11 (end of early learning; top-center), and 36 (end of training; top-right) for a single representative participant. Individual keypress manifold representation clustering in trial 11 (top-center; end of early learning) depicts sub-clustering for the index finger keypress performed at the two different ordinal positions in the sequence (Index_{OP1} and Index_{OP5}), which remains present by trial 36 (top-right). Spatial distribution of regional contributions to decoding (bottom brain surface maps). The surface color heatmap indicates feature importance scores across the brain. Note that decoding contributions shifted from right pre-central cortex at trial 1 (bottom-left) to superior and middle frontal cortex at trials 11 (bottom-center) and 36 (bottom-right). **B. Confusion matrix for 5-class decoding of individual sequence items.** Decoders were trained to classify contextual representations of the keypresses (i.e., 5-class classification of the sequence elements 4-1-2-3-4). Note that the decoding accuracy increased to 94.15% ± SD 4.84% and the misclassification of keypress 4 was significantly reduced (from 141 to 82). **C. Trial-by-trial classification accuracy for 2-class decoder (Index_{OP1} vs. Index_{OP5}).** A decoder trained to differentiate between the two index finger keypresses embedded at different positions (Index_{OP1} at ordinal position 1 vs. Index_{OP5} at ordinal position 5) within the trained sequence becomes progressively more accurately over early learning, stabilizing around 96% by trial 12 (end of early learning). Taken together, these findings indicate that the neural feature space evolves over early learning to incorporate sequence location information.

(**Figure 4A** [↗](#)), which leads to a trial-by-trial increase in 2-class decoding accuracy (**Figure 4C** [↗](#)). Inclusion of contextual information to the hybrid-space decoder also increased classification accuracy of Day 2 Retest trained sequence data (from 87.11% for 4-class to 90.22% for 5-class). As expected, contextualized 5-class decoding of Day 2 Control MEG data for untrained sequences performed at or below chance levels ($\leq 30.22\% \pm \text{SD } 0.44\%$) due to varied ordinal positions of keypress actions across the different sequences. Thus, inclusion of contextual information in the decoding framework only improves decoding accuracy for skill sequences which have been learned through practice.

Neural representation of keypress sequence location diverged during early skill learning

Finally, we used a Euclidian distance measure to evaluate the differentiation of the neural representation manifold of the same action (i.e. - an index-finger keypress) executed within different local sequence contexts (i.e. - ordinal position 1 vs. ordinal position 5). Note that, the neural representation manifolds (i.e. - the reduced dimension hybrid-space features extracted from broadband MEG data) are the features that resulted in the best decoding performance (See Methods, **Figure 3 – figure supplement 2** [↗](#)).

The Euclidian distance between keypress sequence location manifolds of the index finger in its two ordinal positions increased progressively during both rest and practice periods of early learning before stabilizing (**Figure 5A** [↗](#)). Change in this Euclidian distance was more prominent during rest than during practice periods ($t = 4.84, p < 0.001, df = 25$, Cohen's $d = 1.2$; **Figure 5B** [↗](#)), similar to early learning gains (**Figure 1B** [↗](#)) and strongly predicted cumulative micro-offline gains ($r = 0.90, R^2 = 0.82, p < 0.001$; **Figure 5 – figure supplement 1** [↗](#)). Importantly, the differentiation in neural representations was not explained by differences in keypress transition pattern regularity (within-subject; $t = -0.03, p = 0.976$; **Figure 5 – figure supplement 2** [↗](#)) or typing speed (between-subject; $R^2 = 0.028, p = 0.41$; **Figure 5 – figure supplement 3** [↗](#)). We further evaluated individual subject correlations between 1) contextualization changes during rest intervals and micro-offline gains; 2) contextualization changes during practice trials and micro-online gains, and 3) contextualization changes during practice trials and micro-offline gains (**Figure 5 – figure supplement 4** [↗](#)). Within-subject correlations between contextualization changes during rest intervals and micro-offline gains were significantly higher than between 1) online contextualization and micro-online gains ($t = 3.2827, p = 0.0015, df = 25$, Cohen's $d = 1.2$) and 2) online contextualization and micro-offline gains ($t = 3.7021, p = 5.3013e-04, df = 25$, Cohen's $d = 0.69$). Further, when quantifying online changes in contextualization from the first Index_{OP1} to the last Index_{OP5} keypress in the same trial we observed no learning-related trend (**Figure 5 – figure supplement 5, right panel** [↗](#)). Importantly, offline distances were significantly larger than online distances regardless of the measurement approach and neither predicted online learning (**Figure 5 – figure supplement 6** [↗](#)).

The neural representation differences assessed across both rest and practice for the trained sequence (4-1-3-2-4) were retained for *Day 2 Retest* (**Figure 5C** [↗](#)). Comparatively, contextualization was reduced for several untrained sequences controlling for intervening sequence patterns (*Pattern Specificity Control*: 4-2-3-1-4), keypresses performed at the same ordinal positions (1 and 5) with different fingers (*Finger Specificity Control*: 2-1-3-4-2, 1-4-2-3-1 and 2-3-1-4-2) and multiple index finger keypresses occurring at alternative ordinal positions (*Position Specificity Control*: 4-2-4-3-1 and 1-4-3-4-2) (**Figure 5C** [↗](#)).

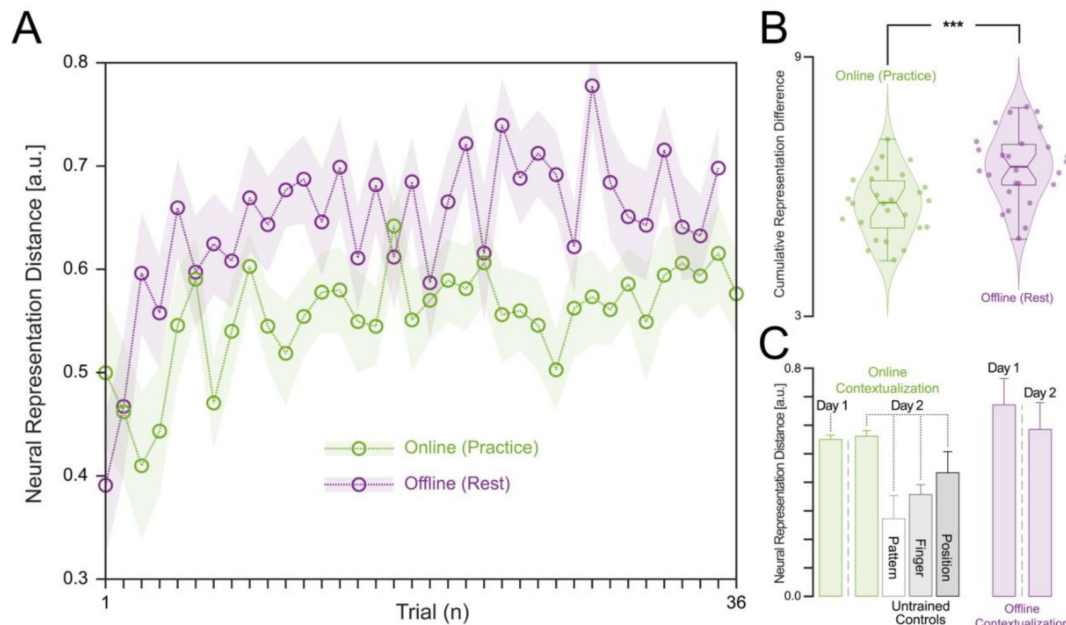


Figure 5

Neural representation distance between index finger keypresses performed at two different ordinal positions within a sequence.

A. Contextualization increases over Early Learning during Day 1 Training. Online (Practice; green line) and offline (Rest; magenta line) neural representation distances between two index finger key presses performed at ordinal positions 1 and 5 of the trained sequence (4-1-3-2-4) are shown for each trial during Day 1 Training. Both online and offline distances between the two index finger representations increase sharply over the Early Learning before stabilizing across later Day 1 Training trials. **B. Contextualization primarily occurs offline during short inter-practice rest periods.** The neural representation difference was significantly greater when assessed offline (right distribution; purple) versus online (left distribution; green) periods ($t = 4.84$, $p < 0.001$, $df = 25$, Cohen's $d = 1.2$). **C. Contextualization was retained after 24 hours and was specific to the trained sequence.** The neural representation differences assessed across both rest and practice for the trained sequence (4-1-3-2-4) were retained for Day 2 Retest. Further, contextualization was significantly reduced for several untrained sequences controlling for: 1) index finger keypresses located at the same ordinal positions 1 and 5 but with a different intervening sequence pattern (*Pattern Specificity Control*: 4-2-3-1-4); 2) both ordinal 1 and 5 position keypresses performed with either the little or ring finger instead of the index finger (*Finger Specificity Control*: 2-1-3-4-2, 1-4-2-3-1 and 2-3-1-4-2); and 3) multiple index finger keypresses occurring at ordinal positions other than 1 and 5 (*Position Specificity Control*: 4-2-4-3-1 and 1-4-3-4-2). The mean online neural representation distance, or degree of contextualization, was substantially lower for the untrained control sequences (51.05% lower for the *Pattern Specificity Control* sequence, 35.80% lower for the *Finger Specificity Control* sequences, and 22.06% lower for the *Position Specificity Control* sequences) compared with the trained sequence. Note that offline contextualization cannot be measured for the Day 2 Control sequences as each sequence was only performed over a single trial.

Discussion

The main findings of this study were that individual sequence action representations differentiate during early skill learning in a manner reflecting the local sequence context in which they are performed, and that the degree of representational differentiation, particularly prominent during rest intervals, predicts skill gains.

Optimizing decoding of sequential finger movements from MEG activity

The initial phase of this study involved optimizing the accuracy of decoding individuated finger keypresses from MEG brain activity. The decoding of individual finger movement execution or intention is a fundamental component of many translational BCI applications^{21–25}. For example, decoding of virtual keypresses can be interfaced with applications for controlling motorized wheelchairs, writing, and sending emails, or even controlling robotic hands or exoskeletons²⁶, and are currently being evaluated in patients with spinal cord and brain lesions^{27,28}. Once these neural signals are characterized, they can be translated into meaningful commands or actions^{26,29}. However, achieving robustness in clinical applications necessitates enhancements in state-of-the-art decoding tools, with a particular focus on improving decoding accuracy^{23,25,30}.

In the present study, we developed a novel hybrid-space decoder that captured the evolving neural representational dynamics over multiple spatial resolutions. This approach combined (1) parcel-space estimates from whole-brain activity and (2) voxel-space estimates from brain regions containing most keypress-related brain activity (see **Methods**). Using this approach, we achieved keypress state decoding accuracy that exceeded 90%, a substantial improvement over accuracies reported before with either non-invasive neuroimaging (i.e. – 43% to 77% for fMRI, MEG, and EEG)^{6,23,31–33} or invasive neural recording decoding of imagined movements (i.e. – 77% to 86% for ECoG and micro-array implant)^{34,35} techniques. Given these findings, the improvement in the keypress decoding accuracy from **Figure 2** (parcel/voxel) to **Figure 3** (parcel/voxel/hybrid with dimension reduction) is likely explained by the capability of the hybrid-space architecture to capture both lower spatially resolved whole-brain and higher spatially resolved regional activity patterns that contribute unique skill-related information to the sequence-embedded keypress state representations³⁶. This approach is reminiscent of the construction of convolutional neural networks (CNNs) used to perform object recognition in image classification applications³⁷. It could also be viewed as a spatial analogue to common time-frequency based analyses such as theta-gamma phase amplitude coupling (θ/γ PAC), which assess interactions between two or more narrow-band spectral features derived from the same time-series data³⁸. Importantly, this approach allowed accurate decoding during skill acquisition before a performance plateau was reached. It is possible that the ability to decode finger movements in the midst of substantial trial-by-trial performance changes as shown here could improve robustness of BCI applications in neurorehabilitation^{22,39}. Accurate estimation of finger movement representations as they evolve during skill learning should also improve detection of neural replay events during wakeful rest or sleep and enhance investigations of the role replay has in supporting skill consolidation⁶.

Best decoding performance was obtained from activity in the bilateral superior and middle frontal cortex and the pre- and post-central regions, all known contributors to skill learning^{18,40}. The contribution of contralateral primary sensorimotor areas to early skill learning has been extensively reported in humans and non-human animals^{6,12,41,42}. Similarly, the involvement of bilateral brain regions to decoding during skill learning of the left hand is not surprising. First, learning with the non-dominant left hand engages substantially more bilateral

neural resources than with the dominant hand^{43,44}. Second, bilateral activation in both frontal and parietal cortices has been extensively reported as essential during skill learning^{45–50}. The frontal lobe is crucial for executive control⁵⁰, motor planning⁴⁶, and working memory^{45,46,51}, while the parietal lobe integrates sensory feedback and visuomotor coordination^{45,46,51–53}, but has been also involved in working memory⁵⁴. Thus, it is not surprising that these regions increasingly contribute to decoding as subjects internalize the sequential task.

Low-frequency oscillations (LFOs) contributed the most to decoding accuracy, consistent with previous reports^{14,55}. LFOs, present during movement onset in the cerebral cortex of animals^{56,57} and humans^{58–60}, encode information about movement trajectories and velocity^{56,57}. They also contain information related to movement timing^{61–63}, preparation^{64,65}, sensorimotor integration⁵⁹, kinematics^{64,65} and may contribute to the precise temporal coordination of movements required for sequencing⁶⁶. Within clinical contexts, LFOs in the frontoparietal regions that were the main contributors to decoding performance in the present study are related to recovery of motor function after brain lesions like stroke^{58,61,67,68}. Our results, in conjunction with those discussed above, suggest that LFOs contain crucial information relevant to decoding of sequence-embedded skill actions.

Neural representations of individual sequence actions are contextualized during early skill learning

Previous work decoded actions from human neural activity when performed in isolation (e.g. individuated finger movement decoding)^{14,31,32,34,35,69–72} or embedded within previously learned sequences^{33,73} near stable plateau performance. To our knowledge, decoding of individual sequence-embedded actions has not been either attempted or reported within the context of rapid performance improvements that characterize early skill learning. We addressed this gap in knowledge by investigating different decoding strategies and identified the approach that rendered the best decoding performance, which in this case was approximately 94% (5-class hybrid-space decoder). We then applied this strategy to investigate neural representations of sequential individual finger movements during the skill learning task. We found that improved 5-class decoding accuracy was linked to trial-by-trial increases in the differentiation between index finger keypresses embedded within the sequence at two different locations throughout early learning (**Figure 4C**), which occurred in parallel to skill performance improvements. Feature importance scores are initially led by the pre-central cortex during early learning followed by an intermediate shift towards post-central cortex by trial 11, which is consistent with the known role of the sensorimotor cortex in early skill acquisition¹². Concurrently, the superior frontal and then middle frontal cortex continue to increase in importance over the Day 1 Training session and represent the two most important features between trials 15 and 36 after the rapid initial performance gains stabilize (**Figure 4 – figure supplement 1**). This is consistent with previous findings that activity patterns within these regions represent hierarchical structures of skill sequences¹⁶.

Neural representations are modified by experience and practice⁴⁰. For example, FMRI studies showed that weeks-long practice of a keyboard task results in representational changes of the skill (finger sequence), but not of the individual sequence action components (individual finger movements) in the primary motor cortex¹⁶. An MEG study showed that previously learned individual sequence-embedded action (i.e. – finger movement) representations display an ordered queuing gradient when preparing to generate a previously learned sequence³³. Thus, representations of previously learned skills following prolonged practice over days and weeks appear to reflect some combination of invariant features of individual action components with higher order representations of the action sequence.

Less is known about changes in neural representations during rapid performance improvements that characterize early skill learning. MEG, which possesses millisecond temporal resolution, is the tool of choice to investigate this short early learning phase⁷⁴. In the present study, we leveraged this feature of MEG to investigate if the representation of the same sequence action (index finger movement) differentiates over early learning when embedded at different ordinal locations within the sequence. We found that practice led to a progressive differentiation of the neural representation of index finger movements reflecting the different contextual positions within the sequence. The finding that the magnitude of representational differentiation predicts skill gains supports the view that contextualization of neural representations contributes to early learning. Further support for this view comes from the finding that adding context information to the decoding algorithm increased decoding accuracy to levels higher than previously reported^{14,31–35,69–73}. A possible neural mechanism supporting contextualization could be the emergence of conjunctive “what–where” representations of procedural memories⁷⁵ with the corresponding modulation of neuronal population dynamics^{76,77}.

Representational contextualization developed predominantly during rest periods of early learning

Early learning defined as the period of practice that encompasses 95% of the maximal skill reached on an initial training session¹ is characterized by rapid and prominent performance improvements¹. Using a protocol that minimizes reactive inhibition effects⁷⁸, learning develops predominantly over periods of rest (micro-offline learning)^{1,79,80}. Retroactive interference immediately following practice periods reduces micro-offline learning indicating stabilization of the motor memory at a scale of seconds⁵, consistent with a form of rapid consolidation^{5,7,80,81}. Supporting this interpretation, hippocampo-neocortical neural replay explains micro-offline learning⁶ and human hippocampal ripples during rest intervals, known markers of neural replay, predict micro-offline as well as total learning^{10,82}. Three lines of evidence suggest that micro-offline gains during early learning are not a result of recovery from motor fatigue. First, we used a protocol already reported to minimize reactive inhibition effects (Pan and Rickard recommended this approach because “...*rapid learning during that period masks any reactive inhibition effect*”⁷⁸). Second, micro-offline learning is unaffected by shortening in half practice intervals’ duration and thus hypothetical fatigue⁵. Finally, learning faster, more fatiguing, skills reduce instead of enhance micro-offline learning⁹⁷.

Representational contextualization during early learning developed to a larger extent during rest than during practice. Importantly, within-subject correlations for contextualization changes during rest intervals and micro-offline gains were significantly higher than between online contextualization and micro-online gains or between online contextualization and micro-offline gains (**Figure 5 – figure supplement 4**). Together with the observation that rapid contextualization was retained the following day, reflecting stability of the skill memory over time, and in line with previous work^{1,83}, our findings suggest that contextualization of action representations contribute to early skill learning, particularly during rest intervals.

Representational contextualization after early learning

The magnitude of contextualization stabilized after early learning. The rapid initial skill gains that characterize early learning are followed by micro-scale fluctuations around skill plateau levels (i.e. following trial 11 in **Figure 1B**). These fluctuations (i.e. - micro-online drops and micro-offline rebounds in the absence of net skill gains) have been attributed to reactive inhibition mechanisms^{1,84}. Similar fluctuations are observed in non-learning contexts, for example when performing non-repeating sequences⁸⁵. Thus, the mechanisms underlying micro-scale changes differ between early learning and after plateau skill has been reached^{1,84}. Evaluation of micro-offline gains within training contexts where reactive inhibition is prominent (e.g. - after early learning or when performing non-repeating sequences^{85–87}) or testing during time intervals within which post-learning consolidation, interference or forgetting may occur⁸⁵.

confounds early learning measurements^{1,78}. Similarly, utilizing the initial practice trials (during which about 40-50% of early learning occurs¹) as “tests” before introducing practice schedule interventions amputates early learning almost in half, markedly distorting its measurement⁸⁵. Our data suggest that contextualization within an initial training session does not improve further after early skill learning.

Limitations

One limitation of this study is that contextualization was investigated for only one finger movement (index finger or digit 4) embedded within a relatively short 5-item skill sequence. It will be useful to find out if representational contextualization of multiple finger movements embedded within longer sequences (e.g. – a short piece of piano music) exhibit similar results. The use of a supervised manifold learning approach (LDA) in this study could be improved in the future by implementing unsupervised strategies more suitable for real-time BCI applications (e.g. - PCA and MDS also substantially improved decoding accuracy in the present study; **Figure 3 – figure supplement 2**). Finally, caution should be used when extrapolating findings during early skill learning to post-plateau performance periods, longer practice timespans, or non-learning situations (e.g. on-repeating groups in⁸⁵) when reactive inhibition plays a more substantive role. Ultimately, it will be important to develop new paradigms allowing one to independently estimate the different coincident or antagonistic features (e.g. - memory consolidation, planning, working memory and reactive inhibition) contributing to micro-online and micro-offline gains during and after early skill learning within a unifying framework.

Summary

In summary, individual sequence action representations contextualize during the initial practice trials of a new skill and the degree of differentiation parallels skill gains. Importantly, the offline neural representational change develops to a larger extent during rest than during practice intervals of early learning in parallel with rapid consolidation of skill. It is possible that the systematic inclusion of contextualized information into sequence skill practice environments could improve learning in areas as diverse as music education, sports training, and rehabilitation of motor skills after brain lesions.

Materials and Methods

Study Participants

The study was approved by the Combined Neuroscience Institutional Review Board of the National Institutes of Health (NIH). A total of thirty-three young and healthy adults (comprising 16 females) with a mean age of 26.6 years (± 0.87 SEM) participated in the study after providing written informed consent and undergoing a standard neurological examination. No participants were actively engaged in playing musical instruments in their daily lives, as per guidelines outlined in prior research^{88,89}. All study scientific data were de-identified and permanently unlinked from all personal identifiable information (PII) before the analysis. These data are publicly available upon request (<https://nih.box.com/v/hcpsSkillLearningData>). Two participants were excluded from the analysis due to MEG system malfunction during data acquisition. An additional 5 subjects were excluded because they failed to generate any correct sequences in two or more consecutive trials. The study was powered to determine the minimum sample size needed to detect a significant change in skill performance following training using a one-sample t-test (two-sided; $\alpha = 0.05$; 95% statistical power; Cohen’s d effect size = 0.8115 calculated from previously acquired data in our lab⁹⁰). The calculated minimum sample size was 22. The included study sample size ($n = 27$) exceeded this minimum.¹

Experimental Setup

Participants practiced a novel procedural motor skill learning task that involved repetitively typing a 5-item numerical sequence (4-1-3-2-4) displayed on a computer screen. They were instructed to perform the task as quickly and accurately as possible using their non-dominant, left hand on a response pad (Cedrus LS-LINE, Cedrus Corp). Each numbered sequence item corresponded to a specific finger keypress: 1 for a little finger keypress, 2 for a ring finger keypress, 3 for a middle finger keypress, and 4 for an index finger keypress. Individual keypress times and identities were recorded and used to assess skill learning and performance.

Participants practiced the skill for 36 trials. Each trial spanned a total of 20 seconds and included a 10 second practice round followed by a 10 second inter-practice rest period. The five-item sequence was displayed on the computer screen for the duration of each practice round and participants were directed to fix their gaze on the sequence. Small asterisks were displayed above a sequence item after each successive keypress, signaling the participants' present position within the sequence. Following the completion of a full sequence iteration, the asterisks were removed. The asterisk display did not provide error feedback as it appeared for both correct and incorrect keypresses. At the end of each practice round, the displayed number sequence was replaced by a string of five "X" symbols displayed on the computer screen, which remained for the duration of the inter-practice rest period. Participants were instructed to focus their gaze on the screen during this time. The behavior in this explicit sequence learning motor tasks is highly generative in nature rather than reactive to stimulus cues as in the serial reaction time task (SRTT). A similar real-world example would be manually inputting a long password into a secure online application in which one intrinsically generates the sequence from memory and receives similar feedback about the password sequence position (also provided as asterisks), which is typically ignored by the user.

On the next day, participants were tested (*Day 2 Retest*) with the same trained sequence (4-1-3-2-4) for 9 trials as well as for 9 different unpracticed control sequences (*Day 2 Control*; 2-1-3-4-2; 4-2-4-3-1; 3-4-2-3-1; 1-4-3-4-2; 3-2-4-3-1; 1-4-2-3-1; 3-2-4-2-1; 2-3-1-4-2; 4-2-3-1-4) each for one trial. The practice schedule structure for Day 2 was same as Day 1, with 10s practice trials interleaved with 10s of rest.

Behavioral data analysis

Skill

Skill, in the context of the present task, is quantified as the *correct sequence typing speed*, (i.e., - the number of correctly typed sequence keypresses per second; kp/s). That is, improvements in the speed/accuracy trade-off equate to greater skill. Keypress transition times (KTT) were calculated as the difference in time between the *keyDown* events recorded for consecutive keypresses. Since the sequence was repeatedly typed within a single trial, individual keypresses were marked as correct if they were members of a 5 consecutive keypress set that matched any possible circular shift of the displayed 5-item sequence. The instantaneous correct sequence speed was calculated as the inverse of the average KTT across a single correct sequence iteration and was updated for each correct keypress. Trial-by-trial skill changes were assessed by computing the median correct sequence typing speed for each trial.

Early Learning

The *early learning* period was defined as the trial range (1 - T trials) over which 95% of the total skill performance was first attained at the group level. We quantified this by fitting the group average trial-by-trial correct sequence speed data with an exponential model of the form:

$$L(t) = C1 + C2 (1 - e^{-kt})$$

Here, the trial number is denoted by t , and $L(t)$ signifies the group-averaged performance at trial t . Parameters $C1$ and $C2$ correspond to the pre-training performance baseline and asymptote, respectively, while k denotes the learning rate. The values for $C1$, $C2$, and k were computed using a constrained nonlinear least-squares method (MATLAB's `lsqcurvefit` function, trust-region-reflective algorithm) and were determined to be 0.5, 0.15, and 0.2, respectively. The early learning trial cut-off, denoted as T , was identified as the first trial where 95% of the learning had been achieved. In this study, T was determined to be trial 11.

Performance improvements during rest (*micro-offline gains*), for each rest period of a trial, were calculated as the net change in performance (instantaneous correct sequence typing speed) from the end of one practice period to the onset of the next (i.e. – over a single inter-practice rest period). *Micro-online gains* (performance improvements during practice periods) were computed as the change in performance between the beginning and end of a single practice trial. Total early learning was derived as the sum of all *micro-online* and *micro-offline* gains over trials 1-11. Cumulative micro-offline gains, micro-online gains, and total early learning were statistically compared using 1-way ANOVAs and post-hoc Tukey tests.

MRI Acquisition

We acquired T1-weighted high-resolution anatomical MRI volumes images (1 mm³ isotropic MPRAGE sequence) for each participant on a 3T MRI scanner (GE Excite HDxt or Siemens Skyra) equipped with a 32-channel head coil. These data allowed for spatial co-registration of an individual participant's brain with the MEG sensors, and individual head models required for surface-based cortical dipole estimation from MEG signals (i.e. – MEG source-space modeling).

MEG Acquisition

We recorded continuous magnetoencephalography (MEG) at a sampling frequency of 600 Hz using a CTF 275 MEG system (CTF Systems, Inc., Canada) while participants were seated in an upright position. The MEG system comprises a whole-head array featuring 275 radial 1st-order gradiometer/SQUID channels housed in a magnetically shielded room (Vacuumschmelze, Germany). Three of the gradiometers (two non-functional and one with high channel noise after visual inspection) were excluded from the analysis resulting in a total of 272 MEG channels for analysis. Synthetic 3rd order gradient balancing was applied to eliminate background noise in real-time data collection. Temporal alignment of behavioral and MEG data was achieved using a TTL trigger. Head position in the scanner coordinate space was monitored using head localization coils at the nasion, left, and right pre-auricular locations. These fiducial positions were co-registered in the participants' T1-MRI coordinate space using a stereotactic neuronavigation system (BrainSight, Rogue Research Inc.). MEG data was acquired starting 6 min before the task (resting-state baseline) and continued through the end of the 12 min training session.

MEG Data Analysis

Preprocessing

MEG data were preprocessed using the FieldTrip⁹¹ and EEGLAB⁹² toolboxes on MATLAB 2022a. Continuous raw MEG data (acquired at 600 Hz) were band-pass filtered between 1-100 Hz with a 4th order noncausal Butterworth filter. 60 Hz line noise was removed with a narrow-band discrete Fourier transform (DFT) notch filter. Independent component analysis (ICA) was used to remove typical MEG signal artifacts related to eye blinks or movement or cardiac pulsation. All recordings were visually inspected and marked to denoise segments containing other large amplitude artifacts due to movements.

Source Reconstruction and Parcellation

For each participant, individual volume conduction models were established to depict the propagation of brain-generated currents through tissue to externally measurable magnetic fields. This was accomplished through a single-shell head corrected-sphere approach based on the brain volume segmentation of their high-resolution T1 MRI. Source models and surface labels from the Desikan-Killiany Atlas¹⁹ were created for each participant using inner-skull and pial layer surfaces obtained through FreeSurfer segmentation and connectome-workbench. Aligning sensor positions in the MEG helmet to individual head space involved warping MEG head coil positions (mean of pre and post recording) to the fiducials of the MRI, applying the same transformation matrix to all MEG sensors.

The individual source, volume conduction model, and sensor positions were then utilized to generate the forward solution at each source dipole location, describing the propagation of source activity from each cortical location on the grid to each MEG sensor. The Linearly Constrained Minimum-Variance (LCMV) beamformer was employed for computing the inverse solution. Each trial of MEG activity contributed to calculating the inverse solution data covariance matrix. The individual sample noise covariance matrix was derived from 6 minutes of pre-training rest MEG data recorded in the same subject during the same session. A total of 15,684 surface-based cortical dipoles (source-space voxels) were estimated. Cortical parcellation involved averaging the time series of all source dipoles within a distinct anatomical boundary defined in the Desikan-Killiany Atlas. To prevent within-parcel source cancellation during averaging, a technique called mean-flipping was applied. This process entailed flipping the sign for all sources with a sign differing from that of the average source.

Feature Selection for Decoding

Features were extracted from the MEG activity at several spatial, oscillatory, and temporal scales.

Oscillatory Analysis

MEG signals were band limited to broadband (1-100 Hz) and standard neural oscillatory frequency bands defined as delta (1-3 Hz), theta (4-7 Hz), alpha (8-14 Hz), beta (15-24 Hz), gamma (25-50 Hz), and high-gamma (51-100 Hz) with a 4th order non-causal Butterworth filter. The decoding analysis was conducted independently for each band of MEG activity.

Spatial Analysis

Decoding was performed at sensor space as well as at source space. For sensor space decoding the feature dimension was 272 (corresponding to the 272 gradiometer channels). At the source space, decoding was executed at both the high-resolution voxel and low-resolution parcel space. The feature dimension for voxel-space decoding was 15,684, representing the total number of independently estimated cortical dipoles. For parcel-space decoding, the feature dimension was 148, derived from spatially averaging the voxel activities within each parcel defined by the

Desikan-Killiany Atlas. In both cases, decoding was carried out across all oscillatory frequency bands (i.e. - broadband, delta, theta, alpha, beta, gamma, and high-gamma) for comprehensive comparison.

Temporal Analysis

Temporal MEG activity corresponding to each keypress was defined by taking a time window of $[t + \Delta t]$, where $t \in [0 : 10 \text{ ms} : 100 \text{ ms}]$ and $\Delta t \in [25 \text{ ms} : 25 \text{ ms} : 350 \text{ ms}]$. In other words, a sliding window of variable width (from 25 ms to 350 ms with 25 ms increments) with onset ranging from the *keyDown* event (i.e. $-t = 0\text{ms}$) to $+100\text{ms}$ after the *keyDown* event (with increments of 10 ms) was used. This approach generated 140 different temporal windows for each keypress for each participant. MEG activity was averaged over each of these time windows and independently analyzed for decoding. The optimal time window was selected for each subject that resulted in best cross-validation performance (**Figure 4 – figure supplement 2** [↗](#)). This window optimization analysis was performed for each frequency band and spatial scale.

Hybrid Spatial Approach

First, we evaluated the decoding performance of each brain region in decoding finger identity. We trained decoders on the voxel activities of each region (intra-parcel decoders) to predict finger identity. Brain regions were then ranked from 1 to 148 based on their decoding accuracy at the group level. In a stepwise manner, we incrementally incorporated voxel activities of brain regions, starting from the top-ranked region. In other words, first, we concatenated the voxel-level features of the highest-ranked region with whole-brain parcel-level features for decoding analysis. Subsequently, we added the voxel-level features of the second-ranked brain region, along with those of the top-ranked region to the parcel-level features for decoding and continued this process. This iterative addition of brain regions was performed until decoding accuracy reached saturation. The optimal feature space comprised all 148 parcel-space features in conjunction with voxel-space features from the top 8 brain regions.

Dimension Reduction

We used several supervised and unsupervised dimension reduction techniques including linear discriminant analysis (LDA), minimum redundant maximum relevance (MRMR), principal component analysis (PCA), Autoencoder, Diffusion maps, factor analysis, large margin nearest neighbor (LMNN), multi-dimensional scaling (MDS), neighbor component analysis (NCA), spatial predictor envelope (SPE) [↗](#) on each of the spatial feature space (sensor, parcel, voxel, and hybrid). Among these techniques, PCA, MDS, MRMR, and LDA emerged as particularly effective in significantly improving decoding performance.

PCA, a method for unsupervised dimensionality reduction, transformed the high-dimensional dataset into a new coordinate system of uncorrelated principal components. These components, capturing the maximum variance in the data, were iteratively added to reconstruct the feature space and execution of decoding. MDS finds a configuration of points in a lower-dimensional space such that the distances between these points reflect the dissimilarities or similarities between the corresponding objects in the original high-dimensional space. MRMR, an approach combining relevance and redundancy metrics, ranked features based on their significance to the target variable and their non-redundancy with other features. The decoding process involved starting with the highest-ranked feature and iteratively incorporating subsequent features until decoding accuracy reached saturation. LDA finds the linear combinations of features (dimensions) that best separate different classes in a dataset. It projects the original features onto a lower-dimensional space (number of classes -1) while preserving the class-discriminatory information. This transformation maximizes the ratio of the between-class variance to the within-class variance. In our study, LDA transformed the features to a 3-dimensional hyperdimensional space that were used for decoding. Dimension reduction was applied to train data and then with the tuned

parameters of the dimension reduction model test data was transformed for decoder metrics evaluation. Decoding accuracies were systematically compared between the original and reduced dimension feature spaces, providing insights into the effectiveness of each dimension reduction technique. By rigorously assessing the impact of dimension reduction on decoding accuracy, the study aimed to identify techniques that not only reduced the computational burden associated with high-dimensional data but also enhanced the discriminative power of the selected features. This comprehensive approach aimed at optimizing the neural representation of each finger keypress for decoding performance across various spatial contexts.

Decoding Analysis

Decoding analysis was conducted for each participant individually, employing a randomized split of the data into training (90%) and test (10%) samples for 8 iterations. For each iteration, to optimize decoder configuration, an 8-fold cross-validation was applied to the training samples, allowing for the fine-tuning of hyperparameters and selection of the most effective model. Across participants, on average, the total number of individual keypresses for the whole duration of training was $219 \pm \text{SD: } 66$ (keypress 1: little), $205 \pm \text{SD: } 66$ (keypress 2: ring), 209 ± 66 (keypress 3: middle), and $426 \pm \text{SD: } 131$ (keypress 4: index). Only keypresses belonging to correctly typed sequence iterations ($94.64\% \pm 4.04\%$ of all keypresses) were considered. The total number of keypresses for keypress 4 was approximately twice that of keypresses 1, 2, and 3, as it was the only action that occurred more than once in the trained sequence (4-1-3-2-4), albeit in two different sequence contexts or ordinal positions. Considering the higher (2x) number of samples for one-class, we independently oversampled the keypresses 1, 2 and 3 to avoid overfitting to the over-represented class. Importantly, oversampling was applied independently for each keypress class, ensuring that validation folds were never oversampled, and training folds did not share common oversampled patterns. The decoder configuration demonstrating the best validation performance was selected for each iteration, and subsequently, test accuracy was evaluated on the independent/unseen test samples. This process was repeated for the 8 different iterations of train-test splitting and the average test accuracy was reported. This rigorous methodology aimed at generalizing decoding performance to ensure robust and reliable results across participants. Further, decoding evaluation was performed on the Day 2 data, for both the trained (*Day 2 Retest*; 9 trials) and untrained sequences (*Day 2 Control*; 9 different single-trial tests).

Machine Learning Classifiers

We employed a diverse set of machine learning decoders, including Naïve Bayes (NB), decision trees (DT), ensembles (EN), k-nearest neighbor (KNN), linear discriminant analysis (LDA), support vector machines (SVM), and artificial neural network (ANN), to train features generated with all possible combinations of spatial, temporal, and oscillatory scales for comprehensive comparative analysis. The hyperparameters of these decoders underwent fine-tuning using Bayesian optimization search.

The Naive Bayes classifier was configured with a normal distribution predictor and Gaussian Kernel, while the KNN classifier had a K value of 4 (for keypress decoding) and utilized the Euclidean distance metric. For decision trees, the maximum number of splits was set to 4 (for keypress decoding), with leaves being merged based on the sum of risk values greater or equal to the risk associated with the parent node. The optimal sequence of pruned trees was estimated, and the predictor selection method was 'Standard CART,' selecting the split predictor that maximizes the split-criterion gain over all possible splits of all predictors. The split criterion used was 'gdi' (Gini's diversity index). Ensembles employed the bagging method with random predictor selections at each split, forming a random forest. The maximum number of learning cycles was set to 100 with a weak learner based on discriminant analysis. For SVM, the RBF kernel was selected through cross-validation (CV), and the 'C' parameter and kernel scale were optimized using Bayesian optimization search. In the case of LDA, the linear coefficient threshold and the amount of regularization were computed based on Bayesian optimization search. The ANN decoder

consisted of one hidden layer with 128 nodes, followed by a sigmoid and a softmax layer, each with 4 nodes (for keypress decoding). Training utilized a scaled conjugate gradient optimizer with backpropagation, employing a learning rate of 0.01 (coarse to fine tuning) for a maximum of 100 epochs, with early stopping validation patience set to 6 epochs.

Additionally, we assessed long short-term memory recurrent neural networks (LSTM-RNN) and bidirectional LSTM-RNN to train the time series of each keypress for decoding. The configuration of these RNNs included two hidden (Bi)LSTM layers of 100 units, followed by three fully connected layers (50, 10, and 4), and a softmax layer with 4 nodes to decode the four keypress classes. The networks were trained using an Adam optimizer with an initial learning rate of 0.001, a learning rate drop factor of 0.1 after 20 epochs, and a maximum epoch of 400. For regularization, 20% dropouts after the (Bi)LSTM layers, 50% dropout after the fully connected layers, and L2 Norm with a gradient threshold value of 0.1 were employed. These hyperparameter values were chosen based on grid search optimization during cross-validation.

Decoding Performance Metrics

Decoding performance was assessed using several metrics, including accuracy (%), which indicates the proportion of correct classifications among all test samples. The confusion matrix provided a detailed comparison of the number of correctly predicted samples for each class against the ground truth. The F1 score, defined as the harmonic mean of the precision (percentage of true predictions that are actually true positive) and recall (percentage of true positives that were correctly predicted as true) scores, was used as a comprehensive metric for all one-versus-all keypress state decoders to assess class-wise performance that accounts for both false-positive and false-negative prediction tendencies^{93,94}. A weighted mean F1 score was then computed across all classes to assess the overall prediction performance of the multi-class model. Test accuracies based on the best decoder performance (LDA in our case) were reported and used for statistical comparisons.

Decoding During Skill Learning Progression

We systematically assessed decoding performance of the 2-class decoder ($\text{Index}_{\text{OP1}}$ vs $\text{Index}_{\text{OP5}}$) at each trial during the skill learning process to identify the relationship between the evolution of differentiated index finger decoding proficiency and the acquired skill. Our approach involved evaluating decoder performance individually for each *Day 1 Training* trial. To mitigate the influence of varying sample sizes in later trials, we ensured an equal number of samples (first k keypresses) in each trial.

We used t-distributed stochastic neighborhood estimation (t-SNE) to visualize the evolution of neural representations corresponding to each keypress at each trial of the learning period. Within t-SNE distributions, index finger keypresses were separately labeled based upon their sequence location (i.e., $\text{Index}_{\text{OP1}}$ and $\text{Index}_{\text{OP5}}$ for ordinal positions 1 and 5, respectively).

Decoding Sequence Elements

Finally, we performed decoding of each contextual action of the sequence (i.e., $\text{Index}_{\text{OP1}}$, Little, Middle, Ring, and $\text{Index}_{\text{OP5}}$). We used the same decoding strategy (90%-10% split for train and test, 8-fold cross validation of training samples to select best decoder configuration, hybrid spatial features, and LDA based dimension reduction) to decode these 5-classes. Note, oversampling was not needed after sub-grouping the index finger keypresses into two separate classes based on their context. Decoding sequence elements were evaluated for both *Day 1 Training*, *Day 2 Retest* and *Day 2 Control* data.

Feature Importance Scores

The relative contribution of source-space voxels and parcels to decoding performance (i.e. – feature importance score) was calculated using minimum redundant maximum relevance (MRMR)⁹⁵ and highlighted in topography plots. MRMR, an approach that combines both relevance and redundancy metrics, ranked individual features based upon their significance to the target variable (i.e. – keypress state identity) prediction accuracy and their non-redundancy with other features.

Neural Representation analysis

We evaluated the *online* (within-trial) and *offline* (between-trial) changes in the neural representation of the contextual actions ($\text{Index}_{\text{OP1}}$ and $\text{Index}_{\text{OP5}}$) for each trial during training. For offline differentiation, we evaluated the Euclidian distance between the hybrid spatial features of the last index finger keypress of a trial ($\text{Index}_{\text{OP5}}$) to the first index finger keypress ($\text{Index}_{\text{OP1}}$) of the subsequent trial, mirroring the approach used to calculate micro-offline gains in skill. This offline distance provided insights on the net change in contextual representation of the index finger keypress during each interspersed rest interval. For online differentiation, we calculated the mean Euclidian distance between $\text{Index}_{\text{OP1}}$ and $\text{Index}_{\text{OP5}}$ of all the correctly typed sequences within a practice trial. Online differentiation informed on the net change in the contextual representation of the index finger keypress within each practice trial. Cumulative offline and online representation distances across participants were compared using 2-sample *t*-tests. Additionally, we computed trial-by-trial differences in offline and online representations during early learning, exploring their relationships with cumulative micro-offline and micro-online gains in skill, respectively, through regression analysis and Pearson correlation analysis. Linear regression models were trained utilizing the *fitlm* function in MATLAB. The model employed M-estimation, formulating estimating equations and solving them through the Iteratively Reweighted Least Squares (IRLS) method⁹⁶. Key metrics such as the square root of the mean squared error (RMSE), which estimates the standard deviation of the prediction error distribution, the coefficient of explained variance (R^2), the F-statistic as a test statistic for the F-test on the regression model, examining whether the model significantly outperforms a degenerate model consisting only of a constant term, and the *p*-value for the F-test on the model were computed and compared across different models. This multifaceted approach aimed to uncover the nuanced dynamics of neural representation changes in response to skill acquisition.

As a control analysis, we also computed the difference in neural representation between $\text{Index}_{\text{OP1}}$ and $\text{Index}_{\text{OP5}}$ on *Day 2 Retest* data for the same sequence (4-1-3-2-4) as well as for different *Day 2 Control* untrained sequences where the same action was performed at ordinal positions 1 and 5 (2-1-3-4-2; 1-4-2-3-1; 2-3-1-4-2; 4-2-3-1-4). Finally, we assessed for specificity of contextualization to the trained sequence, by evaluating differentiation between index finger keypress representations performed at two different positions within untrained sequences (4-2-4-3-1 and 1-4-3-4-2). The cumulative differences across participants were compared with 2-sample *t*-tests.

Supplementary Materials

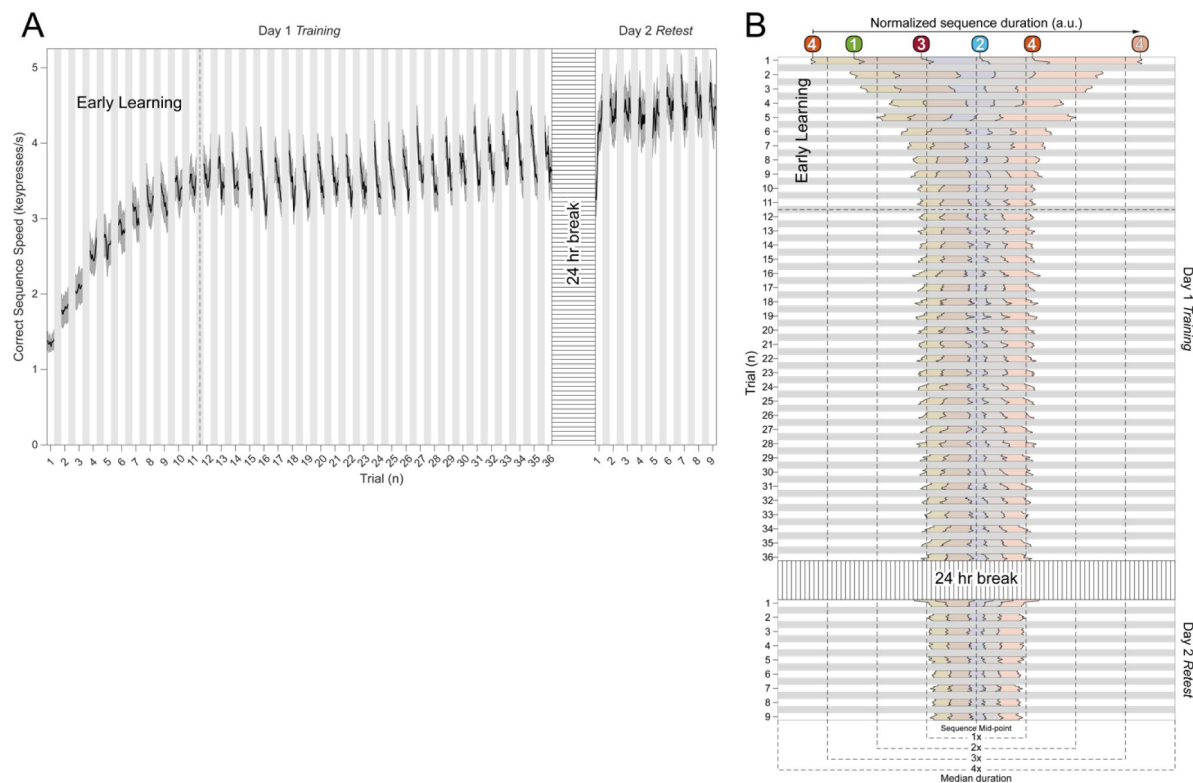


Figure 1 – figure supplement 1

Behavioral performance during skill learning.

A. Total Skill Learning over Day 1 Training (36 trials) and Day 2 Retest (9 trials). As reported previously¹³, participants on average reached 95% of peak performance during *Day 1 Training* by trial 11. Note that after trial 11 performance stabilizes around a plateau through trial 36. Following a 24-hour break, participants displayed an upward shift in performance during the *Day 2 Retest* – indicative of an overnight skill consolidation effect. **B. Keypress transition time (KTT) variability.** Distribution of KTTs normalized to the median correct sequence time for each participant and centered on the mid-point for each full sequence iteration during early learning. Note that the initial variability of the five component transitions in the sequence (i.e. – 4-1, 1-3, 3-2, 2-4, 4-4) stabilize by trial 6 in the early learning period and remain stable throughout the rest of *Day 1 Training* (through trial 36) and *Day 2 Retest*.

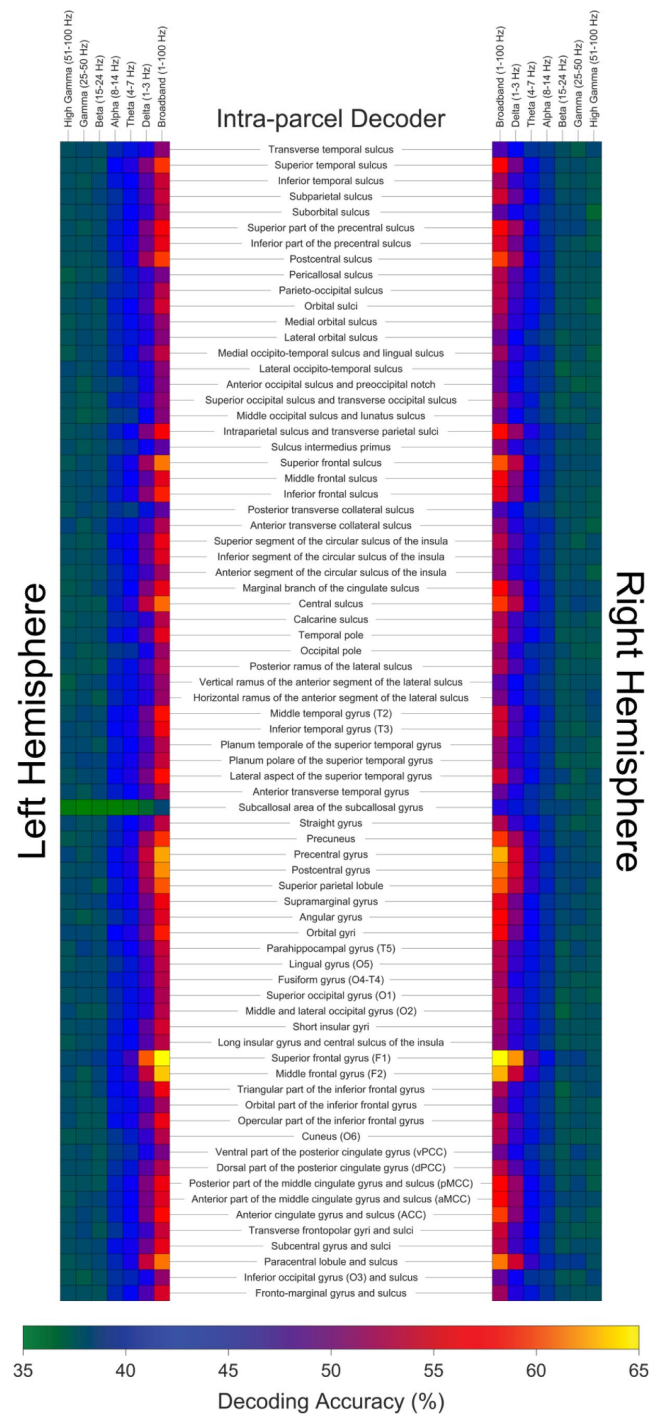


Figure 2 – figure supplement 1

Oscillatory contributions at individual brain regions.

Decoding performance of each individual brain region (intra-parcel decoder performance) at each oscillatory level is shown for both left and right hemisphere as a heatmap. Optimal decoding performances were obtained from bilateral superior frontal (Left: $68.77\% \pm \text{SD } 7.6\%$; Right: $67.52\% \pm \text{SD } 6.78\%$), middle frontal (Left: $63.41\% \pm \text{SD } 7.58\%$; Right: $62.78\% \pm \text{SD } 76.94\%$), pre-central (Left: $62.37\% \pm \text{SD } 6.32\%$; Right: $62.69\% \pm \text{SD } 5.94\%$), and post-central (Left: $61.71\% \pm \text{SD } 6.62\%$; Right: $61.09\% \pm \text{SD } 6.2\%$) brain regions. Superior parietal, central, paracentral, anterior-cingulate, and precuneus regions also showed greater ($> 60\%$) decoding performance. For Delta band, only superior frontal regions showed $> 60\%$ decoding performance.

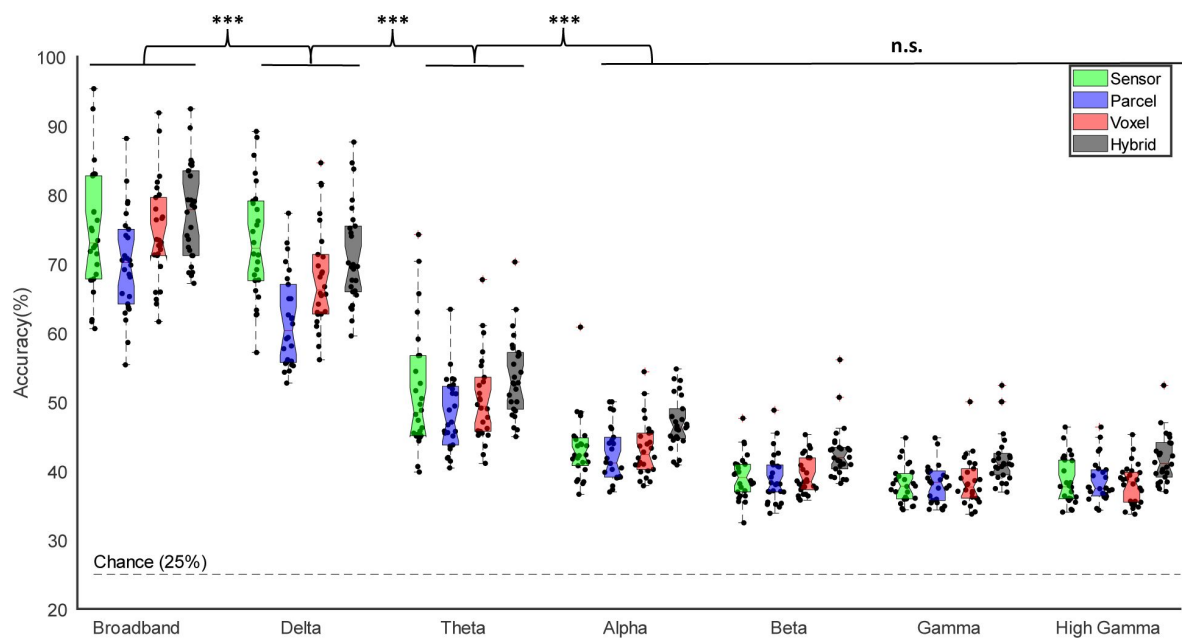


Figure 3—figure supplement 1

Contribution of whole-brain oscillatory frequencies to decoding.

Accuracy was highest when decoders were trained on broadband activity, closely followed by delta band activity, across whole-brain -parcel, -voxel, and -hybrid space. Sensor-space decoder accuracy did not differ statistically when trained on either broadband or delta-band activity. Hybrid approach resulted in best decoding accuracy for each frequency range. Interestingly, accuracy with sensor space decoders was comparable to accuracy with parcel and voxel space decoders. Dots depict decoding accuracy for each participant. “***” indicates $p < 0.001$, “**” indicates $p < 0.01$, and “n.s.” denotes no statistical significance (i.e. - $p > 0.05$).

Figure 3 – figure supplement 2

Comparison of different dimensionality reduction techniques.

Dimensionality reduction was applied to the input features for each approach [parcel (N=148)/voxel(N=15684)/hybrid(N=1295)] [1]. The results with principal component analysis (PCA, in green), multi-dimensional scaling (MDS, in blue), minimum redundant maximum relevance algorithm (MRMR, in red), linear discriminant analysis (LDA, in black) are shown in comparison to performance obtained using all input features (in magenta). For parcel space, all these approaches increased the mean decoding accuracy with PCA and LDA showing statistically significant improvement [1-way ANOVA: $F = 13.05$, $p < 0.001$; post hoc Tukey tests: $p = 0.032$ (PCA), $p < 0.001$ (LDA), $p > 0.05$ (MDS, MRMR)]. At the voxel space, there was no statistically significant improvement with either of the approaches ($p > 0.05$). MRMR showed the highest improvement but not statistically significant with post hoc Tukey tests ($p = 0.14$). With LDA the performance dropped significantly. For hybrid space, all the dimensionality reduction techniques were significant in improving decoding performance [1-way ANOVA: $F = 21.32$, post hoc Tukey tests: $p < 0.05$] and the best improvement was seen with LDA.

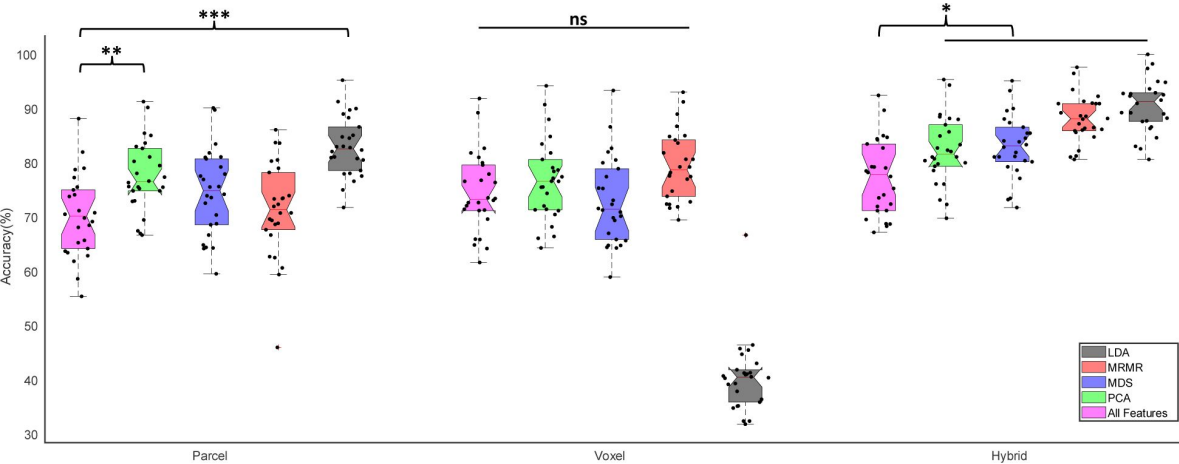


Figure 3 – figure supplement 3

Confusion matrices for decoding performance on Day 2 Retest (A) and Day 2 Control (B) data.

Note that, the hybrid decoding strategy generalized to Day 2 data with 87.11% keypress decoding accuracy for the trained sequence (Day 2 Retest) and 79.44% accuracy for decoding keypresses embedded within untrained control sequences (Day 2 Control).

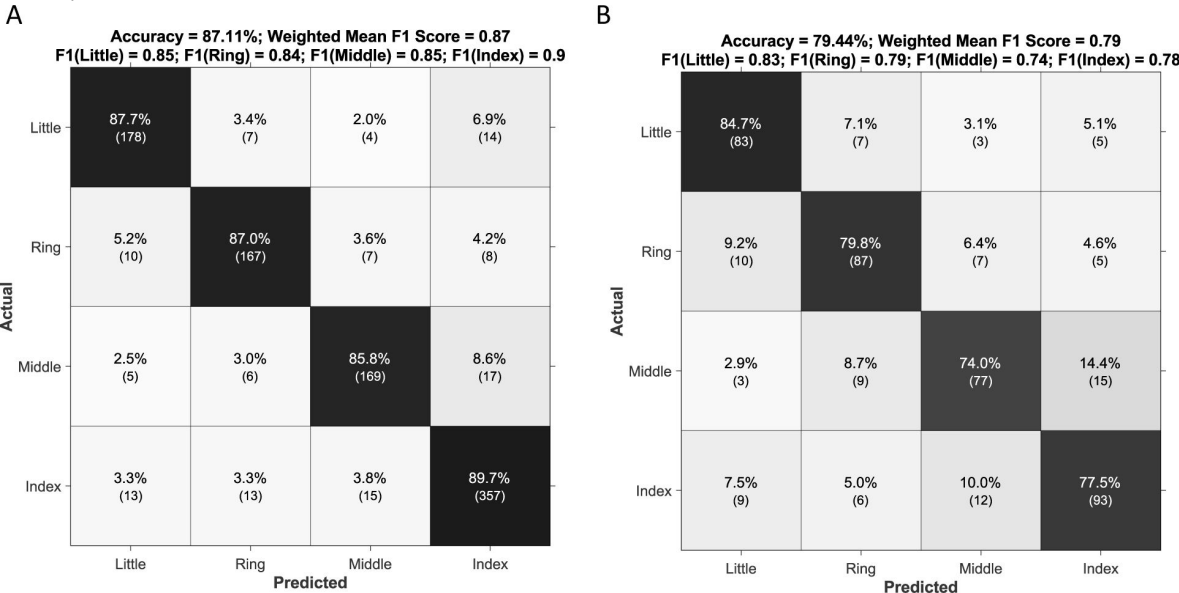


Figure 3 – figure supplement 4

Comparison of different decoder methods.

Performance for different decoders used is shown for each participant. The results show that linear discriminant analysis classifier (LDA) was the optimal choice. Results from LDA decoder analyses were used for all subjects and results reported in the manuscript.

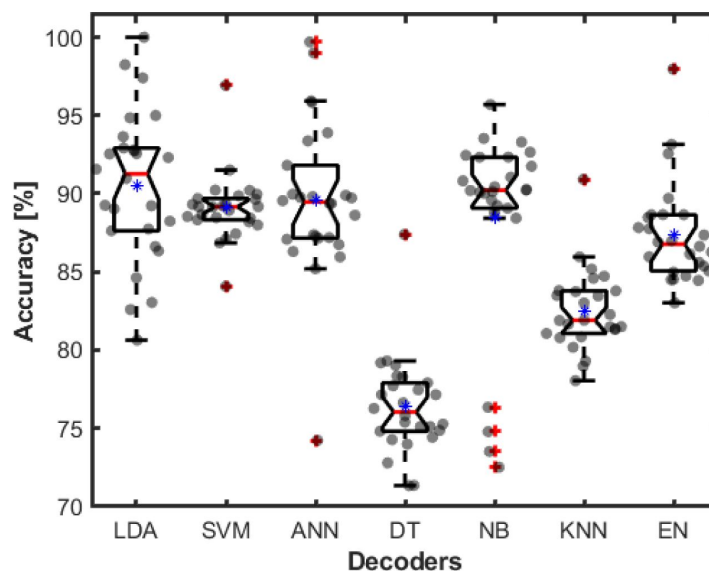
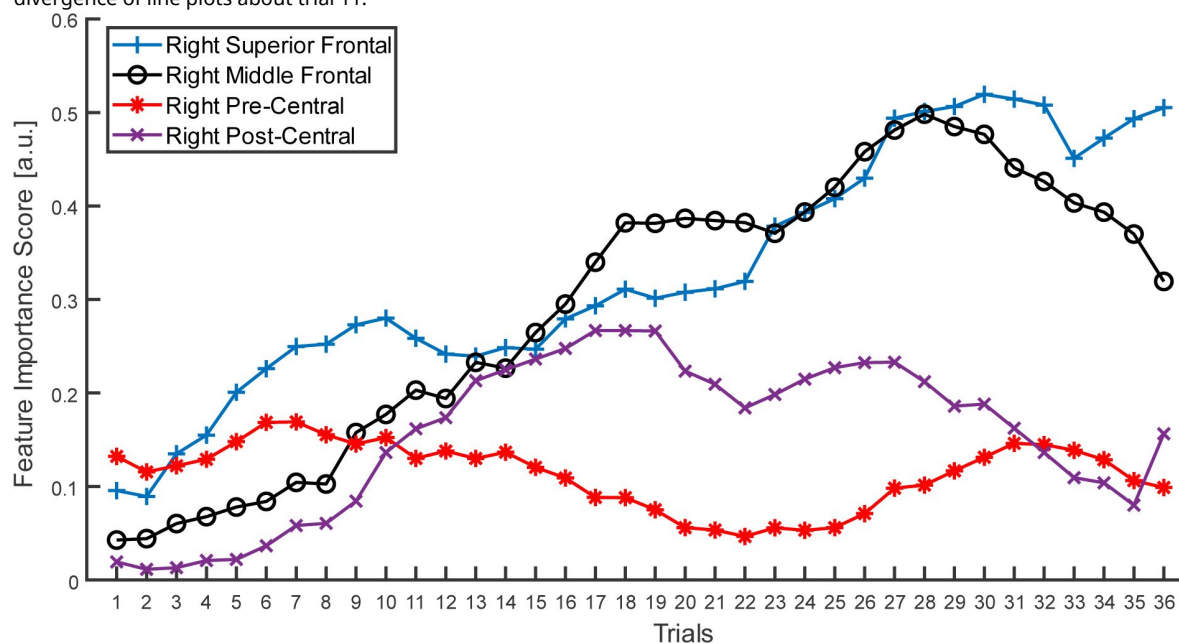


Figure 4 – figure supplement 1

Quantification of regional trial-by-trial feature importance score during skill learning.

The quantification of regional importance in decoding for each trial is shown for the regions that showed highest decoding accuracy, i.e., superior frontal, middle frontal, pre-central, and post-central cortex. Please note that, the feature importance score was higher for pre-central cortex which shifted to middle frontal cortex during later trials, as can be seen with the divergence of line plots about trial 11.



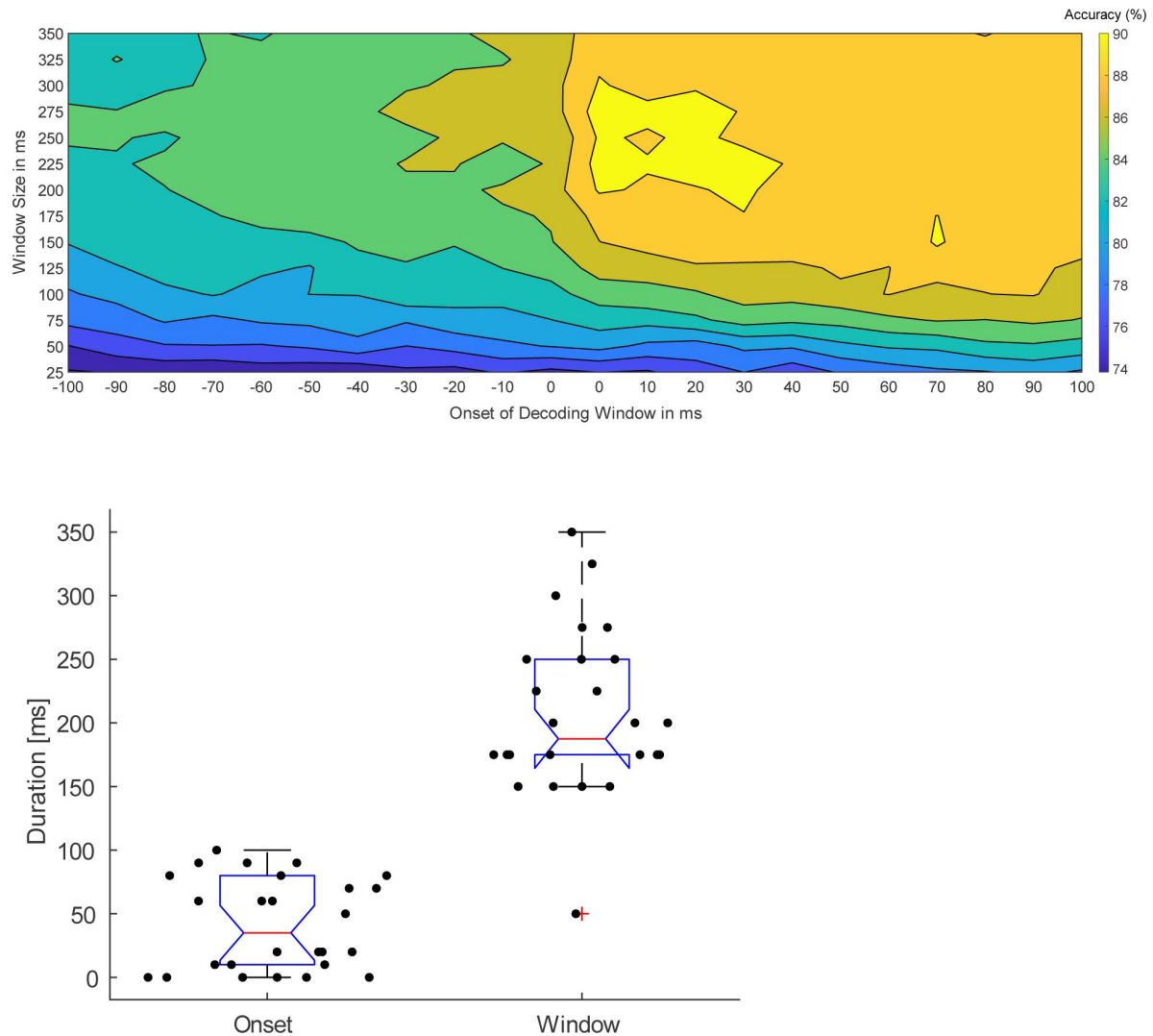


Figure 4 – figure supplement 2

Average decoding accuracies across participants with varying window parameters (top panel). X-axis represents the onset of window for decoding analysis with respect to keypress onset (0). Y-axis represents the window size. The heatmap color denotes the decoding accuracy for all window size/location pairings. Note that, the best decoding accuracy across subjects is obtained by taking a window starting from 0 (i.e., onset of keypress) with a window size of 200ms. The windowing parameters used for each subject in reported results are shown in the bottom panel.

Figure 5. figure supplement 1.

Relationship between offline neuronal representational change and micro-offline learning.

Cumulative micro-offline gains, i.e., the net gain in skill during inter-practice rest periods increased over time during early learning. Offline representation difference, i.e., the net change in the neural representations of keypress 4 over the inter-practice rest intervals between different ordinal positions in the sequence (context) increased over time during early learning. A linear regression analysis showed a strong temporal relationship (correlation coefficient (r) = 0.9034 and coefficient of variance explained (R^2) = 0.82) between amount of contextualization during rest and cumulative micro-offline gains during rest, signifying the effect of contextualization in sequential skill learning.

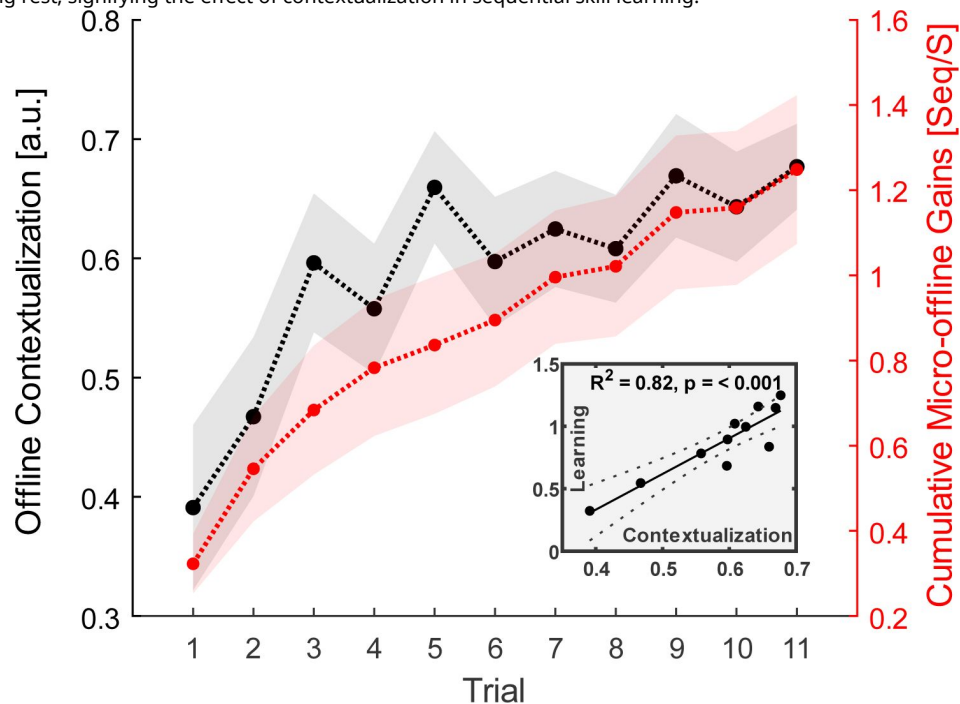


Figure 5 – figure supplement 2

Online versus offline changes in keypress transition patterns. A. Trial-by-trial Euclidian distance between keypress transition patterns (i.e. – relative share of each keypress transition across the full sequence duration) for the first and last sequence iteration within a single trial (online; green) and last sequence iteration of the current trial versus the first sequence iteration of the subsequent trial (offline; magenta). B. Cumulative online (green; left) and offline (magenta; right) pattern distances recorded over all forty-five trials covering Days 1 and 2. Note that cumulative online and offline distances are not significantly different ($t = -0.03$, $p = 0.976$).

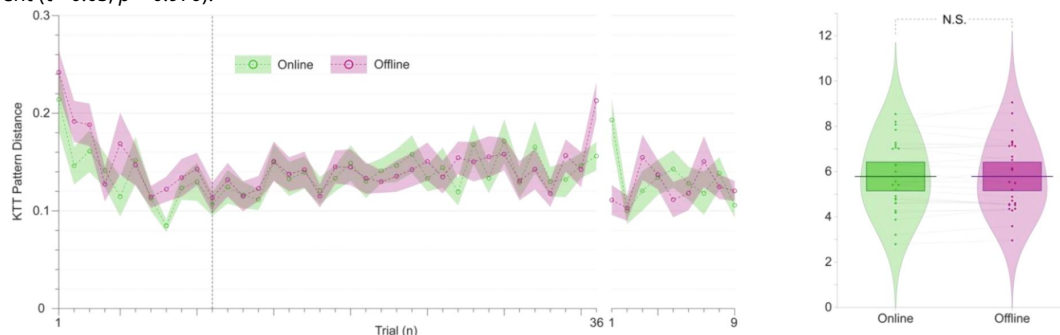


Figure 5 – figure supplement 3

Relationship between contextualization and absolute speed.

A. Relationship between maximum speed and corresponding contextualization: The maximum typing speed and the degree of contextualization was related using a linear regression analysis that showed no significant relationship between maximum typing speed and degree of contextualization ($R^2 = 0.028$, $p = 0.41$). Here, each dot represents the maximum speed attained and the corresponding degree of contextualization of each participant. Thus, people with higher typing speed did not show higher degree of contextualization. **B. Relationship between typing speed and degree of contextualization at each trial.** We performed a regression analysis for each trial to relate the degree of contextualization at each trial and the typing speed at that trial. The violin plot represents the distribution of R^2 values obtained with regression analysis and here each dot represents a trial. Note that, there was no significant relationship at any trial (mean $R^2 = 0.06$; $p > 0.05$).

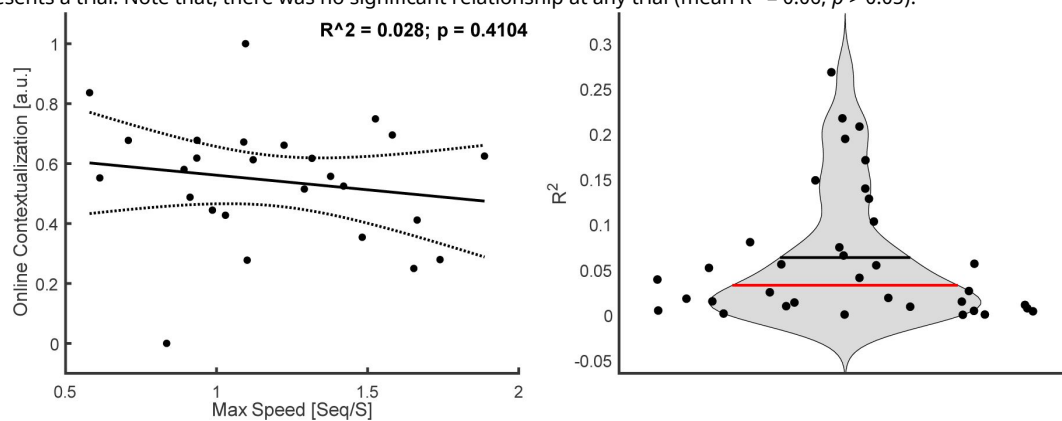


Figure 5 – figure supplement 4

Distribution of individual subject correlation coefficients between contextualization changes occurring during practice or rest with micro-online and micro-offline performance gains.

The correlation distributions were significantly higher for the relationship between contextualization changes during rest and micro-offline gains than for contextualization changes during practice with either micro-online or micro-offline gains.

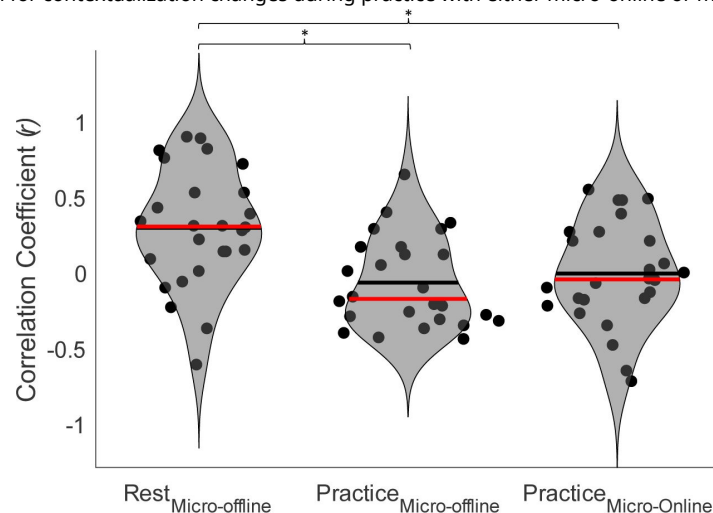


Figure 5 – figure supplement 5

Trial by trial trend of offline (left panel) and online (middle and right panels) contextualization changes.

Offline contextualization changes were assessed by calculating the distance between neural representations for the last Index_{OP5} keypress in the previous trial and the first Index_{OP1} keypress in the present trial. Two different approaches were used to characterize online contextualization changes. The analysis included in the reviewed manuscript (middle panel) calculated the distance between Index_{OP1} and Index_{OP5} for each correct sequence, which was then averaged across the trial. This approach is limited by the lack of control for the passage of time when making online versus offline comparisons. Thus, the second approach controlled for the passage of time by calculating distance between the representations associated with the first Index_{OP1} keypress and the last Index_{OP5} keypress within the same trial. Note that while the first approach showed an increase online contextualization trend with practice, the second approach did not.

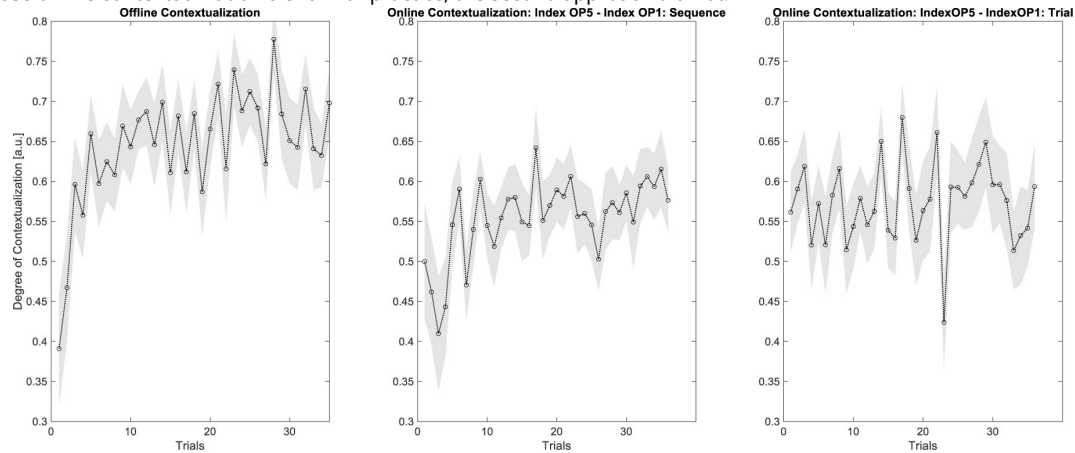
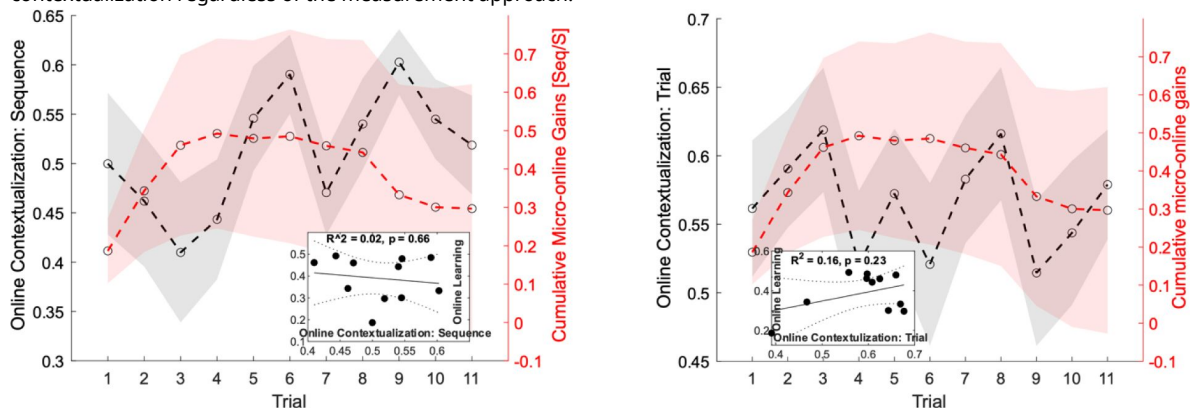


Figure 5 – figure supplement 6

Online contextualization versus micro-online learning.

The relationship between online contextualization and online learning is shown for both within-sequence (left) and across-sequence (right) distance calculation. There was no significant relationship between online learning and online contextualization regardless of the measurement approach.



Data Availability

All de-identified and permanently unlinked from all personal identifiable information (PII) data are publicly available. ([Box Repository](#)).

Acknowledgements

We thank Ms. Tasneem Malik, Ms. Michele Richman, and NIMH MEG Core Facility staff for their support. This work utilized the computational resources of the NIH HPC Biowulf cluster (<http://hpc.nih.gov> ). This research was supported by the Intramural Research Program of the NINDS, NIH.

References

1. Bönstrup M., et al. 2019) **A rapid form of offline consolidation in skill learning** *Current Biology* **29**:1346–1351
2. Schmidt R. A., Lee T. D., Winstein C., Wulf G., Zelaznik H. N. 2018) **Motor Control and Learning: A Behavioral Emphasis** *Human kinetics*
3. Doyon J., Benali H. 2005) **Reorganization, and plasticity in the adult brain during learning of motor skills** *Curr Opin Neurobiol* **15**:161–167
4. Dehaene S., Meyniel F., Wacongne C., Wang L., Pallier C. 2015) **The neural representation of sequences: from transition probabilities to algebraic patterns and linguistic trees** *Neuron* **88**:2–19
5. Bönstrup M., Iturrate I., Hebart M. N., Censor N., Cohen L. G. 2020) **Mechanisms of offline motor learning at a microscale of seconds in large-scale crowdsourced data** *NPJ Sci Learn* **5**:1–10
6. Buch E. R., Claudino L., Quentin R., Bönstrup M., Cohen L. G. 2021) **Consolidation of human skill linked to waking hippocampo-neocortical replay** *Cell Rep* **35**:109193
7. Jacobacci F., et al. 2020) **Rapid hippocampal plasticity supports motor sequence learning** *Proceedings of the National Academy of Sciences* **117**:23898–23903
8. Deleglise A., et al. 2023) **Human motor sequence learning drives transient changes in network topology and hippocampal connectivity early during memory consolidation** *Cerebral Cortex* **33**:6120–6131
9. Chen S., Epps J. 2014) **Using task-induced pupil diameter and blink rate to infer cognitive load** *Hum Comput Interact* **29**:390–413
10. Sjøgård M., et al. 2024) **Hippocampal ripples mediate motor learning during brief rest breaks in humans** *bioRxiv* :2024–2025
11. Buzsáki G. 2015) **Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning** *Hippocampus* **25**:1073–1188
12. Kami A., et al. 1995) **Functional MRI evidence for adult motor cortex plasticity during motor skill learning** *Nature* **377**:155–158
13. Song S., Cohen L. G. 2014) **Practice and sleep form different aspects of skill** *Nat Commun* **5**:3407
14. Natraj N., Silversmith D. B., Chang E. F., Ganguly K. 2022) **Compartmentalized dynamics within a common multi-area mesoscale manifold represent a repertoire of human hand movements** *Neuron* **110**:154–174
15. Ghilardi M. F., Moisello C., Silvestri G., Ghez C., Krakauer J. W. 2009) **Learning of a sequential motor skill comprises explicit and implicit components that consolidate differently**

16. Yokoi A., Diedrichsen J. 2019) **Neural organization of hierarchical motor sequence representations in the human neocortex** *Neuron* **103**:1178–1190
17. Beukema P., Diedrichsen J., Verstynen T. D. 2019) **Binding during sequence learning does not alter cortical representations of individual actions** *Journal of Neuroscience* **39**:6968–6977
18. Hikosaka O., et al. 1999) **Parallel neural networks for learning sequential procedures** *Trends Neurosci* **22**:464–471
19. Destrieux C., Fischl B., Dale A., Halgren E. 2010) **Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature** *Neuroimage* **53**:1–15
20. Van Der Maaten L., Postma E. O., van den Herik H. J. 2009) **Dimensionality reduction: A comparative review** *Journal of Machine Learning Research* **10**:13
21. Merino E. C., Faes A., Van Hulle M. M. 2023) **The role of distinct ECoG frequency features in decoding finger movement** *J Neural Eng* **20**:066014
22. Liu C., et al. 2023) **Decoding the EEG patterns induced by sequential finger movement for brain-computer interfaces** *Front Neurosci* **17**
23. Lee H. S., et al. 2022) **Individual finger movement decoding using a novel ultra-high-density electroencephalography-based brain-computer interface system** *Front Neurosci* **16**:1009878
24. Zhao Y., et al. 2022) **Decoding finger movement patterns from microscopic neural drive information based on deep learning** *Med Eng Phys* **104**:103797
25. Yao L., Zhu B., Shoaran M. 2022) **Fast and accurate decoding of finger movements from ECoG through Riemannian features and modern machine learning techniques** *J Neural Eng* **19**:016037
26. Buch E., et al. 2008) **Think to move: a neuromagnetic brain-computer interface (BCI) system for chronic stroke** *Stroke* **39**:910–917
27. Vogel J., et al. 2014) **Continuous control of the dlr light-weight robot iii by a human with tetraplegia using the braingate2 neural interface system** In: *Experimental Robotics: The 12th International Symposium on Experimental Robotics* Springer pp. 125–136
28. Rubin D. B., Hochberg L. R. 2023) **BrainGate: An Intracortical Brain-Computer Interface for the Restoration of Communication and Functional Independence for People with Paralysis** In: *2023 11th International Winter Conference on Brain-Computer Interface (BCI)* IEEE pp. 1–3
29. Birbaumer N., Cohen L. G. 2007) **Brain-computer interfaces: communication and restoration of movement in paralysis** *J Physiol* **579**:621–636
30. Birbaumer N., Murguialday A. R., Cohen L. 2008) **Brain-computer interface in paralysis** *Curr Opin Neurol* **21**:634–638
31. Liao K., Xiao R., Gonzalez J., Ding L. 2014) **Decoding individual finger movements from one hand using human EEG signals** *PLoS One* **9**:e85192

32. Quandt F., et al. 2012) **Single trial discrimination of individual finger movements on one hand: a combined MEG and EEG study** *Neuroimage* **59**:3316–3324
33. Kornysheva K., et al. 2019) **Neural competitive queuing of ordinal structure underlies skilled sequential action** *Neuron* **101**:1166–1180
34. Shenoy P., Miller K. J., Ojemann J. G., Rao R. P. N. 2007) **Finger movement classification for an electrocorticographic BCI** In: *2007 3rd International IEEE/EMBS Conference on Neural Engineering* IEEE pp. 192–195
35. Onaran I., Ince N. F., Cetin A. E., Abosch A. 2011) **A hybrid SVM/HMM based system for the state detection of individual finger movements from multichannel ECoG signals** In: *2011 5th International IEEE/EMBS Conference on Neural Engineering* IEEE pp. 457–460
36. Munn B. R., et al. 2024) **Multiscale organization of neuronal activity unifies scale-dependent theories of brain function** *Cell*
37. Srinivas S., et al. 2016) **A taxonomy of deep convolutional neural nets for computer vision** *Front Robot AI* **2**:36
38. Lisman J. E., Jensen O. 2013) **The theta-gamma neural code** *Neuron* **77**:1002–1016
39. Presacco A., Forrester L. W., Contreras-Vidal J. L. 2012) **Decoding intra-limb and inter-limb kinematics during treadmill walking from scalp electroencephalographic (EEG) signals** *IEEE Transactions on neural systems and rehabilitation engineering* **20**:212–219
40. Dayan E., Cohen L. G. 2011) **Neuroplasticity subserving motor skill learning** *Neuron* **72**:443–454
41. Classen J., Liepert J., Wise S. P., Hallett M., Cohen L. G. 1998) **Rapid plasticity of human cortical movement representation induced by practice** *J Neurophysiol* **79**:1117–1123
42. Kleim J. A., Barbay S., Nudo R. J. 1998) **Functional reorganization of the rat motor cortex following motor skill learning** *J Neurophysiol* **80**:3321–3325
43. Sawamura D., et al. 2019) **Acquisition of chopstick-operation skills with the non-dominant hand and concomitant changes in brain activity** *Sci Rep* **9**:20397
44. Lee S. H., Jin S. H., An J. 2019) **The difference in cortical activation pattern for complex motor skills: A functional near-infrared spectroscopy study** *Sci Rep* **9**:14066
45. Shadmehr R., Holcomb H. H. 1997) **Neural correlates of motor memory consolidation** *Science (1979)* **277**:821–825
46. Toni I., Ramnani N., Josephs O., Ashburner J., Passingham R. E. 2001) **Learning arbitrary visuomotor associations: temporal dynamic of brain activity** *Neuroimage* **14**:1048–1057
47. Doyon J., et al. 2002) **Experience-dependent changes in cerebellar contributions to motor sequence learning** *Proceedings of the National Academy of Sciences* **99**:1017–1022
48. Grafton S. T., et al. 1992) **Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET** *Journal of Neuroscience* **12**:2542–2548

49. Kennerley S. W., Sakai K., Rushworth M. F. S. 2004) **Organization of action sequences and the role of the pre-SMA** *J Neurophysiol* **91**:978–993
50. Hardwick R. M., Rottschy C., Miall R. C., Eickhoff S. B. 2013) **A quantitative meta-analysis and review of motor learning in the human brain** *Neuroimage* **67**:283–297
51. Wolpert D. M., Goodbody S. J., Husain M. 1998) **Maintaining internal representations: the role of the human superior parietal lobe** *Nat Neurosci* **1**:529–533
52. Andersen R. A., Buneo C. A. 2002) **Intentional maps in posterior parietal cortex** *Annu Rev Neurosci* **25**:189–220
53. Buneo C. A., Andersen R. A. 2006) **The posterior parietal cortex: sensorimotor interface for the planning and online control of visually guided movements** *Neuropsychologia* **44**:2594–2606
54. Grover S., Wen W., Viswanathan V., Gill C. T., Reinhart R. M. G. 2022) **Long-lasting, dissociable improvements in working memory and long-term memory in older adults with repetitive neuromodulation** *Nat Neurosci* :1–10
55. Reddy L., et al. 2021) **Theta-phase dependent neuronal coding during sequence learning in human single neurons** *Nat Commun* **12**:4839
56. Bansal A. K., Vargas-Irwin C. E., Truccolo W., Donoghue J. P. 2011) **Relationships among low-frequency local field potentials, spiking activity, and three-dimensional reach and grasp kinematics in primary motor and ventral premotor cortices** *J Neurophysiol* **105**:1603–1619
57. Mollazadeh M., et al. 2011) **Spatiotemporal variation of multiple neurophysiological signals in the primary motor cortex during dexterous reach-to-grasp movements** *Journal of Neuroscience* **31**:15531–15543
58. Bönstrup M., et al. 2019) **Low-frequency brain oscillations track motor recovery in human stroke** *Ann Neurol* **86**:853–865
59. Cruikshank L. C., Singhal A., Hueppelsheuser M., Caplan J. B. 2012) **Theta oscillations reflect a putative neural mechanism for human sensorimotor integration** *J Neurophysiol* **107**:65–77
60. Tomassini A., Ambrogioni L., Medendorp W. P., Maris E. 2017) **Theta oscillations locked to intended actions rhythmically modulate perception** *eLife* **6**:e25618
61. Ramanathan D. S., et al. 2018) **Low-frequency cortical activity is a neuromodulatory target that tracks recovery after stroke** *Nat Med* **24**:1257–1267
62. Hall T. M., Nazarpour K., Jackson A. 2014) **Real-time estimation and biofeedback of single-neuron firing rates using local field potentials** *Nat Commun* **5**:5462
63. Stefanics G., et al. 2010) **Phase entrainment of human delta oscillations can mediate the effects of expectation on reaction speed** *Journal of Neuroscience* **30**:13578–13585
64. Flint R. D., Ethier C., Oby E. R., Miller L. E., Slutzky M. W. 2012) **Local field potentials allow accurate decoding of muscle activity** *J Neurophysiol* **108**:18–24
65. Krasoulis A., Hall T. M., Vijayakumar S., Jackson A., Nazarpour K. 2014) **Generalizability of EMG decoding using local field potentials** In: *2014 36th Annual International Conference of the IEEE*

66. Churchland M. M., et al. 2012) **Neural population dynamics during reaching** *Nature* **487**:51–56
67. Frohlich J., Toker D., Monti M. M. 2021) **Consciousness among delta waves: a paradox?** *Brain* **144**:2257–2277
68. Kim J., et al. 2022) **Recovery of consolidation after sleep following stroke— interaction of slow waves, spindles, and GABA** *Cell Rep* **38**
69. Shen G., et al. 2014) **Decoding the individual finger movements from single-trial functional magnetic resonance imaging recordings of human brain activity** *European Journal of Neuroscience* **39**:2071–2082
70. Alazrai R., Alwanni H., Daoud M. I. 2019) **EEG-based BCI system for decoding finger movements within the same hand** *Neurosci Lett* **698**:113–120
71. Xiao R., Ding L. 2013) **Evaluation of EEG features in decoding individual finger movements from one hand** *Comput Math Methods Med* **2013**:243257
72. Stankevich L. A., et al. 2016) **EEG pattern decoding of rhythmic individual finger imaginary movements of one hand** *Hum Physiol* **42**:32–42
73. Nambu I., et al. 2015) **Decoding sequential finger movements from preparatory activity in higher-order motor regions: a functional magnetic resonance imaging multi-voxel pattern analysis** *European Journal of Neuroscience* **42**:2851–2859
74. Baillet S. 2017) **Magnetoencephalography for brain electrophysiology and imaging** *Nat Neurosci* **20**:327–339
75. Komorowski R. W., Manns J. R., Eichenbaum H. 2009) **Robust conjunctive item–place coding by hippocampal neurons parallels learning what happens where** *Journal of Neuroscience* **29**:9918–9929
76. Georgopoulos A. P. 1994) **Population activity in the control of movement** *Int Rev Neurobiol* **103**
77. Georgopoulos A. P., Kalaska J. F., Caminiti R., Massey J. T. 1982) **On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex** *J Neurosci* **2**:1527–1537
78. Pan S. C., Rickard T. C. 2015) **Sleep and motor learning: Is there room for consolidation?** *Psychol Bull* **141**:812
79. Jacobacci F., et al. 2020) **Rapid hippocampal plasticity supports motor sequence learning** *Proceedings of the National Academy of Sciences* **117**:23898–23903
80. Brooks E., Wallis S., Hendrikse J., Coxon J. 2024) **Micro-consolidation occurs when learning an implicit motor sequence, but is not influenced by HIIT exercise** *NPJ Sci Learn* **9**:23
81. Robertson E. M. 2019) **Skill memory: mind the ever-decreasing gap for offline processing** *Current Biology* **29**:R287–R289

82. Chen P.-C., Stritzelberger J., Walther K., Hamer H., Staresina B. 2024) **Hippocampal ripples during offline periods predict human motor sequence learning** *bioRxiv* :2010–2024
83. Johnson B. P., et al. 2023) **Generalization of procedural motor sequence learning after a single practice trial** *NPJ Sci Learn* **8**:45
84. Rickard T. C., Cai D. J., Rieth C. A., Jones J., Ard M. C. 2008) **Sleep does not enhance motor sequence learning** *J Exp Psychol Learn Mem Cogn* **34**:834
85. Das A., Karagiorgis A., Diedrichsen J., Stenner M.-P., Azanon E. 2024) **“Micro-offline gains” convey no benefit for motor skill learning** *bioRxiv* :2024–2027
86. Gupta M. W., Rickard T. C. 2024) **Comparison of online, offline, and hybrid hypotheses of motor sequence learning using a quantitative model that incorporate reactive inhibition** *Sci Rep* **14**:4661
87. Gupta M. W., Rickard T. C. 2022) **Dissipation of reactive inhibition is sufficient to explain post-rest improvements in motor sequence learning** *NPJ Sci Learn* **7**:25
88. Ruiz M. H., Jabusch H.-C., Altenmüller E. 2009) **Detecting wrong notes in advance: neuronal correlates of error monitoring in pianists** *Cerebral cortex* **19**:2625–2639
89. Maidhof C., Rieger M., Prinz W., Koelsch S. 2009) **Nobody is perfect: ERP effects prior to performance errors in musicians indicate fast monitoring processes** *PLoS One* **4**:e5032
90. Censor N., Horovitz S. G., Cohen L. G. 2014) **Interference with existing memories alters offline intrinsic functional brain connectivity** *Neuron* **81**:69–76
91. Oostenveld R., Fries P., Maris E., Schoffelen J.-M. 2011) **FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data** *Comput Intell Neurosci* **2011**:1–9
92. Delorme A., Makeig S. 2004) **EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis** *J Neurosci Methods* **134**:9–21
93. Rijsbergen V. 1979) **Information retrieval; Butterworth, 1978** *J. librariansh* **11**:237
94. Schütze H., Manning C. D., Raghavan P. 2008) **Introduction to information retrieval** Cambridge University Press
95. Ding C., Peng H. 2005) **Minimum redundancy feature selection from microarray gene expression data** *J Bioinform Comput Biol* **3**:185–205
96. Holland P. W., Welsch R. E. 1977) **Robust regression using iteratively reweighted least-squares** *Communications in Statistics-theory and Methods* **6**:813–827
97. Iwane F., Buch R., Cohen L. 2024) **Sequence regularity determines if early learning gains occur during practice or rest. Program No. PST369.21. 2024 Neuroscience Meeting Planner** Chicago, IL: Society for Neuroscience

Author information

Debadatta Dash

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0000-0002-0543-0304](https://orcid.org/0000-0002-0543-0304)

Fumiaki Iwane

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0000-0002-9659-4127](https://orcid.org/0000-0002-9659-4127)

William Hayward

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0000-0002-9344-4203](https://orcid.org/0000-0002-9344-4203)

Roberto Salamanca-Giron

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0009-0008-5743-6805](https://orcid.org/0009-0008-5743-6805)

Marlene Bonstrup

Department of Neurology, University of Leipzig Medical Center, Leipzig, Germany

ORCID iD: [0000-0002-7237-1359](https://orcid.org/0000-0002-7237-1359)

Ethan Buch

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0000-0002-5443-8222](https://orcid.org/0000-0002-5443-8222)

For correspondence: ethan.buch@nih.gov

Leonardo G Cohen

Human Cortical Physiology and Neurorehabilitation Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, United States

ORCID iD: [0000-0002-1705-8773](https://orcid.org/0000-0002-1705-8773)

For correspondence: cohenl@ninds.nih.gov

Editors

Reviewing Editor

Juan Alvaro Gallego

Imperial College London, London, United Kingdom

Senior Editor

Michael Frank

Brown University, Providence, United States of America

Reviewer #1 (Public review):**Summary:**

This study addresses the issue of rapid skill learning and whether individual sequence elements (here: finger presses) are differentially represented in human MEG data. The authors use a decoding approach to classify individual finger elements, and accomplish an accuracy of around 94%. A relevant finding is that the neural representations of individual finger elements dynamically change over the course of learning. This would be highly relevant for any attempts to develop better brain machine interfaces - one now can decode individual elements within a sequence with high precision, but these representations are not static but develop over the course of learning.

Strengths:

The work follows a large body of work from the same group on the behavioural and neural foundations of sequence learning. The behavioural task is well established a neatly designed to allow for tracking learning and how individual sequence elements contribute. The inclusion of short offline rest periods between learning epochs has been influential because it has revealed that a lot, if not most of the gains in behaviour (ie speed of finger movements) occur in these so-called micro-offline rest periods.

The authors use a range of new decoding techniques, and exhaustively interrogate their data in different ways, using different decoding approaches. Regardless of the approach, impressively high decoding accuracies are observed, but when using a hybrid approach that combines the MEG data in different ways, the authors observe decoding accuracies of individual sequence elements from the MEG data of up to 94%.

Weaknesses:

A formal analysis and quantification of how head movement may have contributed to the results should be included in the paper or supplemental material. The type of correlated head movements coming from vigorous key presses aren't necessarily visible to the naked eye, and even if arms etc are restricted, this will not preclude shoulder, neck or head movement necessarily; if ICA was conducted, for example, the authors are in the position to show the components that relate to such movement; but eye-balling the data would not seem sufficient. The related issue of eye movements is addressed via classifier analysis. A formal analysis which directly accounts for finger/eye movements in the same analysis as the main result (ie any variance related to these factors) should be presented.

This reviewer recommends inclusion of a formal analysis that the intra-vs inter parcels are indeed completely independent. For example, the authors state that the inter-parcel features reflect "lower spatially resolved whole-brain activity patterns or global brain dynamics". A formal quantitative demonstration that the signals indeed show "complete independence" (as claimed by the authors) and are orthogonal would be helpful

<https://doi.org/10.7554/eLife.102475.2.sa3>

Reviewer #2 (Public review):**Summary:**

The current paper consists of two parts. The first part is the rigorous feature optimization of the MEG signal to decode individual finger identity performed in a sequence (4-1-3-2-4; 1~4 corresponds to little~index fingers of the left hand). By optimizing various parameters for the MEG signal, in terms of (i) reconstructed source activity in voxel- and parcel-level resolution

and their combination, (ii) frequency bands, and (iii) time window relative to press onset for each finger movement, as well as the choice of decoders, the resultant "hybrid decoder" achieved extremely high decoding accuracy (~95%). This part seems driven almost by pure engineering interest in gaining as high decoding accuracy as possible.

In the second part of the paper, armed with the successful 'hybrid decoder,' the authors asked more scientific questions about how neural representation of individual finger movement that is embedded in a sequence, changes during a very early period of skill learning and whether and how such representational change can predict skill learning. They assessed the difference in MEG feature patterns between the first and the last press 4 in sequence 41324 at each training trial and found that the pattern differentiation progressively increased over the course of early learning trials. Additionally, they found that this pattern differentiation specifically occurred during the rest period rather than during the practice trial. With a significant correlation between the trial-by-trial profile of this pattern differentiation and that for accumulation of offline learning, the authors argue that such "contextualization" of finger movement in a sequence (e.g., what-where association) underlies the early improvement of sequential skill. This is an important and timely topic for the field of motor learning and beyond.

Strengths:

Each part has its own strength. For the first part, the use of temporally rich neural information (MEG signal) has a significant advantage over previous studies testing sequential representations using fMRI. This allowed the authors to examine the earliest period (= the first few minutes of training) of skill learning with finer temporal resolution. Through the optimization of MEG feature extraction, the current study achieved extremely high decoding accuracy (approx. 94%) compared to previous works. For the second part, the finding of the early "contextualization" of the finger movement in a sequence and its correlation to early (offline) skill improvement is interesting and important. The comparison between "online" and "offline" pattern distance is a neat idea.

Weaknesses:

Despite the strengths raised, the specific goal for each part of the current paper, i.e., achieving high decoding accuracy and answering the scientific question of early skill learning, seems not to harmonize with each other very well. In short, the current approach, which is solely optimized for achieving high decoding accuracy, does not provide enough support and interpretability for the paper's interesting scientific claim. This reminds me of the accuracy-explainability tradeoff in machine learning studies (e.g., Linardatos et al., 2020). More details follow.

There are a number of different neural processes occurring before and after a key press, such as planning of upcoming movement and ahead around premotor/parietal cortices, motor command generation in primary motor cortex, sensory feedback related processes in sensory cortices, and performance monitoring/evaluation around the prefrontal area. Some of these may show learning-dependent change and others may not.

Given the use of whole-brain MEG features with a wide time window (up to ~200 ms after each key press) under the situation of 3~4 Hz (i.e., 250~330 ms press interval) typing speed, these different processes in different brain regions could have contributed to the expression of the "contextualization," making it difficult to interpret what really contributed to the "contextualization" and whether it is learning related. Critically, the majority of data used for decoder training has the chance of such potential overlap of signal, as the typing speed almost reached a plateau already at the end of the 11th trial and stayed until the 36th trial. Thus, the decoder could have relied on such overlapping features related to the future presses. If that is the case, a gradual increase in "contextualization" (pattern separation)

during earlier trials makes sense, simply because the temporal overlap of the MEG feature was insufficient for the earlier trials due to slower typing speed.

Several direct ways to address the above concern, at the cost of decoding accuracy to some degree, would be either using the shorter temporal window for the MEG feature or training the model with the early learning period data only (trials 1 through 11) to see if the main results are unaffected would be some example.

<https://doi.org/10.7554/eLife.102475.2.sa2>

Reviewer #3 (Public review):

Summary:

One goal of this paper is to introduce a new approach for highly accurate decoding of finger movements from human magnetoencephalography data via dimension reduction of a "multi-scale, hybrid" feature space. Following this decoding approach, the authors aim to show that early skill learning involves "contextualization" of the neural coding of individual movements, relative to their position in a sequence of consecutive movements. Furthermore, they aim to show that this "contextualization" develops primarily during short rest periods interspersed with skill training, and correlates with a performance metric which the authors interpret as an indicator of offline learning.

Strengths:

A strength of the paper is the innovative decoding approach, which achieves impressive decoding accuracies via dimension reduction of a "multi-scale, hybrid space". This hybrid-space approach follows the neurobiologically plausible idea of concurrent distribution of neural coding across local circuits as well as large-scale networks. A further strength of the study is the large number of tested dimension reduction techniques and classifiers.

Weaknesses:

A clear weakness of the paper lies in the authors' conclusions regarding "contextualization". Several potential confounds, which partly arise from the experimental design (mainly the use of a single sequence) and which are described below, question the neurobiological implications proposed by the authors, and provide a simpler explanation of the results. Furthermore, the paper follows the assumption that short breaks result in offline skill learning, while recent evidence, described below, casts doubt on this assumption.

Specifically:

The authors interpret the ordinal position information captured by their decoding approach as a reflection of neural coding dedicated to the local context of a movement (Figure 4). One way to dissociate ordinal position information from information about the moving effectors is to train a classifier on one sequence, and test the classifier on other sequences that require the same movements, but in different positions (Kornysheva et al., Neuron 2019). In the present study, however, participants trained to repeat a single sequence (4-1-3-2-4). As a result, ordinal position information is potentially confounded by the fixed finger transitions around each of the two critical positions (first and fifth press). Across consecutive correct sequences, the first keypress in a given sequence was always preceded by a movement of the index finger (=last movement of the preceding sequence), and followed by a little finger movement. The last keypress, on the other hand, was always preceded by a ring finger movement, and followed by an index finger movement (=first movement of the next sequence). Figure 4 - supplement 2 shows that finger identity can be decoded with high accuracy (>70%) across a large time window around the time of the keypress, up to at least {plus minus}100 ms (and likely beyond, given that decoding accuracy is still high at the

boundaries of the window depicted in that figure). This time window approaches the keypress transition times in this study. Given that distinct finger transitions characterized the first and fifth keypress, the classifier could thus rely on persistent (or "lingering") information from the preceding finger movement, and/or "preparatory" information about the subsequent finger movement, in order to dissociate the first and fifth keypress. Currently, the manuscript provides little evidence that the context information captured by the decoding approach is more than a by-product of temporally extended, and therefore overlapping, but independent neural representations of consecutive keypresses that are executed in close temporal proximity - rather than a neural representation dedicated to context. During the review process, the authors pointed out that a "mixing" of temporally overlapping information from consecutive keypresses, as described above, should result in systematic misclassifications and therefore be detectable in the confusion matrices in Figures 3C and 4B, which indeed do not provide any evidence that consecutive keypresses are systematically confused. However, such absence of evidence (of systematic misclassification) should be interpreted with caution, and, of course, provides no evidence of absence. The authors also pointed out that such "mixing" would hamper the discriminability of the two ordinal positions of the index finger, given that "ordinal position 5" is systematically followed by "ordinal position 1". This is a valid point which, however, cannot rule out that "contextualization" nevertheless reflects the described "mixing".

During the review process, the authors responded to my concern that training of a single sequence introduces the potential confound of "mixing" described above, which could have been avoided by training on several sequences, as in Kornysheva et al. (Neuron 2019), by arguing that Day 2 in their study did include control sequences. However, the authors' findings regarding these control sequences are fundamentally different from the findings in Kornysheva et al. (2019), and do not provide any indication of effector-independent ordinal information in the described contextualization - but, actually, the contrary. In Kornysheva et al. (Neuron 2019), ordinal, or positional, information refers purely to the rank of a movement in a sequence. In line with the idea of competitive queuing, Kornysheva et al. (2019) have shown that humans prepare for a motor sequence via a simultaneous representation of several of the upcoming movements, weighted by their rank in the sequence. Importantly, they could show that this gradient carries information that is largely devoid of information about the order of specific effectors involved in a sequence, or their timing, in line with competitive queuing. They showed this by training a classifier to discriminate between the five consecutive movements that constituted one specific sequence of finger movements (five classes: 1st, 2nd, 3rd, 4th, 5th movement in the sequence) and then testing whether that classifier could identify the rank (1st, 2nd, 3rd, etc) of movements in another sequence, in which the fingers moved in a different order, and with different timings. Importantly, this approach demonstrated that the graded representations observed during preparation were largely maintained after this cross-decoding, indicating that the sequence was represented via ordinal position information that was largely devoid of information about the specific effectors or timings involved in sequence execution. This result differs completely from the findings in the current manuscript. Dash et al. report a drop in detected ordinal position information (degree of contextualization in figure 5C) when testing for contextualization in their novel, untrained sequences on Day 2, indicating that context and ordinal information as defined in Dash et al. is not at all devoid of information about the specific effectors involved in a sequence. In this regard, a main concern in my public review, as well as the second reviewer's public review, is that Dash et al. cannot tell apart, by design, whether there is truly contextualization in the neural representation of a sequence (which they claim), or whether their results regarding "contextualization" are explained by what they call "mixing" in their author response, i.e., an overlap of representations of consecutive movements, as suggested as an alternative explanation by Reviewer 2 and myself.

Such temporal overlap of consecutive, independent finger representations may also account for the dynamics of "ordinal coding"/"contextualization", i.e., the increase in 2-class decoding

accuracy, across Day 1 (Figure 4C). As learning progresses, both tapping speed and the consistency of keypress transition times increase (Figure 1), i.e., consecutive keypresses are closer in time, and more consistently so. As a result, information related to a given keypress is increasingly overlapping in time with information related to the preceding and subsequent keypresses. The authors seem to argue that their regression analysis in Figure 5 - figure supplement 3 speaks against any influence of tapping speed on "ordinal coding" (even though that argument is not made explicitly in the manuscript). However, Figure 5 - figure supplement 3 shows inter-individual differences in a between-subject analysis (across trials, as in panel A, or separately for each trial, as in panel B), and, therefore, says little about the within-subject dynamics of "ordinal coding" across the experiment. A regression of trial-by-trial "ordinal coding" on trial-by-trial tapping speed (either within-subject, or at a group-level, after averaging across subjects) could address this issue. Given the highly similar dynamics of "ordinal coding" on the one hand (Figure 4C), and tapping speed on the other hand (Figure 1B), I would expect a strong relationship between the two in the suggested within-subject (or group-level) regression. Furthermore, learning should increase the number of (consecutively) correct sequences, and, thus, the consistency of finger transitions. Therefore, the increase in 2-class decoding accuracy may simply reflect an increasing overlap in time of increasingly consistent information from consecutive keypresses, which allows the classifier to dissociate the first and fifth keypress more reliably as learning progresses, simply based on the characteristic finger transitions associated with each. In other words, given that the physical context of a given keypress changes as learning progresses - keypresses move closer together in time, and are more consistently correct - it seems problematic to conclude that the mental representation of that context changes. To draw that conclusion, the physical context should remain stable (or any changes to the physical context should be controlled for).

A similar difference in physical context may explain why neural representation distances ("differentiation") differ between rest and practice (Figure 5). The authors define "offline differentiation" by comparing the hybrid space features of the last index finger movement of a trial (ordinal position 5) and the first index finger movement of the next trial (ordinal position 1). However, the latter is not only the first movement in the sequence, but also the very first movement in that trial (at least in trials that started with a correct sequence), i.e., not preceded by any recent movement. In contrast, the last index finger of the last correct sequence in the preceding trial includes the characteristic finger transition from the fourth to the fifth movement. Thus, there is more overlapping information arising from the consistent, neighbouring keypresses for the last index finger movement, compared to the first index finger movement of the next trial. A strong difference (larger neural representation distance) between these two movements is, therefore, not surprising, given the task design, and this difference is also expected to increase with learning, given the increase in tapping speed, and the consequent stronger overlap in representations for consecutive keypresses. Furthermore, initiating a new sequence involves pre-planning, while ongoing practice relies on online planning (Ariani et al., eNeuro 2021), i.e., two mental operations that are dissociable at the level of neural representation (Ariani et al., bioRxiv 2023).

A further complication in interpreting the results stems from the visual feedback that participants received during the task. Each keypress generated an asterisk shown above the string on the screen. It is not clear why the authors introduced this complicating visual feedback in their task, besides consistency with their previous studies. The resulting systematic link between the pattern of visual stimulation (the number of asterisks on the screen) and the ordinal position of a keypress makes the interpretation of "contextual information" that differentiates between ordinal positions difficult. During the review process, the authors reported a confusion matrix from a classification of asterisks position based on eye tracking data recorded during the task, and concluded that the classifier performed at chance level and gaze was, thus, apparently not biased by the visual stimulation. However, the confusion matrix showed a huge bias that was difficult to interpret (a very strong tendency to predict one of the five asterisk positions, despite chance-level

performance). Without including additional information for this analysis (or simply the gaze position as a function of the number of asterisk on the screen) in the manuscript, this important control analysis cannot be properly assessed, and is not available to the public.

The authors report a significant correlation between "offline differentiation" and cumulative micro-offline gains. However, this does not address the question whether there is a trial-by-trial relation between the degree of "contextualization" and the amount of micro-offline gains - i.e., the question whether performance changes (micro-offline gains) are less pronounced across rest periods for which the change in "contextualization" is relatively low. The single-subject correlation between contextualization changes "during" rest and micro-offline gains (Figure 5 - figure supplement 4) addresses this question, however, the critical statistical test (are correlation coefficients significantly different from zero) is not included. Given the displayed distribution, it seems unlikely that correlation coefficients are significantly above zero.

The authors follow the assumption that micro-offline gains reflect offline learning. However, there is no compelling evidence in the literature, and no evidence in the present manuscript, that micro-offline gains (during any training phase) reflect offline learning. Instead, emerging evidence in the literature indicates that they do not (Das et al., bioRxiv 2024), and instead reflect transient performance benefits when participants train with breaks, compared to participants who train without breaks, however, these benefits vanish within seconds after training if both groups of participants perform under comparable conditions (Das et al., bioRxiv 2024). During the review process, the authors argued that differences in the design between Das et al. (2024) on the one hand (Experiments 1 and 2), and the study by Bönstrup et al. (2019) on the other hand, may have prevented Das et al. (2024) from finding the assumed (lasting) learning benefit by micro-offline consolidation. However, the Supplementary Material of Das et al. (2024) includes an experiment (Experiment S1) whose design closely follows the early learning phase of Bönstrup et al. (2019), and which, nevertheless, demonstrates that there is no lasting benefit of taking breaks for the acquired skill level, despite the presence of micro-offline gains.

Along these lines, the authors' claim, based on Bönstrup et al. 2020, that "retroactive interference immediately following practice periods reduces micro-offline learning", is not supported by that very reference. Citing Bönstrup et al. (2020), "Regarding early learning dynamics (trials 1-5), we found no differences in microscale learning parameters (micro-online/offline) or total early learning between both interference groups." That is, contrary to Dash et al.'s current claim, Bönstrup et al. (2020) did not find any retroactive interference effect on the specific behavioral readout (micro-offline gains) that the authors assume to reflect consolidation.

The authors conclude that performance improves, and representation manifolds differentiate, "during" rest periods (see, e.g., abstract). However, micro-offline gains (as well as offline contextualization) are computed from data obtained during practice, not rest, and may, thus, just as well reflect a change that occurs "online", e.g., at the very onset of practice (like pre-planning) or throughout practice (like fatigue, or reactive inhibition). That is, the definition of micro-offline gains (as well as offline contextualization) conflates online and "offline" processes. This becomes strikingly clear in the recent Nature paper by Griffin et al. (2025), who computed micro-offline gains as the difference in average performance across the first five sequences in a practice period (a block, in their terminology) and the last five sequences in the previous practice period. Averaging across sequences in this way minimises the chance to detect online performance changes, and inflates changes in performance "offline". The problem that "offline" gains (or contextualization) is actually computed from data entirely generated online, and therefore subject to processes that occur online, is inherent in the very definition of micro-offline gains, whether, or not, they computed from averaged performance.

A simple control analysis based on shuffled class labels could lend further support to the authors' complex decoding approach. As a control analysis that completely rules out any source of overfitting, the authors could test the decoder after shuffling class labels. Following such shuffling, decoding accuracies should drop to chance-level for all decoding approaches, including the optimized decoder. This would also provide an estimate of actual chance-level performance (which is informative over and beyond the theoretical chance level). During the review process, the authors reported this analysis to the reviewers. Given that readers may consider following the presented decoding approach in their own work, it would have been important to include that control analysis in the manuscript to convince readers of its validity.

Furthermore, the authors' approach to cortical parcellation raises questions regarding the information carried by varying dipole orientations within a parcel (which currently seems to be ignored?) and the implementation of the mean-flipping method (given that there are two dimensions - space and time - it is unclear what the authors refer to when they talk about the sign of the "average source", line 477).

<https://doi.org/10.7554/eLife.102475.2.sa1>

Author response:

The following is the authors' response to the original reviews.

eLife Assessment

This valuable study investigates how the neural representation of individual finger movements changes during the early period of sequence learning. By combining a new method for extracting features from human magnetoencephalography data and decoding analyses, the authors provide incomplete evidence of an early, swift change in the brain regions correlated with sequence learning, including a set of previously unreported frontal cortical regions. The addition of more control analyses to rule out that head movement artefacts influence the findings, and to further explain the proposal of offline contextualization during short rest periods as the basis for improvement performance would strengthen the manuscript.

We appreciate the Editorial assessment on our paper's strengths and novelty. We have implemented additional control analyses to show that neither task-related eye movements nor increasing overlap of finger movements during learning account for our findings, which are that contextualized neural representations in a network of bilateral frontoparietal brain regions actively contribute to skill learning. Importantly, we carried out additional analyses showing that contextualization develops predominantly during rest intervals.

Public Reviews:

Reviewer #1 (Public review):

Summary:

This study addresses the issue of rapid skill learning and whether individual sequence elements (here: finger presses) are differentially represented in human MEG data. The authors use a decoding approach to classify individual finger elements and accomplish an accuracy of around 94%. A relevant finding is that the neural representations of individual finger elements dynamically change over the course of learning. This would be highly relevant for any attempts to develop better brain machine interfaces - one now

can decode individual elements within a sequence with high precision, but these representations are not static but develop over the course of learning.

Strengths:

The work follows a large body of work from the same group on the behavioural and neural foundations of sequence learning. The behavioural task is well established and neatly designed to allow for tracking learning and how individual sequence elements contribute. The inclusion of short offline rest periods between learning epochs has been influential because it has revealed that a lot, if not most of the gains in behaviour (ie speed of finger movements) occur in these so-called micro-offline rest periods. The authors use a range of new decoding techniques, and exhaustively interrogate their data in different ways, using different decoding approaches. Regardless of the approach, impressively high decoding accuracies are observed, but when using a hybrid approach that combines the MEG data in different ways, the authors observe decoding accuracies of individual sequence elements from the MEG data of up to 94%.

We have previously showed that neural replay of MEG activity representing the practiced skill was prominent during rest intervals of early learning, and that the replay density correlated with micro-offline gains (Buch et al., 2021). These findings are consistent with recent reports (from two different research groups) that hippocampal ripple density increases during these inter-practice rest periods, and predict offline learning gains (Chen et al., 2024; Sjøgård et al., 2024). However, decoder performance in our earlier work (Buch et al., 2021) left room for improvement. Here, we reported a strategy to improve decoding accuracy that could benefit future studies of neural replay or BCI using MEG.

Weaknesses:

There are a few concerns which the authors may well be able to resolve. These are not weaknesses as such, but factors that would be helpful to address as these concern potential contributions to the results that one would like to rule out. Regarding the decoding results shown in Figure 2 etc, a concern is that within individual frequency bands, the highest accuracy seems to be within frequencies that match the rate of keypresses. This is a general concern when relating movement to brain activity, so is not specific to decoding as done here. As far as reported, there was no specific restraint to the arm or shoulder, and even then it is conceivable that small head movements would correlate highly with the vigor of individual finger movements. This concern is supported by the highest contribution in decoding accuracy being in middle frontal regions - midline structures that would be specifically sensitive to movement artefacts and don't seem to come to mind as key structures for very simple sequential keypress tasks such as this - and the overall pattern is remarkably symmetrical (despite being a unimanual finger task) and spatially broad. This issue may well be matching the time course of learning, as the vigor and speed of finger presses will also influence the degree to which the arm/shoulder and head move. This is not to say that useful information is contained within either of the frequencies or broadband data. But it raises the question of whether a lot is dominated by movement "artefacts" and one may get a more specific answer if removing any such contributions.

Reviewer #1 expresses concern that the combination of the low-frequency narrow-band decoder results, and the bilateral middle frontal regions displaying the highest average intra-parcel decoding performance across subjects is suggestive that the decoding results could be driven by head movement or other artefacts.

Head movement artefacts are highly unlikely to contribute meaningfully to our results for the following reasons. First, in addition to ICA denoising, all "recordings were visually inspected

and marked to denoise segments containing other large amplitude artifacts due to movements” (see Methods). Second, the response pad was positioned in a manner that minimized wrist, arm or more proximal body movements during the task. Third, while online monitoring of head position was not performed for this study, it was assessed at the beginning and at the end of each recording. The head was restrained with an inflatable air bladder, and head movement between the beginning and end of each scan did not exceed 5mm for all participants included in the study.

The Reviewer states a concern that “it is conceivable that small head movements would correlate highly with the vigor of individual finger movements”. We agree that despite the steps taken above, it is possible that minor head movements could still contribute to some remaining variance in the MEG data in our study. However, such correlations between small head movements and finger movements could only meaningfully contribute to decoding performance if: (A) they were consistent and pervasive throughout the recording (which might not be the case if the head movements were related to movement vigor and vigor changed over time); and (B) they systematically varied between different finger movements, and also between the same finger movement performed at different sequence locations (see 5-class decoding performance in Figure 4B). The possibility of any head movement artefacts meeting all these conditions is unlikely. Alternatively, for this task design a much more likely confound could be the contribution of eye movement artefacts to the decoder performance (an issue raised by Reviewer #3 in the comments below).

Remember from Figure 1A in the manuscript that an asterisk marks the current position in the sequence and is updated at each keypress. Since participants make very few performance errors, the position of the asterisk on the display is highly correlated with the keypress being made in the sequence. Thus, it is possible that if participants are attending to the visual feedback provided on the display, they may generate eye movements that are systematically related to the task. Since we did record eye movements simultaneously with the MEG recordings (EyeLink 1000 Plus; Fs = 600 Hz), we were able to perform a control analysis to address this question. For each keypress event during trials in which no errors occurred (which is the same time-point that the asterisk position is updated), we extracted three features related to eye movements: 1) the gaze position at the time of asterisk position update (triggered by a *KeyDown* event), 2) the gaze position 150ms later, and 3) the peak velocity of the eye movement between the two positions. We then constructed a classifier from these features with the aim of predicting the location of the asterisk (ordinal positions 1-5) on the display. As shown in the confusion matrix below (Author response image 1), the classifier failed to perform above chance levels (overall cross-validated accuracy = 0.21817):

Author response image 1.

Confusion matrix showing that three eye movement features fail to predict asterisk position on the task display above chance levels (Fold 1 test accuracy = 0.21718; Fold 2 test accuracy = 0.22023; Fold 3 test accuracy = 0.21859; Fold 4 test accuracy = 0.22113; Fold 5 test accuracy = 0.21373; Overall cross-validated accuracy = 0.2181). Since the ordinal position of the asterisk on the display is highly correlated with the ordinal position of individual keypresses in the sequence, this analysis provides strong evidence that keypress decoding performance from MEG features is not explained by systematic relationships between finger movement behavior and eye movements (i.e. – behavioral artefacts) (end of figure legend).

1	5566	5437	5177	5018	4709
2	25	27	19	36	49
3	69	58	58	67	83
4		1			1
5	226	166	190	199	357
	1	2	3	4	5

True Class

Remember that the task display does not provide explicit feedback related to performance, only information about the present position in the sequence. Thus, it is possible that participants did not actively attend to the feedback. In fact, inspection of the eye position data revealed that on majority of trials, participants displayed random-walk-like gaze patterns around a central fixation point located near the center of the screen. Thus, participants did not attend to the asterisk position on the display, but instead intrinsically generated the action sequence. A similar realworld example would be manually inputting a long password into a secure online application. In this case, one intrinsically generates the sequence from memory and receives similar feedback about the password sequence position (also provided as asterisks) as provided in the study task – feedback which is typically ignored by the user.

The minimal participant engagement with the visual task display observed in this study highlights another important point – that the behavior in explicit sequence learning motor tasks is highly generative in nature rather than reactive to stimulus cues as in the serial reaction time task (SRTT). This is a crucial difference that must be carefully considered when designing investigations and comparing findings across studies.

We observed that initial keypress decoding accuracy was predominantly driven by contralateral primary sensorimotor cortex in the initial practice trials before transitioning to bilateral frontoparietal regions by trials 11 or 12 as performance gains plateaued. The contribution of contralateral primary sensorimotor areas to early skill learning has been extensively reported in humans and non-human animals (Buch et al., 2021; Classen et al., 1998; Karni et al., 1995; Kleim et al., 1998). Similarly, the increased involvement of bilateral frontal and parietal regions to decoding during early skill learning in the non-dominant hand is well known. Enhanced bilateral activation in both frontal and parietal cortex during skill learning has been extensively reported (Doyon et al., 2002; Grafton et al., 1992; Hardwick et al., 2013; Kennerley et al., 2004; Shadmehr & Holcomb, 1997; Toni, Ramnani, et al., 2001), and appears to be even more prominent during early fine motor skill learning in the non-dominant hand (Lee et al., 2019; Sawamura et al., 2019). The frontal regions identified in these studies are known to play crucial roles in executive control (Battaglia-Mayer & Caminiti, 2019), motor planning (Toni, Thoenissen, et al., 2001), and working memory (Andersen & Buneo, 2002; Buneo & Andersen, 2006; Shadmehr & Holcomb, 1997; Toni, Ramnani, et al., 2001; Wolpert et al., 1998) processes, while the same parietal regions are known to integrate multimodal sensory feedback and support visuomotor transformations (Andersen & Buneo, 2002; Buneo & Andersen, 2006; Shadmehr & Holcomb, 1997; Toni, Ramnani, et al., 2001; Wolpert et al., 1998), in addition to working memory (Grover et al., 2022). Thus, it is not surprising that these regions increasingly contribute to decoding as subjects internalize the

sequential task. We now include a statement reflecting these considerations in the revised Discussion.

A somewhat related point is this: when combining voxel and parcel space, a concern is whether a degree of circularity may have contributed to the improved accuracy of the combined data, because it seems to use the same MEG signals twice - the voxels most contributing are also those contributing most to a parcel being identified as relevant, as parcels reflect the average of voxels within a boundary. In this context, I struggled to understand the explanation given, ie that the improved accuracy of the hybrid model may be due to "lower spatially resolved whole-brain and higher spatially resolved regional activity patterns".

We disagree with the Reviewer's assertion that the construction of the hybrid-space decoder is circular for the following reasons. First, the base feature set for the hybrid-space decoder constructed for all participants includes whole-brain spatial patterns of MEG source activity averaged within parcels. As stated in the manuscript, these 148 inter-parcel features reflect "lower spatially resolved whole-brain activity patterns" or global brain dynamics. We then independently test how well spatial patterns of MEG source activity for all voxels distributed within individual parcels can decode keypress actions. Again, the testing of these intra-parcel spatial patterns, intended to capture "higher spatially resolved regional brain activity patterns", is completely independent from one another and independent from the weighting of individual inter-parcel features. These intra-parcel features could, for example, provide additional information about muscle activation patterns or the task environment. These approximately 1150 intra-parcel voxels (on average, within the total number varying between subjects) are then combined with the 148 inter-parcel features to construct the final hybrid-space decoder. In fact, this varied spatial filter approach shares some similarities to the construction of convolutional neural networks (CNNs) used to perform object recognition in image classification applications (Srinivas et al., 2016). One could also view this hybrid-space decoding approach as a spatial analogue to common timefrequency based analyses such as theta-gamma phase amplitude coupling (θ/γ PAC), which assess interactions between two or more narrow-band spectral features derived from the same time-series data (Lisman & Jensen, 2013).

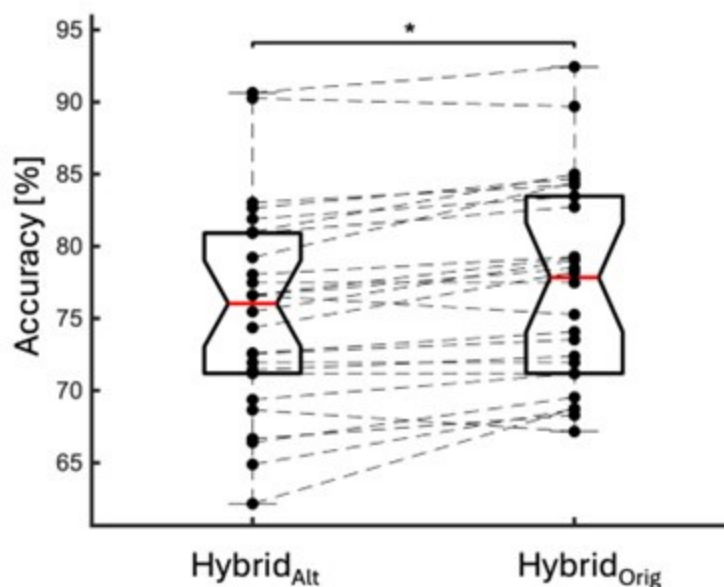
We directly tested this hypothesis – that spatially overlapping *intra-* and *inter-parcel* features portray different information – by constructing an alternative hybrid-space decoder (Hybrid_{Alt}) that excluded average inter-parcel features which spatially overlapped with intra-parcel voxel features, and comparing the performance to the decoder used in the manuscript (Hybrid_{Orig}). The prediction was that if the overlapping parcel contained similar information to the more spatially resolved voxel patterns, then removing the parcel features ($n=8$) from the decoding analysis should not impact performance. In fact, despite making up less than 1% of the overall input feature space, removing those parcels resulted in a significant drop in overall performance greater than 2% ($78.15\% \pm 7.03\%$ SD for Hybrid_{Orig} vs. $75.49\% \pm 7.17\%$ for Hybrid_{Alt}; Wilcoxon signed rank test, $z = 3.7410$, $p = 1.8326e-04$; Author response image 2).

Author response image 2.

Comparison of decoding performances with two different hybrid approaches.

Hybrid_{Alt}: Intra-parcel voxel-space features of top ranked parcels and inter-parcel features of remaining parcels. Hybrid_{Orig}: Voxel-space features of top ranked parcels and whole-brain parcel-space features (i.e. – the version used in the manuscript). Dots represent decoding accuracy for individual subjects. Dashed lines indicate the trend in performance change across participants. Note, that Hybrid_{Orig} (the approach used in our manuscript) significantly outperforms the Hybrid_{Alt} approach, indicating that the excluded parcel features provide

unique information compared to the spatially overlapping intra-parcel voxel patterns (end of figure legend).



Firstly, there will be a relatively high degree of spatial contiguity among voxels because of the nature of the signal measured, i.e. nearby individual voxels are unlikely to be independent. Secondly, the voxel data gives a somewhat misleading sense of precision; the inversion can be set up to give an estimate for each voxel, but there will not just be dependence among adjacent voxels, but also substantial variation in the sensitivity and confidence with which activity can be projected to different parts of the brain. Midline and deeper structures come to mind, where the inversion will be more problematic than for regions along the dorsal convexity of the brain, and a concern is that in those midline structures, the highest decoding accuracy is seen.

We agree with the Reviewer that some inter-parcel features representing neighboring (or spatially contiguous) voxels are likely to be correlated, an important confound in connectivity analyses (Colclough et al., 2015; Colclough et al., 2016), not performed in our investigation.

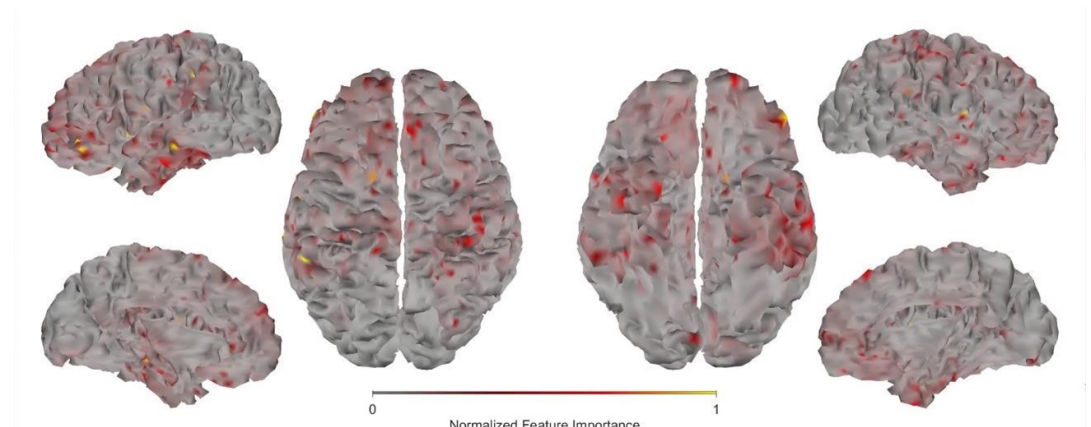
In our study, correlations between adjacent voxels effectively reduce the dimensionality of the input feature space. However, as long as there are multiple groups of correlated voxels within each parcel (i.e. – the rank is greater than 1), the intra-parcel spatial patterns could meaningfully contribute to the decoder performance, as shown by the following results:

First, we obtained higher decoding accuracy with voxel-space features ($74.51\% \pm 7.34\%$ SD) compared to parcel space features ($68.77\% \pm 7.6\%$; Figure 3B), indicating individual voxels carry more information in decoding the keypresses than the averaged voxel-space features or parcel space features. Second, individual voxels within a parcel showed varying feature importance scores in decoding keypresses (Author response image 3). This finding shows that correlated voxels form mini subclusters that are much smaller spatially than the parcel they reside within.

Author response image 3.:

Feature importance score of individual voxels in decoding keypresses: MRMR was used to rank the individual voxel space features in decoding keypresses and the min-max normalized

MRMR score was mapped to a structural brain surface. Note that individual voxels within a parcel showed different contribution to decoding (end of figure legend).



Some of these concerns could be addressed by recording head movement (with enough precision) to regress out these contributions. The authors state that head movement was monitored with 3 fiducials, and their time courses ought to provide a way to deal with this issue. The ICA procedure may not have sufficiently dealt with removing movement-related problems, but one could eg relate individual components that were identified to the keypresses as another means for checking. An alternative could be to focus on frequency ranges above the movement frequencies. The accuracy for those still seems impressive and may provide a slightly more biologically plausible assessment.

We have already addressed the issue of movement related artefacts in the first response above. With respect to a focus on frequency ranges above movement frequencies, the Reviewer states the “accuracy for those still seems impressive and may provide a slightly more biologically plausible assessment”. First, it is important to note that cortical delta-band oscillations measured with local field potentials (LFPs) in macaques is known to contain important information related to end-effector kinematics (Bansal et al., 2011; Mollazadeh et al., 2011) muscle activation patterns (Flint et al., 2012) and temporal sequencing (Churchland et al., 2012) during skilled reaching and grasping actions. Thus, there is a substantial body of evidence that low-frequency neural oscillatory activity in this range contains important information about the skill learning behavior investigated in the present study. Second, our own data shows (which the Reviewer also points out) that significant information related to the skill learning behavior is also present in higher frequency bands (see Figure 2A and Figure 3—figure supplement 1). As we pointed out in our earlier response to questions about the hybrid space decoder architecture (see above), it is likely that different, yet complimentary, information is encoded across different temporal frequencies (just as it is encoded across different spatial frequencies) (Heusser et al., 2016). Again, this interpretation is supported by our data as the highest performing classifiers in all cases (when holding all parameters constant) were always constructed from broadband input MEG data (Figure 2A and Figure 3—figure supplement 1).

One question concerns the interpretation of the results shown in Figure 4. They imply that during the course of learning, entirely different brain networks underpin the behaviour. Not only that, but they also include regions that would seem rather unexpected to be key nodes for learning and expressing relatively simple finger sequences, such as here. What then is the biological plausibility of these results? The authors seem to circumnavigate this issue by moving into a distance metric that captures the (neural network) changes over the course of learning, but the discussion seems

detached from which regions are actually involved; or they offer a rather broad discussion of the anatomical regions identified here, eg in the context of LFOs, where they merely refer to "frontoparietal regions".

The Reviewer notes the shift in brain networks driving keypress decoding performance between trials 1, 11 and 36 as shown in Figure 4A. The Reviewer questions whether these shifts in brain network states underpinning the skill are biologically plausible, as well as the likelihood that bilateral superior and middle frontal and parietal cortex are important nodes within these networks.

First, previous fMRI work in humans assessed changes in functional connectivity patterns while participants performed a similar sequence learning task to our present study (Bassett et al., 2011). Using a dynamic network analysis approach, Bassett et al. showed that flexibility in the composition of individual network modules (i.e. – changes in functional brain region membership of orthogonal brain networks) is up-regulated in novel learning environments and explains differences in learning rates across individuals. Thus, consistent with our findings, it is likely that functional brain networks rapidly reconfigure during early learning of novel sequential motor skills.

Second, frontoparietal network activity is known to support motor memory encoding during early learning (Albouy et al., 2013; Albouy et al., 2012). For example, reactivation events in the posterior parietal (Qin et al., 1997) and medial prefrontal (Euston et al., 2007; Molle & Born, 2009) cortex (MPFC) have been temporally linked to hippocampal replay, and are posited to support memory consolidation across several memory domains (Frankland & Bontempi, 2005), including motor sequence learning (Albouy et al., 2015; Buch et al., 2021; F. Jacobacci et al., 2020). Further, synchronized interactions between MPFC and hippocampus are more prominent during early as opposed to later learning stages (Albouy et al., 2013; Gais et al., 2007; Sterpenich et al., 2009), perhaps reflecting “redistribution of hippocampal memories to MPFC” (Albouy et al., 2013). MPFC contributes to very early memory formation by learning association between contexts, locations, events and adaptive responses during rapid learning (Euston et al., 2012). Consistently, coupling between hippocampus and MPFC has been shown during initial memory encoding and during subsequent rest (van Kesteren et al., 2010; van Kesteren et al., 2012). Importantly, MPFC activity during initial memory encoding predicts subsequent recall (Wagner et al., 1998). Thus, the spatial map required to encode a motor sequence memory may be “built under the supervision of the prefrontal cortex” (Albouy et al., 2012), also engaged in the development of an abstract representation of the sequence (Ashe et al., 2006). In more abstract terms, the prefrontal, premotor and parietal cortices support novice performance “by deploying attentional and control processes” (Doyon et al., 2009; Hikosaka et al., 2002; Penhune & Steele, 2012) required during early learning (Doyon et al., 2009; Hikosaka et al., 2002; Penhune & Steele, 2012). The dorsolateral prefrontal cortex DLPFC specifically is thought to engage in goal selection and sequence monitoring during early skill practice (Schendan et al., 2003), all consistent with the schema model of declarative memory in which prefrontal cortices play an important role in encoding (Morris, 2006; Tse et al., 2007). Thus, several prefrontal and frontoparietal regions contributing to long term learning (Berlot et al., 2020) are also engaged in early stages of encoding. Altogether, there is strong biological support for the involvement of bilateral prefrontal and frontoparietal regions to decoding during early skill learning. We now address this issue in the revised manuscript.

If I understand correctly, the offline neural representation analysis is in essence the comparison of the last keypress vs the first keypress of the next sequence. In that sense, the activity during offline rest periods is actually not considered. This makes the nomenclature somewhat confusing. While it matches the behavioural analysis, having only key presses one can't do it in any other way, but here the authors actually do have recordings of brain activity during offline rest. So at the very least calling it offline neural

representation is misleading to this reviewer because what is compared is activity during the last and during the next keypress, not activity during offline periods. But it also seems a missed opportunity - the authors argue that most of the relevant learning occurs during offline rest periods, yet there is no attempt to actually test whether activity during this period can be useful for the questions at hand here.

We agree with the Reviewer that our previous “offline neural representation” nomenclature could be misinterpreted. In the revised manuscript we refer to this difference as the “offline neural representational change”. Please, note that our previous work did link offline neural activity (i.e. – 16-22 Hz beta power (Bonstrup et al., 2019) and neural replay density (Buch et al., 2021) during inter-practice rest periods) to observed micro-offline gains.

Reviewer #2 (Public review):

Summary

Dash et al. asked whether and how the neural representation of individual finger movements is "contextualized" within a trained sequence during the very early period of sequential skill learning by using decoding of MEG signal. Specifically, they assessed whether/how the same finger presses (pressing index finger) embedded in the different ordinal positions of a practiced sequence (4-1-3-2-4; here, the numbers 1 through 4 correspond to the little through the index fingers of the non-dominant left hand) change their representation (MEG feature). They did this by computing either the decoding accuracy of the index finger at the ordinal positions 1 vs. 5 (index_OP1 vs index_OP5) or pattern distance between index_OP1 vs. index_OP5 at each training trial and found that both the decoding accuracy and the pattern distance progressively increase over the course of learning trials. More interestingly, they also computed the pattern distance for index_OP5 for the last execution of a practice trial vs. index_OP1 for the first execution in the next practice trial (i.e., across the rest period). This "off-line" distance was significantly larger than the "on-line" distance, which was computed within practice trials and predicted micro-offline skill gain. Based on these results, the authors conclude that the differentiation of representation for the identical movement embedded in different positions of a sequential skill ("contextualization") primarily occurs during early skill learning, especially during rest, consistent with the recent theory of the "micro-offline learning" proposed by the authors' group. I think this is an important and timely topic for the field of motor learning and beyond.

Strengths

The specific strengths of the current work are as follows. First, the use of temporally rich neural information (MEG signal) has a large advantage over previous studies testing sequential representations using fMRI. This allowed the authors to examine the earliest period (= the first few minutes of training) of skill learning with finer temporal resolution. Second, through the optimization of MEG feature extraction, the current study achieved extremely high decoding accuracy (approx. 94%) compared to previous works. As claimed by the authors, this is one of the strengths of the paper (but see my comments). Third, although some potential refinement might be needed, comparing "online" and "offline" pattern distance is a neat idea.

Weaknesses

Along with the strengths I raised above, the paper has some weaknesses. First, the pursuit of high decoding accuracy, especially the choice of time points and window length (i.e., 200 msec window starting from 0 msec from key press onset), casts a shadow on the interpretation of the main result. Currently, it is unclear whether the decoding results simply reflect behavioral change or true underlying neural change. As shown in

the behavioral data, the key press speed reached 3~4 presses per second already at around the end of the early learning period (11th trial), which means inter-press intervals become as short as 250-330 msec. Thus, in almost more than 60% of training period data, the time window for MEG feature extraction (200 msec) spans around 60% of the inter-press intervals. Considering that the preparation/cueing of subsequent presses starts ahead of the actual press (e.g., Kornysheva et al., 2019) and/or potential online planning (e.g., Ariani and Diedrichsen, 2019), the decoder likely has captured these future press information as well as the signal related to the current key press, independent of the formation of genuine sequential representation (e.g., "contextualization" of individual press). This may also explain the gradual increase in decoding accuracy or pattern distance between index_OP1 vs. index_OP5 (Figure 4C and 5A), which co-occurred with performance improvement, as shorter inter-press intervals are more favorable for the dissociating the two index finger presses followed by different finger presses. The compromised decoding accuracies for the control sequences can be explained in similar logic. Therefore, more careful consideration and elaborated discussion seem necessary when trying to both achieve high-performance decoding and assess early skill learning, as it can impact all the subsequent analyses.

The Reviewer raises the possibility that (given the windowing parameters used in the present study) an increase in "contextualization" with learning could simply reflect faster typing speeds as opposed to an actual change in the underlying neural representation.

We now include a new control analysis that addresses this issue as well as additional re-examination of previously reported results with respect to this issue – all of which are inconsistent with this alternative explanation that "contextualization" reflects a change in mixing of keypress related MEG features as opposed to a change in the underlying representations themselves. As correct sequences are generated at higher and higher speeds over training, MEG activity patterns related to the planning, execution, evaluation and memory of individual keypresses overlap more in time. Thus, increased overlap between the "4" and "1" keypresses (at the start of the sequence) and "2" and "4" keypresses (at the end of the sequence) could artefactually increase contextualization distances even if the underlying neural representations for the individual keypresses remain unchanged. One must also keep in mind that since participants repeat the sequence multiple times within the same trial, a majority of the index finger keypresses are performed adjacent to one another (i.e. - the "4-4" transition marking the end of one sequence and the beginning of the next). Thus, increased overlap between consecutive index finger keypresses as typing speed increased should increase their similarity and mask contextualization related changes to the underlying neural representations.

We addressed this question by conducting a new multivariate regression analysis to directly assess whether the neural representation distance score could be predicted by the 4-1, 2-4 and 4-4 keypress transition times observed for each complete correct sequence (both predictor and response variables were z-score normalized within-subject). The results of this analysis also affirmed that the possible alternative explanation that contextualization effects are simple reflections of increased mixing is not supported by the data (Adjusted R^2 = 0.00431; F = 5.62). We now include this new negative control analysis in the revised manuscript.

We also re-examined our previously reported classification results with respect to this issue. We reasoned that if mixing effects reflecting the ordinal sequence structure is an important driver of the contextualization finding, these effects should be observable in the distribution of decoder misclassifications. For example, "4" keypresses would be more likely to be misclassified as "1" or "2" keypresses (or vice versa) than as "3" keypresses. The confusion matrices presented in Figures 3C and 4B and Figure 3—figure supplement 3A display a

distribution of misclassifications that is inconsistent with an alternative mixing effect explanation of contextualization.

Based upon the increased overlap between adjacent index finger keypresses (i.e. – “4-4” transition), we also reasoned that the decoder tasked with separating individual index finger keypresses into two distinct classes based upon sequence position, should show decreased performance as typing speed increases. However, Figure 4C in our manuscript shows that this is not the case. The 2-class hybrid classifier actually displays improved classification performance over early practice trials despite greater temporal overlap. Again, this is inconsistent with the idea that the contextualization effect simply reflects increased mixing of individual keypress features.

In summary, both re-examination of previously reported data and new control analyses all converged on the idea that the proximity between keypresses does not explain contextualization.

We do agree with the Reviewer that the naturalistic, generative, self-paced task employed in the present study results in overlapping brain processes related to planning, execution, evaluation and memory of the action sequence. We also agree that there are several tradeoffs to consider in the construction of the classifiers depending on the study aim. Given our aim of optimizing keypress decoder accuracy in the present study, the set of trade-offs resulted in representations reflecting more the latter three processes, and less so the planning component. Whether separate decoders can be constructed to tease apart the representations or networks supporting these overlapping processes is an important future direction of research in this area. For example, work presently underway in our lab constrains the selection of windowing parameters in a manner that allows individual classifiers to be temporally linked to specific planning, execution, evaluation or memory-related processes to discern which brain networks are involved and how they adaptively reorganize with learning. Results from the present study (Figure 4—figure supplement 2) showing hybrid-space decoder prediction accuracies exceeding 74% for temporal windows spanning as little as 25ms and located up to 100ms prior to the *KeyDown* event strongly support the feasibility of such an approach.

Related to the above point, testing only one particular sequence (4-1-3-2-4), aside from the control ones, limits the generalizability of the finding. This also may have contributed to the extremely high decoding accuracy reported in the current study.

The Reviewer raises a question about the generalizability of the decoder accuracy reported in our study. Fortunately, a comparison between decoder performances on Day 1 and Day 2 datasets does provide insight into this issue. As the Reviewer points out, the classifiers in this study were trained and tested on keypresses performed while practicing a specific sequence (4-1-3-2-4). The study was designed this way as to avoid the impact of interference effects on learning dynamics. The cross-validated performance of classifiers on MEG data collected within the same session was 90.47% overall accuracy (4-class; Figure 3C). We then tested classifier performance on data collected during a separate MEG session conducted approximately 24 hours later (Day 2; see Figure 3 — figure supplement 3). We observed a reduction in overall accuracy rate to 87.11% when tested on MEG data recorded while participants performed the same learned sequence, and 79.44% when they performed several previously unpracticed sequences. Both changes in accuracy are important with regards to the generalizability of our findings. First, 87.11% performance accuracy for the trained sequence data on Day 2 (a reduction of only 3.36%) indicates that the hybrid-space decoder performance is robust over multiple MEG sessions, and thus, robust to variations in SNR across the MEG sensor array caused by small differences in head position between scans. This indicates a substantial advantage over sensor-space decoding approaches. Furthermore, when tested on data from unpracticed sequences, overall performance dropped an additional

7.67%. This difference reflects the performance bias of the classifier for the trained sequence, possibly caused by high-order sequence structure being incorporated into the feature weights. In the future, it will be important to understand in more detail how random or repeated keypress sequence training data impacts overall decoder performance and generalization. We strongly agree with the Reviewer that the issue of generalizability is extremely important and have added a new paragraph to the Discussion in the revised manuscript highlighting the strengths and weaknesses of our study with respect to this issue.

In terms of clinical BCI, one of the potential relevance of the study, as claimed by the authors, it is not clear that the specific time window chosen in the current study (up to 200 msec since key press onset) is really useful. In most cases, clinical BCI would target neural signals with no overt movement execution due to patients' inability to move (e.g., Hochberg et al., 2012). Given the time window, the surprisingly high performance of the current decoder may result from sensory feedback and/or planning of subsequent movement, which may not always be available in the clinical BCI context. Of course, the decoding accuracy is still much higher than chance even when using signal before the key press (as shown in Figure 4 Supplement 2), but it is not immediately clear to me that the authors relate their high decoding accuracy based on post-movement signal to clinical BCI settings.

The Reviewer questions the relevance of the specific window parameters used in the present study for clinical BCI applications, particularly for paretic patients who are unable to produce finger movements or for whom afferent sensory feedback is no longer intact. We strongly agree with the Reviewer that any intended clinical application must carefully consider the specific input feature constraints dictated by the clinical cohort, and in turn impose appropriate and complimentary constraints on classifier parameters that may differ from the ones used in the present study. We now highlight this issue in the Discussion of the revised manuscript and relate our present findings to published clinical BCI work within this context.

One of the important and fascinating claims of the current study is that the "contextualization" of individual finger movements in a trained sequence specifically occurs during short rest periods in very early skill learning, echoing the recent theory of micro-offline learning proposed by the authors' group. Here, I think two points need to be clarified. First, the concept of "contextualization" is kept somewhat blurry throughout the text. It is only at the later part of the Discussion (around line #330 on page 13) that some potential mechanism for the "contextualization" is provided as "what-and-where" binding. Still, it is unclear what "contextualization" actually is in the current data, as the MEG signal analyzed is extracted from 0-200 msec after the keypress. If one thinks something is contextualizing an action, that contextualization should come earlier than the action itself.

The Reviewer requests that we: 1) more clearly define our use of the term “contextualization” and 2) provide the rationale for assessing it over a 200ms window aligned to the *KeyDown* event. This choice of window parameters means that the MEG activity used in our analysis was coincident with, rather than preceding, the actual keypresses. We define contextualization as the differentiation of representation for the identical movement embedded in different positions of a sequential skill. That is, representations of individual action elements progressively incorporate information about their relationship to the overall sequence structure as the skill is learned. We agree with the Reviewer that this can be appropriately interpreted as “what-and-where” binding. We now incorporate this definition in the Introduction of the revised manuscript as requested.

The window parameters for optimizing accurate decoding individual finger movements were determined using a grid search of the parameter space (a sliding window of variable width

between 25-350 ms with 25 ms increments variably aligned from 0 to +100ms with 10ms increments relative to the KeyDown event). This approach generated 140 different temporal windows for each keypress for each participant, with the final parameter selection determined through comparison of the resulting performance between each decoder. Importantly, the decision to optimize for decoding accuracy placed an emphasis on keypress representations characterized by the most consistent and robust features shared across subjects, which in turn maximize statistical power in detecting common learning-related changes. In this case, the optimal window encompassed a 200ms epoch aligned to the *KeyDown* event ($t_0 = 0$ ms). We then asked if the representations (i.e. – spatial patterns of combined parcel- and voxel-space activity) of the same digit at two different sequence positions changed with practice within this optimal decoding window. Of course, our findings do not rule out the possibility that contextualization can also be found before or even after this time window, as we did not directly address this issue in the present study. Future work in our lab, as pointed out above, are investigating contextualization within different time windows tailored specifically for assessing sequence skill action planning, execution, evaluation and memory processes.

The second point is that the result provided by the authors is not yet convincing enough to support the claim that "contextualization" occurs during rest. In the original analysis, the authors presented the statistical significance regarding the correlation between the "offline" pattern differentiation and micro-offline skill gain (Figure 5. Supplement 1), as well as the larger "offline" distance than "online" distance (Figure 5B). However, this analysis looks like regressing two variables (monotonically) increasing as a function of the trial. Although some information in this analysis, such as what the independent/dependent variables were or how individual subjects were treated, was missing in the Methods, getting a statistically significant slope seems unsurprising in such a situation. Also, curiously, the same quantitative evidence was not provided for its "online" counterpart, and the authors only briefly mentioned in the text that there was no significant correlation between them. It may be true looking at the data in Figure 5A as the online representation distance looks less monotonically changing, but the classification accuracy presented in Figure 4C, which should reflect similar representational distance, shows a more monotonic increase up to the 11th trial. Further, the ways the "online" and "offline" representation distance was estimated seem to make them not directly comparable. While the "online" distance was computed using all the correct press data within each 10 sec of execution, the "offline" distance is basically computed by only two presses (i.e., the last index_OP5 vs. the first index_OP1 separated by 10 sec of rest). Theoretically, the distance between the neural activity patterns for temporally closer events tends to be closer than that between the patterns for temporally far-apart events. It would be fairer to use the distance between the first index_OP1 vs. the last index_OP5 within an execution period for "online" distance, as well.

The Reviewer suggests that the current data is not enough to show that contextualization occurs during rest and raises two important concerns: 1) the relationship between online contextualization and micro-online gains is not shown, and 2) the online distance was calculated differently from its offline counterpart (i.e. - instead of calculating the distance between last Index_{OP5} and first Index_{OP1} from a single trial, the distance was calculated for each sequence within a trial and then averaged).

We addressed the first concern by performing individual subject correlations between 1) contextualization changes during rest intervals and micro-offline gains; 2) contextualization changes during practice trials and micro-online gains, and 3) contextualization changes during practice trials and micro-offline gains (Figure 5 – figure supplement 4). We then statistically compared the resulting correlation coefficient distributions and found that within-subject correlations for contextualization changes during rest intervals and micro-

offline gains were significantly higher than online contextualization and micro-online gains ($t = 3.2827$, $p = 0.0015$) and online contextualization and micro-offline gains ($t = 3.7021$, $p = 5.3013 \times 10^{-4}$). These results are consistent with our interpretation that micro-offline gains are supported by contextualization changes during the inter-practice rest periods.

With respect to the second concern, we agree with the Reviewer that one limitation of the analysis comparing online versus offline changes in contextualization as presented in the original manuscript, is that it does not eliminate the possibility that any differences could simply be explained by the passage of time (which is smaller for the online analysis compared to the offline analysis). The Reviewer suggests an approach that addresses this issue, which we have now carried out. When quantifying online changes in contextualization from the first Index_{OP1} the last Index_{OP5} keypress in the same trial we observed no learning-related trend (Figure 5 – figure supplement 5, right panel). Importantly, offline distances were significantly larger than online distances regardless of the measurement approach and neither predicted online learning (Figure 5 – figure supplement 6).

A related concern regarding the control analysis, where individual values for max speed and the degree of online contextualization were compared (Figure 5 Supplement 3), is whether the individual difference is meaningful. If I understood correctly, the optimization of the decoding process (temporal window, feature inclusion/reduction, decoder, etc.) was performed for individual participants, and the same feature extraction was also employed for the analysis of representation distance (i.e., contextualization). If this is the case, the distances are individually differently calculated and they may need to be normalized relative to some stable reference (e.g., 1 vs. 4 or average distance within the control sequence presses) before comparison across the individuals.

The Reviewer makes a good point here. We have now implemented the suggested normalization procedure in the analysis provided in the revised manuscript.

Reviewer #3 (Public review):

Summary:

One goal of this paper is to introduce a new approach for highly accurate decoding of finger movements from human magnetoencephalography data via dimension reduction of a "multiscale, hybrid" feature space. Following this decoding approach, the authors aim to show that early skill learning involves "contextualization" of the neural coding of individual movements, relative to their position in a sequence of consecutive movements. Furthermore, they aim to show that this "contextualization" develops primarily during short rest periods interspersed with skill training and correlates with a performance metric which the authors interpret as an indicator of offline learning.

Strengths:

A clear strength of the paper is the innovative decoding approach, which achieves impressive decoding accuracies via dimension reduction of a "multi-scale, hybrid space". This hybrid-space approach follows the neurobiologically plausible idea of the concurrent distribution of neural coding across local circuits as well as large-scale networks. A further strength of the study is the large number of tested dimension reduction techniques and classifiers (though the manuscript reveals little about the comparison of the latter).

We appreciate the Reviewer's comments regarding the paper's strengths.

A simple control analysis based on shuffled class labels could lend further support to this complex decoding approach. As a control analysis that completely rules out any source

of overfitting, the authors could test the decoder after shuffling class labels. Following such shuffling, decoding accuracies should drop to chance level for all decoding approaches, including the optimized decoder. This would also provide an estimate of actual chance-level performance (which is informative over and beyond the theoretical chance level). Furthermore, currently, the manuscript does not explain the huge drop in decoding accuracies for the voxel-space decoding (Figure 3B). Finally, the authors' approach to cortical parcellation raises questions regarding the information carried by varying dipole orientations within a parcel (which currently seems to be ignored?) and the implementation of the mean-flipping method (given that there are two dimensions - space and time - what do the authors refer to when they talk about the sign of the "average source", line 477?).

The Reviewer recommends that we: 1) conduct an additional control analysis on classifier performance using shuffled class labels, 2) provide a more detailed explanation regarding the drop in decoding accuracies for the voxel-space decoding following LDA dimensionality reduction (see Fig 3B), and 3) provide additional details on how problems related to dipole solution orientations were addressed in the present study.

In relation to the first point, we have now implemented a random shuffling approach as a control for the classification analyses. The results of this analysis indicated that the chance level accuracy was 22.12% ($\pm$ SD 9.1%) for individual keypress decoding (4-class classification), and 18.41% ($\pm$ SD 7.4%) for individual sequence item decoding (5-class classification), irrespective of the input feature set or the type of decoder used. Thus, the decoding accuracy observed with the final model was substantially higher than these chance levels.

Second, please note that the dimensionality of the voxel-space feature set is very high (i.e. – 15684). LDA attempts to map the input features onto a much smaller dimensional space (number of classes – 1; e.g. – 3 dimensions, for 4-class keypress decoding). Given the very high dimension of the voxel-space input features in this case, the resulting mapping exhibits reduced accuracy. Despite this general consideration, please refer to Figure 3—figure supplement 3, where we observe improvement in voxel-space decoder performance when utilizing alternative dimensionality reduction techniques.

The decoders constructed in the present study assess the average spatial patterns across time (as defined by the windowing procedure) in the input feature space. We now provide additional details in the Methods of the revised manuscript pertaining to the parcellation procedure and how the sign ambiguity problem was addressed in our analysis.

Weaknesses:

A clear weakness of the paper lies in the authors' conclusions regarding "contextualization". Several potential confounds, described below, question the neurobiological implications proposed by the authors and provide a simpler explanation of the results. Furthermore, the paper follows the assumption that short breaks result in offline skill learning, while recent evidence, described below, casts doubt on this assumption.

We thank the Reviewer for giving us the opportunity to address these issues in detail (see below).

The authors interpret the ordinal position information captured by their decoding approach as a reflection of neural coding dedicated to the local context of a movement (Figure 4). One way to dissociate ordinal position information from information about the moving effectors is to train a classifier on one sequence and test the classifier on

other sequences that require the same movements, but in different positions (Kornysheva et al., 2019). In the present study, however, participants trained to repeat a single sequence (4-1-3-2-4). As a result, ordinal position information is potentially confounded by the fixed finger transitions around each of the two critical positions (first and fifth press). Across consecutive correct sequences, the first keypress in a given sequence was always preceded by a movement of the index finger (=last movement of the preceding sequence), and followed by a little finger movement. The last keypress, on the other hand, was always preceded by a ring finger movement, and followed by an index finger movement (=first movement of the next sequence). Figure 4 - Supplement 2 shows that finger identity can be decoded with high accuracy (>70%) across a large time window around the time of the key press, up to at least ± 100 ms (and likely beyond, given that decoding accuracy is still high at the boundaries of the window depicted in that figure). This time window approaches the keypress transition times in this study. Given that distinct finger transitions characterized the first and fifth keypress, the classifier could thus rely on persistent (or "lingering") information from the preceding finger movement, and/or "preparatory" information about the subsequent finger movement, in order to dissociate the first and fifth keypress. Currently, the manuscript provides no evidence that the context information captured by the decoding approach is more than a by-product of temporally extended, and therefore overlapping, but independent neural representations of consecutive keypresses that are executed in close temporal proximity - rather than a neural representation dedicated to context.

Such temporal overlap of consecutive, independent finger representations may also account for the dynamics of "ordinal coding"/"contextualization", i.e., the increase in 2-class decoding accuracy, across Day 1 (Figure 4C). As learning progresses, both tapping speed and the consistency of keypress transition times increase (Figure 1), i.e., consecutive keypresses are closer in time, and more consistently so. As a result, information related to a given keypress is increasingly overlapping in time with information related to the preceding and subsequent keypresses. The authors seem to argue that their regression analysis in Figure 5 - Figure Supplement 3 speaks against any influence of tapping speed on "ordinal coding" (even though that argument is not made explicitly in the manuscript). However, Figure 5 - Figure Supplement 3 shows inter-individual differences in a between-subject analysis (across trials, as in panel A, or separately for each trial, as in panel B), and, therefore, says little about the within-subject dynamics of "ordinal coding" across the experiment. A regression of trial-by-trial "ordinal coding" on trial-by-trial tapping speed (either within-subject or at a group-level, after averaging across subjects) could address this issue. Given the highly similar dynamics of "ordinal coding" on the one hand (Figure 4C), and tapping speed on the other hand (Figure 1B), I would expect a strong relationship between the two in the suggested within-subject (or group-level) regression. Furthermore, learning should increase the number of (consecutively) correct sequences, and, thus, the consistency of finger transitions. Therefore, the increase in 2-class decoding accuracy may simply reflect an increasing overlap in time of increasingly consistent information from consecutive keypresses, which allows the classifier to dissociate the first and fifth keypress more reliably as learning progresses, simply based on the characteristic finger transitions associated with each. In other words, given that the physical context of a given keypress changes as learning progresses - keypresses move closer together in time and are more consistently correct - it seems problematic to conclude that the mental representation of that context changes. To draw that conclusion, the physical context should remain stable (or any changes to the physical context should be controlled for).

The issues raised by Reviewer #3 here are similar to two issues raised by Reviewer #2 above. We agree they must both be carefully considered in any evaluation of our findings.

As both Reviewers pointed out, the classifiers in this study were trained and tested on keypresses performed while practicing a specific sequence (4-1-3-2-4). The study was designed this way as to avoid the impact of interference effects on learning dynamics. The cross-validated performance of classifiers on MEG data collected within the same session was 90.47% overall accuracy (4class; Figure 3C). We then tested classifier performance on data collected during a separate MEG session conducted approximately 24 hours later (Day 2; see Figure 3—supplement 3). We observed a reduction in overall accuracy rate to 87.11% when tested on MEG data recorded while participants performed the same learned sequence, and 79.44% when they performed several previously unpracticed sequences. This classification performance difference of 7.67% when tested on the Day 2 data could reflect the performance bias of the classifier for the trained sequence, possibly caused by mixed information from temporally close keypresses being incorporated into the feature weights.

Along these same lines, both Reviewers also raise the possibility that an increase in “ordinal coding/contextualization” with learning could simply reflect an increase in this mixing effect caused by faster typing speeds as opposed to an actual change in the underlying neural representation. The basic idea is that as correct sequences are generated at higher and higher speeds over training, MEG activity patterns related to the planning, execution, evaluation and memory of individual keypresses overlap more in time. Thus, increased overlap between the “4” and “1” keypresses (at the start of the sequence) and “2” and “4” keypresses (at the end of the sequence) could artefactually increase contextualization distances even if the underlying neural representations for the individual keypresses remain unchanged (assuming this mixing of representations is used by the classifier to differentially tag each index finger press). If this were the case, it follows that such mixing effects reflecting the ordinal sequence structure would also be observable in the distribution of decoder misclassifications. For example, “4” keypresses would be more likely to be misclassified as “1” or “2” keypresses (or vice versa) than as “3” keypresses. The confusion matrices presented in Figures 3C and 4B and Figure 3—figure supplement 3A in the previously submitted manuscript do not show this trend in the distribution of misclassifications across the four fingers.

Following this logic, it’s also possible that if the ordinal coding is largely driven by this mixing effect, the increased overlap between consecutive index finger keypresses during the 4-4 transition marking the end of one sequence and the beginning of the next one could actually mask contextualization-related changes to the underlying neural representations and make them harder to detect. In this case, a decoder tasked with separating individual index finger keypresses into two distinct classes based upon sequence position might show decreased performance with learning as adjacent keypresses overlapped in time with each other to an increasing extent. However, Figure 4C in our previously submitted manuscript does not support this possibility, as the 2-class hybrid classifier displays improved classification performance over early practice trials despite greater temporal overlap.

As noted in the above reply to Reviewer #2, we also conducted a new multivariate regression analysis to directly assess whether the neural representation distance score could be predicted by the 4-1, 2-4 and 4-4 keypress transition times observed for each complete correct sequence (both predictor and response variables were z-score normalized within-subject). The results of this analysis affirmed that the possible alternative explanation put forward by the Reviewer is not supported by our data (Adjusted $R^2 = 0.00431$; $F = 5.62$). We now include this new negative control analysis result in the revised manuscript.

Finally, the Reviewer hints that one way to address this issue would be to compare MEG responses before and after learning for sequences typed at a fixed speed. However, given that the speed-accuracy trade-off should improve with learning, a comparison between unlearned and learned skill states would dictate that the skill be evaluated at a very low fixed speed. Essentially, such a design presents the problem that the post-training test is evaluating the representation in the unlearned behavioral state that is not representative of the acquired

skill. Thus, this approach would miss most learning effects on a task in which speed is the main learning metrics.

A similar difference in physical context may explain why neural representation distances ("differentiation") differ between rest and practice (Figure 5). The authors define "offline differentiation" by comparing the hybrid space features of the last index finger movement of a trial (ordinal position 5) and the first index finger movement of the next trial (ordinal position 1). However, the latter is not only the first movement in the sequence but also the very first movement in that trial (at least in trials that started with a correct sequence), i.e., not preceded by any recent movement. In contrast, the last index finger of the last correct sequence in the preceding trial includes the characteristic finger transition from the fourth to the fifth movement. Thus, there is more overlapping information arising from the consistent, neighbouring keypresses for the last index finger movement, compared to the first index finger movement of the next trial. A strong difference (larger neural representation distance) between these two movements is, therefore, not surprising, given the task design, and this difference is also expected to increase with learning, given the increase in tapping speed, and the consequent stronger overlap in representations for consecutive keypresses. Furthermore, initiating a new sequence involves pre-planning, while ongoing practice relies on online planning (Ariani et al., eNeuro 2021), i.e., two mental operations that are dissociable at the level of neural representation (Ariani et al., bioRxiv 2023).

The Reviewer argues that the comparison of last finger movement of a trial and the first in the next trial are performed in different circumstances and contexts. This is an important point and one we tend to agree with. For this task, the first sequence in a practice trial is pre-planned before the first keypress is performed. This occurs in a somewhat different context from the sequence iterations that follow, which involve temporally overlapping planning, execution and evaluation processes. The Reviewer is concerned about a difference in the temporal mixing effect issue raised above between the first and last keypresses performed in a trial. Please, note that since neural representations of individual actions are competitively queued during the pre-planning period in a manner that reflects the ordinal structure of the learned sequence (Kornysheva et al., 2019), mixing effects are most likely present also for the first keypress in a trial.

Separately, the Reviewer suggests that contextualization during early learning may reflect preplanning or online planning. This is an interesting proposal. Given the decoding time-window used in this investigation, we cannot dissect separate contributions of planning, memory and sensory feedback to contextualization. Taking advantage of the superior temporal resolution of MEG relative to fMRI tools, work under way in our lab is investigating decoding time-windows more appropriate to address each of these questions.

Given these differences in the physical context and associated mental processes, it is not surprising that "offline differentiation", as defined here, is more pronounced than "online differentiation". For the latter, the authors compared movements that were better matched regarding the presence of consistent preceding and subsequent keypresses (online differentiation was defined as the mean difference between all first vs. last index finger movements during practice). It is unclear why the authors did not follow a similar definition for "online differentiation" as for "micro-online gains" (and, indeed, a definition that is more consistent with their definition of "offline differentiation"), i.e., the difference between the first index finger movement of the first correct sequence during practice, and the last index finger of the last correct sequence. While these two movements are, again, not matched for the presence of neighbouring keypresses (see the argument above), this mismatch would at least be the same across "offline differentiation" and "online differentiation", so they would be more comparable.

This is the same point made earlier by Reviewer #2, and we agree with this assessment. As stated in the response to Reviewer #2 above, we have now carried out quantification of online contextualization using this approach and included it in the revised manuscript. We thank the Reviewer for this suggestion.

A further complication in interpreting the results regarding "contextualization" stems from the visual feedback that participants received during the task. Each keypress generated an asterisk shown above the string on the screen, irrespective of whether the keypress was correct or incorrect. As a result, incorrect (e.g., additional, or missing) keypresses could shift the phase of the visual feedback string (of asterisks) relative to the ordinal position of the current movement in the sequence (e.g., the fifth movement in the sequence could coincide with the presentation of any asterisk in the string, from the first to the fifth). Given that more incorrect keypresses are expected at the start of the experiment, compared to later stages, the consistency in visual feedback position, relative to the ordinal position of the movement in the sequence, increased across the experiment. A better differentiation between the first and the fifth movement with learning could, therefore, simply reflect better decoding of the more consistent visual feedback, based either on the feedback-induced brain response, or feedback-induced eye movements (the study did not include eye tracking). It is not clear why the authors introduced this complicated visual feedback in their task, besides consistency with their previous studies.

We strongly agree with the Reviewer that eye movements related to task engagement are important to rule out as a potential driver of the decoding accuracy or contextualization effect. We address this issue above in response to a question raised by Reviewer #1 about the impact of movement related artefacts on our findings.

First, the assumption the Reviewer makes here about the distribution of errors in this task is incorrect. On average across subjects, $2.32\% \pm 1.48\%$ (mean $\pm$ SD) of all keypresses performed were errors, which were evenly distributed across the four possible keypress responses. While errors increased progressively over practice trials, they did so in proportion to the increase in correct keypresses, so that the overall ratio of correct-to-incorrect keypresses remained stable over the training session. Thus, the Reviewer's assumptions that there is a higher relative frequency of errors in early trials, and a resulting systematic trend phase shift differences between the visual display updates (i.e. – a change in asterisk position above the displayed sequence) and the keypress performed is not substantiated by the data. To the contrary, the asterisk position on the display and the keypress being executed remained highly correlated over the entire training session. We now include a statement about the frequency and distribution of errors in the revised manuscript.

Given this high correlation, we firmly agree with the Reviewer that the issue of eye movement related artefacts is still an important one to address. Fortunately, we did collect eye movement data during the MEG recordings so were able to investigate this. As detailed in the response to Reviewer #1 above, we found that gaze positions and eye-movement velocity time-locked to visual display updates (i.e. – a change in asterisk position above the displayed sequence) did not reflect the asterisk location above chance levels (Overall cross-validated accuracy = 0.21817; see Author response image 1). Furthermore, an inspection of the eye position data revealed that most participants on most trials displayed random walk gaze patterns around a center fixation point, indicating that participants did not attend to the asterisk position on the display. This is consistent with intrinsic generation of the action sequence, and congruent with the fact that the display does not provide explicit feedback related to performance. As pointed out above, a similar real-world example would be manually inputting a long password into a secure online application. In this case, one intrinsically generates the sequence from memory and receives similar feedback about the

password sequence position (also provided as asterisks), which is typically ignored by the user.

The minimal participant engagement with the visual display in this explicit sequence learning motor task (which is highly generative in nature) contrasts markedly with behavior observed when reactive responses to stimulus cues are needed in the serial reaction time task (SRTT). This is a crucial difference that must be carefully considered when comparing findings across studies using the two sequence learning tasks.

The authors report a significant correlation between "offline differentiation" and cumulative microoffline gains. However, it would be more informative to correlate trial-by-trial changes in each of the two variables. This would address the question of whether there is a trial-by-trial relation between the degree of "contextualization" and the amount of micro-offline gains - are performance changes (micro-offline gains) less pronounced across rest periods for which the change in "contextualization" is relatively low? Furthermore, is the relationship between micro-offline gains and "offline differentiation" significantly stronger than the relationship between micro-offline gains and "online differentiation"?

In response to a similar issue raised above by Reviewer #2, we now include new analyses comparing correlation magnitudes between (1) "online differentiation" vs micro-online gains, (2) "online differentiation" vs micro-offline gains and (3) "offline differentiation" and micro-offline gains (see Figure 5 – figure supplement 4, 5 and 6). These new analyses and results have been added to the revised manuscript. Once again, we thank both Reviewers for this suggestion.

The authors follow the assumption that micro-offline gains reflect offline learning.

We disagree with this statement. The original (Bonstrup et al., 2019) paper clearly states that micro-offline gains do not necessarily reflect offline learning in some cases and must be carefully interpreted based upon the behavioral context within which they are observed. Further, the paper lays out the conditions under which one can have confidence that micro-offline gains reflect offline learning. In fact, the excellent meta-analysis of (Pan & Rickard, 2015), which re-interprets the benefits of sleep in overnight skill consolidation from a "reactive inhibition" perspective, was a crucial resource in the experimental design of our initial study (Bonstrup et al., 2019), as well as in all our subsequent work. Pan & Rickard state:

"Empirically, reactive inhibition refers to performance worsening that can accumulate during a period of continuous training (Hull, 1943). It tends to dissipate, at least in part, when brief breaks are inserted between blocks of training. If there are multiple performance-break cycles over a training session, as in the motor sequence literature, performance can exhibit a scalloped effect, worsening during each uninterrupted performance block but improving across blocks (Brawn et al., 2010; Rickard et al., 2008). Rickard, Cai, Rieth, Jones, and Ard (2008) and Brawn, Fenn, Nusbaum, and Margoliash (2010) (Brawn et al., 2010; Rickard et al., 2008) demonstrated highly robust scalloped reactive inhibition effects using the commonly employed 30 s–30 s performance break cycle, as shown for Rickard et al.'s (2008) massed practice sleep group in Figure 2. The scalloped effect is evident for that group after the first few 30 s blocks of each session. The absence of the scalloped effect during the first few blocks of training in the massed group suggests that rapid learning during that period masks any reactive inhibition effect."

Crucially, Pan & Rickard make several concrete recommendations for reducing the impact of the reactive inhibition confound on offline learning studies. One of these recommendations was to reduce practice times to 10s (most prior sequence learning studies up until that point had employed 30s long practice trials). They state:

“The traditional design involving 30 s-30 s performance break cycles should be abandoned given the evidence that it results in a reactive inhibition confound, and alternative designs with reduced performance duration per block used instead (Pan & Rickard, 2015 . One promising possibility is to switch to 10 s performance durations for each performance-break cycle Instead (Pan & Rickard, 2015 . That design appears sufficient to eliminate at least the majority of the reactive inhibition effect (Brawn et al., 2010; Rickard et al., 2008 .”

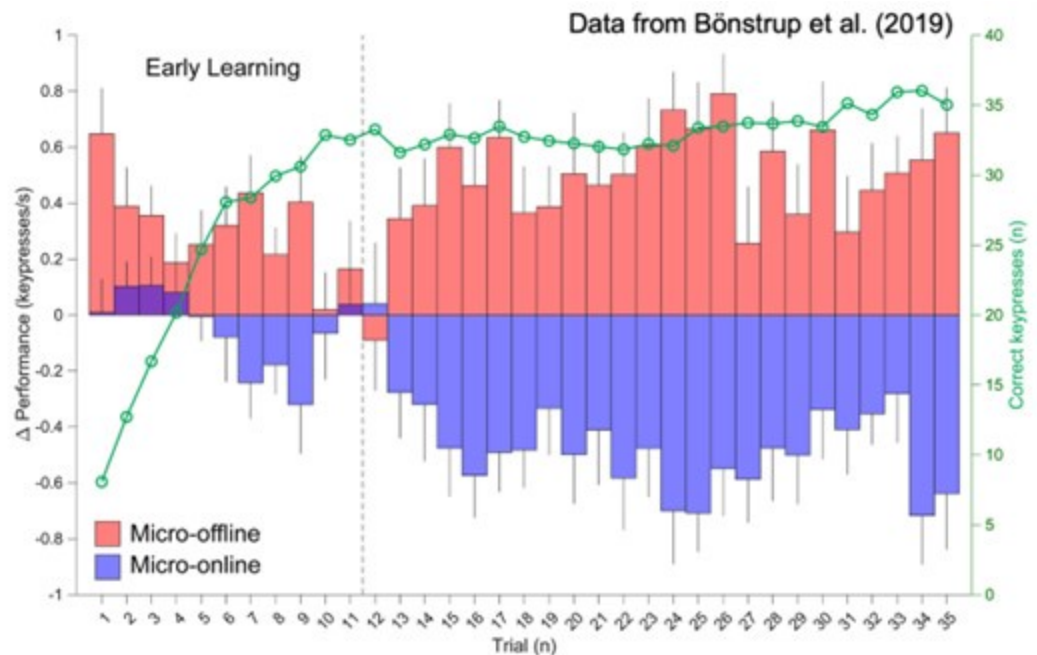
We mindfully incorporated recommendations from (Pan & Rickard, 2015) into our own study designs including 1) utilizing 10s practice trials and 2) constraining our analysis of micro-offline gains to early learning trials (where performance monotonically increases and 95% of overall performance gains occur), which are prior to the emergence of the “scalloped” performance dynamics that are strongly linked to reactive inhibition effects.

However, there is no direct evidence in the literature that micro-offline gains really result from offline learning, i.e., an improvement in skill level.

We strongly disagree with the Reviewer’s assertion that “there is no direct evidence in the literature that micro-offline gains really result from offline learning, i.e., an improvement in skill level.” The initial (Bonstrup et al., 2019) report was followed up by a large online crowd-sourcing study (Bonstrup et al., 2020). This second (and much larger) study provided several additional important findings supporting our interpretation of micro-offline gains in cases where the important behavioral conditions clarified above were met (see Author response image 4 below for further details on these conditions).

Author response image 4.

This Figure shows that micro-offline gains observed in learning and nonlearning contexts are attributed to different underlying causes. Micro-offline and online changes relative to overall trial-by-trial learning. This figure is based on data from (Bonstrup et al., 2019). During early learning, micro-offline gains (red bars) closely track trial-by-trial performance gains (green line with open circle markers), with minimal contribution from micro-online gains (blue bars). The stated conclusion in Bonstrup et al. (2019) is that micro-offline gains only during this Early Learning stage reflect rapid memory consolidation (see also (Bonstrup et al., 2020)). After early learning, about practice trial 11, skill plateaus. This plateau skill period is characterized by a striking emergence of coupled (and relatively stable) micro-online drops and micro-offline increases. Bonstrup et al. (2019) as well as others in the literature (Brooks et al., 2024; Gupta & Rickard, 2022; Florencia Jacobacci et al., 2020), argue that micro-offline gains during the plateau period likely reflect recovery from inhibitory performance factors such as reactive inhibition or fatigue, and thus must be excluded from analyses relating micro-offline gains to skill learning. The *Non-repeating* groups in Experiments 3 and 4 from Das et al. (2024) suffer from a lack of consideration of these known confounds (end of Fig legend).



Evidence documented in that paper (Bonstrup et al., 2020) showed that micro-offline gains during *early* skill learning were: 1) replicable and generalized to subjects learning the task in their daily living environment ($n=389$); 2) equivalent when significantly shortening practice period duration, thus confirming that they are not a result of recovery from performance fatigue ($n=118$); 3) reduced (along with learning rates) by retroactive interference applied immediately after each practice period relative to interference applied after passage of time ($n=373$), indicating stabilization of the motor memory at a microscale of several seconds consistent with rapid consolidation; and 4) not modified by random termination of the practice periods, ruling out a contribution of predictive motor slowing ($N = 71$) (Bonstrup et al., 2020). Altogether, our findings were strongly consistent with the interpretation that micro-offline gains reflect memory consolidation supporting early skill learning. This is precisely the portion of the learning curve (Pan & Rickard, 2015) refer to when they state “... rapid learning during that period masks any reactive inhibition effect”.

This interpretation is further supported by brain imaging evidence linking known memory-related networks and consolidation mechanisms to micro-offline gains. First, we reported that the density of fast hippocampo-neocortical skill memory replay events increases approximately three-fold during early learning inter-practice rest periods with the density explaining differences in the magnitude of micro-offline gains across subjects (Buch et al., 2021). Second, Jacobacci et al. (2020) independently reproduced our original behavioral findings and reported BOLD fMRI changes in the hippocampus and precuneus (regions also identified in our MEG study (Buch et al., 2021)) linked to micro-offline gains during early skill learning. These functional changes were coupled with rapid alterations in brain microstructure in the order of minutes, suggesting that the same network that operates during rest periods of early learning undergoes structural plasticity over several minutes following practice (Deleglise et al., 2023). Crucial to this point, Chen et al. (2024) and Sjøgård et al (2024) provided direct evidence from intracranial EEG in humans linking sharp-wave ripple density during rest periods (which are known markers for neural replay (Buzsaki, 2015)) in the human hippocampus (80-120 Hz) to micro-offline gains during early skill learning.

Thus, there is now substantial converging evidence in humans across different indirect noninvasive and direct invasive recording techniques linking hippocampal activity, neural

replay dynamics and offline performance gains in skill learning.

On the contrary, recent evidence questions this interpretation (Gupta & Rickard, npj Sci Learn 2022; Gupta & Rickard, Sci Rep 2024; Das et al., bioRxiv 2024). Instead, there is evidence that micro-offline gains are transient performance benefits that emerge when participants train with breaks, compared to participants who train without breaks, however, these benefits vanish within seconds after training if both groups of participants perform under comparable conditions (Das et al., bioRxiv 2024).

The recent work of (Gupta & Rickard, 2022, 2024) does not present any data that directly opposes our finding that early skill learning (Bonstrup et al., 2019) is expressed as micro-offline gains during rest breaks. These studies are an extension of the Rickard et al (2008) paper that employed a massed (30s practice followed by 30s breaks) vs spaced (10s practice followed by 10s breaks) experimental design to assess if recovery from reactive inhibition effects could account for performance gains measured after several minutes or hours. Gupta & Rickard (2022) added two additional groups (30s practice/10s break and 10s practice/10s break as used in the work from our group). The primary aim of the study was to assess whether it was more likely that changes in performance when retested 5 minutes after skill training (consisting of 12 practice trials for the massed groups and 36 practice trials for the spaced groups) had ended reflected memory consolidation effects or recovery from reactive inhibition effects. The Gupta & Rickard (2024) follow-up paper employed a similar design with the primary difference being that participants performed a fixed number of sequences on each trial as opposed to trials lasting a fixed duration. This was done to facilitate the fitting of a quantitative statistical model to the data.

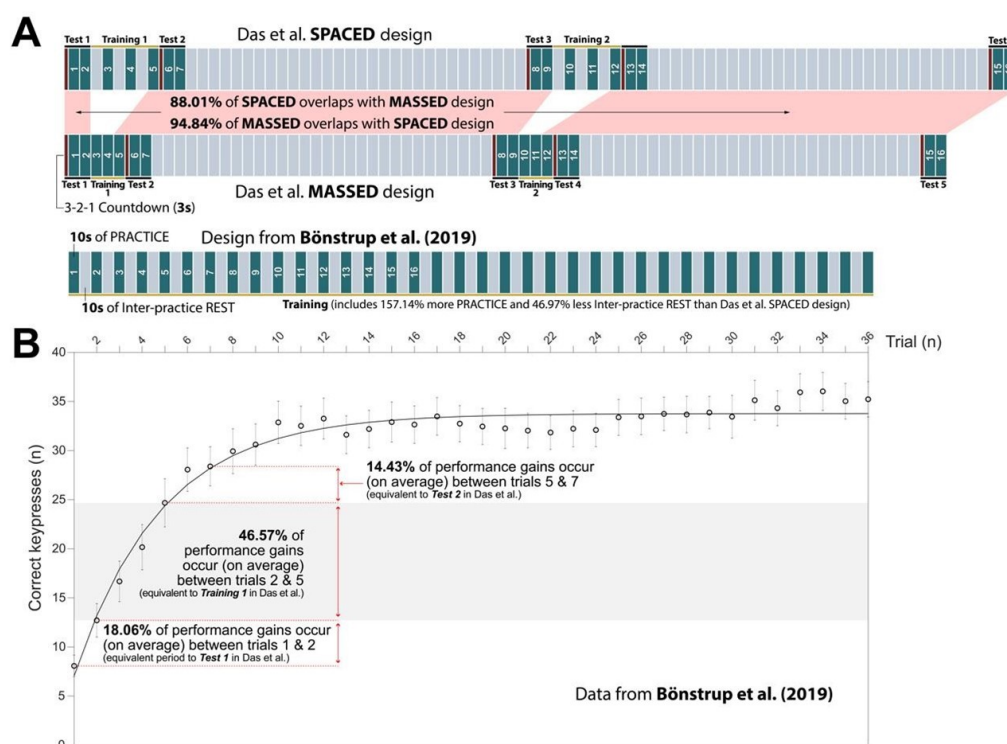
To reiterate, neither study included any analysis of micro-online or micro-offline gains and did not include any comparison focused on skill gains during early learning trials (only at retest 5 min later). Instead, Gupta & Rickard (2022), reported evidence for reactive inhibition effects for all groups over much longer training periods than early learning. In fact, we reported the same findings for trials following the early learning period in our original 2019 paper (Bonstrup et al., 2019) (Author response image 4). Please, note that we also reported that cumulative microoffline gains over early learning did not correlate with overnight offline consolidation measured 24 hours later (Bonstrup et al., 2019) (see the Results section and further elaboration in the Discussion). We interpreted these findings as indicative that the mechanisms underlying offline gains over the micro-scale of seconds during early skill learning versus over minutes or hours very likely differ.

In the recent preprint from (Das et al., 2024), the authors make the strong claim that “micro-offline gains during early learning do not reflect offline learning” which is not supported by their own data. The authors hypothesize that if “micro-offline gains represent offline learning, participants should reach higher skill levels when training with breaks, compared to training without breaks”. The study utilizes a spaced vs. massed practice groups between-subjects design inspired by the reactive inhibition work from Rickard and others to test this hypothesis.

Crucially, their design incorporates only a small fraction of the training used in other investigations to evaluate early skill learning (Bonstrup et al., 2020; Bonstrup et al., 2019; Brooks et al., 2024; Buch et al., 2021; Deleglise et al., 2023; F. Jacobacci et al., 2020; Mylonas et al., 2024). A direct comparison between the practice schedule designs for the spaced and massed groups in Das et al., and the training schedule all participants experienced in the original Bonstrup et al. (2019) paper highlights this issue as well as several others (Author response image 5):

Author response image 5.

This figure shows (A) Comparison of Das et al. Spaced & Massed group training session designs, and the training session design from the original (Bonstrup et al., 2019) paper. Similar to the approach taken by Das et al., all practice is visualized as 10-second practice trials with a variable number (either 0, 1 or 30) of 10-second-long inter-practice rest intervals to allow for direct comparisons between designs. The two key takeaways from this comparison are that (1) the intervention differences (i.e. – practice schedules) between the Massed and Spaced groups from the Das et al. report are extremely small (less than 12% of the overall session schedule) (gaps in the red shaded area) and (2) the overall amount of practice is much less than compared to the design from the original Bönstrup report (Bonstrup et al., 2019) (which has been utilized in several subsequent studies). (B) Group-level learning curve data from Bönstrup et al. (2019) (Bonstrup et al., 2019) is used to estimate the performance range accounted for by the equivalent periods covering *Test 1*, *Training 1* and *Test 2* from Das et al (2024). Note that the intervention in the Das et al. study is limited to a period covering less than 50% of the overall learning range (end of figure legend).



Participants in the original (Bonstrup et al., 2019) experienced 157.14% more practice time and 46.97% less inter-practice rest time than the Spaced group in the Das et al. study (Author response image 5). Thus, the overall amount of practice and rest differ substantially between studies, with much more limited training occurring for participants in Das et al.

In addition, the training interventions (i.e. – the practice schedule differences between the Spaced and Massed groups) were designed in a manner that minimized any chance of effectively testing their hypothesis. First, the interventions were applied over an extremely short period relative to the length of the total training session (5% and 12% of the total training session for Massed and Spaced groups, respectively; see gaps in the red shaded area in Author response image 5). Second, the intervention was applied during a period in which only half of the known total learning occurs. Specifically, we know from Bönstrup et al. (2019)

that only 46.57% of the total performance gains occur in the practice interval covered by Das et al *Training 1* intervention. Thus, early skill learning as evaluated by multiple groups (Bonstrup et al., 2020; Bonstrup et al., 2019; Brooks et al., 2024; Buch et al., 2021; Deleglise et al., 2023; F. Jacobacci et al., 2020; Mylonas et al., 2024), is in the Das et al experiment amputated to about half.

Furthermore, a substantial amount of learning takes place during Das et al's *Test 1* and *Test 2* periods (32.49% of total gains combined). The fact that substantial learning is known to occur over both the *Test 1* (18.06%) and *Test 2* (14.43%) intervals presents a fundamental problem described by Pan and Rickard (Pan & Rickard, 2015). They reported that averaging over intervals where substantial performance gains occur (i.e. – performance is not stable) inject crucial artefacts into analyses of skill learning:

“A large amount of averaging has the advantage of yielding more precise estimates of each subject's pretest and posttest scores and hence more statistical power to detect a performance gain. However, calculation of gain scores using that strategy runs the risk that learning that occurs during the pretest and (or) posttest periods (i.e., online learning is incorporated into the gain score (Rickard et al., 2008; Robertson et al., 2004).”

The above statement indicates that the *Test 1* and *Test 2* performance scores from Das et al. (2024) are substantially contaminated by the learning rate within these intervals. This is particularly problematic if the intervention design results in different *Test 2* learning rates between the two groups. This in fact, is apparent in their data (Figure 1C,E of the Das et al., 2024 preprint) as the *Test 2* learning rate for the Spaced group is negative (indicating a unique interference effect observable only for this group). Specifically, the Massed group continues to show an increase in performance during *Test 2* and 4 relative to the last 10 seconds of practice during *Training 1* and 2, respectively, while the Spaced group displays a marked decrease. This post-training performance decrease for the Spaced group is in stark contrast to the monotonic performance increases observed for both groups at all other time-points. One possible cause could be related to the structure of the *Test* intervals, which include 20 seconds of uninterrupted practice. For the Spaced group, this effectively is a switch to a Massed practice environment (i.e., two 10-secondlong practice trials merged into one long trial), which interferes with greater *Training 1* interval gains observed for the Space group. Interestingly, when statistical comparisons between the groups are made at the time-points when the intervention is present (Figure 1E) then the stated hypothesis, “If micro-offline gains represent offline learning, participants should reach higher skill levels when training with breaks, compared to training without breaks”, is confirmed.

In summary, the experimental design and analyses used by Das et al does not contradict the view that early skill learning is expressed as micro-offline gains during rest breaks. The data presented by Gupta and Rickard (2022, 2024) and Das et al. (2024) is in many ways more confirmatory of the constraints employed by our group and others with respect to experimental design, analysis and interpretation of study findings, rather than contradictory. Still, it does highlight a limitation of the current micro-online/offline framework, which was originally only intended to be applied to early skill learning over spaced practice schedules when reactive inhibition effects are minimized (Bonstrup et al., 2019; Pan & Rickard, 2015). Extrapolation of this current framework to postplateau performance periods, longer timespans, or non-learning situations (e.g. – the Nonrepeating groups from Das et al. (2024)), when reactive inhibition plays a more substantive role, is not warranted. Ultimately, it will be important to develop new paradigms allowing one to independently estimate the different coincident or antagonistic features (e.g. - memory consolidation, planning, working memory and reactive inhibition) contributing to micro-online and micro-offline gains during and after early skill learning within a unifying framework.

Recommendations for the authors:

Reviewer #1 (Recommendations for the authors):

(1) I found Figure 2B too small to be useful, as the actual elements of the cells are very hard to read.

We have removed the grid colormap panel (top-right) from Figure 2B. All of this colormap data is actually a subset of data presented in Figure 2 – figure supplement 1, so can still be found there.

Reviewer #2 (Recommendations for the authors):

(1) Related to the first point in my concerns, I would suggest the authors compare decoding accuracy between correct presses followed by correct vs. incorrect presses. This would clarify if the decoder is actually taking the MEG signal for subsequent press into account. I would also suggest the authors use pre-movement MEG features and post-movement features with shorter windows and compare each result with the results for the original post-movement MEG feature with a longer window.

The present study does not contain enough errors to perform the analysis proposed by the Reviewer. As noted above, we did re-examine our data and now report a new control regression analysis, all of which indicate that the proximity between keypresses does not explain contextualization effects.

(2) I was several times confused by the author's use of "neural representation of an action" or "sequence action representations" in understanding whether these terms refer to representation on the level of whole-brain, region (as defined by the specific parcellation used), or voxels. In fact, what is submitted to the decoder is some complicated whole-brain MEG feature (i.e., the "neural representation"), which is a hybrid of voxel and parcel features that is further dimension-reduced and not immediately interpretable. Clarifying this point early in the text and possibly using some more sensible terms, such as adding "brain-wise" before the "sequence action representation", would be the most helpful for the readers.

We now clarified this terminology in the revised manuscript.

(3) Although comparing many different ways in feature selection/reduction, time window selection, and decoder types is undoubtedly a meticulous work, the current version of the manuscript seems still lacking some explanation about the details of these methodological choices, like which decoding method was actually used to report the accuracy, whether or not different decoding methods were chosen for individual participants' data, how training data was selected (is it all of the correct presses in Day 1 data?), whether the frequency power or signal amplitude was used, and so on. I would highly appreciate these additional details in the Methods section.

The reported accuracies were based on linear discriminant analysis classifier. A comparison of different decoders (Figure 3 – figure supplement 4) shows LDA was the optimal choice.

Whether or not different decoding methods were chosen for individual participants' data

We selected the same decoder (LDA) performance to report the final accuracy.

How training data was selected (is it all of the correct presses in Day 1 data?),

Decoder training was conducted as a randomized split of the data (all correct keypresses of Day 1) into training (90%) and test (10%) samples for 8 iterations.

Whether the frequency power or signal amplitude was used

Signal amplitude was used for feature calculation.

(4) In terms of the Methods, please consider adding some references about the 'F1 score', the 'feature importance score,' and the 'MRMR-based feature ranking,' as the main readers of the current paper would not be from the machine learning community. Also, why did the LDA dimensionality reduction reduce accuracy specifically for the voxel feature?

We have now added the following statements to the Methods section that provide more detailed descriptions and references for these metrics:

“The F1 score, defined as the harmonic mean of the precision (percentage of true predictions that are actually true positive) and recall (percentage of true positives that were correctly predicted as true) scores, was used as a comprehensive metric for all one-versus-all keypress state decoders to assess class-wise performance that accounts for both false-positive and false-negative prediction tendencies [REF]. A weighted mean F1 score was then computed across all classes to assess the overall prediction performance of the multi-class model.”

and

“Feature Importance Scores

The relative contribution of source-space voxels and parcels to decoding performance (i.e. – feature importance score) was calculated using minimum redundant maximum relevance (MRMR) and highlighted in topography plots. MRMR, an approach that combines both relevance and redundancy metrics, ranked individual features based upon their significance to the target variable (i.e. – keypress state identity) prediction accuracy and their non-redundancy with other features.”

As stated in the Reviewer responses above, the dimensionality of the voxel-space feature set is very high (i.e. – 15684). LDA attempts to map the input features onto a much smaller dimensional space (number of classes-1; e.g. – 3 dimensions for 4-class keypress decoding). It is likely that the reduction in accuracy observed only for the voxel-space feature was due to the loss of relevant information during the mapping process that resulted in reduced accuracy. This reduction in accuracy for voxel-space decoding was specific to LDA. Figure 3—figure supplement 3 shows that voxel-space decoder performance actually improved when utilizing alternative dimensionality reduction techniques.

(5) Paragraph 9, lines #139-142: "Notably, decoding associated with index finger keypresses (executed at two different ordinal positions in the sequence) exhibited the highest number of misclassifications of all digits (N = 141 or 47.5% of all decoding errors; Figure 3C), raising the hypothesis that the same action could be differentially represented when executed at different learning state or sequence context locations."

This does not seem to be a fair comparison, as the index finger appears twice as many as the other fingers do in the sequence. To claim this, proper statistical analysis needs to be done taking this difference into account.

We thank the Reviewer for bringing this issue to our attention. We have now corrected this comparison to evaluate relative false negative and false positive rates between individual

keypress state decoders, and have revised this statement in the manuscript as follows:

“Notably, decoding of index finger keypresses (executed at two different ordinal positions in the sequence) exhibited the highest false negative (0.116 per keypress) and false positive (0.043 per keypress) misclassification rates compared with all other digits (false negative rate range = [0.067 0.114]; false positive rate range = [0.020 0.037]; Figure 3C), raising the hypothesis that the same action could be differentially represented when executed within different contexts (i.e. - different learning states or sequence locations).”

(6) Finally, the authors could consider acknowledging in the Discussion that the contribution of micro-offline learning to genuine skill learning is still under debate (e.g., Gupta and Rickard, 2023; 2024; Das et al., bioRxiv, 2024).

We have added a paragraph in the Discussion that addresses this point.

Reviewer #3 (Recommendations for the authors):

In addition to the additional analyses suggested in the public review, I have the following suggestions/questions:

(1) Given that the authors introduce a new decoding approach, it would be very helpful for readers to see a distribution of window sizes and window onsets eventually used across individuals, at least for the optimized decoder.

We have now included a new supplemental figure (Figure 4 – figure Supplement 2) that provides this information.

(2) Please explain in detail how you arrived at the (interpolated?) group-level plot shown in Figure 1B, starting from the discrete single-trial keypress transition times. Also, please specify what the shading shows.

Instantaneous correct sequence speed (skill measure) was quantified as the inverse of time (in seconds) required to complete a single iteration of a correctly generated full 5-item sequence. Individual keypress responses were labeled as members of correct sequences if they occurred within a 5-item response pattern matching any possible circular shifts of the 5-item sequence displayed on the monitor (41324). This approach allowed us to quantify a measure of skill within each practice trial at the resolution of individual keypresses. The dark line indicates the group mean performance dynamics for each trial. The shaded region indicates the 95% confidence limit of the mean (see Methods).

(3) Similarly, please explain how you arrived at the group-level plot shown in Figure 1C. What are the different colored lines (rows) within each trial? How exactly did the authors reach the conclusion that KTT variability stabilizes by trial 6?

Figure 1C provides additional information to the correct sequence speed measure above, as it also tracks individual transition speed composition over learning. Figure 1C, thus, represents both changes in overall correct sequence speed dynamics (indicated by the overall narrowing of the horizontal speed lines moving from top to bottom) and the underlying composition of the individual transition patterns within and across trials. The coloring of the lines is a shading convention used to discriminate between different keypress transitions. These curves were sampled with 1ms resolution, as in Figure 1B. Addressing the underlying keypress transition patterns requires within-subject normalization before averaging across subjects. The distribution of KTTs was normalized to the median correct sequence time for each participant and centered on the mid-point for each full sequence iteration during early learning.

(4) Maybe I missed it, but it was not clear to me which of the tested classifiers was eventually used. Or was that individualized as well? More generally, a comparison of the different classifiers would be helpful, similar to the comparison of dimension reduction techniques.

We have now included a new supplemental figure that provides this information.

(5) Please add df and effect sizes to all statistics.

Done.

(6) Please explain in more detail your power calculation.

The study was powered to determine the minimum sample size needed to detect a significant change in skill performance following training using a one-sample t-test (two-sided; $\alpha = 0.05$; 95% statistical power; Cohen's D effect size = 0.8115 calculated from previously acquired data in our lab). The calculated minimum sample size was 22. The included study sample size ($n = 27$) exceeded this minimum.

This information is now included in the revised manuscript.

(7) The cut-off for the high-pass filter is unusually high and seems risky in terms of potential signal distortions (de Cheveigne, Neuron 2019). Why did the authors choose such a high cut-off?

The 1Hz high-pass cut-off frequency for the 1-150Hz band-pass filter applied to the continuous raw MEG data during preprocessing has been used in multiple previous MEG publications (Barratt et al., 2018; Brookes et al., 2012; Higgins et al., 2021; Seedat et al., 2020; Vidaurre et al., 2018).

(8) "Furthermore, the magnitude of offline contextualization predicted skill gains while online contextualization did not", lines 336/337 - where is that analysis?

Additional details pertaining to this analysis are now provided in the Results section (Figure 5 – figure supplement 4).

(9) How were feature importance scores computed?

We have now added a new subheading in the Methods section with a more detailed description of how feature importance scores were computed.

(10) Please add x and y ticks plus tick labels to Figure 5 - Figure Supplement 3, panel A

Done

(11) Line 369, what does "comparable" mean in this context?

The sentence in the "Study Participants" part of the Methods section referred to here has now been revised for clarity.

(12) In lines 496/497, please specify what $t=0$ means (KeyDown event, I guess?).

Yes, the *KeyDown* event occurs at $t = 0$. This has now been clarified in the revised manuscript.

(13) Please specify consistent boundaries between alpha- and beta-bands (they are currently not consistent in the Results vs. Methods (14/15 Hz or 15/16 Hz)).

We thank the Reviewer for alerting us to this discrepancy caused by a typographic error in the Methods. We have now corrected this so that the alpha (8-14 Hz) and beta-band (15-24 Hz) frequency limits are described consistently throughout the revised manuscript.

References

- Albouy, G., Fogel, S., King, B. R., Laventure, S., Benali, H., Karni, A., Carrier, J., Robertson, E. M., & Doyon, J. (2015). Maintaining vs. enhancing motor sequence memories: respective roles of striatal and hippocampal systems. *Neuroimage*, 108, 423-434. <https://doi.org/10.1016/j.neuroimage.2014.12.049>
- Albouy, G., King, B. R., Maquet, P., & Doyon, J. (2013). Hippocampus and striatum: dynamics and interaction during acquisition and sleep-related motor sequence memory consolidation. *Hippocampus*, 23(11), 985-1004. <https://doi.org/10.1002/hipo.22183>
- Albouy, G., Sterpenich, V., Vandewalle, G., Darsaud, A., Gais, S., Rauchs, G., Desseilles, M., Boly, M., Dang-Vu, T., Baletau, E., Degueldre, C., Phillips, C., Luxen, A., & Maquet, P. (2012). Neural correlates of performance variability during motor sequence acquisition. *NeuroImage*, 60(1), 324-331. <https://doi.org/10.1016/j.neuroimage.2011.12.049>
- Andersen, R. A., & Buneo, C. A. (2002). Intentional maps in posterior parietal cortex. *Annu Rev Neurosci*, 25, 189-220. <https://doi.org/10.1146/annurev.neuro.25.112701.142922> [pii]
- Ashe, J., Lungu, O. V., Basford, A. T., & Lu, X. (2006). Cortical control of motor sequences. *Curr Opin Neurobiol*, 16(2), 213-221. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16563734
- Bansal, A. K., Vargas-Irwin, C. E., Truccolo, W., & Donoghue, J. P. (2011). Relationships among low-frequency local field potentials, spiking activity, and three-dimensional reach and grasp kinematics in primary motor and ventral premotor cortices. *J Neurophysiol*, 105(4), 1603-1619. <https://doi.org/10.1152/jn.00532.2010>
- Barratt, E. L., Francis, S. T., Morris, P. G., & Brookes, M. J. (2018). Mapping the topological organisation of beta oscillations in motor cortex using MEG. *NeuroImage*, 181, 831-844. <https://doi.org/10.1016/j.neuroimage.2018.06.041>
- Bassett, D. S., Wymbs, N. F., Porter, M. A., Mucha, P. J., Carlson, J. M., & Grafton, S. T. (2011). Dynamic reconfiguration of human brain networks during learning. *Proc Natl Acad Sci U S A*, 108(18), 7641-7646. <https://doi.org/10.1073/pnas.1018985108>
- Battaglia-Mayer, A., & Caminiti, R. (2019). Corticocortical Systems Underlying High-Order Motor Control. *J Neurosci*, 39(23), 4404-4421. <https://doi.org/10.1523/JNEUROSCI.2094-18.2019>
- Berlot, E., Popp, N. J., & Diedrichsen, J. (2020). A critical re-evaluation of fMRI signatures of motor sequence learning. *Elife*, 9. <https://doi.org/10.7554/eLife.55241>
- Bonstrup, M., Iturrate, I., Hebart, M. N., Censor, N., & Cohen, L. G. (2020). Mechanisms of offline motor learning at a microscale of seconds in large-scale crowdsourced data. *NPJ Sci Learn*, 5, 7. <https://doi.org/10.1038/s41539-020-0066-9>
- Bonstrup, M., Iturrate, I., Thompson, R., Cruciani, G., Censor, N., & Cohen, L. G. (2019). A Rapid Form of Offline Consolidation in Skill Learning. *Curr Biol*, 29(8), 1346-1351 e1344. <https://doi.org/10.1016/j.cub.2019.02.049>

- Brawn, T. P., Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2010). Consolidating the effects of waking and sleep on motor-sequence learning. *J Neurosci*, 30(42), 13977-13982. <https://doi.org/10.1523/JNEUROSCI.3295-10.2010>
- Brookes, M. J., Woolrich, M. W., & Barnes, G. R. (2012). Measuring functional connectivity in MEG: a multivariate approach insensitive to linear source leakage. *NeuroImage*, 63(2), 910-920. <https://doi.org/10.1016/j.neuroimage.2012.03.048>
- Brooks, E., Wallis, S., Hendrikse, J., & Coxon, J. (2024). Micro-consolidation occurs when learning an implicit motor sequence, but is not influenced by HIIT exercise. *NPJ Sci Learn*, 9(1), 23. <https://doi.org/10.1038/s41539-024-00238-6>
- Buch, E. R., Claudino, L., Quentin, R., Bonstrup, M., & Cohen, L. G. (2021). Consolidation of human skill linked to waking hippocampo-neocortical replay. *Cell Rep*, 35(10), 109193. <https://doi.org/10.1016/j.celrep.2021.109193>
- Buneo, C. A., & Andersen, R. A. (2006). The posterior parietal cortex: sensorimotor interface for the planning and online control of visually guided movements. *Neuropsychologia*, 44(13), 2594-2606. <https://doi.org/10.1016/j.neuropsychologia.2005.10.011>
- Buzsaki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus*, 25(10), 1073-1188. <https://doi.org/10.1002/hipo.22488>
- Chen, P.-C., Stritzelberger, J., Walther, K., Hamer, H., & Staresina, B. P. (2024). Hippocampal ripples during offline periods predict human motor sequence learning. *bioRxiv*, 2024.2010.2006.614680. <https://doi.org/10.1101/2024.10.06.614680>
- Churchland, M. M., Cunningham, J. P., Kaufman, M. T., Foster, J. D., Nuyujukian, P., Ryu, S. I., & Shenoy, K. V. (2012). Neural population dynamics during reaching. *Nature*, 487(7405), 51-56. <https://doi.org/10.1038/nature11129>
- Classen, J., Liepert, J., Wise, S. P., Hallett, M., & Cohen, L. G. (1998). Rapid plasticity of human cortical movement representation induced by practice. *J Neurophysiol*, 79(2), 1117-1123. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9463469
- Colclough, G. L., Brookes, M. J., Smith, S. M., & Woolrich, M. W. (2015). A symmetric multivariate leakage correction for MEG connectomes. *NeuroImage*, 117, 439-448. <https://doi.org/10.1016/j.neuroimage.2015.03.071>
- Colclough, G. L., Woolrich, M. W., Tewarie, P. K., Brookes, M. J., Quinn, A. J., & Smith, S. M. (2016). How reliable are MEG resting-state connectivity metrics? *NeuroImage*, 138, 284-293. <https://doi.org/10.1016/j.neuroimage.2016.05.070>
- Das, A., Karagiorgis, A., Diedrichsen, J., Stenner, M.-P., & Azanon, E. (2024). “Micro-offline gains” convey no benefit for motor skill learning. *bioRxiv*, 2024.2007.2011.602795. <https://doi.org/10.1101/2024.07.11.602795>
- Deleglise, A., Donnelly-Kehoe, P. A., Yeffal, A., Jacobacci, F., Jovicich, J., Amaro, E., Jr., Armony, J. L., Doyon, J., & Della-Maggiore, V. (2023). Human motor sequence learning drives transient changes in network topology and hippocampal connectivity early during memory consolidation. *Cereb Cortex*, 33(10), 6120-6131. <https://doi.org/10.1093/cercor/bhac489>
- Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., Lehericy, S., & Benali, H. (2009). Contributions of the basal ganglia and functionally related brain structures to motor learning. [Review]. *Behavioural brain research*, 199(1), 61-75. <https://doi.org/10.1016/j.bbr.2008.11.012>

- Doyon, J., Song, A. W., Karni, A., Lalonde, F., Adams, M. M., & Ungerleider, L. G. (2002). Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc Natl Acad Sci U S A*, 99(2), 1017-1022. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11805340
- Euston, D. R., Gruber, A. J., & McNaughton, B. L. (2012). The role of medial prefrontal cortex in memory and decision making. *Neuron*, 76(6), 1057-1070. <https://doi.org/10.1016/j.neuron.2012.12.002>
- Euston, D. R., Tatsuno, M., & McNaughton, B. L. (2007). Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. *Science*, 318(5853), 1147-1150. <https://doi.org/10.1126/science.1148979>
- Flint, R. D., Ethier, C., Oby, E. R., Miller, L. E., & Slutzky, M. W. (2012). Local field potentials allow accurate decoding of muscle activity. *J Neurophysiol*, 108(1), 18-24. <https://doi.org/10.1152/jn.00832.2011>
- Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nat Rev Neurosci*, 6(2), 119-130. <https://doi.org/10.1038/nrn1607>
- Gais, S., Albouy, G., Boly, M., Dang-Vu, T. T., Darsaud, A., Desseilles, M., Rauchs, G., Schabus, M., Sterpenich, V., Vandewalle, G., Maquet, P., & Peigneux, P. (2007). Sleep transforms the cerebral trace of declarative memories. *Proc Natl Acad Sci U S A*, 104(47), 18778-18783. <https://doi.org/10.1073/pnas.0705454104>
- Grafton, S. T., Mazziotta, J. C., Presty, S., Friston, K. J., Frackowiak, R. S., & Phelps, M. E. (1992). Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET. *J Neurosci*, 12(7), 2542-2548.
- Grover, S., Wen, W., Viswanathan, V., Gill, C. T., & Reinhart, R. M. G. (2022). Long-lasting, dissociable improvements in working memory and long-term memory in older adults with repetitive neuromodulation. *Nat Neurosci*, 25(9), 1237-1246. <https://doi.org/10.1038/s41593-022-01132-3>
- Gupta, M. W., & Rickard, T. C. (2022). Dissipation of reactive inhibition is sufficient to explain post-rest improvements in motor sequence learning. *NPJ Sci Learn*, 7(1), 25. <https://doi.org/10.1038/s41539-022-00140-z>
- Gupta, M. W., & Rickard, T. C. (2024). Comparison of online, offline, and hybrid hypotheses of motor sequence learning using a quantitative model that incorporate reactive inhibition. *Sci Rep*, 14(1), 4661. <https://doi.org/10.1038/s41598-024-52726-9>
- Hardwick, R. M., Rottschy, C., Miall, R. C., & Eickhoff, S. B. (2013). A quantitative metaanalysis and review of motor learning in the human brain. *NeuroImage*, 67, 283-297. <https://doi.org/10.1016/j.neuroimage.2012.11.020>
- Heusser, A. C., Poeppel, D., Ezzyat, Y., & Davachi, L. (2016). Episodic sequence memory is supported by a theta-gamma phase code. *Nat Neurosci*, 19(10), 1374-1380. <https://doi.org/10.1038/nn.4374>
- Higgins, C., Liu, Y., Vidaurre, D., Kurth-Nelson, Z., Dolan, R., Behrens, T., & Woolrich, M. (2021). Replay bursts in humans coincide with activation of the default mode and parietal alpha networks. *Neuron*, 109(5), 882-893 e887. <https://doi.org/10.1016/j.neuron.2020.12.007>
- Hikosaka, O., Nakamura, K., Sakai, K., & Nakahara, H. (2002). Central mechanisms of motor skill learning. *Curr Opin Neurobiol*, 12(2), 217-222. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12015240

- Jacobacci, F., Armony, J. L., Yeffal, A., Lerner, G., Amaro, E., Jr., Jovicich, J., Doyon, J., & Della-Maggiore, V. (2020). Rapid hippocampal plasticity supports motor sequence learning. *Proc Natl Acad Sci U S A*, 117(38), 23898-23903. <https://doi.org/10.1073/pnas.2009576117>
- Jacobacci, F., Armony, J. L., Yeffal, A., Lerner, G., Amaro Jr, E., Jovicich, J., Doyon, J., & DellaMaggiore, V. (2020). Rapid hippocampal plasticity supports motor sequence learning. *Proceedings of the National Academy of Sciences*, 117(38), 23898-23903. Karni, A., Meyer, G., Jezzeard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature*, 377(6545), 155-158. <https://doi.org/10.1038/377155a0>
- Kennerley, S. W., Sakai, K., & Rushworth, M. F. (2004). Organization of action sequences and the role of the pre-SMA. *J Neurophysiol*, 91(2), 978-993. <https://doi.org/10.1152/jn.00651.2003> [pii]
- Kleim, J. A., Barbay, S., & Nudo, R. J. (1998). Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol*, 80, 3321-3325.
- Kornysheva, K., Bush, D., Meyer, S. S., Sadnicka, A., Barnes, G., & Burgess, N. (2019). Neural Competitive Queuing of Ordinal Structure Underlies Skilled Sequential Action. *Neuron*, 101(6), 1166-1180 e1163. <https://doi.org/10.1016/j.neuron.2019.01.018>
- Lee, S. H., Jin, S. H., & An, J. (2019). The difference in cortical activation pattern for complex motor skills: A functional near- infrared spectroscopy study. *Sci Rep*, 9(1), 14066. <https://doi.org/10.1038/s41598-019-50644-9>
- Lisman, J. E., & Jensen, O. (2013). The theta-gamma neural code. *Neuron*, 77(6), 1002-1016. <https://doi.org/10.1016/j.neuron.2013.03.007>
- Mollazadeh, M., Aggarwal, V., Davidson, A. G., Law, A. J., Thakor, N. V., & Schieber, M. H. (2011). Spatiotemporal variation of multiple neurophysiological signals in the primary motor cortex during dexterous reach-to-grasp movements. *J Neurosci*, 31(43), 15531-15543. <https://doi.org/10.1523/JNEUROSCI.2999-11.2011>
- Molle, M., & Born, J. (2009). Hippocampus whispering in deep sleep to prefrontal cortex--for good memories? *Neuron*, 61(4), 496-498. <https://doi.org/10.1016/j.neuron.2009.02.002>
- Morris, R. G. M. (2006). Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. [Review]. *The European journal of neuroscience*, 23(11), 2829-2846. <https://doi.org/10.1111/j.1460-9568.2006.04888.x>
- Mylonas, D., Schapiro, A. C., Verfaellie, M., Baxter, B., Vangel, M., Stickgold, R., & Manoach, D. S. (2024). Maintenance of Procedural Motor Memory across Brief Rest Periods Requires the Hippocampus. *J Neurosci*, 44(14). <https://doi.org/10.1523/JNEUROSCI.1839-23.2024>
- Pan, S. C., & Rickard, T. C. (2015). Sleep and motor learning: Is there room for consolidation? *Psychol Bull*, 141(4), 812-834. <https://doi.org/10.1037/bul0000009>
- Penhune, V. B., & Steele, C. J. (2012). Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. *Behav. Brain Res.*, 226(2), 579-591. <https://doi.org/10.1016/j.bbr.2011.09.044>
- Qin, Y. L., McNaughton, B. L., Skaggs, W. E., & Barnes, C. A. (1997). Memory reprocessing in corticocortical and hippocampocortical neuronal ensembles. *Philos Trans R Soc Lond B Biol Sci*, 352(1360), 1525-1533. <https://doi.org/10.1098/rstb.1997.0139>

- Rickard, T. C., Cai, D. J., Rieth, C. A., Jones, J., & Ard, M. C. (2008). Sleep does not enhance motor sequence learning. *J Exp Psychol Learn Mem Cogn*, 34(4), 834-842. <https://doi.org/10.1037/0278-7393.34.4.834>
- Robertson, E. M., Pascual-Leone, A., & Miall, R. C. (2004). Current concepts in procedural consolidation. *Nat Rev Neurosci*, 5(7), 576-582. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15208699
- Sawamura, D., Sakuraba, S., Suzuki, Y., Asano, M., Yoshida, S., Honke, T., Kimura, M., Iwase, Y., Horimoto, Y., Yoshida, K., & Sakai, S. (2019). Acquisition of chopstick-operation skills with the non-dominant hand and concomitant changes in brain activity. *Sci Rep*, 9(1), 20397. <https://doi.org/10.1038/s41598-019-56956-0>
- Schendan, H. E., Searl, M. M., Melrose, R. J., & Stern, C. E. (2003). An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. *Neuron*, 37(6), 1013-1025. [https://doi.org/10.1016/s0896-6273\(03\)00123-5](https://doi.org/10.1016/s0896-6273(03)00123-5)
- Seedat, Z. A., Quinn, A. J., Vidaurre, D., Liuzzi, L., Gascoyne, L. E., Hunt, B. A. E., O'Neill, G. C., Pakenham, D. O., Mullinger, K. J., Morris, P. G., Woolrich, M. W., & Brookes, M. J. (2020). The role of transient spectral 'bursts' in functional connectivity: A magnetoencephalography study. *NeuroImage*, 209, 116537. <https://doi.org/10.1016/j.neuroimage.2020.116537>
- Shadmehr, R., & Holcomb, H. H. (1997). Neural correlates of motor memory consolidation. *Science*, 277, 821-824.
- Sjøgård, M., Baxter, B., Mylonas, D., Driscoll, B., Kwok, K., Tolosa, A., Thompson, M., Stickgold, R., Vangel, M., Chu, C., & Manoach, D. S. (2024). Hippocampal ripples mediate motor learning during brief rest breaks in humans. *bioRxiv*. <https://doi.org/10.1101/2024.05.02.592200>
- Srinivas, S., Sarvadevabhatla, R. K., Mopuri, K. R., Prabhu, N., Kruthiventi, S. S. S., & Babu, R. V. (2016). A Taxonomy of Deep Convolutional Neural Nets for Computer Vision [Technology Report]. *Frontiers in Robotics and AI*, 2. <https://doi.org/10.3389/frobt.2015.00036>
- Sterpenich, V., Albouy, G., Darsaud, A., Schmidt, C., Vandewalle, G., Dang Vu, T. T., Desseilles, M., Phillips, C., Degueldre, C., Balteau, E., Collette, F., Luxen, A., & Maquet, P. (2009). Sleep promotes the neural reorganization of remote emotional memory. *J Neurosci*, 29(16), 5143-5152. <https://doi.org/10.1523/JNEUROSCI.0561-09.2009>
- Toni, I., Ramnani, N., Josephs, O., Ashburner, J., & Passingham, R. E. (2001). Learning arbitrary visuomotor associations: temporal dynamic of brain activity. *Neuroimage*, 14(5), 10481057. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11697936
- Toni, I., Thoenissen, D., & Zilles, K. (2001). Movement preparation and motor intention. *NeuroImage*, 14(1 Pt 2), S110-117. <https://doi.org/10.1006/nimg.2001.0841>
- Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P. A., Wood, E. R., Witter, M. P., & Morris, R. G. (2007). Schemas and memory consolidation. *Science*, 316(5821), 76-82. <https://doi.org/10.1126/science.1135935>
- van Kesteren, M. T., Fernandez, G., Norris, D. G., & Hermans, E. J. (2010). Persistent schemadependent hippocampal-neocortical connectivity during memory encoding and postencoding rest in humans. *Proc Natl Acad Sci U S A*, 107(16), 7550-7555. <https://doi.org/10.1073/pnas.0914892107>
- van Kesteren, M. T., Ruiter, D. J., Fernandez, G., & Henson, R. N. (2012). How schema and novelty augment memory formation. *Trends Neurosci*, 35(4), 211-219. <https://doi.org/10.1016/j.trends.2012.02.007>

.tins.2012.02.001

Vidaurre, D., Hunt, L. T., Quinn, A. J., Hunt, B. A. E., Brookes, M. J., Nobre, A. C., & Woolrich, M. W. (2018). Spontaneous cortical activity transiently organises into frequency specific phase-coupling networks. *Nat Commun*, 9(1), 2987. <https://doi.org/10.1038/s41467-01805316-z>

Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, A. M., Rosen, B. R., & Buckner, R. L. (1998). Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. [Comment]. *Science (New York, N.Y.)*, 281(5380), 1188-1191. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9712582&retmode=ref&cmd=prlinks>

Wolpert, D. M., Goodbody, S. J., & Husain, M. (1998). Maintaining internal representations: the role of the human superior parietal lobe. *Nat Neurosci*, 1(6), 529-533. <https://doi.org/10.1038/2245>

<https://doi.org/10.7554/eLife.102475.2.sa0>