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Competitive state of movements during planning predicts sequence performance

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New & Noteworthy

Sequence planning is an integral part of motor sequence control. Here, we demonstrate that the competitive state of sequential movements during sequence planning can be read out behaviourally through movement probes. We show that position-dependent differences in movement availability during planning reflect sequence preparedness and skill, but not the timing of the planned sequence. Behavioural access to the preparatory state of movements may serve as a marker of sequence planning capacity.

Abstract

Humans can learn and produce skilled movement sequences from memory, yet the nature of sequence planning is not well understood. Previous computational and neurophysiological work suggests that movements in a sequence are planned as parallel graded activations and selected for output through competition. However, the relevance of this planning pattern to sequence production fluency and accuracy, as opposed to the temporal structure of sequences, is unclear. To resolve this question, we assessed the relative availability of constituent movements behaviourally during the preparation of motor sequences from memory. In three separate multi-session experiments, healthy participants were trained to retrieve and produce 4-element finger press sequences with particular timing according to an abstract sequence cue. We evaluated reaction time (RT) and error rate as markers of movement availability to constituent movement probes. Our results demonstrate that longer preparation time produces more pronounced differences in availability between adjacent sequence elements, whilst no effect was found for sequence speed or temporal grouping. Further, participants with larger position-dependent differences in movement availability tended to initiate correct sequences faster and with a higher temporal accuracy. Our results suggest that competitive pre-activation during sequence planning is established gradually during sequence planning and predicts sequence skill, rather than the temporal structure of the motor sequence.

Keywords:

motor planning; sequence control; competitive queuing; reaction time; error rate

Introduction

Producing movement sequences from memory fluently is an essential capacity of primates, in particular humans. It enables a skilled and flexible interaction with the world for a range of everyday activities - from tool-use, speech, and gestural communication, to sports and music. Key to fluent sequence production is sequence planning before the initiation of the first movement (Lashley 1951; Rosenbaum 1985), with longer preparation time benefitting sequence execution, i.e., reducing initiation time after a *Go* cue and improving accuracy (Ariani and Diedrichsen 2019). However, the underlying nature and content of sequence planning is still debated (Remington et al. 2018).

Different computational accounts of sequence control make contrasting predictions with regard to the content of sequence planning. Models postulating a purely serial control of motor sequences suggest that a well-learnt sequence is a cohesive entity, rather than a series of individual movements, e.g. individual strokes when drawing a geometrical figure or finger presses playing the piano (Goudar and Buonomano 2018; Laje and Buonomano 2013). They predict that sequence planning activity reflects bringing the neural trajectory towards the correct neural state of sequence initiation from which it cascades serially through a learnt trajectory. Sequence planning would therefore entail the preparation of the state occupied by the first movement, e.g. using a null-state to allow preparation without premature initiation, as shown empirically for reaching movements (Kaufman et al. 2014; O'Shea and Shenoy 2016).

In contrast, models postulating parallel sequence control, such as competitive queuing models (Houghton 1990), propose simultaneous control of the items, here movements, in a sequence. They predict that preparatory neural activity pre-activates sequence movements *concurrently*. Specifically, the neural activation pattern for each movement is weighted according to its temporal position in the respective sequence (Burgess and Hitch 1999; Hartley and Houghton 1996), resulting in a position-dependent pre-activation *gradient* for each upcoming movement in the sequence. Indirect support for parallel and independent neural control of sequential movements stems from observations of serial recall including transposition of neighbouring sequence items and items occupying the same position in different chunks (Glasspool

and Houghton 2005; Hartley and Houghton 1996; Henson 1998), and excitability of forthcoming movements during sequence production (Behmer et al. 2018).

Direct neurophysiological support for the parallel control of sequence movements has been provided in the context of well-trained finger sequences (Kornysheva et al. 2019; Pinet et al. 2019), saccades (Basu and Murthy 2020), drawing of geometrical shapes (Averbeck et al. 2002). Specifically, during planning, the probability of neural patterns associated with each movement in the sequence was highest for the first, and lowest for the fourth and fifth movements of the planned sequence. This effect could not be explained by a graded pre-pressing of the corresponding fingers according to their order and was observed at the trial-by-trial level, suggesting that this competitive pre-activation is not an artefact of trial averaging (Kornysheva et al. 2019). Importantly, the ordered pre-activation gradient of sequence movements during planning was relevant to subsequent execution. In particular, the quality and strength of this gradient was predictive of sequence production accuracy such that participants with stronger pre-activation differences between the sequence items during planning were more accurate during sequence production. Together, these data suggest that skilled sequence production involves an orderly parallel planning of several movements in advance before sequence initiation and predicts better sequence performance.

While the pre-activation gradient during planning has been shown to predict subsequent execution, it remains unclear what this preparatory pattern reflects – the skill of sequence production (fluency of initiation and accuracy of the sequence execution), or the temporal structure of the sequence (speed and temporal grouping). Most competitive queuing models assume the presence of a temporal or positional context layer and that the activity gradients are learned by associations of the latter to each sequence item in the parallel planning layer, e.g. through Hebbian learning (Burgess and Hitch 1999). The form of activity in the context layer can be as simple as a decaying start signal (Page and Norris 1998), a combination of start and end signals (Houghton 1990, 2018) or a sequence of overlapping states (Burgess and Hitch 1999, 2006). Although primarily encoding serial order of sequence items, models utilizing overlapping states can implement effects of temporal grouping or sequence rhythm (Burgess and Hitch 1999; Hartley et al. 2016) making timing an intrinsic

property of the competitive queuing of sequential movements. Likewise, a separate timing process (Kornysheva et al. 2013; Kornysheva and Diedrichsen 2014; Medina et al. 2005; Spencer et al. 2009; Ullén and Bengtsson 2003; Zeid and Bullock 2019) may modulate the parallel planning of the serial order of items, e.g. in the parallel planning layer. In both cases, the competitive pre-activation gradient of movements during planning would reflect the temporal grouping or temporal proximity of movements in the upcoming sequence, with movements closer together in time having more similar levels of pre-activation than those that are further apart (Burgess and Hitch 1999). In contrast, sequence timing may not impact the competitive pre-activation of sequential movements during planning and interact with the latter during execution only.

In order to investigate the nature of sequence planning and its relation to subsequent execution, we developed a behavioural paradigm to capture the preparatory state of each constituent movement of a well-learned sequence during planning. Following training, participants prepared a motor sequence from memory following an abstract visual stimulus associated with a particular sequence of finger presses performed with a particular speed or temporal grouping. In half of the trials during the test phase, the *Go* cue was replaced by a finger press cue prompting the production of movements associated with different positions in the sequence. We used behavioural availability for fast and correct execution of the presses in these *Probe* trials (RT and error rate) as behavioural markers of the relative pre-activation of upcoming movements during sequence planning.

We hypothesized that if competitive queuing during planning primarily reflected the accuracy of the sequence plan (Averbeck et al. 2002; Kornysheva et al. 2019), but not its timing, we would predict a gradual differentiation of the position-dependent pre-activation gradient with longer sequence preparation time. Accordingly, we would observe an increase of position-dependent differences in press availability across preparation durations of 500, 1000 and 1500 ms, despite matched speed and temporal grouping of sequence production. Further, participants with a more pronounced gradient would be more fluent and accurate, specifically show more rapid sequence initiation of correct sequences after the *Go* cue, more accurate timing and fewer finger press errors.

Alternatively, if the gradient reflected the timing of the sequence during planning, movements planned to be executed closer in time would show smaller position-dependent differences relative to movements further apart. Accordingly, sequences twice as fast (speed manipulation) would result in more similar levels of availability of movements in neighbouring sequence positions. Further, the latter would be modulated by irregular inter-press-intervals (IPI) with shorter versus longer IPIs being accompanied by smaller versus larger differences in position-dependent availability during planning, respectively (temporal grouping manipulation).

We report that during the 1.5 seconds of sequence retrieval and preparation from memory the behavioural availability of sequential movements decreases on average with their planned serial position, up to the last but one. Specifically, movement probes associated with later sequence positions were progressively more likely to lead to erroneous presses during planning, and correct presses were executed more slowly. This characteristic preparatory gradient of movement availability increased with preparation duration rendering movements pre-planned to occur in later compared to earlier sequence positions progressively less available. Across participants, the size of this gradient during preparation correlated with more fluent initiation and temporally accurate sequence production. Contrary to the timing hypothesis, we found no reliable effect of sequence speed or temporal grouping on movement availability during planning. Based on this data, we propose that sequence planning involves a competitive pre-activation gradient of sequential movements during sequence planning which operates independently of sequence timing and facilitates skilled sequence performance.

Materials and Methods

Participants

Data were collected from a total of 55 right-handed University students (Experiment 1: *N* = 19, 11 females; *M* = 24.2 years, *SD* = 4.1; Experiment 2: *N*=18, 11 females; *M* = 24.2 years, *SD* = 4.5; Experiment 3: *N* = 18, 9 females; *M* = 20.8 years, *SD* = 2.4). Four additional participants were tested but excluded from analysis based on their sequence production finger error rate (cf. Participant exclusion criteria). They were hypothesis-naive and had no previous exposure in performing a similar

experimental task. All participants had normal or corrected-to-normal vision and reported no history of neurological or psychiatric disorders or hearing problems. Handedness was evaluated through the online Handedness Questionnaire (http://www.brainmapping.org/shared/Edinburgh.php) adapted from the Edinburgh Handedness Inventory (Oldfield 1971) (Experiment 1, *M* = 88.4, *SD* = 9.4; Experiment 2, *M* = 90.6, *SD* = 9.7; Experiment 3, *M* = 90, *SD* = 11.8). All participants provided written informed consent before participation and were debriefed after completing the study. They were compensated either monetarily or with course credits at the end of the experiment. All procedures were approved by the Bangor University School of Psychology Research Ethics Committee (Ethics Review Board Approval Code 2017-16100-A14320).

Apparatus

For all three experiments participants were seated in a quiet room in front of a 19-inch LCD monitor (LG Flatron L1953HR, 1280 x 1024 pixels), wearing headphones for noise isolation. All instructions, visual stimuli and feedback were precisely timed by the monitor's refresh rate (60Hz) and controlled by Cogent 2000 (v1.29) (http://www.vislab.ucl.ac.uk/cogent.php) through a custom-written MATLAB program (v9.2 R2017a, The MathWorks, Inc., Natick, Massachusetts, United States). In Experiments 1 and 2, a Pyka 5-button fiber optic device (Current Designs) was used to record the responses. A customized foam channel stabilized the cable and a thin anti-slip mat, placed underneath the response device, prevented from sliding during the task. The response device was positioned horizontally and adjusted for each participant to ensure good control over the target buttons as well as arm and wrist comfort. Participants were instructed to place the right index, middle, ring and little fingers on the respective target buttons of the device. Experiment 3 used an identical experimental set-up with the exception that responses were recorded using a computer keyboard. Here, participants were instructed to place their right thumb in addition to the rest of the right-hand fingers on the designated keyboard keys. For hand stabilization and comfort their wrist was positioned on a rest cushion.

Experimental design

All three experiments employed a visually cued motor learning task adapted from Kornysheva et al. (2019). Experiments 1 and 2 involved the recording of

sequential and single button presses produced with the four fingers (index, middle, ring and little) of the right hand. Experiment 3 additionally required single presses with the thumb. In all experiments, participants were trained to associate a visual cue (an abstract fractal shape, henceforth *Sequence* cue) with a four-element finger sequence produced with a specific timing. The paradigm employed two main trial types: *Sequence* and *Probe* (single press) trials. *Sequence* trials were further divided into visually instructed and memory-guided trials. Instructed *Sequence* trials involved the presentation of four visual digit cues (index, middle, ring and little) at specified intervals comprising a unique target sequence. These were only used during training in the first two days, and during two refresher blocks on the third day (Figure 1a). The test phase on the third day involved sequence production without visual guidance (Figure 1b). *Probe* trials involved the production of only one visual digit cue (*Probe* cue) corresponding to one of the serial positions in the target sequence (Figure 1c).

Experiment 1 – Preparation duration. All participants were trained to produce two different finger sequences comprising four presses with target IPIs of 800 ms (slow timing). Two additional sequences served as practice sequences to impose familiarization with the task. All sequences were randomly generated offline for each participant through a custom-written MATLAB code for each participant. The sequence generation process excluded sequences with ascending and descending digit triplets and identical finger positions.

All trial types started with a *Sequence* cue. The *Sequence* cue had a fixed duration of 400 ms followed by a fixation cross, the latency of which varied depending on the delay period between the *Sequence* cue and *Go* cue onsets. The resultant short (500 ms), intermediate (1000 ms), and long (1500 ms) delay periods comprised the three preparation duration conditions employed in the task. After the delay period, a black right-hand stimulus appeared as the *Go* cue.

In an instructed *Sequence* trial, the *Go* cue was presented on a grey background for 2400 ms. A white circle appeared on top of the corresponding finger digits of the hand stimulus sequentially to guide the participants throughout the execution of the sequence. The time intervals between the digit cues formed the target timing of the sequence and defined its duration of 2400 ms. To achieve finger and temporal accuracy during training, participants were asked to “synchronise” the correct

finger presses with the digit cues until the completion of the sequence. As the first digit cue of a sequence appeared at the same time as the *Go* cue, immediate initiation of the sequence was emphasized in the instructions. In a memory-guided *Sequence* trial, the *Go* cue was presented on a green background, remaining on the screen for 2400 ms. Memory-guided *Sequence* trials were devoid of finger digit cues, requiring participants to produce the upcoming target sequence from memory. Participants were instructed to initiate the sequence as quickly as possible and produce the sequence according to its target finger order and timing. In a *Probe* trial, after the delay period, the *Go* cue was replaced with a *Probe* cue, namely a single digit cue, displayed for 1000 ms. The *Probe* cue prompted a single press with a corresponding finger as fast and accurately as possible. Participants were encouraged to avoid premature responses (before the *Go* cue) in all trial types. Following the *Go* cue in any trial type, a fixation cross (1000 ms) and, subsequently, feedback (1000 ms) were presented on the screen. The duration of a *Sequence* trial was 5.4 s, while a *Probe* trial had a duration of 4 s, including feedback. The inter-trial-interval (ITI) was fixed at 800 ms.

The experiment consisted of two 90 min long training sessions (Days 1 and 2) and a test session (Day 3) which took place over three consecutive days. Day 1 commenced with a practice block which involved two instructed and two memory-guided *Sequence* trials for each of the target sequences, and two randomly selected *Probe* trials, with randomly chosen preparation durations. Over the three days, participants serially underwent a pre-training (2 blocks), a training (36 blocks), a post-training (2 blocks) and a test phase (2 refresher training blocks + 16 test blocks), completing a total of 58 blocks. To assess sequence planning and execution from memory only data from the test phase is presented here.

Participants were naïve as to the structure of the transition from the training through to the test phase and which block type they were administered (Figure 1a). The training phase was organized in three stages: 12 blocks of 288 instructed *Sequence* and 72 *Probe* trials (stage A, 80% instructed *Sequence* and 20% *Probe* trials in each block), 12 blocks of 144 instructed, 144 memory-guided *Sequence* and 72 *Probe* trials (stage B, 40% for each *Sequence* type and 20% *Probe* trials in each block), and 12 blocks of 288 memory-guided *Sequence* and 72 *Probe* trials (stage C, 80% memory-guided *Sequence* and 20% *Probe* trials in each block). Each training

block (3 min long) consisted of 30 trials. On each training block there was a 20% occurrence of *Probe* trials (6 in each block) comprising a total of 216 throughout the training blocks. All *Probe* trial conditions (24; 2 sequences × 3 preparation durations × 4 digits) were counterbalanced across the training blocks. The test phase (Day 3) started with two refresher training blocks (stage B, 40% for each *Sequence* type and 20% *Probe* trials in each block) and immediately progressed to 16 blocks of 48 trials each, in which 24 memory-guided *Sequence* and 24 *Probe* trials were randomly presented (test, 50% memory-guided *Sequence* and 50% *Probe* trials). Duration of each test block was 4.4 min. The preparation duration conditions were counterbalanced across the two target sequences in memory-guided *Sequence* and *Probe* trials in each block. This resulted in a total of 128 memory-guided *Sequence* trials per preparation duration condition, across blocks. In *Probe* trials, each *Probe* cue was combined with the three preparation duration conditions resulting in 32 trials per digit cue per preparation duration condition. The test phase had a total of 768 trials (384 memory-guided *Sequence* and 384 *Probe* trials). Overall, the participants underwent 2004 trials excluding the practice trials.

Preparation duration (foreperiod) effects on RT have been associated with carry-over effects from preceding to current trials, and may bias our RT findings if trial history is unbalanced (Langner et al. 2018; Steinborn and Langner 2012). Post-hoc, we examined the preparation duration conditions in both *Probe* trials and memory-guided *Sequence* trials (cf. Supplemental Figure S1a, b). The mean preparation duration of preceding trials (previous, *n-1*, or two trials previously, *n-2*) did not vary depending on the serial position associated with the target sequence in any of the preparation durations of a current trial (*n*) (4 x 3 repeated-measures ANOVAs: Position x Preparation duration *n-1*, F (6, 108) = .88, p = .511, ηp^2 = .05; Position x Preparation duration *n-2*, F (6, 108) = 1.14, p = .344, ηp^2 = .06). Equally, analysis of the sequence production trials revealed that preparation duration of a current trial did not vary with the mean preparation duration of preceding trials (one-way repeated-measures ANOVAs: Preparation duration *n-1*, F (2, 36) = 2.53, p = .093, ηp^2 = .12; Preparation duration *n-2*, F (2, 36) = .36, p = .701, ηp^2 = .02). This demonstrates a balanced design in which the foreperiod length history up to two previous trials was unlikely to bias RT or error rates on the current trial.

Experiment 2 – Sequence timing. Procedures for Experiment 2 were identical to Experiment 1 except that the delay period was fixed at 1500 ms and participants were trained in associating three target sequences. Each featured a unique *Sequence* cue associated with one finger order instructed to be performed at three target IPIs: slow (800-800-800 ms), fast (400-400-400 ms) and irregular (400-1600-400 ms), comprising the three timing conditions. The timing manipulation was used to test the effect of temporal proximity and grouping on the pre-activation of movements during preparation. The relative compression and expansion of target IPIs by a scaling factor of 2 in the fast and irregular timing conditions relative to the baseline condition (long preparation duration and slow timing conditions) is in line with previous work on motor timing (Wang et al., 2018). Although participants were trained to produce specific IPI durations imposed by the target IPIs, relative timing, i.e., temporal IPI modulations relative to the baseline condition, was key to evaluating the influence of timing at the group and individual levels. Thus, relative timing was calculated offline from memory-guided *Sequence* trials (test phase) as each IPI duration (1^{st}, 2^{nd}, 3^{rd}) relative to the mean produced IPI duration in the baseline condition (in percent). Accordingly, relative temporal error, was defined as the mean absolute deviation from the target IPI per trial in percent.

In a *Sequence* trial, the *Go* cue remained on the screen for 3000 ms while in a *Probe* trial, the *Probe* cue for 1000 ms. This was followed by a fixation cross (1000 ms) and feedback (1000 ms) with a varying ITI of 500, 900 and 1300 ms. As a result, a *Sequence* trial was 6.5 min long and a *Probe* trial 4.5 min long. The participants underwent the same structure of training and test sessions as in Experiment 1. The timing conditions were equally matched to the number of all trial types in each block. Overall, in this experiment participants completed 2016 trials over 58 blocks.

Experiment 3 – Sequence timing and control movement. Procedures for Experiment 3 were identical to Experiment 1, except the introduction of additional *Probe* trials that cued the thumb. Thumb presses were not part of any target finger sequence. Thus, they served as a control condition to obtain reaction times and error rates for unplanned movements. Across each training stage, there were 60 *Probe* trials, while the test phase (30 blocks × 26 trials) contained 360 memory-guided *Sequence* trials (120 trials per timing condition), 360 *Probe* trials (30 trials per digit per

timing condition), and 60 thumb *Probe* trials (20 trials per timing condition). Overall, participants completed 1990 trials over 72 blocks, excluding the practice block.

Feedback. In all experiments, a points system was designed to reward fast initiation and accurate performance and avoid any performance drift in blocks with motor production from memory. To incentivize the participants to gain as many points as possible on each trial, we offered an extra monetary reward (£10) to those two with the highest total points. In *Sequence* trials, points (0-10) could be awarded based on three performance criteria: finger press accuracy, sequence initiation reaction time (RT), i.e., response from *Go* cue to the first press, and temporal error (deviation from the target IPIs). Points in each *Sequence* trial were the sum of the points for initiation RT and mean temporal error, multiplied by finger press accuracy points (0 or 1). If at least one incorrect press or an incorrect number of presses was recorded (< 4 or > 4), 0 points were given on that trial, regardless of initiation RT and temporal error. Points gained from the initiation RT component of the sequence, were defined by tolerance RT windows of 0-200, 200-360, 360-480, 480-560, 560-600 ms resulting in 5, 4, 3, 2 and 1 points, respectively. For late (> 600) responses, 0 points were given. Mean temporal error was calculated for each trial as deviation of presses from target timing in percent of the respective target IPI to account for the scalar variability of timing (Jazayeri and Shadlen 2010; Rakitin et al. 1998). Thresholds for mean absolute percentage deviation across all correct presses were set at 10, 20, 30, 40 and 50 % assigning 5, 4, 3, 2 and 1 points, respectively. Mean temporal error above 50 % resulted in 0 points.

Points (0-5) in each *Probe* trial were calculated based on finger press accuracy (0 or 1) and RT utilizing the same tolerance RT windows. In the case of an incorrect press or incorrect number of presses (< 1 or > 1), 0 points were given regardless of the RT length. The points were displayed on the screen after each *Probe* trial whilst after a *Sequence* trial they were presented above a schematic visual feedback.

Schematic feedback provided information on both finger press accuracy and temporal error performance only after each *Sequence* trial. An '*x*' or a '-' symbol was shown for every correct or incorrect press, respectively. For early presses, the respective symbol was displayed below the midline (target timing), while for late presses it was displayed above. For orientation, the lines above and below (upper and

lower border) corresponded to timing deviations as large as the target IPI itself (100%). Timing deviation was only shown for second, third and fourth presses of the sequence. The first symbol reflected the first press and was always positioned on the midline, representing the starting point of the sequence. Participants were instructed to adjust their performance by keeping the '*x*' symbols as close to the midline as possible. Deviation from the target onset (presented or assumed) rather than the interval timing encouraged participants to synchronise with the instructed sequences during training, however, may have contributed to a tendency to compress the overall sequence length during the memory-guided *Sequence* trials.

Participant exclusion criteria

In each experiment, mean finger error rate (percent error trials out of total trials) during sequence production from memory (memory-guided *Sequence* trials; test phase) above three standard deviations of the group mean performance was considered as outlier performance. This was to ensure that participants reached a comparable skill level in sequence production. Additionally, it allowed for a sufficient number of trials for RT analysis per participant, which included correct trials only. Data exclusion was blind to the individual *Probe* trial performance and, thus, independent of the measures analysed to test our hypotheses. In Experiment 1, the data of one participant was excluded who showed 53.1% finger error in the short, 54.7% in the intermediate and 53.9% in the long preparation duration conditions. Two participants' data sets were removed from Experiment 2, one with 25% finger error in the slow timing and 18.8% in the irregular timing conditions, whilst the other showed 44.5% finger error in the fast timing condition. The data of one participant was excluded from Experiment 3 due to 12.5% finger error in the fast timing condition. Overall, the data of 19 participants were analysed for Experiment 1, 18 participants for Experiment 2, and 18 participants for Experiment 3.

Data analysis

Data analyses were performed using custom written code in MATLAB (v9.2 R2017a, The MathWorks, Inc., Natick, Massachusetts, United States), and SPSS v22.0 (IBM Corp., Armonk, N.Y., USA).

Sequence planning. Median reaction time (RT; correct trials only) and mean error rate in *Probe* trials were used as dependent measures for assessing the availability of movements corresponding to different sequence positions during planning. First, we tested for the RT and error rate increases from 1st to 2nd, 2nd to 3rd and 3rd to 4th positions in each experiment. These were tested in the baseline condition common across the three experiments (long preparation duration and slow timing conditions). One-tailed paired samples t-tests were performed on the raw RTs and error rates, based on the one-sided hypothesis of an increase with position number. The position-dependent differences for error were further examined in the lower and upper RT quartiles to test for position-dependent increases of press error depending on response speed.

Second, to test for the interaction of factors Position (1st, 2nd, 3rd, 4th) and Preparation duration (short / 500 ms, intermediate / 1000 ms, long / 1500 ms) in *Probe* trials of Experiment 1, the raw RTs and error rates were submitted to two-way repeated measures ANOVAs. Using the same test, we assessed the interaction of the factors Position and sequence Timing (slow, fast, irregular) in Experiments 2 and 3. Significant interaction effects were investigated using planned repeated contrasts, to determine the changes relative to baseline that were driving the interaction. To evaluate the RT and error rate for the control movement (Experiment 3), we used two-tailed paired samples t-tests (control *vs* 4th position).

Third, we calculated the increase of RT and error rate for each probed position relative to the first position in each condition (in %) for each participant. This enabled us to quantify and visualise the relative position-dependent increases in each condition (Figure 2). Further, we calculated the average relative RT and error differences between adjacent positions (mean difference across 1st minus 2nd, 2nd minus 3rd, 3rd minus 4th) in the baseline condition for each participant as markers of the movements' pre-activation gradient size during sequence planning. One-way repeated measures ANOVAs in each experiment were used to assess modulations of the latter by the

experimental conditions (Preparation duration in Experiment 1 and Timing in Experiments 1 and 2). To test for the association between these measures and sequence performance (initiation RT of correct sequences, relative temporal error, and finger error rate) six one-tailed Pearson's correlation analyses were performed across experiments (N = 55). Further, a median split was calculated based on each performance measure for raw mean RTs and error rates for each position in the baseline condition. These were subjected to three mixed ANOVAs (Position × Group) to test for the position-dependent differences in movement availability during planning depending on performance (N = 55).

Finally, we looked at the percent of presses associated with the 1st, 2nd, 3rd, and 4th positions of the planned sequence in erroneous *Probe* trials, for each probed position separately (four one-way repeated measures ANOVAs; N = 55).

Sequence production. Only the memory-guided *Sequence* trials (test phase) were used for analysing the components of sequence production. First, relative timing (percent duration of each IPI relative to the mean produced IPI in the baseline condition) was subjected to a 3 x 3 repeated measures ANOVA, for each experiment, depending on IPI (1st, 2nd, 3rd) and Preparation duration (Experiment 1) or sequence Timing (Experiments 2 and 3). Finally, to evaluate the fluency and accuracy of sequence production, we calculated sequence initiation RT (online recording of *Go* cue to first press latency), relative temporal error (deviation from target IPI) and finger press error (percent trials with incorrect presses). These constituted the three performance measures to reflect skill in sequence execution and were analysed for each experiment separately in nine one-way repeated measures ANOVAs to assess modulations of skill by Preparation duration or Timing.

The error data of both *Probe* and *Sequence* trials were arcsine transformed (Winer et al. 1991) before they were submitted to the ANOVA models and t-tests due to violation of normality. Partial eta squared ratios and Cohen's *d* are reported as measures of effect sizes in the corresponding tests.

Results

Availability of movements during sequence planning is dependent on their position in the planned sequence

In all three experiments, participants were trained for two days to associate abstract visual cues with four-element finger sequences. They were instructed to produce the sequences with a particular temporal structure (Timing: slow, fast, irregular) following a brief preparation period (Preparation duration: short / 500ms, intermediate / 1000 ms, long / 1500 ms). In half of the trials in the test phase (Day 3), a *Probe* cue instructed participants to respond with the corresponding finger press as quickly and accurately as possible at the end of the planning phase (Figure 1c). This allowed us to probe the availability of movement associated with each position of the planned sequence (1^{st} - 4^{th}) for accurate and fast execution. Based on our previous neurophysiological findings (Kornysheva et al. 2019) in a similar task that showed a graded pre-activation of movements during planning according to their sequential position, we hypothesized that the behavioural availability of movements during planning will be position-dependent. Specifically, we predicted a significant increase in RT and error rate for probed movements from 1^{st} to 2^{nd} and 2^{nd} to 3^{rd} positions. Based on our neurophysiological results we did not expect an increase in movement availability from penultimate to final position (here: 3^{rd} to 4^{th}), but the latter has been previously observed in the context of a drawing sequence task in non-human primates (Averbeck et al. 2002). Additionally, we included probes for a control movement (Experiment 3) to reveal whether the movement associated with the last position of the planned sequence is more accurately and quickly selected and executed than a movement that is not part of the sequence. A higher behavioural availability of the last position movement would suggest that the sequence movements are more pre-activated, albeit to a different level, rather than activated and inhibited relative to a baseline movement. Position-dependent RT and press error increases were analysed from trials in the experimental condition which constituted the baseline in all three experiments (long preparation duration - 1500 ms - and slow timings).

Reaction times to movement probes. Figure 2a shows the percent RT increase relative to the RT for the movements associated with the first position, respectively (cf. Supplemental Figure S2a for raw RT values; Supplemental Table S1a for statistics).

Experiment 1 revealed a significant RT increase from 1st to 2nd position (paired samples t-test: t (18) = -7.45, p < .001, d = 1.32, one-tailed) but not from 2nd to 3rd position (t (18) = .05, p =.479, d = .01) or from 3rd to 4th position (t (18) = -.72, p = .241, d = .09). Experiment 2 replicated the RT results from Experiment 1 revealing a significant RT increase from 1st to 2nd position (t (17) = -6.45, p < .001, d = 1.60), but not from 2nd to 3rd (t (17) = -.63, p = .267, d = .16) or 3rd to 4th position (t (17) = -.25, p = .404, d = .05). Experiment 3 showed a significant RT increase from 1st to 2nd position (t (17) = -4.61, p < .001, d = 1.03) and, unlike the Experiments 1 and 2, also from 2nd to 3rd position (t (17) = -2.41, p = .014, d = .40). As in Experiments 1 and 2, the RT increase from 3rd to 4th position was not significant (t (17) = -.21, p = .417, d = .04). To further investigate whether the inconsistent mean RT increase for probes from 2nd to 3rd position would be resolved with higher power, a pooled analysis across the three experiments was performed (N = 55). This revealed a marginal RT increase from 2nd to 3rd position (t (54) = -1.55, p = .063, d = .15), suggesting that this overall increase was highly variable across subjects. Finally, the RT of the control movement was significantly higher than the movement associated with the last position (4th) of the planned sequence (paired samples t-test: t (17) = 3.04, p = .007, d = .86, two-tailed).

Across experiments, the present RT data shows that during sequence planning, correct finger presses associated with earlier positions in a sequence can be selected and executed quicker than those associated with later positions, suggesting a position-dependent pre-activation gradient. In particular, the latter can switch flexibly trial-by-trial, depending on which finger sequence is retrieved and planned in a particular trial. The data also suggests that the availability is modulated up to three positions ahead, with RT increases for later positions becoming less consistent across subjects. Finally, although the movement associated with the last position was the slowest to execute on average, it was still faster than a control movement not featuring in the planned sequence.

Error rates to movement probes. Figure 2b shows the percent press error increase relative to the error rates for the movements associated with the first position, respectively (cf. Supplemental Figure S2b for raw press error rates; Supplemental Table S1a for statistics). Experiment 1 revealed significant error increases from 1st to 2nd position (paired samples t-test: t (18) = -6.65, p <.001, d = 1.83, one-tailed) and

from 2nd to 3rd position (t (18) = -1.93, p = .035, d = .27), and no significant increase from 3rd to 4th position (t (18) = -1.24, p = .116, d = .21). Experiment 2 replicated the significant error increase from 1st to 2nd cf. position (t (17) = -5.51, p < .001, d = 1.57) and from 2nd to 3rd position (t (17) = -2.05, p = .029, d = .43). In contrast, the difference from 3rd to 4th position showed no significant increase, but an unexpected decrease of errors (t (17) = 2.60, p = .010, d = .54). Experiment 3 again replicated the significant error increases from 1st to 2nd position (t (17) = -7.77, p < .001, d = 1.83) and from 2nd to 3rd position (t (17) = -1.88, p = .039, d = .58), whilst there was no significant difference between the 3rd and 4th positions (t (17) = .77, p = .227, d = .20). The control movement did not show a significant increase in errors compared to the 4th position (paired samples t-test: t (17) = -.81, p = .430, d = .26, two-tailed).

The error rate data from all experiments indicate that during sequence planning, movement probes associated with earlier positions in a sequence are more likely to lead to correct finger presses than those associated with later positions, which are more prone to erroneous finger presses. Like RT, error rate data points to a position-dependent pre-activation gradient for movements associated with the first three positions in the sequence, but respective error increases between the first 3 positions appear to be more pronounced and consistent across participants, particularly for increases from 2nd to 3rd position. Further, it shows that movements associated with the last (4th) position are equally error prone as a sequence irrelevant control movement, although the former is still faster to execute when selected correctly. Taken together, our findings advocate the presence of a preparatory pre-activation gradient which renders movements associated with later sequence positions less available for correct selection and fast execution. They point to the planning of up to three constituent movements in advance within a brief preparation period and retrieval from memory. This pre-activation level does not increase linearly with movement positions but falls off and becomes more variable across participants for movements associated with later positions. The variability of the gradient during planning across participants is examined below in the context of skilled performance.

Position-dependent differences in movement availability are modulated by preparation duration, not timing

Next, we examined whether the position-dependent availability for correct movement selection and fast execution during planning is modulated by the time to prepare a sequence, or the planned sequence timing.

Preparation duration. According to our accuracy hypothesis, a more accurate plan of the sequence progressively established across preparation durations of 500-1500 ms would lead to an expansion of the pre-activation gradient (Kornysheva et al. 2019). In Experiment 1 (cf. Supplemental Table S1b for statistics), we found a large significant interaction of Position and Preparation duration for error rates (4 × 3 repeated measures ANOVA of raw press error rates: F (6, 108) = 3.35, p = .005, ηp^2 = .16). The latter was driven by a significant error rate increase for 2nd relative to 1st sequence positions with longer preparation duration (500 *vs* 1500 ms preparation duration, F (1, 18) = 15.89, p = .001, ηp^2 = .47). This contrast was also significant for RTs (F (1, 18) = 5.89, p = .026, ηp^2 = .25), although the interaction between Position and Preparation duration for RTs did not reach significance (4 × 3 repeated measures ANOVA of raw RTs: F (6, 108) = 2.07, p = .063, ηp^2 = .10). This shows that the increase in RT and error rate from 1st to 2nd position became more pronounced with longer preparation durations, an effect which drove the significant interaction.

Importantly, both the relative RT and error differences became more pronounced with longer preparation duration conditions (one-way repeated measures ANOVA of: Relative RT differences - Experiment 1, F (2, 36) = 4.38, p = .020, ηp^2 = .20; Relative error differences - Experiment 1, F (2, 36) = 3.46, p = .042, ηp^2 = .16; cf. Supplemental Table S1c for statistics). Thus, more time to prepare the sequence made the probed movements associated with later positions less available for correct selection and fast execution, and vice versa. This suggests that the pre-activation state of the planned movements became more differentiated according to position and the pre-activation gradient expanded across the sequence retrieval and preparation period.

Timing. According to the timing hypothesis, movements in a sequence that are closer in time should have more similar levels of pre-activation, and vice versa, leading to a contraction and expansion of the pre-activation gradient for each action. Contrary to the timing hypothesis, the interaction between Position and Timing (cf. Supplemental Table S1b for statistics) did not reach significance, neither for RTs, nor

for error rate increases (4 × 3 repeated measures ANOVA of: Raw RTs - Experiment 2, F (3.27, 55.54) = 2.30, p = .082, ηp^2 = .12, Greenhouse-Geisser corrected, χ^2 (20) = 42.61, p = .003; Experiment 3, F (3.87, 65.79) = .98, p = .426, ηp^2 = .05, Greenhouse-Geisser corrected, χ^2 (20) = 34.06, p = .028; Raw error rates - Experiment 2, F (6, 102) = 1.86, p = .095, ηp^2 = .10; Experiment 3, F (6, 102) = 1.02, p = .416, ηp^2 = .06). This finding was corroborated by an absent effect of Timing on either the relative RT or the relative error differences (one-way repeated measures ANOVA of: Relative RT differences - Experiment 2, F (1.48, 25.23) = .68, p = .475, ηp^2 = .04, Greenhouse-Geisser corrected, χ^2 (2) = 6.83, p = .033; Experiment 3, F (2, 34) = 1.92, p = .162, ηp^2 = .10; Relative error differences - Experiment 2, F (2, 34) = .00, p = .999, ηp^2 = .00; Experiment 3, F (1.27, 21.52) = 1.50, p = .241, ηp^2 = .08, Greenhouse-Geisser corrected, χ^2 (2) = 13.87, p = .001; cf. Supplemental Table S1c for statistics). We investigated whether the results may be contaminated by participants that considerably deviated in their relative temporal error performance (memory-guided *Sequence* trials; test phase). Therefore, we performed the same analyses after removing outlier participants that showed little modulation of timing during sequence production (cf. Supplemental Figure S3). However, without these outliers, the interaction between Position and Timing was still not significant. Overall, these analyses indicate that preparing a sequence that is twice as fast, or temporally grouped, did not impact the position-dependent pre-activation gradient of movements during sequence planning.

Position-dependent modulation of press error during planning is revealed through fast responses to probes

Next, we sought to determine whether the characteristic position-dependent increases in press errors in *Probe* trials were driven by automatic responses to *Probe* cues, or by deliberate movement selection. To investigate this question, we analysed the position-dependent error increases for the first versus last RT distribution quartiles in each participant (baseline condition: 1500 ms preparation duration and slow timing). Figure 2c (cf. Supplemental Table S1a for statistics) illustrates the press error increases relative to the first position for fast and slow RT quartiles. In fast response trials, we found significant error increases up to the 3rd position in Experiments 1 and 3 (paired samples t-tests: Experiment 1, 1st to 2nd position, t (18) = -6.54, p <.001, d = .54, one-tailed; 2nd to 3rd position, t (18) = -2.87, p = .005, d = .40; 3rd to 4th position, t (18) = 3.12, p = .003, d = .48; Experiment 3, 1st to 2nd position, t (17) = -6.59, p <.001, d = 2.12; 2nd to 3rd position, t (17) = -1.82, p = .043, d = .55; 3rd to 4th position, t (17) = 1.63, p = .061, d = .35) and up to the 2nd position in Experiment 2 (1st to 2nd position, t (17) = -6.99, p <.001, d = 1.57; 2nd to 3rd position, t (17) = -.93, p = .184, d = .43; 3rd to 4th position, t (17) = 1.43, p = .085, d = .54). In contrast, in slow response trials, errors did not increase significantly with position (Experiment 1, 1st to 2nd position, t (18) = .59, p = .281, d = .20); 2nd to 3rd position, t (18) = -.55, p = .294, d = .19; 3rd to 4th position, t (18) = -.60, p = .277, d = .16; Experiment 2, 1st to 2nd position, t (17) = -.57, p = .290, d = .20; 2nd to 3rd position, (t (17) = .00, p = .500, d = .00; 3rd to 4th position, t (17) = .57, p = .290, d = .20; Experiment 3, 1st to 2nd position, t (17) = -.34, p = .368, d = .08; 2nd to 3rd position, t (17) = .15, p = .443, d = .04; 3rd to 4th position, t (17) = .54, p = .299, d = .17). The control movement did not show more errors than the 4th position in either fast or slow RT as in the main results (Fast RTs, t (17) = -.95, p = .353, d = .28, two-tailed; Slow RTs, t (17) = .10, p = .922, d = .03).

These results demonstrate that the position-dependent availability of movements for correct selection following movement *Probe* cues is driven by automatic responses rather than by a cognitive selection process.

Incorrect presses to movement probes during planning are dominated by the movement in the first sequence position

We investigated whether incorrect presses in Probe trials were associated with specific positions of the planned sequence on that trial (Figure 3; cf. Supplemental Table S2 for statistics). This was undertaken for each probed position separately and across all three experiments. Results for 1st position (Figure 3, upper left) did not yield significant differences among the press rate for 2nd, 3rd, and 4th positions (one-way repeated measures ANOVA: F (2, 108) = .63, p = .535, ηp^2 = .01). In contrast, probing the movements associated with 2nd, 3rd, and 4th positions revealed that participants consistently selected the 1st position more frequently. Specifically, when the 2nd position was probed (Figure 3, upper right), there was a significant difference among 1st, 3rd, and 4th erroneously pressed positions (F (1.38, 74.36) = 84.70, p < .001, ηp^2 = .61, Greenhouse-Geisser corrected, χ^2 (2) = 31.92, p < .001; 1st position higher than 3rd position, p < .001; 1st position higher than 4th position, p < .001; 3rd position higher than 4th position, p = .007). Similarly, the press rate for the 1st position when the 3rd position was probed (Figure 3, lower left) was higher than the 2nd and 4th pressed positions (F (1.34, 72.50) = 84.90, p < .001, ηp^2 = .61, Greenhouse-Geisser corrected, χ^2 (2) = 35.65, p < .001; 1st position higher than 2nd position, p < .001; 1st position higher than 4th position, p < .001; 2nd position marginally lower than 4th position, p = .069). The 4th probed position (Figure 3, lower right) produced higher 1st position presses (F (1.54, 83.34) = 42.95, p < .001, ηp^2 = .44, Greenhouse-Geisser corrected, χ^2 (2) = 18.60, p < .001; 1st position higher than 2nd position, p < .001; 1st position higher than 3rd position, p < .001; 2nd position not significantly higher than 3rd position, p = 1.000).

The distribution of erroneous presses shows that the movement availability was highly biased towards the production of the movement in the first position in each respective sequence upon retrieval and planning of the cued sequence.

Greater position-dependent differences in movement availability during planning predict better performance

Position-dependent pre-activation differences between sequential movement patterns during planning have been shown to predict the participants' subsequent performance accuracy (Kornysheva et al. 2019). Specifically, the distance (i.e.,

672 difference) between the neural pattern probabilities of consecutive movements during
673 planning predicted more skilled sequence execution. Accordingly, we predicted that
674 larger position-dependent differences in availability of movements for correct selection
675 and fast execution during planning would correlate with a more skilled performance
676 during sequence execution. Position-dependent differences in availability of
677 movements was considered a proxy measure for the pre-activation gradient size (cf.
678 *relative RT and error differences* in Data analysis, Methods). We took faster initiation
679 of correct sequences after the *Go* cue, as well as reduced relative temporal errors and
680 finger errors as markers of a more skilled performance. Correlation analyses were
681 performed on group data (N = 55) obtained from trials in the baseline condition present
682 in all experiments (long preparation duration and slow timing conditions; Figure 4a, b;
683 cf. Supplemental Figure S4 for raw RT and error differences; Supplemental Table S3a
684 for statistics). Results showed that participants with larger relative RT and error
685 differences during planning initiated correct sequences faster (Relative RT differences:
686 r = -.39, p = .002; Relative error differences: r = -.54, p < .001, one-tailed). Larger
687 relative RT differences during planning were also correlated with lower relative
688 temporal error (r = -.35, p = .005). This association did not hold up for the relative error
689 differences (r = -.05, p = .356). Thus, the latter may be a less sensitive predictor for
690 temporal accuracy than the relative RT differences. In contrast to our predictions, we
691 did not find an association with finger error (Relative RT differences: r = .08, p = .273;
692 Relative error differences: r = .12, p = .196). This was likely due to ceiling effects in
693 finger press accuracy performance attributable to the limited number of trained finger
694 sequences.

695 To inspect the position-dependent slopes in movement availability based on
696 sequence performance, we performed median split-based initiation RT, relative
697 temporal error, and finger error (Figure 4 insets; cf. Supplemental Table S3b for
698 statistics). Participants with faster initiation RTs exhibited larger position-dependent
699 RT differences (Figure 4a, inset) compared to those with slower initiation RTs (mixed
700 ANOVA with median split of initiation RT: Main effect of Group, F (1, 53) = 33.63, p
701 < .001, ηp^2 = .39; Position × Group, F (3, 159) = 5.70, p = .001, ηp^2 = .10). Equally, the
702 position-dependent press error differences (Figure 4b, inset) were steeper for
703 participants with fast initiation RTs (mixed ANOVA with median split of initiation RT:

Main effect of Group, F (1, 53) = 10.77, p = .002, ηp^2 = .17; Position × Group, F (3, 159) = 3.90, p = .010, ηp^2 = .07). Median splits by relative temporal error or finger error did not show differences in movement availability during planning, confirming further that this relationship is either more subtle (temporal error) or absent (finger error).

Together, these analyses show that behavioural markers of a more expanded pre-activation gradient can predict faster initiation of correct finger sequences and improved relative temporal, but not finger accuracy during production.

Next, we conducted a series of extended analyses focussing on sequence production. These additional analyses examined whether participants – on average – produced the sequences from memory with accurate relative timing, and whether preparation time and sequence timing conditions changed performance, i.e., speed of correct sequence initiation, as well as temporal and finger accuracy.

Participants produced sequences from memory with correct relative timing

Participants were trained to either retain the same (Experiment 1) or consistently modulate (Experiments 2 and 3) the relative timing during sequence production across sequence conditions. On average, participants produced the sequences with timing relative to the target inter-press-intervals (IPI) (Figure 5a; cf. Supplemental Table S4 for statistics; Supplemental Figure S5 for mean absolute press timing per trial).

The mean relative timing of finger presses in Experiment 1 was nearly identical across preparation duration conditions (Figure 5a, left). Nevertheless, we detected a small but significant interaction between IPI and Preparation duration (3 × 3 repeated measures ANOVA: F (4, 72) = 2.53, p = .048, ηp^2 = .12), explained by IPI modulations of 9 ms across conditions. Post-hoc comparisons (Bonferroni-corrected for nine tests) revealed a significant shortening of the 1st interval in the short preparation duration (p = .002) and of the 1st (p = .002) and 3rd (p = .004) intervals in the intermediate compared to the long preparation duration. This shows that there was a tendency to slightly compress the 1st and 3rd intervals with shorter preparation time. If there were a timing confound on sequence planning duration in Experiment 1, the timing effect should have been vastly amplified by the experimental modulation of timing requiring

the doubling or halving of IPIs in Experiments 2 and 3. However, we did not observe any strong and consistent effect of the latter on sequence planning.

Experiment 2 (Figure 5a, middle) showed a large significant interaction of IPI and Timing (3 × 3 repeated measures ANOVA: F (1.26, 21.42) = 59.49, p < .001, ηp^2= .78, Greenhouse-Geisser corrected, χ^2 (9) = 97.83, p < .001), in line with the task instructions. The pairwise comparisons (Bonferroni-corrected for nine tests) of the produced IPIs confirmed that the participants modulated their relative timing according to the target IPI structure. In accordance with the cued sequence, the 1st IPI was significantly longer in the slow than in the fast (p < .001) and the irregular timing conditions (p < .001), while it did not differ in the fast *vs* irregular timing conditions (p = 1.000). The 2nd IPI length increased slightly, yet proportionally for both the slow and fast timing conditions, retaining the significant difference (p < .001), and doubled in length in the irregular relative to the slow timing condition (p < .001). The 3rd IPI exhibited a very similar profile to the 1st IPI (slow *vs* fast, p < .001; slow *vs* irregular, p < .001), but its length decreased slightly in the fast compared to the irregular timing condition (p = .027). Experiment 3 (Figure 5a, right) replicated the findings of Experiment 2 showing a significant interaction of IPI and Timing (3 × 3 repeated measures ANOVA: F (1.56, 26.49) = 17.37, p < .001, ηp^2 = .51, Greenhouse-Geisser corrected, χ^2 (9) = 61.31, p < .001). Again, post-hoc pairwise comparisons (Bonferroni-corrected for nine tests) confirmed that the 1st IPI in the slow timing was longer than that in the fast (p = .001) and irregular (p = .003) timing conditions, while no difference was found between the fast and irregular timing conditions (p = 1.000). The 2nd IPI was significantly longer in the slow compared to the fast timing condition (p = .001), but shorter compared to the irregular timing condition (p = .005). Similarly, the 2nd IPI in the fast timing was half as long than in the irregular timing condition (p < .001). The 3rd IPI was twice as long in the slow compared to the fast timing condition (p < .001). It did not show a significant shortening for the irregular timing when compared to the slow timing condition (p = 1.000) and showed only a marginally significant difference between the fast and irregular timing conditions (p = .096).

Overall, these results demonstrate that, on average, participants produced the finger sequences from memory with accurate relative timing across conditions.

Longer preparation durations shortened initiation of correct sequences

We found a significant difference in sequence initiation RT with Preparation duration (one-way repeated measures ANOVA: Experiment 1, F (1.38, 24.88) = 52.81, p < .001, ηp^2 = .75, Greenhouse-Geisser corrected, χ^2 (2) = 10.07, p = .006) (Figure 5b, left; cf. Supplemental Table S4 for statistics). Pairwise comparisons (Bonferroni-corrected for three tests) confirmed that sequence initiation RT was significantly faster for the intermediate (1000 ms) and long (1500 ms) preparation duration than following a short (500 ms) preparation duration (intermediate *vs* short, p < .001; long *vs* short, p < .001). Further, sequence initiation RT following a long preparation duration was significantly faster as compared to the intermediate preparation duration (p = .005). In experiments with single movements the effect of variable preparation duration on RT is known as the foreperiod effect (Foley 1959; Vallesi et al. 2007). It can be accounted for by generic motor preparedness due to heightened temporal expectation (hazard rate) for longer preparation durations (Bueti et al. 2010), and includes carry-over effects across trials (Langner et al. 2018; Steinborn and Langner 2012) (cf. Supplemental Figure S6 for preparation duration effects of preceding trials in Experiment 1). However, the effect on initiation RT reported here cannot be attributed to *general* temporal preparedness alone. In contrast to classical foreperiod paradigms the current paradigm involves a *Sequence* cue at the start of the foreperiod, instead of a neutral warning signal. Therefore, a facilitation of initiation RT will reflect the state of sequence preparedness that increases with longer durations (Ariani and Diedrichsen 2019; Sternberg et al. 1978), not just non-specific effects of temporal expectation.

There was no main effect of Timing on sequence initiation RT in Experiment 2 (one-way repeated measures ANOVA: F (1.41, 23.92) = 1.70, p = .207, ηp^2 = .09, Greenhouse-Geisser corrected, χ^2 (2) = 8.76, p = .013), but a main effect of Timing in Experiment 3 (one-way repeated measures ANOVA: F (1.29, 21.99) = 11.59, p = .001, ηp^2 = .41, Greenhouse-Geisser corrected, χ^2 (2) = 12.63, p = .002). As explained by pairwise comparisons (Bonferroni-corrected for three tests), participants in Experiment 3 were slower at initiating a sequence of slow timing when compared to fast timing (p = .006) and irregular timing (p = .010). There was no difference in initiation RT between the fast and the irregular timing conditions (p = .118). This effect was not consistent across Experiments 2 and 3, but present at the mean level in both experiments. This

implies that sequences with a slow isochronous timing structure were less prepared for initiation following a *Go* cue compared to sequences that started with two presses in short succession (fast and irregular timing structures), which may be more prone to a rushed initiation.

Sequences involving irregular inter-press-intervals were produced with less accurate timing

Next, we established whether preparation duration (Experiment 1) and sequence timing (Experiments 2 and 3) modulated relative temporal error during sequence production (Figure 5b, middle; cf. Supplemental Table S4 for statistics). In Experiment 1, mean relative temporal error did not differ among the three preparation duration conditions (one-way repeated measures ANOVA: F (2, 36) = .11, p = .901, ηp^2 = .01). Here relative temporal performance may have been compensated in the short preparation duration condition by slower initiation RT (cf. above). In Experiment 2, there was a significant effect of Timing (one-way repeated measures ANOVA: F (2, 34) = 28.23, p < .001, ηp^2 = .62). Pairwise comparisons (Bonferroni-corrected for three tests) revealed that participants performed at a lower relative temporal error when producing a sequence of slow timing compared to irregular timing (p < .001) and a sequence of fast timing compared to irregular timing (p < .001), while there was no difference between sequences in the slow *vs* fast timing conditions (p = 1.000). Experiment 3 replicated the main effect of Timing (one-way repeated measures ANOVA: F (1.45, 24.72) = 7.06, p = .007, ηp^2 = .29, Greenhouse-Geisser corrected, χ^2 (2) = 7.53, p = .023). In line with the findings of Experiment 2, there were less relative temporal errors in the slow timing (p = .049) and fast timing (p =. 008) conditions when compared to the irregular timing condition. Again, there was no significant difference in relative temporal performance between the two isochronous conditions (slow *vs* fast, p = 1.000). In sum, the production of sequences which consisted of non-isochronous IPIs (irregular timing condition) as opposed to equal IPI lengths (isochronous timing conditions; slow, fast) were associated with decreased relative temporal accuracy.

Finger press accuracy in sequences produced from memory was matched across conditions

In the test phase, participants produced finger press sequences entirely from memory. Neither Preparation duration (one-way repeated measures ANOVA: Experiment 1, F (2, 36) = .23, p = .795, ηp^2 = .01), nor Timing (one-way repeated measures ANOVA: Experiment 2, F (2, 34) = .02, p = .984, ηp^2 = .00; Experiment 3, F (2, 34) = .96, p = .394, ηp^2 = .05) affected finger error during sequence production (Figure 5b, right; cf. Supplemental Table S4 for statistics). This means that participants prepared the finger order of cued sequences with the same accuracy, regardless of the preparation time or temporal structure of the planned sequence. Note that finger error in sequence production was higher in Experiment 1 than in Experiments 2 and 3. This is likely due to Experiment 1 involving sequences of two different finger sequences on a trial-by-trial basis, whereas Experiments 2 and 3 involved the same finger sequence performed with different timing.

Discussion

Sequence planning is central to skilled action control, however its content and structure is poorly understood (Bullock 2004; Remington et al. 2018). Neurophysiological findings have demonstrated that a trained movement sequence is pre-planned by establishing a competitive pre-activation gradient of movement patterns according to their serial position, and that the quality of this neural pattern during planning predicts subsequent performance (Averbeck et al. 2002; Basu and Murthy 2020; Kornysheva et al. 2019; Pinet et al. 2019). Here we report a putative behavioural marker of this competitive pre-activation gradient. During a short retrieval and preparation period, we measured the behavioural availability of each constituent movement of the planned sequence for accurate and fast production. Our findings show that behavioural availability is dependent on the sequence position the respective movements are associated with, mirroring the pre-activation gradient observed in neurophysiological studies (Averbeck et al. 2002; Kornysheva et al. 2019) as predicted by competitive queuing (CQ) models (Bullock 2004; Burgess and Hitch 1999; Hartley et al. 2016; Hartley and Houghton 1996). Critically, a stronger differentiation between the state of movements assigned to consecutive sequence positions correlated with markers of skilled production – the speed of correct sequence initiation and the temporal production accuracy. In contrast, the latter did not reliably

reflect the sequence production speed, or the inter-press-interval pattern of the planned sequence.

Sequence planning markedly contrasts with mechanisms for non-sequential movement planning involving multiple movement options: In the latter, a cued set of possible movements triggers equal activity increase in cortical populations tuned to the respective movements, and the preparatory competition is only resolved once a cue specifies the target movement (Cisek and Kalaska 2005). In contrast, sequence planning established a fine-tuned gradient of movement pre-activations, with the latter switching flexibly on a trial-by-trial basis, in line with the retrieved sequence. Notably, movements that were part of the planned sequence were executed faster than a control movement which was not part of the retrieved sequence (Figure 2a, right). This suggests that all constituent movements were concurrently pre-activated above a passive baseline, albeit to a different degree depending on their position in the planned sequence.

Our study provides a measure of the competitive state of constituent movements *prior to* sequence production. This is complementary to previous behavioural work which supports the presence of competitive queuing of sequence presses *during* production, such as accuracy and RT curves obtained from sequence execution (Rhodes et al. 2004; Verwey and Abrahamse 2012), or on-the-fly movement planning following sequence initiation, assessed behaviourally (Behmer and Crump 2017) and through measures of cortico-spinal excitability (Behmer et al. 2018). Gilbert and colleagues have employed a paradigm at the interface between sequence preparation and production to characterize the competitive queuing profile of the respective sequential movements – silent rehearsal (Gilbert et al. 2017). Here participants were asked to listen to sequences of spoken digits and silently rehearse the items during a retention interval. They received explicit instructions to rehearse the sequence at the same pace as active production. After an unpredictable delay, a tone prompted the report of an item being rehearsed at that moment and revealed graded overlapping probabilities of neighbouring items, suggesting potential CQ during internal rehearsal. In contrast to the latter study, our paradigm did not enable active rehearsal during preparation: First, our participants retrieved the sequence entirely from memory without a sensory instruction period which might have facilitated active

entrainment with the sequence prior to planning. Second, the period for sequence retrieval and planning was comparatively brief (ranging from 500 to 1500 ms after *Sequence* cue onset) and not sufficient to cycle through the full sequence at the rate participants employed for active production. In addition, if the observed CQ gradient were somehow driven by silent rehearsal at the target rate, it would have been more pronounced for the fast sequences, as more of the planned sequence could fit into the preparation phase. However, there was no significant difference between relative availability of probed movements for fast and slow sequences.

Whilst active motor rehearsal at scale during the short preparation phase is unlikely, an alternative *serial* preparation mechanism may be related to rapid sequence replay. The latter has been observed in the hippocampus during navigation tasks (Ólafsdóttir et al. 2018) and perceptual sequence encoding (Liu et al. 2019), as well as in the motor cortex in the context of motor sequence learning tasks (Eichenlaub et al. 2020). Replay has been shown to involve fast sweeps through the neural patterns associated with the sequence during wakeful rest and planning (preplay) (Dragoi and Tonegawa 2011; Drieu and Zugaro 2019; Jafarpour et al. 2014; Ólafsdóttir et al. 2018), and is characterized by a multifold temporal compression (Eichenlaub et al. 2020; Kurth-Nelson et al. 2016; Liu et al. 2019; Michelmann et al. 2019). How replay could translate into a parallel pre-activation of serial movements reported here is uncertain. One possibility is that serial sweeps during motor sequence planning involve fast repeated replay fragments (Davidson et al. 2009; Michelmann et al. 2019) of different length during preparation, starting with the first elements – e.g. 1^{st}-2^{nd}-3^{rd}, 1^{st}-2^{nd}, 1^{st}, 1^{st}-2^{nd}-3^{rd}-4^{th}, 1^{st}-2^{nd} etc. This would produce an overall bias towards the pre-activation of earlier rather than later parts of the planned sequence. This, in turn, may be translated into a cumulative ramping activity for each constituent movement by a separate downstream neuronal mechanism during the preparation period (Cisek and Kalaska 2005; Li et al. 2016). Analysis of the 'sequenceness' of the corresponding neural patterns (Eichenlaub et al. 2020; Liu et al. 2019) during preparation should shed light on the presence of preplay and its possible relationship to the competitive pre-activation of movements during planning (Kornysheva et al. 2019).

Characteristic differences in press error rate to movement probes were revealed through faster rather than slower responses after the *Probe* cue (Figure 2c).

This suggests that the competitive pre-activation gradient established during the short phase of sequence retrieval and planning is driven by a rapid automatic process and is not a result of slow deliberation or higher-level decision making. Contrary to a prominent account of sequence learning (Krakauer and Mazzoni 2011; Wong and Krakauer 2019), we suggest that the reported behavioural differences in sequence press availability reflect mechanisms of rapid and automatic planning for the production of discrete motor sequences from memory.

Remarkably, longer preparation reinforced the competitive pre-activation making responses to movement probes associated with later sequence positions even slower and more inaccurate relative to those associated with earlier positions. This is counterintuitive in the context of single movement performance gains from longer foreperiod durations (Niemi and Näätänen 1981). Here, a pure foreperiod effect would dictate general benefits for RT and error rate with longer preparation durations (Steinborn et al. 2008). In contrast, we found relative benefits and costs of the latter to be position-dependent. The reported differences in movement availability became more striking the longer time participants had to prepare, e.g., the error rate for probed movements associated with later positions increased further with longer foreperiods – these movements became even harder to retrieve. The pre-activation gradient expansion with longer preparation suggests a dynamic refinement of the plan for sequence production during retrieval and planning. We propose that the primacy gradient (Grossberg 1978a, 1978b) in the parallel planning layer of CQ models expands dynamically during each sequence preparation phase enhancing the organisation of sequential movements with preparation time.

Furthermore, participants exhibiting more pronounced differences in availability of movements associated with neighbouring sequence positions during planning exhibited both faster initiation times and a more accurate temporal execution of the sequence after the *Go* cue, particularly when looking at position-dependent differences in RT. These findings strengthen the interpretation that an ordered competitive pre-activation of movements during planning pre-empts subsequent fluency and temporal accuracy of the sequence (Kornysheva et al. 2019). The individual differences in planning are likely driven by differences in sequence learning,

957 which are associated with an expansion of the “planning horizon” with practice (Ariani
958 et al. 2020).
959 Yet, we did not replicate the association of the planning gradient with finger error
960 probability found in the latter study. This may be due to a smaller pool of timing and
961 finger order sequences that the participants had to learn relative to the previous
962 paradigm, and the presence of only one finger order (paired with different sequence
963 timings) in Experiments 2 and 3. This facilitated finger accuracy to reach ceiling levels
964 in a substantial number of participants. Future experiments should resolve an
965 association with finger accuracy through the inclusion of a larger pool of trained
966 sequences to provoke more frequent finger errors. Alternatively, reaching, drawing or
967 force production tasks would allow to quantify more fine-grained deviations from target
968 at overall high ordinal accuracy levels of sequence production.
969 In contrast to preparation duration, doubling the speed of sequence production
970 did not change the relative behavioural availability of sequential movements during
971 planning. This suggests invariance of the pre-activation gradient across sequences
972 produced at different time scales. This transfer across speed profiles is in line with the
973 presence of flexible motor timing and temporal scaling in dynamic neuronal
974 populations (Goudar and Buonomano 2018; Wang et al. 2018). Here the assumption
975 is that a separate neural process controls the speed of a sequence during execution,
976 e.g. through the strength of an external input to the network involved in the generation
977 of timed behaviour (Wang et al. 2018). We found that preparing a sequence of the
978 same length with an irregular compared to an isochronous interval structure was
979 associated with a slight tendency for a dampened CQ gradient during sequence
980 planning. However, this non-significant trend is unlikely to be the effect of temporal
981 grouping, as the irregular interval sequence was characterized by a significant
982 increase in temporal interval production error (Figure 5b, middle), associated with
983 timing complexity – the sequencing of two different (non-isochronous) constituent
984 temporal intervals rather than just one (isochronous). Instead, we hypothesize that
985 longer preparation time (above 1500 ms) would have benefitted the participants and
986 enhanced the relative pre-activation gradient, in line with Experiment 1, facilitating the
987 formation of a more accurate plan for this more temporally complex sequence.

Our empirical data on the pre-ordering of sequential movements does not support the integration of movement order with movement timing prior to sequence execution. The weighting of the availability of each movement appears to be entirely driven by its position in the planned sequence and correlated with the fluency of correct sequence initiation. Given that participants could on average correctly modulate the relative timing of the sequences, a separate preparation process for the speed and timing of the respective sequence must be assumed. The latter may take place concurrently or at different time points during preparation (Bortoletto et al. 2011; Bortoletto and Cunnington 2010; Maslovat et al. 2018). In previous work, we proposed a drift-diffusion based model which contains input from separate modules that activate movement order and timing (Kornysheva et al. 2013). This model was based on behavioural sequence learning data demonstrating that sequence timing is encoded independently of the movement order, but requires multiplicative, rather than additive integration with each movement. This enables trained sequence timing to be transferred to new sequences, but only after the movement order has been acquired, reconciling previous experimental findings (Kornysheva and Diedrichsen 2014; O'Reilly et al. 2008; Shin and Ivry 2003; Ullén and Bengtsson 2003; Zeid and Bullock 2019).

Recently, Zeid and Bullock proposed how such plans may be generated in the context of CQ models (Zeid and Bullock 2019). The authors propose that two separate CQ modules could operate in parallel - one controlling the item order and the other controlling the sequence of inter-press-intervals that define a rhythmic pattern, including separate parallel planning and competitive choice layers. While this model is in line with neurophysiological and imaging evidence for a separate control of timing for sequence generation (Bengtsson et al. 2004, 2005; Crowe et al. 2014; Friston and Buzsáki 2016; Kornysheva and Diedrichsen 2014; Merchant et al. 2013), empirical support for timing being implemented via a CQ process for temporal intervals is still lacking. Behavioural paradigms are unlikely to be valuable in this context, as it is impossible to probe the planning of inter-press-interval sequences decoupled from the effector. However neurophysiological recordings in monkeys and humans may shed further light on the organisation of interval patterns prior to production: If temporal intervals in a sequence are competitively queued, we should expect neuronal

populations preferentially tuned to temporal intervals of different durations, e.g. as found in the medial premotor cortex (Crowe et al. 2014; Merchant et al. 2013), to be pre-activated in parallel during planning according to their respective position in the sequence, and transfer across effectors.

Alternatively, timing of discrete movements in a sequence may be controlled during execution only through the acquired cyclical dynamics of neuronal population activity. Specifically, isochronous sequences involving the same movement have been associated with circular population trajectories where each interval cycle is shifted forward along a sequence position or "tapping manifold" resulting in a helical population trajectory (Balasubramaniam et al. 2020; Russo et al. 2020). Here the interval duration has been linked to the amplitude size of the trajectory loops thus controlling the speed of isochronous tapping sequences. The sequence position or "tapping manifold" may be the readout of a competitive queuing process and thus serve as a potential interface between position, interval, and movement identity. However, it remains unclear whether such a cyclical procession of population activity is also utilised for the production of sequences with non-isochronous intervals and sequences involving multiple movements.

Conclusions

In sum, our findings indicate that the behavioural availability of movements during a brief period of retrieval and planning reflects the subsequent movement order, such that movements associated with later positions are less available for fast and accurate execution. Crucially, the competitive state of the movements appears to be invariant to the exact timing of the sequence. Instead, it is dynamically established during sequence planning and predicts the individual's subsequent sequence production fluency and accuracy. The current behavioural paradigm could provide a straightforward and cost-effective way to assess the organisation of movements during sequence planning across trials in individual participants, in addition to neurophysiological approaches requiring access to neuroimaging, electrophysiology and computational resources for advanced neural pattern analysis (Averbeck et al. 2002; Kornysheva et al. 2019). This behavioural readout of the state of movements before execution may serve to advance our understanding of the neural processes

associated with disorders affecting the fluent production of motor sequences, such as stuttering, dyspraxia, and task-dependent dystonia (Craig-McQuaide et al. 2014; Howell 2007; Ingham et al. 2018; Miller 1988; Sadnicka et al. 2018).

Supplemental Figure S1: https://doi.org/10.6084/m9.figshare.13688131
Supplemental Figure S2: https://doi.org/10.6084/m9.figshare.13227953
Supplemental Figure S3: https://doi.org/10.6084/m9.figshare.13168514
Supplemental Figure S4: https://doi.org/10.6084/m9.figshare.13168628
Supplemental Figure S5: https://doi.org/10.6084/m9.figshare.13168649
Supplemental Figure S6: https://doi.org/10.6084/m9.figshare.13675330
Supplemental Table S1: https://doi.org/10.6084/m9.figshare.13673605
Supplemental Table S2: https://doi.org/10.6084/m9.figshare.13673668
Supplemental Table S3: https://doi.org/10.6084/m9.figshare.13673734
Supplemental Table S4: https://doi.org/10.6084/m9.figshare.13673800

References

Ariani G, **Diedrichsen J**. Sequence learning is driven by improvements in motor planning. *J Neurophysiol* 121: 2088–2100, 2019. https://doi.org/10.1152/jn.00041.2019

Ariani G, **Kordjazi N**, **Pruszynski JA**, **Diedrichsen J**. The planning horizon for movement sequences. *bioRxiv* , 2020. https://doi.org/10.1101/2020.07.15.204529

Averbeck BB, **Chafee M V.**, **Crowe DA**, **Georgopoulos AP**. Parallel processing of serial movements in prefrontal cortex. *Proc Natl Acad Sci U S A* 99: 13172–13177, 2002. https://doi.org/10.1073/pnas.162485599

Balasubramaniam R, **Haegens S**, **Jazayeri M**, **Merchant H**, **Sternad D**, **Song J-H**. Neural encoding and representation of time for sensorimotor control and learning. *J Neurosci* , 2020. https://doi.org/10.1523/JNEUROSCI.1652-20.2020

Basu D, **Murthy A**. Parallel programming of saccades in the macaque frontal eye field: Are sequential motor plans coactivated? *J Neurophysiol* 123: 107–119, 2020. https://doi.org/10.1152/jn.00545.2018

Behmer LP, **Crump MJC**. The dynamic range of response set activation during action sequencing. *J Exp Psychol Hum Percept Perform* 43: 537–554, 2017. https://doi.org/10.1037/xhp0000335

Behmer LP, **Jantzen KJ**, **Martinez S**, **Walls R**, **Amir-Brownstein E**, **Jaye A**, **Leytze M**, **Lucier K**, **Crump MJC**. Parallel regulation of past, present, and future actions during sequencing. *J Exp Psychol Hum Percept Perform* 44: 1147–1152, 2018. https://doi.org/10.1037/xhp0000507

Bengtsson SL, **Ehrsson HH**, **Forssberg H**, **Ullén F**. Dissociating brain regions controlling the temporal and ordinal structure of learned movement sequences. *Eur J Neurosci* 19: 2591–2602, 2004. https://doi.org/10.1111/j.0953-816X.2004.03269.x

Bengtsson SL, **Ehrsson HH**, **Forssberg H**, **Ullén F**. Effector-independent voluntary timing: Behavioural and neuroimaging evidence. *Eur J Neurosci* 22: 3255–3265, 2005. https://doi.org/10.1111/j.1460-9568.2005.04517.x

Bortoletto M, **Cook A**, **Cunnington R**. Motor timing and the preparation for sequential actions. *Brain Cogn* 75: 196–204, 2011. https://doi.org/10.1016/j.bandc.2010.11.016

Bortoletto M, **Cunnington R**. Motor timing and motor sequencing contribute

differently to the preparation for voluntary movement. *Neuroimage* 49: 3338–3348, 2010. https://doi.org/10.1016/j.neuroimage.2009.11.048

Bueti D, **Bahrami B**, **Walsh V**, **Rees G**. Encoding of temporal probabilities in the human brain. *J Neurosci* 30: 4343–4352, 2010. https://doi.org/10.1523/JNEUROSCI.2254-09.2010

Bullock D. Adaptive neural models of queuing and timing in fluent action. *Trends Cogn Sci* 8: 426–433, 2004. https://doi.org/10.1016/j.tics.2004.07.003

Burgess N, **Hitch GJ**. Memory for serial order: A network model of the phonological loop and its timing. *Psychol Rev* 106: 551–581, 1999. https://doi.org/10.1037/0033-295X.106.3.551

Burgess N, **Hitch GJ**. A revised model of short-term memory and long-term learning of verbal sequences. *J Mem Lang* 55: 627–652, 2006. https://doi.org/10.1016/j.jml.2006.08.005

Cisek P, **Kalaska JF**. Neural correlates of reaching decisions in dorsal premotor cortex: Specification of multiple direction choices and final selection of action. *Neuron* 45: 801–814, 2005. https://doi.org/10.1016/j.neuron.2005.01.027

Craig-McQuaide A, **Akram H**, **Zrinzo L**, **Tripoliti E**. A review of brain circuitries involved in stuttering. *Front Hum Neurosci* 8: 1–20, 2014. https://doi.org/10.3389/fnhum.2014.00884

Crowe DA, **Zarco W**, **Bartolo R**, **Merchant H**. Dynamic representation of the temporal and sequential structure of rhythmic movements in the primate medial premotor cortex. *J Neurosci* 34: 11972–11983, 2014. https://doi.org/10.1523/JNEUROSCI.2177-14.2014

Davidson TJ, **Kloosterman F**, **Wilson MA**. Hippocampal replay of extended experience. *Neuron* 63: 497–507, 2009. https://doi.org/10.1016/j.neuron.2009.07.027

Dragoi G, **Tonegawa S**. Preplay of future place cell sequences by hippocampal cellular assemblies. *Nature* 469: 397–401, 2011. https://doi.org/10.1038/nature09633

Drieu C, **Zugaro M**. Hippocampal sequences during exploration: Mechanisms and functions. *Front. Cell. Neurosci.* 13: 232, 2019. https://doi.org/10.3389/fncel.2019.00232

Eichenlaub JB, **Jarosiewicz B**, **Saab J**, **Franco B**, **Kelemen J**, **Halgren E**, **Hochberg LR**, **Cash SS**. Replay of learned neural firing sequences during rest in

human motor cortex. *Cell Rep* 31: 107581, 2020. https://doi.org/10.1016/j.celrep.2020.107581

Foley PJ. The foreperiod and simple reaction time. *Can J Psychol* 13: 20–22, 1959. https://doi.org/10.1037/h0083759

Friston K, **Buzsáki G**. The functional anatomy of time: What and when in the brain. *Trends Cogn. Sci.* 20: 500–511, 2016. https://doi.org/10.1016/j.tics.2016.05.001

Gilbert RA, **Hitch GJ**, **Hartley T**. Temporal precision and the capacity of auditory–verbal short-term memory. *Q J Exp Psychol* 70: 2403–2418, 2017. https://doi.org/10.1080/17470218.2016.1239749

Glasspool DW, **Houghton G**. Serial order and consonant-vowel structure in a graphemic output buffer model. *Brain Lang* 94: 304–330, 2005. https://doi.org/10.1016/j.bandl.2005.01.006

Goudar V, **Buonomano D V.** Encoding sensory and motor patterns as time-invariant trajectories in recurrent neural networks. *Elife* 7: e31134, 2018. https://doi.org/10.7554/eLife.31134

Grossberg S. Behavioral contrast in short term memory: Serial binary memory models or parallel continuous memory models? *J Math Psychol* 17: 199–219, 1978a. https://doi.org/10.1016/0022-2496(78)90016-0

Grossberg S. A theory of human memory: Self-organization and performance of sensory-motor codes, maps, and plans. In: *Progress in Theoretical Biology*. 1978b, p. 233–374. https://doi.org/10.1007/978-94-009-7758-7_13

Hartley T, **Houghton G**. A linguistically constrained model of short-term memory for nonwords. *J Mem Lang* 35: 1–31, 1996. https://doi.org/10.1006/jmla.1996.0001

Hartley T, **Hurlstone MJ**, **Hitch GJ**. Effects of rhythm on memory for spoken sequences: A model and tests of its stimulus-driven mechanism. *Cogn Psychol* 87: 135–178, 2016. https://doi.org/10.1016/j.cogpsych.2016.05.001

Henson RNA. Short-term memory for serial order: The Start-End Model. *Cogn Psychol* 36: 73–137, 1998. https://doi.org/10.1006/cogp.1998.0685

Houghton G. The problem of serial order: A neural network model of sequence learning and recall. *Curr Res Nat Lang Gener* 287–319, 1990.

Houghton G. Action and perception in literacy: A common-code for spelling and

reading. *Psychol Rev* 125: 83–116, 2018. https://doi.org/10.1037/rev0000084

Howell P. A model of serial order problems in fluent, stuttered and agrammatic speech. *Hum Mov Sci* 26: 728–741, 2007. https://doi.org/10.1016/j.humov.2007.07.004

Ingham RJ, **Ingham JC**, **Euler HA**, **Neumann K**. Stuttering treatment and brain research in adults: A still unfolding relationship. *J. Fluency Disord.* 55: 106–119, 2018. https://doi.org/10.1016/j.jfludis.2017.02.003

Jafarpour A, **Fuentemilla L**, **Horner AJ**, **Penny W**, **Duzel E**. Replay of very early encoding representations during recollection. *J Neurosci* 34: 242–248, 2014. https://doi.org/10.1523/JNEUROSCI.1865-13.2014

Jazayeri M, **Shadlen MN**. Temporal context calibrates interval timing. *Nat Neurosci* 13: 1020–1026, 2010. https://doi.org/10.1038/nn.2590

Kaufman MT, **Churchland MM**, **Ryu SI**, **Shenoy K V.** Cortical activity in the null space: Permitting preparation without movement. *Nat Neurosci* 17: 440–448, 2014. https://doi.org/10.1038/nn.3643

Kornysheva K, **Bush D**, **Meyer SS**, **Sadnicka A**, **Barnes G**, **Burgess N**. Neural competitive queuing of ordinal structure underlies skilled sequential action. *Neuron* 101: 1166-1180.e3, 2019. https://doi.org/10.1016/j.neuron.2019.01.018

Kornysheva K, **Diedrichsen J**. Human premotor areas parse sequences into their spatial and temporal features. *Elife* 3: e03043, 2014. https://doi.org/10.7554/eLife.03043

Kornysheva K, **Sierk A**, **Diedrichsen J**. Interaction of temporal and ordinal representations in movement sequences. *J Neurophysiol* 109: 1416–1424, 2013. https://doi.org/10.1152/jn.00509.2012

Krakauer JW, **Mazzoni P**. Human sensorimotor learning: Adaptation, skill, and beyond. *Curr. Opin. Neurobiol.* 21: 636–644, 2011. https://doi.org/10.1016/j.conb.2011.06.012

Kurth-Nelson Z, **Economides M**, **Dolan RJ**, **Dayan P**. Fast sequences of non-spatial state representations in humans. *Neuron* 91: 194–204, 2016. https://doi.org/10.1016/j.neuron.2016.05.028

Laje R, **Buonomano D V**. Robust timing and motor patterns by taming chaos in recurrent neural networks. *Nat Neurosci* 16: 925–933, 2013.

https://doi.org/10.1038/nn.3405

Langner R, **Steinborn MB**, **Eickhoff SB**, **Huestegge L**. When specific action biases meet nonspecific preparation: Event repetition modulates the variable-foreperiod effect. *J Exp Psychol Hum Percept Perform* 44: 1313–1323, 2018. https://doi.org/10.1037/xhp0000561

Lashley K. The problem of serial order in behavior. In: *Cerebral mechanisms in behavior*. 1951, p. 112–146.

Li N, **Daie K**, **Svoboda K**, **Druckmann S**. Robust neuronal dynamics in premotor cortex during motor planning. *Nature* 532: 459–464, 2016. https://doi.org/10.1038/nature17643

Liu Y, **Dolan RJ**, **Kurth-Nelson Z**, **Behrens TEJ**. Human replay spontaneously reorganizes experience. *Cell* 178: 640-652.e14, 2019. https://doi.org/10.1016/j.cell.2019.06.012

Maslovat D, **Chua R**, **Klapp ST**, **Franks IM**. Preparation of timing structure involves two independent sub-processes. *Psychol Res* 82: 981–996, 2018. https://doi.org/10.1007/s00426-017-0877-3

Medina JF, **Carey MR**, **Lisberger SG**. The representation of time for motor learning. *Neuron* 45: 157–67, 2005. https://doi.org/10.1016/j.neuron.2004.12.017

Merchant H, **Pérez O**, **Zarco W**, **Gámez J**. Interval tuning in the primate medial premotor cortex as a general timing mechanism. *J Neurosci* 33: 9082–9096, 2013. https://doi.org/10.1523/JNEUROSCI.5513-12.2013

Michelmann S, **Staresina BP**, **Bowman H**, **Hanslmayr S**. Speed of time-compressed forward replay flexibly changes in human episodic memory. *Nat. Hum. Behav.* 3: 143–154, 2019. https://doi.org/10.1038/s41562-018-0491-4

Miller N. The assessment of limb dyspraxia. *Clin Rehabil* 2: 177–181, 1988. https://doi.org/10.1177/026921558800200301

Niemi P, **Näätänen R**. Foreperiod and simple reaction time. *Psychol Bull* 89: 133–162, 1981. https://doi.org/10.1037/0033-2909.89.1.133

O'Reilly JX, **McCarthy KJ**, **Capizzi M**, **Nobre AC**. Acquisition of the temporal and ordinal structure of movement sequences in incidental learning. *J Neurophysiol* 99: 2731–2735, 2008. https://doi.org/10.1152/jn.01141.2007

O'Shea DJ, **Shenoy K V.** The importance of planning in motor learning. *Neuron*

2016. https://doi.org/10.1016/j.neuron.2016.11.003

Ólafsdóttir HF, **Bush D**, **Barry C**. The role of hippocampal replay in memory and planning. *Curr. Biol.* 28: R37–R50, 2018. https://doi.org/10.1016/j.cub.2017.10.073

Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9: 97–113, 1971. https://doi.org/10.1016/0028-3932(71)90067-4

Page MPA, **Norris D**. The primacy model: A new model of immediate serial recall. *Psychol Rev* 105: 761–781, 1998. https://doi.org/10.1037/0033-295x.105.4.761-781

Pinet S, **Dell GS**, **Alario FX**. Tracking keystroke sequences at the cortical level reveals the dynamics of serial order production. *J Cogn Neurosci* , 2019. https://doi.org/10.1162/jocn_a_01401

Rakitin BC, **Penney TB**, **Gibbon J**, **Malapani C**, **Hinton SC**, **Meck WH**. Scalar expectancy theory and peak-interval timing in humans. *J Exp Psychol Anim Behav Process* 24: 15–33, 1998. https://doi.org/10.1037//0097-7403.24.1.15

Remington ED, **Egger SW**, **Narain D**, **Wang J**, **Jazayeri M**. A dynamical systems perspective on flexible motor timing. *Trends Cogn Sci* 22: 938–952, 2018. https://doi.org/10.1016/j.tics.2018.07.010

Rhodes BJ, **Bullock D**, **Verwey WB**, **Averbeck BB**, **Page MPA**. Learning and production of movement sequences: Behavioral, neurophysiological, and modeling perspectives. *Hum Mov Sci* 23: 699–746, 2004. https://doi.org/10.1016/j.humov.2004.10.008

Rosenbaum DA. Motor rogramming: A review and scheduling theory. In: *Motor Behavior*. 1985, p. 1–33. https://doi.org/10.1007/978-3-642-69749-4_1

Russo AA, **Khajeh R**, **Bittner SR**, **Perkins SM**, **Cunningham JP**, **Abbott LF**, **Churchland MM**. Neural trajectories in the supplementary motor area and primary motor cortex exhibit distinct geometries, compatible with different classes of computation. *Neuron* 107: 745-758.e6, 2020. https://doi.org/10.1016/j.neuron.2020.05.020

Sadnicka A, **Kornysheva K**, **Rothwell JC**, **Edwards MJ**. A unifying motor control framework for task-specific dystonia. *Nat Rev Neurol* 14: 116–124, 2018.

https://doi.org/10.1038/nrneurol.2017.146

Shin JC, **Ivry RB**. Spatial and temporal sequence learning in patients with Parkinson's disease or cerebellar lesions. *J Cogn Neurosci* 15: 1232–1243, 2003. https://doi.org/10.1162/089892903322598175

Spencer RMC, **Karmarkar U**, **Ivry RB**. Evaluating dedicated and intrinsic models of temporal encoding by varying context. *Philos Trans R Soc Lond B Biol Sci* 364: 1853–63, 2009. https://doi.org/10.1098/rstb.2009.0024

Steinborn MB, **Langner R**. Arousal modulates temporal preparation under increased time uncertainty: Evidence from higher-order sequential foreperiod effects. *Acta Psychol (Amst)* 139, 2012. https://doi.org/10.1016/j.actpsy.2011.10.010

Steinborn MB, **Rolke B**, **Bratzke D**, **Ulrich R**. Sequential effects within a short foreperiod context: Evidence for the conditioning account of temporal preparation. *Acta Psychol (Amst)* 129: 297–307, 2008. https://doi.org/10.1016/j.actpsy.2008.08.005

Sternberg S, **Monsell S**, **Knoll RL**, **Wright CE**. The latency and duration of rapid movement sequences: Comparisons of speech and typewriting. In: *Information Processing in Motor Control and Learning*. 1978, p. 117–152. https://doi.org/10.1016/B978-0-12-665960-3.50011-6

Ullén F, **Bengtsson SL**. Independent processing of the temporal and ordinal structure of movement sequences. *J Neurophysiol* 90: 3725–3735, 2003. https://doi.org/10.1152/jn.00458.2003

Vallesi A, **Shallice T**, **Walsh V**. Role of the prefrontal cortex in the foreperiod effect: TMS evidence for dual mechanisms in temporal preparation. *Cereb Cortex* 17: 466–474, 2007. https://doi.org/10.1093/cercor/bhj163

Verwey WB, **Abrahamse EL**. Distinct modes of executing movement sequences: Reacting, associating, and chunking. *Acta Psychol (Amst)* 140: 274–282, 2012. https://doi.org/10.1016/j.actpsy.2012.05.007

Wang J, **Narain D**, **Hosseini EA**, **Jazayeri M**. Flexible timing by temporal scaling of cortical responses. *Nat Neurosci* 21: 102–112, 2018. https://doi.org/10.1038/s41593-017-0028-6

Winer BJ, **Brown DR**, **Michels KM**. *Statistical principles in experimental design*. 3rd ed. New York: McGraw-Hill, 1991.

Wong AL, **Krakauer JW**. Why are sequence representations in primary motor cortex so elusive? *Neuron* 103: 956–958, 2019. https://doi.org/10.1016/j.neuron.2019.09.011

Zeid O, **Bullock D**. Moving in time: Simulating how neural circuits enable rhythmic enactment of planned sequences. *Neural Networks* 120: 86–107, 2019. https://doi.org/10.1016/j.neunet.2019.08.006

Figure legends

Figure 1 | Design and experimental conditions. a. The first two days integrated the three training stages. Participants progressed from entirely instructed sequence production trials (stage A) to blocks of mixed trials (stage B) and, finally, to producing the target sequences from memory during the last stage of the training (stage C). All training stages incorporated a fixed percentage of *Probe* trials, randomized in each block, to ensure a degree of familiarity with single-press *Probe* cues. In the test phase (Day 3), participants underwent two refresher blocks (stage B) and, subsequently, an equal number of memory-guided *Sequence* trials and *Probe* trials (test). **b.** Test phase: After training, participants were prompted to produce 4-element finger sequences from memory following a *Go* cue. Each finger order or timing corresponded to a unique abstract visual *Sequence* cue presented for up to 1500 ms before the *Go* cue (preparation period). Experiment 1 cued the production of sequences with two different finger orders and isochronous timing (slow). Here, we manipulated the duration of the preparation period (500, 1000, 1500 ms). In Experiments 2 and 3, the *Sequence* cues had a fixed preparation duration of 1500 ms and prompted the production of sequences with the same finger order but a different timing (slow, fast, irregular). In all three experiments, the target IPIs, illustrated in ms, were used to train participants to develop a relative timing proportionate to the target timing. Participants received visual feedback in each trial on the accuracy of the finger order and their timing. Points were based on finger press accuracy, initiation reaction time (RT), and temporal accuracy (cf. Materials and Methods). **c.** Test phase: In all experiments, we introduced *Probe* trials, in which, following the preparation period, the *Go* cue was replaced with a *Probe* cue. That prompted a particular finger digit to be pressed, corresponding to each sequence position or a control movement which did not feature in any sequence production. The *Probe* condition was used to obtain the RT and error rate for each position at the end of the preparation period. The participants received points for accurate presses and fast RTs.

Figure 2 | Position-dependent movement availability during sequence planning. a. RTs for each probed sequence position relative to the first position. **b.** Press errors for each probed sequence position relative to the first position. (cf. raw RT and press error graphs in Supplemental Figure S2a, b). Both relative RT and press error were calculated from RTs and press error rates, respectively, obtained in *Probe* trials prompting the production of a movement associated with the 1st – 4th press position of the planned sequence (Experiments 1, 2 and 3) or a control movement not present in any sequence (Experiment 3). Black inset violin plots illustrate the position-dependent increases of raw RT and raw press error in the baseline condition (Dur: 1500 ms, T: slow), from 1st to 2nd, 2nd to 3rd, and 3rd to 4th positions. Grey inset violin plots illustrate the difference between 4th position and control across sequence conditions, as indicated by the brackets. **c.** Relative press error in lower ('Fast RT') and upper ('Slow RT') RT quartiles. Error bars in line graphs represent standard errors. In inset violin plots, solid white lines represent the median, and lower and upper dashed white lines represent the 25th and 75th percentiles, respectively. Significance asterisks over the black inset violin plots indicate one-tailed increases (position-dependent increases in RT and error rate), whereas the asterisks over the grey

inset violin plots represent significance for a two-tailed test (increases or decreases in availability relative to control movement). | * P ≤ 0.05 | ** P ≤ 0.01 | *** P ≤ 0. 001 |

Figure 3 | Pattern of press errors for probed movements associated with different sequence positions. Incorrect presses per probed position across experiments are shown in percent of all responses. | * P ≤ 0.05 | ** P ≤ 0.01 | *** P ≤ 0.001 |

Figure 4 | Correlation of performance with position-dependent differences in movement availability during planning. The mean difference between adjacent positions (1st - 2nd, 2nd - 3rd, 3rd - 4th) based on RTs and press errors relative to the first position (*Probe* trials) was taken as a proxy for the pre-activation gradient size during preparation, with steeper (larger) differences reflecting a more expanded gradient (cf. raw RT and error differences in Supplemental Figure S4). **a.** Correlations between relative position-dependent differences in RT in *Probe* trials and each of the performance measures (initiation RT, relative temporal error, and finger error). **b.** Correlations between relative position-dependent differences in error rate in *Probe* trials and each of the performance measures (initiation RT, relative temporal error, and finger error). Inset graphs in each panel illustrate relative position-dependent RT (**a**) and press error (**b**) increases during planning for participants with faster *vs* slower initiation RT and lower *vs* higher relative temporal error performance (median splits). Error bars represent standard errors. All correlations are one-tailed, in line with one-sided predictions regarding the beneficial effect of a differentiated pre-activation of sequence movements during planning.

Figure 5 | Sequence production. **a.** Relative timing as a function of inter-press interval (IPI) production of a slow, twice as fast and an irregular sequence. Both the produced (solid lines) and target IPIs (dashed lines) were normalized across trials relative to the baseline condition (Dur: 1500 ms, T: slow). Error bars represent standard errors. **b.** Sequence initiation RT (*Go* cue to first press latency), relative temporal error, and finger error (proportion of trials with incorrect presses) in each experimental condition (preparation duration, Experiment 1; timing, Experiments 2 and 3). Solid white lines represent the median, and lower and upper dashed white lines represent the 25^{th} and 75^{th} percentiles, respectively. | * P ≤ 0.05 | ** P ≤ 0.01 | *** P ≤ 0.001 |

Figure 1

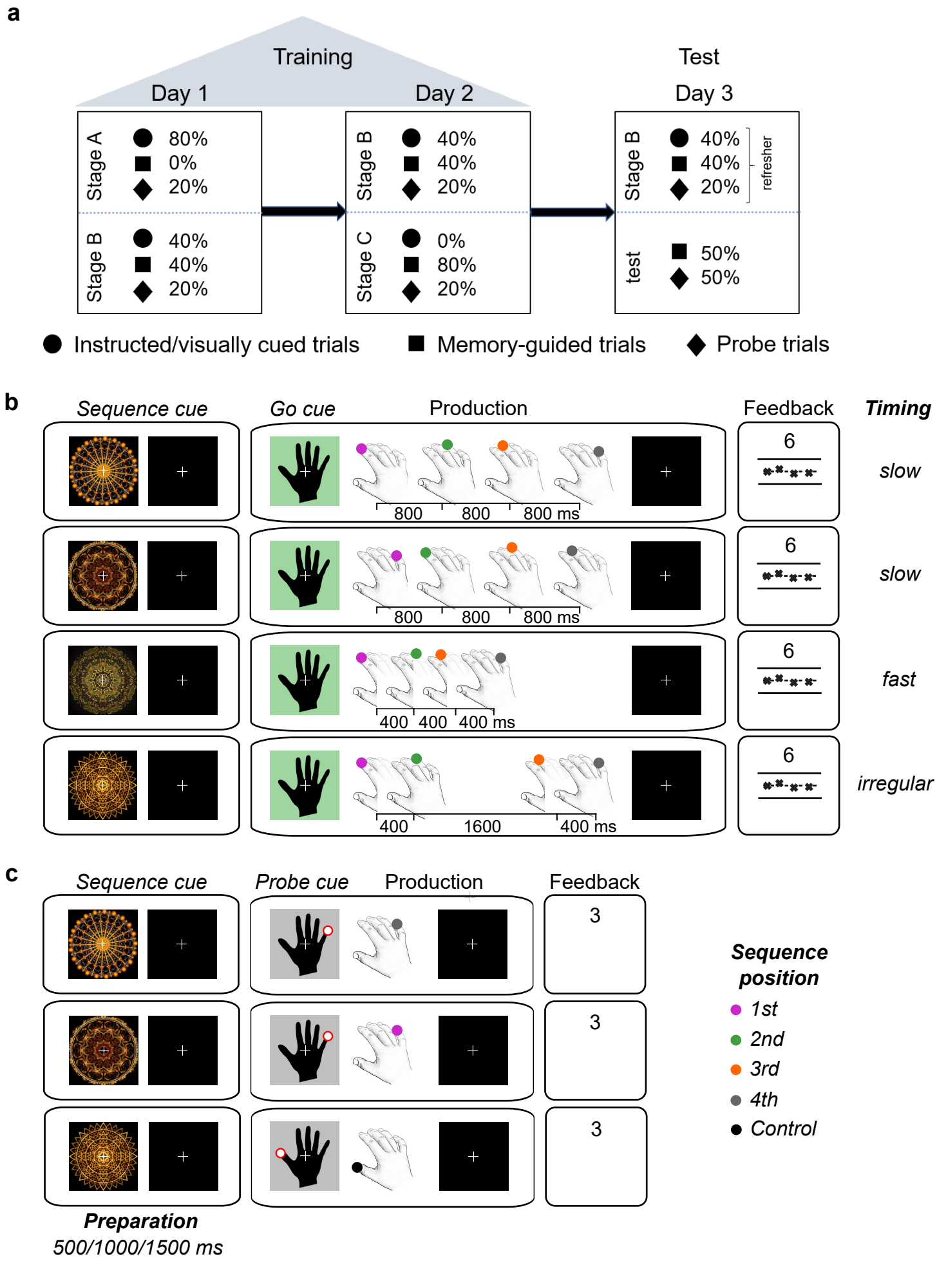

a
Training
Test
Day 1
Day 2
Day 3
Stage A
80%
0%
20%
Stage B
40%
40%
20%
Stage B
40%
40%
20%
Stage C
0%
80%
20%
Stage B
40%
40%
20%
refresher
test
50%
50%
Instructed/visually cued trials
Memory-guided trials
Probe trials
b
Sequence cue
Go cue
Production
Feedback
Timing
800
800
800 ms
6
slow
800
800
800 ms
6
slow
400
400
400 ms
6
fast
400
1600
400 ms
6
irregular
c
Sequence cue
Probe cue
Production
Feedback
3
3
3
Preparation
500/1000/1500 ms
Sequence position
1st
2nd
3rd
4th
Control

Figure 2

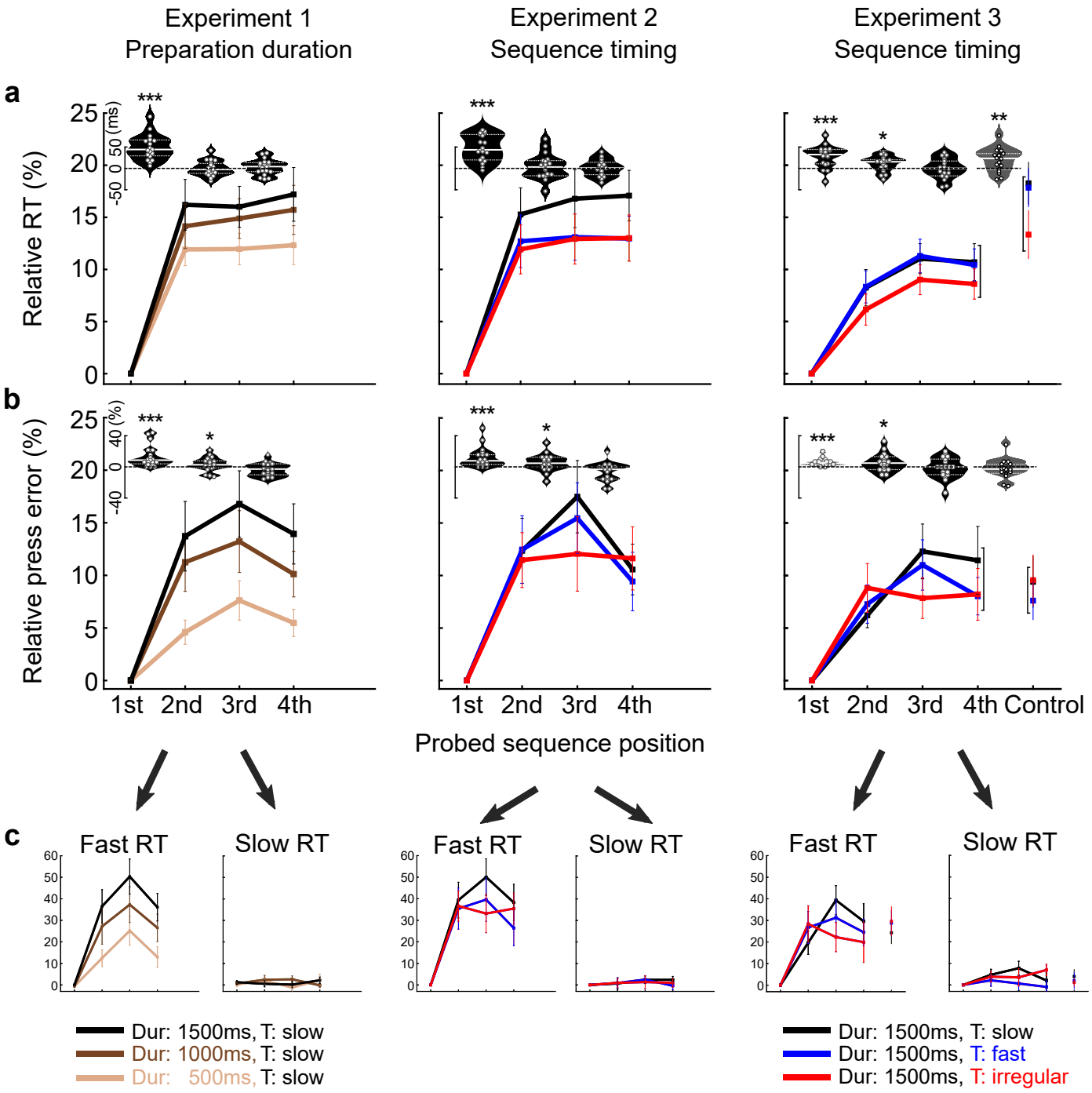

Experiment 1
Preparation duration
Experiment 2
Sequence timing
Experiment 3
Sequence timing
a
Relative RT (%)
b
Relative press error (%)
1st 2nd 3rd 4th
1st 2nd 3rd 4th Control
Probed sequence position
c
Fast RT
Slow RT
Dur: 1500ms, T: slow
Dur: 1000ms, T: slow
Dur: 500ms, T: slow
Dur: 1500ms, T: slow
Dur: 1500ms, T: fast
Dur: 1500ms, T: irregular

Figure 3

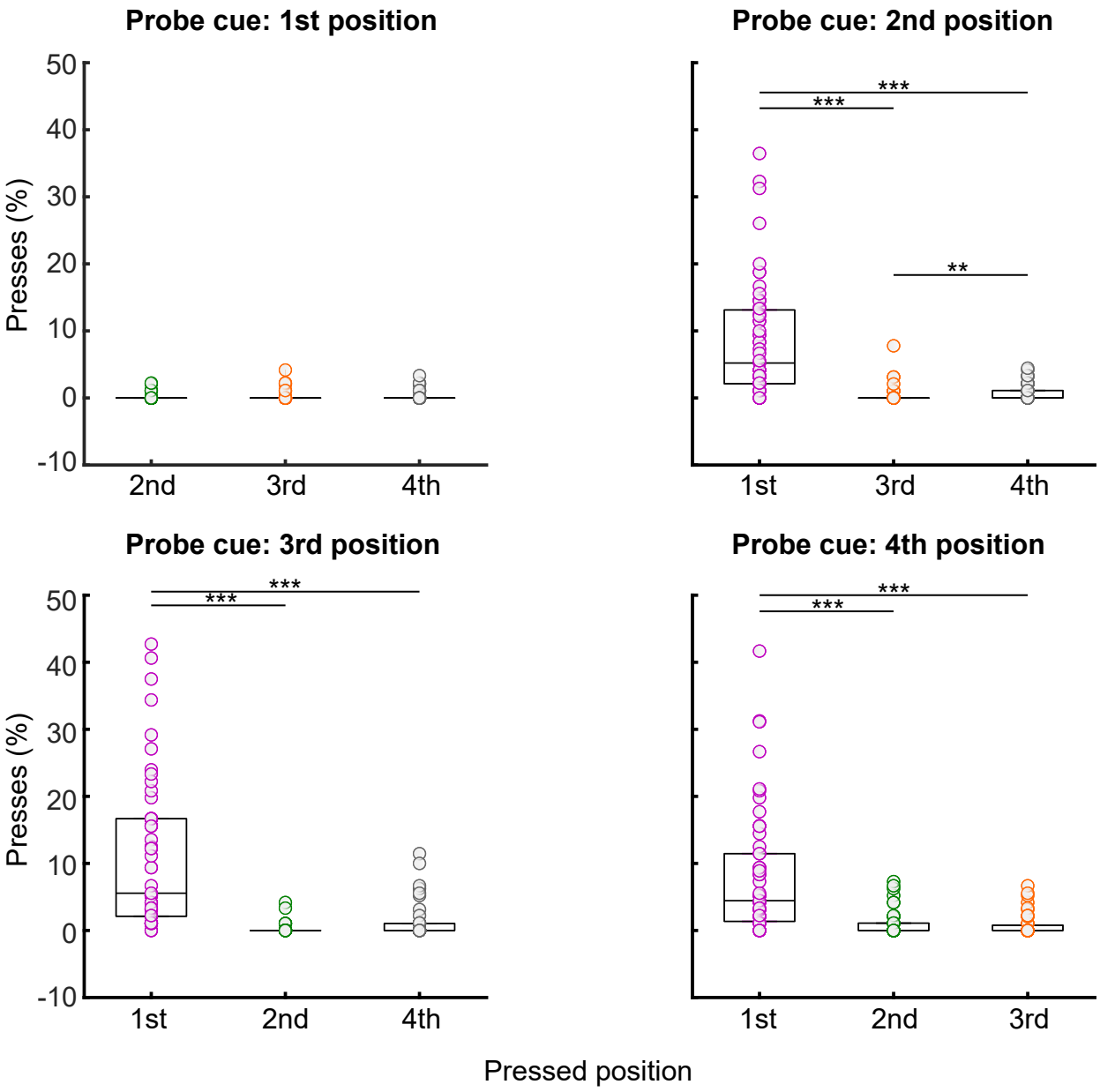

Probe cue: 1st position
Probe cue: 2nd position
Probe cue: 3rd position
Probe cue: 4th position
Presses (%)
50
40
30
20
10
0
-10
2nd
3rd
4th
1st
***
**
Pressed position

Figure 4

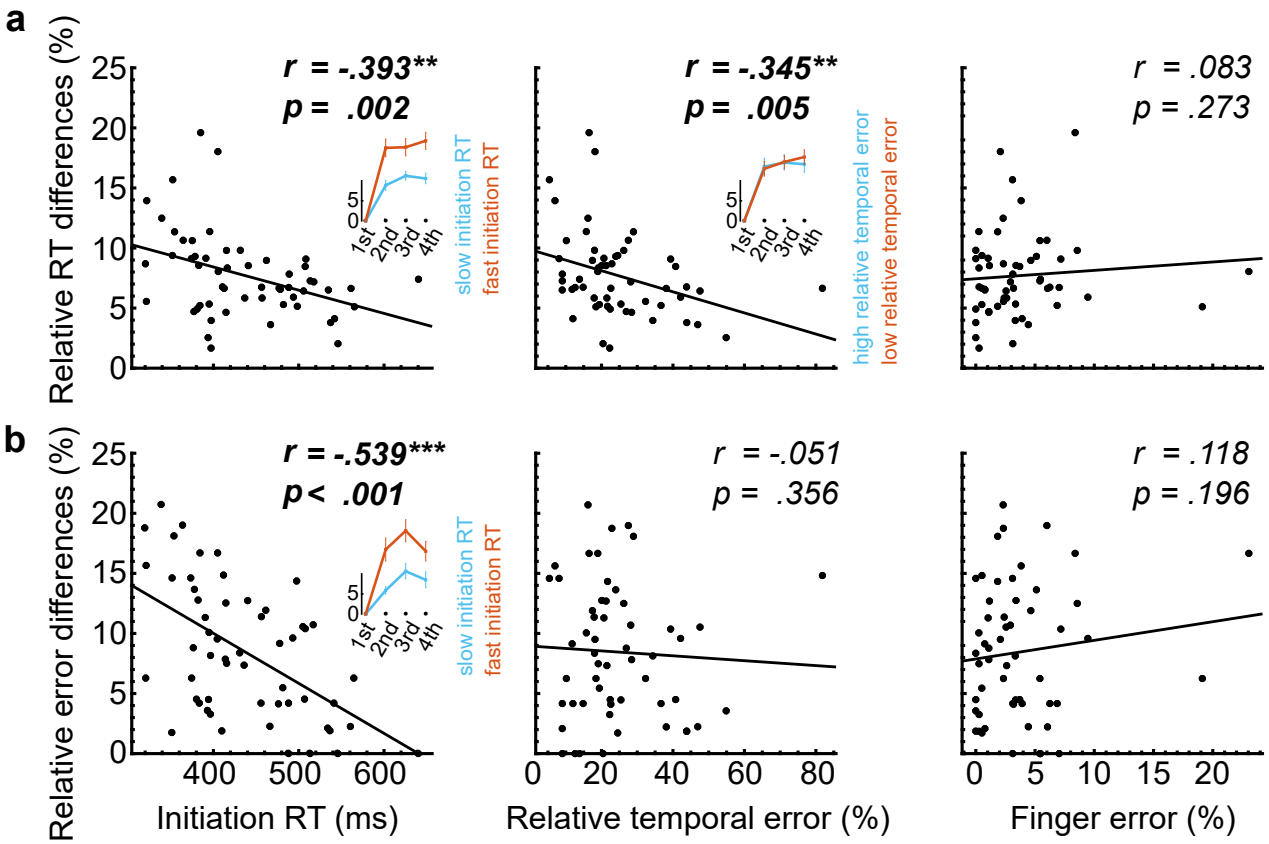
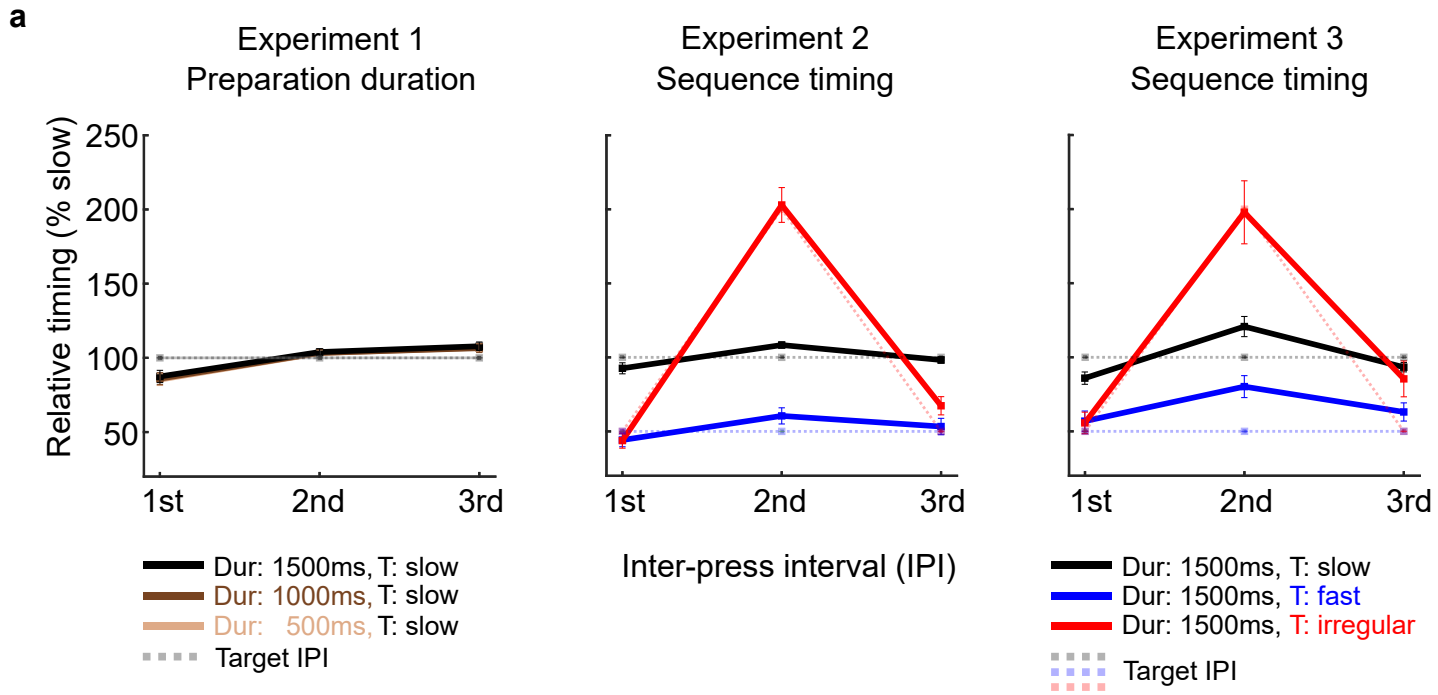
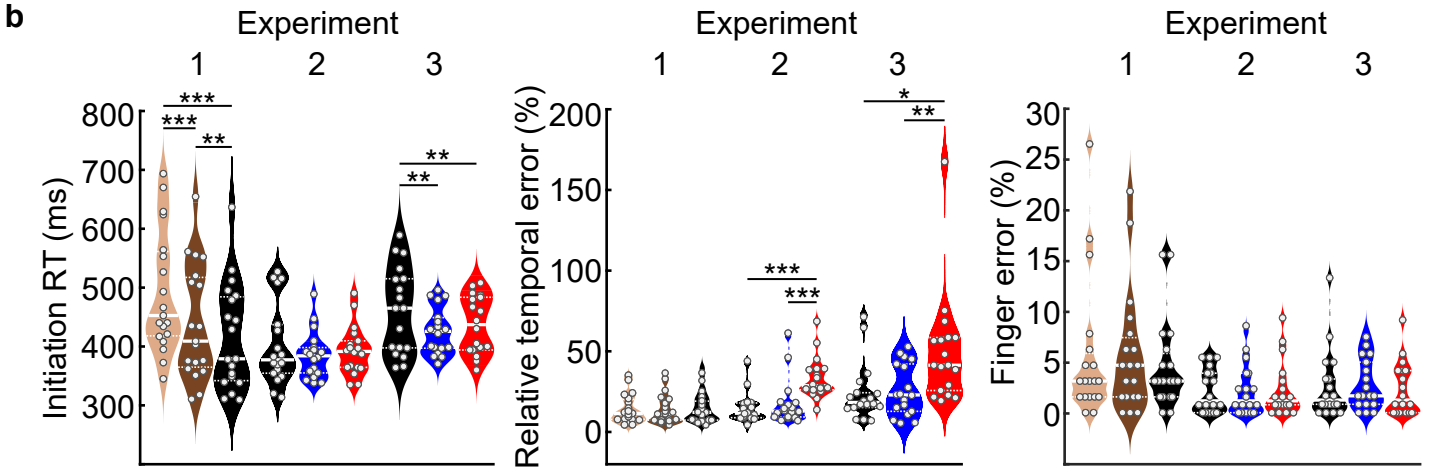

a
b
Relative RT differences (%)
Relative error differences (%)
r = -.393**
p = .002
r = -.345**
p = .005
r = .083
p = .273
r = -.539***
p < .001
r = -.051
p = .356
r = .118
p = .196
slow initiation RT
fast initiation RT
high relative temporal error
low relative temporal error
1st
2nd
3rd
4th
Initiation RT (ms)
Relative temporal error (%)
Finger error (%)

Figure 5


a
Experiment 1
Preparation duration
Experiment 2
Sequence timing
Experiment 3
Sequence timing
Relative timing (% slow)
250
200
150
100
50
1st
2nd
3rd
Inter-press interval (IPI)
Dur: 1500ms, T: slow
Dur: 1000ms, T: slow
Dur: 500ms, T: slow
Target IPI
Dur: 1500ms, T: slow
Dur: 1500ms, T: fast
Dur: 1500ms, T: irregular
Target IPI


b
Experiment
1
2
3
Initiation RT (ms)
800
700
600
500
400
300
***
***
**
**
**
Relative temporal error (%)
200
150
100
50
0
***
***
*
**
Finger error (%)
30
25
20
15
10
5
0