“Micro-offline gains” convey no benefit for motor skill learning

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Abstract

While practising a new motor skill, resting for a few seconds can improve performance immediately after the rest. This improvement has been interpreted as rapid offline learning[1,2] ("micro-offline gains", MOG), supported by neural replay of the trained movement sequence during rest[3]. Here, we provide evidence that MOG reflect transient performance benefits, partially mediated by motor planning, and not replay-mediated offline learning. In five experiments, participants trained to produce a sequence of finger movements as many times as possible during fixed-duration practice periods. When participants trained during 10-second practice periods, each followed by a 10-second rest period, they produced more correct keypresses during training than participants who trained without taking breaks. However, this benefit vanished within seconds after the end of training, when both groups performed under comparable conditions, revealing similar levels of skill acquisition. This challenges the idea that MOG reflect offline learning, which, if present, should result in sustained performance benefits, compared to training without breaks. Furthermore, sequence-specific replay was not necessary for MOG, given that we observed persistent MOG when participants produced sequences that never repeated, preventing any effect of (sequence-specific) replay on the performance of subsequent (novel) sequences. Importantly, we observed diminished MOG when participants could not pre-plan the first few movements of an upcoming practice period. We conclude that "micro-offline gains" represent short-lived performance benefits that are partially driven by motor pre-planning, rather than replay-mediated offline learning.

Results

Bönstrup et al.[1,2] have shown that performance during motor sequence learning improves immediately after a short rest period. In their studies, participants trained to repeat a sequence of five finger movements as often and as accurately as possible during 10-second practice periods, which alternated with 10-second rest periods. While the time needed to produce a full sequence remained similar from the beginning to the end of each practice period, it decreased from the end of one practice period to the beginning of the next practice period, demonstrating a performance benefit across rest. Bönstrup et al.[1,2] interpreted these "micro-offline gains" (MOG) as behavioral evidence for micro-consolidation during short rest from motor skill learning. This interpretation has gained increasing acceptance in recent years[4–8]. In a similar paradigm, Buch et al.[3] proposed that MOG are supported by highly compressed neural replay of the trained sequence of movements during rest, which they decoded from human magnetoencephalography (MEG) data, and which correlated with the size of MOG. Here, we provide evidence that MOG result neither from sequence-specific replay, nor from offline learning. Instead, MOG represent short-lived performance benefits, partially driven by motor pre-planning.

MOG do not reflect offline learning

If MOG represent offline learning, participants should reach higher skill levels when training with breaks, compared to training without breaks.

We tested this idea in a lab-based study (Experiment 1, N=85 across two groups, see also **Supplemental Information (SI) Figure S1** for an additional experiment), and confirmed results in a bigger cohort via an online study (Experiment 2, N=358 across two groups). As in Bönstrup et al[1], right-handed participants trained to produce a sequence of five finger movements as often and as accurately as possible (4-1-3-2-4, where "1" represents the little finger and "4" the index finger; see **Figure 1A**) with their left hand. At several time points before and during training, we assessed skill level in separate test sessions.

We assigned participants to two groups who differed in their training schedule. Both groups first completed a baseline test (T1) of 20 s, followed by a first block of training. One group ('With Breaks') trained via three 10-second practice periods, each preceded by a 10-second rest period. In the other group ('No Breaks'), the baseline test transitioned seamlessly into a continuous 30-second practice period without rest. In both groups, training was immediately followed by a 3-second countdown, shown on the screen, which prepared participants for the upcoming 20-second test session (T2). The countdown prompted participants to stop all ongoing movement and prepare for the upcoming test. This ensured that both groups started the test session under comparable conditions. After the test, there was a break of 5 minutes, followed by a retention test of 20 s (T3). Participants then completed a second block of training (30 s of practice; with or without breaks, as per group), followed by another 20-second test session (T4), and, after another 5-minute break, a final 20-second retention test (T5). Like the first test session, all following test sessions were cued on the screen by a 3-second countdown. Every 20-second test was basically a continuous practice period during which participants performed the same '4-1-3-2-4' sequence as often and as accurately as they could.

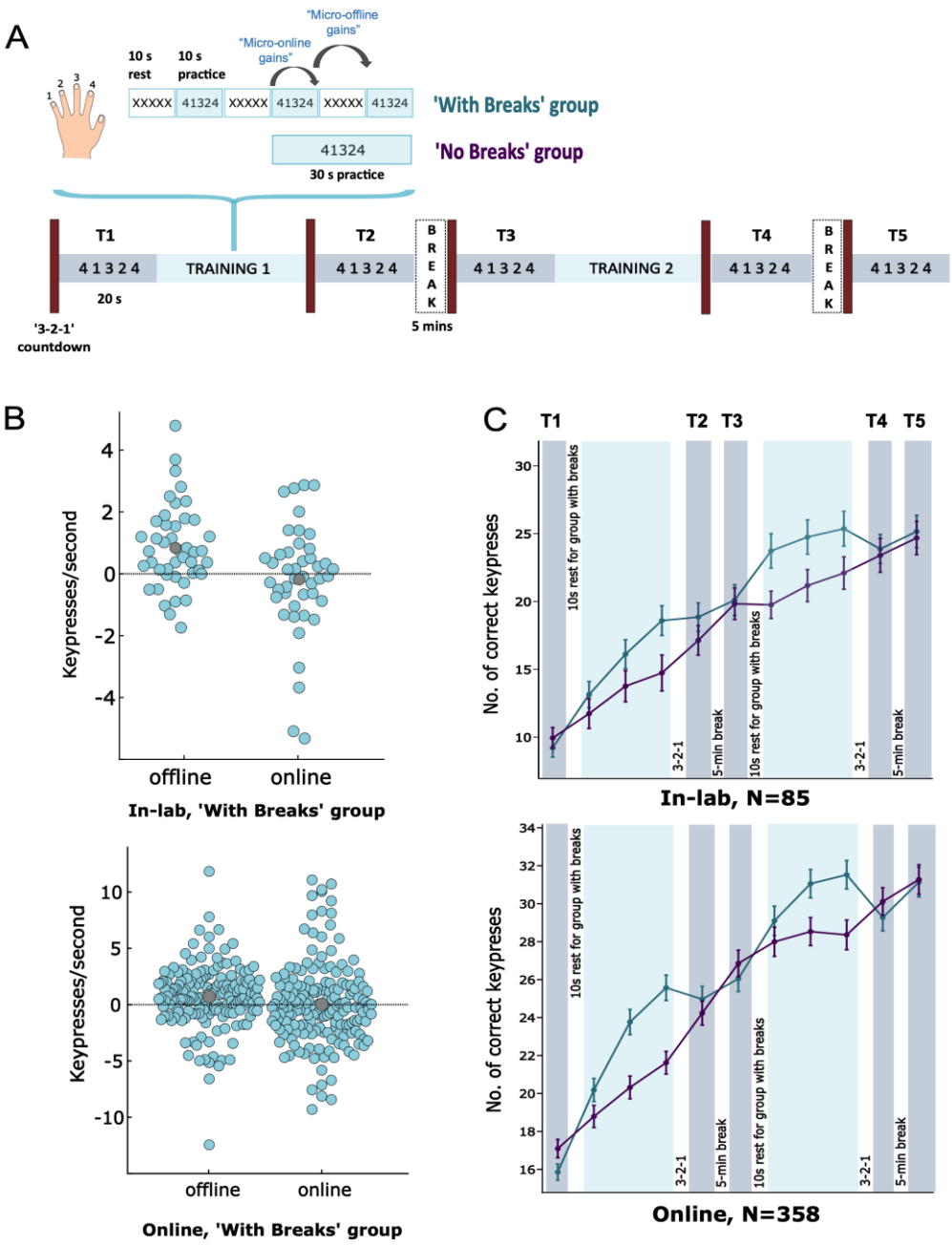

Figure 1. Experiment 1 (in-lab study) and 2 (online study). (A) Experimental design for both groups. One group ('No Breaks') of participants were instructed to perform the sequence 4-1-3-2-4 throughout a continuous training of 30 s, whereas the other group ('With Breaks') performed the same sequence in three 10-second practice periods, separated by three 10-second rest periods. Five identical practice periods of 20 s each, served as test periods at different times of the experiment. (B) Participants who trained with breaks exhibited on average significant 'micro-offline gains' (test against zero), whereas 'micro-online gains' were around zero. (C) The entire experiment was binned into 10-second periods to allow for between-group comparison of training performance. For the 20-second test sessions, the two resulting 10-second bins were averaged. In B and C, the top row shows results from the in-lab study, and the bottom row shows results from the online study. In the online study, the group with breaks produced a significantly larger number of correct keypresses in the last two bins of the first training, but this benefit vanished in the next 10-second bin, right after the 3-second countdown.

Similarly, the last bin of the second training also showed significantly better performance for the participants with breaks, however, this benefit dissipated again after the 3-second countdown. Refer to the Supplemental Information for results from a combined analysis of the two datasets. Error bars represent SEM.

We first confirmed that the group who trained with breaks exhibited MOG: We observed significant performance improvements, evident as faster keypresses from the end of one practice period (i.e., last correct sequence) to the beginning of the next practice period (i.e., first correct sequence), both in the in-lab study ($t(43)=3.922$, $p<.001$, $d=0.591$, $BF_{10}=84.730$; **Figure 1B top**) and in the online study ($t(178)=3.526$, $p<.001$, $d=0.264$, $BF_{10}=30.992$; **Figure 1B bottom**; one-sample t tests against zero). MOG were also significant when we analysed them separately for the first and second training sessions (see **SI Figure S2**).

Importantly, despite the occurrence of MOG in the group who trained with breaks, both groups achieved similar performance, evident as similar numbers of correct keypresses, during all 20-second tests, as shown in **Figure 1C**. A 2x5 ANOVA (two groups and five test sessions) revealed a main effect of test session (in-lab study: $F(2.9, 238.3)=250.075$, $p<.001$, $\eta^2_{partial}=0.751$; online study: $F(3.2, 1141.4)=591.542$, $p<.001$, $\eta^2_{partial}=0.624$) indicating that both groups improved across tests. There was no effect of group (in-lab study: $F(1,83)=0.093$, $p=.761$, $\eta^2_{partial}=0.001$; online study: $F(1, 356)=0.3$, $p=.584$, $\eta^2_{partial}=0.00084$). There was a significant, but small, group x bin interaction effect in the online study ($F(3.2, 1141.4)=2.924$, $p=.03$, $\eta^2_{partial}=0.008$), which was absent in the lab-based study ($F(2.9, 238.3)=0.776$, $p=.504$, $\eta^2_{partial}=0.009$). Most importantly, separate group comparisons for each test session revealed that there were no performance differences between groups in any of the test sessions (**Figure 1C**), for both the lab-based and online studies (all $p_{corr}>0.6$, all $BF_{10}\leq0.35$; detailed statistical results are available in **SI Table S2**). This demonstrates that there were no detectable group differences in the two retention tests conducted after the 5-minute breaks, nor in the tests conducted immediately after training, regardless of the training schedule or the occurrence of MOG.

To compare learning during training between groups, we computed the number of correct keypresses every 10 seconds. We then conducted two 2x4 ANOVA, each with two groups and four time points: three 10 s training bins plus a baseline, which corresponded to the average of the two 10-second bins in the test session immediately preceding training (T1 for the first training block, and T3 for the second training block). We observed a main effect of time point for the first training block (in-lab study: F(2.4,199.8)=78.545, p<.001, $\eta^2_{partial}$=0.486; online study: F(2.9,1036.9)=189.8, p<.001, $\eta^2_{partial}$=0.348), as well as the second training block (in-lab study: F(2.8,229.7)=24.835, p<.001, $\eta^2_{partial}$=0.23; online study: F(2.9,1029.6)=58.703, p<.001, $\eta^2_{partial}$=0.142), indicating that performance improved with learning. Additionally, there was a group by time point interaction, indicating that this improvement differed between groups, both for the first training block (in-lab study: F(2.4,199.8)=7.604, p<.001, $\eta^2_{partial}$=0.084; online study: F(2.9,1036.9)=27.2, p<.001, $\eta^2_{partial}$=0.071), and the second training block (in-lab study: F(2.8,229.7)=6.59, p<.001, $\eta^2_{partial}$=0.074; online study: F(2.9,1029.6)=17.482, p<.001, $\eta^2_{partial}$=0.047). Post hoc t-tests for each time point revealed no significant differences between groups in the in-lab study, after adjusting for multiple comparisons (all p_{corr}≥.1, all BF_{10} between 1.048 and 2.049; **Figure 1C**). In the online study, the group with breaks significantly outperformed the group without breaks at the penultimate and last time point of the first training block (both p_{corr}≤ .05, BF_{10}≥6, d>0.3), as well as at the last time point of the second training block (**SI Table S1**).

Thus, taking short breaks during training resulted in immediate performance benefits while training (**Figure 1B-C**), as reflected in both a larger number of correct keypresses produced across training sessions, and the emergence of MOG during breaks. However, after the 3-second countdown, the benefit of the group who trained with breaks disappeared, revealing that they did not acquire a higher skill level than the group who trained without breaks (**Figure 1C**). An additional replication of these results is reported in **SI Figure S1**.

MOG do not require sequence-specific replay

If MOG are supported by sequence-specific replay, as previously proposed[3], MOG should disappear when sequences never repeat, so that any replay of previously trained sequences cannot take effect on the performance of later trained sequences. We tested this in Experiment 3 by comparing MOG in participants who practised a single, repeating sequence '4-1-3-2-4' (Repeating group, N=24 participants) with MOG in participants who produced five-element sequences that never repeated (Non-repeating group; N=19 participants; **Figure 2A**). In both groups, 10-second practice periods alternated with 10-second rest periods. To avoid group differences in prior knowledge about the first upcoming sequence of each new practice period, which could affect MOG, we cued the first sequence of the upcoming practice period on the screen throughout the preceding rest period (advance information).

As expected, the Repeating group improved performance more than the Non-repeating group (**Figure 2B left**), as shown by a significant group x trial interaction effect on the number of correct keypresses ($F(3.582,146.861)=13.745$, $p<.001$ $\eta^2_{partial}=0.251$). Importantly, however, we found significant MOG in both groups (Repeating group: $t(23)=3.533$, $p_{corr}=.002$, $d=0.721$, $BF_{10}=21.222$, Non-repeating group: $t(18)=4.515$, $p_{corr}=.002$, $d=1.036$, $BF_{10}=117.138$). Furthermore, there was clear evidence that MOG were not larger in the Repeating group, compared to the Non-repeated group ($t(41)=-1.902$, $p=.97$, $d=-0.584$, $BF_{10}=0.119$, one-sided test). Instead, the performance difference between the Repeating and Non-repeating groups arose during ongoing practice, i.e., in 'micro-online gains'[1] ($t(41)=2.999$, $p=.002$, $d=-0.921$, $BF_{10}=18.063$, one-sided test). Thus, the effects of sequence-specific learning on performance likely occurred during practice, clearly contradicting the idea that sequence-specific replay causes MOG. Interestingly, the 'micro-online gains' for the Non-repeating group were negative, an issue to which we return in the discussion.

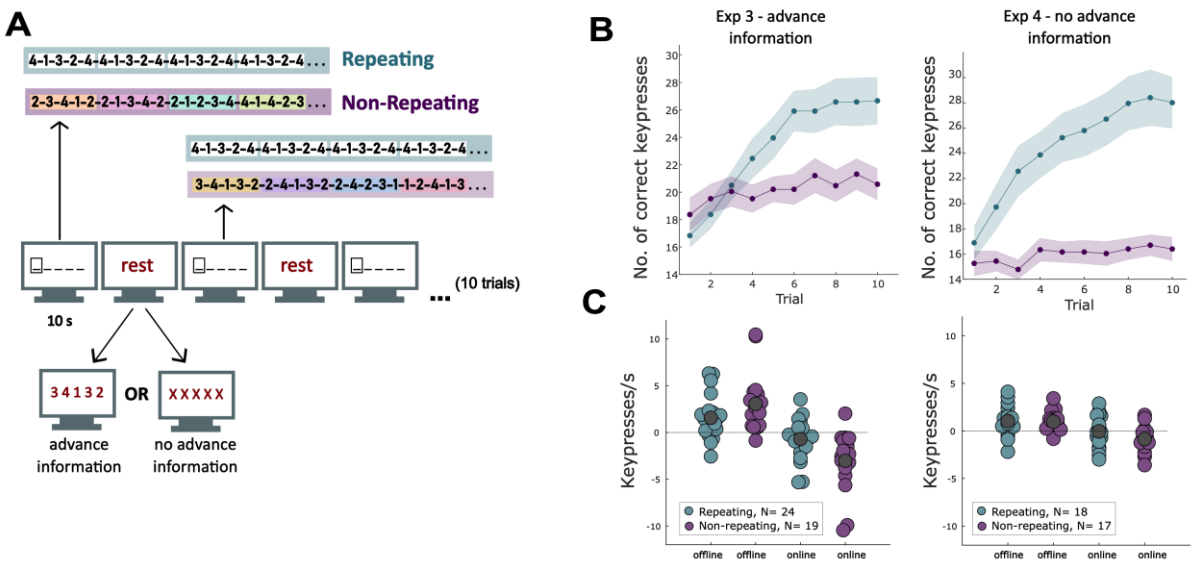

Figure 2. Experiment 3 (advance information) and Experiment 4 (no advance information). (A) Experimental design: Participants saw a string of five numbers in 10-second practice periods. Similar to a Serial Reaction Time Task (SRTT), the sequences moved left with each press, with the next keypress highlighted within a square. The Repeating group saw the same 5-element sequence repeatedly (4-1-3-2-4), allowing sequence-specific replay to take effect on performance, whereas the Non-repeating group performed non-repeating, 5-element sequences, such that sequence-specific replay of any previously encountered sequences could not benefit subsequent performance. Note that the hyphen between consecutive 5-element sequences is only for illustration purposes, while participants encountered strings of numbers continuously, without any separation into discrete sequences. A 10-second rest period preceded every practice period, which showed in red the first sequence of the next practice period (advance information, Experiment 3), or 'XXXXX' (no advance information, Experiment 4), for both groups throughout the rest. A trial is defined as a 10-second practice period, together with its following 10-second rest period. (B) The 'Repeating' group learnt to perform significantly more keypresses compared to the 'Non-Repeating' group across 10 trials, in both experiments. (C) Both groups exhibited significant 'micro-offline gains', in both experiments.

MOG partially reflect motor pre-planning

One possible reason why MOG occur similarly in both Repeating and Non-Repeating groups is that participants in both groups can pre-plan the first few elements of the upcoming sequence during the rest period. Such pre-planning has been shown to accelerate the first 3-4 keypresses of a sequence, after which execution slows down as the subsequent keypresses need to be planned online[9,10]. Such an initial speed-up due to this pre-planning could give rise to MOG (which are computed as the difference in performance from the end of one practice period to the beginning of the next). To test this idea, we

repeated the previous experiment, but omitted any advance information during rest (Experiment 4, N=18 and N=17 participants in the Repeating and Non-repeating groups, respectively). As a result, the Non-Repeating group could not plan movements until the sequence was revealed, at the onset of a new practice period.

Again, the Repeating group improved more in performance than the Non-repeating group (**Figure 2B right**), as shown by a significant group x time point interaction ($F(4.7,155.3)=12.058$, $p<.001$, $\eta^2_{partial}=0.268$). Both groups again showed significant MOG ($t(17)=2.848$, $p_{corr}=.011$, $d=0.671$, $BF_{10}=4.84$ for the Repeating group; $t(16)=4.021$, $p_{corr}=.002$, $d=0.975$, $BF_{10}=38.26$ for the Non-repeating group; one-sample t-tests against zero). There was no difference in the magnitude of MOG between Repeating and Non-repeating groups ($t(33)=0.146$, $p_{corr}=.885$, $d=0.049$, $BF_{10}=0.328$). Critically, however, comparing the Non-repeating groups between Experiment 3 and 4 (i.e., with vs. without advance information, **Figure 2C**), we found that MOG were reduced when the rest could not be used for pre-planning ($U=248$, $p_{corr}=.017$, $d=0.459$, $BF_{10}=1.811$; Mann-Whitney U to account for unequal variances between the two experiment groups).

This indicates that MOG are sensitive to explicit cueing of upcoming movements during rest, and, therefore, may partially reflect motor pre-planning. However, MOG persisted to some degree also in the Non-repeating group of Experiment 4 (without advance information). One possible reason for this is that participants pre-planned the first few movements during the reaction time period (the period at the beginning of a practice period, between first presentation of the sequence cue, and the first keypress in response to this cue; reaction time was not taken into account when calculating the tapping speed of the first sequence[1]). Indeed, the initiation of the first movement in the Non-repeating group in Experiment 4 was slower than for the Non-repeating group in Experiment 3 ($t(34)=4.995$, $p<.001$, $d=1.668$, $BF_{10}=970.765$; independent-samples t-test).

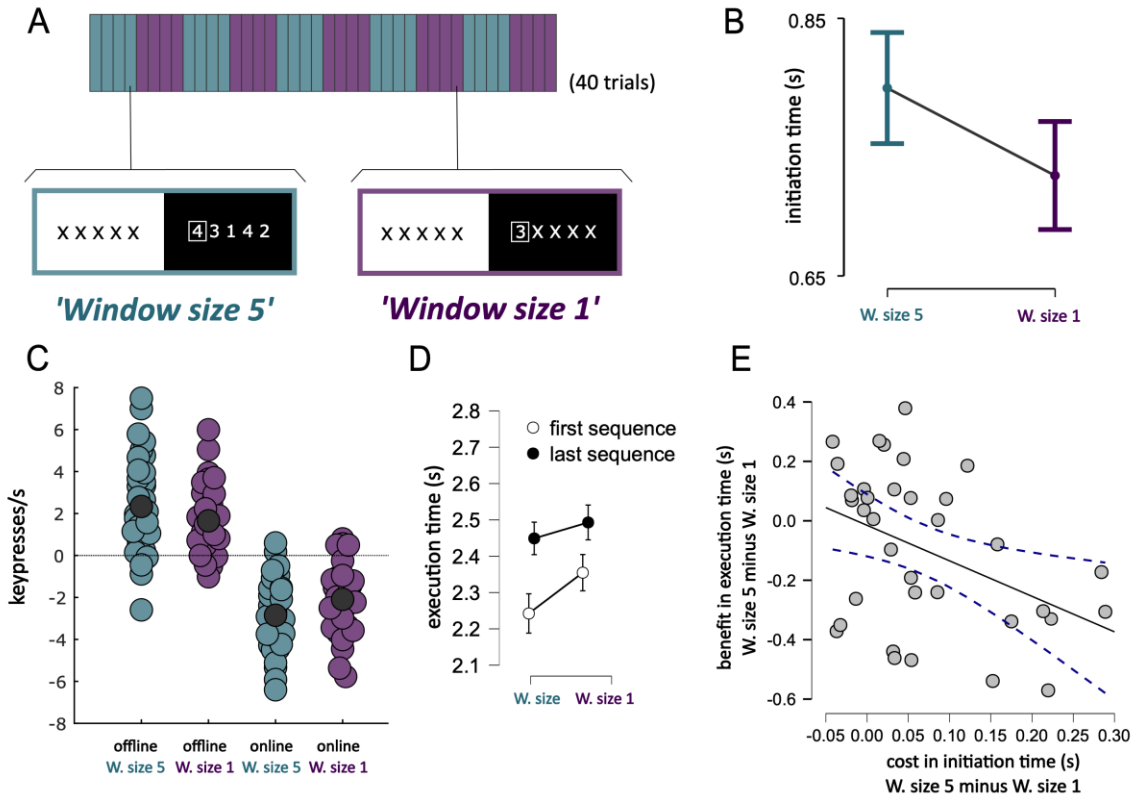

Figure 3. Experiment 5 (A) Within-subject design: The two conditions (Window size 5 and Window size 1) alternated every four trials, where a trial is defined as a 10-second practice period, together with the subsequent 10-second rest period. In every 10-second practice period, novel, i.e., non-repeating, 5-element sequences were shown, similar to Experiment 4. As in an SRTT, the sequences moved left with each press, with the next keypress highlighted within a square. In the 'Window size 5' trials, practice periods always showed 5 numbers on the screen at the same time, allowing participants to pre-plan up to 5 finger movements. This pre-planning could only occur at the start of the 10-second practice period, as each practice period was preceded by a 10-second rest period showing 'XXXXX'. In the 'Window size 1' practice periods, on the other hand, only the single next number was shown, followed by four X's (updated with every response). This prevented planning of more than a single finger movement at a time. (B) Participants initiated their first response significantly later in the 'Window size 5' trials, compared to the 'Window size 1' trials, indicating that they took time to pre-plan several upcoming finger movements before initiating the very first response. (C) Participants exhibited significant MOG in both conditions, but MOG were significantly lower for the 'Window size 1' trials. (D) A larger window size improved performance of the first, but not last, sequence in practice periods. (E) We observed a significant negative correlation between the benefit in execution time for the first sequence in a practice period in 'Window size 5' trials, compared to 'Window size 1' trials, and the cost in the time needed to initiate the first response in that sequence. This suggests that taking extra time to pre-plan speeds sequence execution.

We tested the role of pre-planning directly in a final within-subject experiment (Experiment 5, N=35, **Figure 3A**), in which we prevented any pre-planning of more than a single movement in one

condition ('Window size 1'), while allowing for pre-planning of up to five movements at the onset of a practice period in the other condition ('Window size 5'). Participants produced non-repeating 5-element sequences in 10-second practice periods, separated by 10-second rest periods. All sequences were novel (i.e., non-repeating). During practice periods in half of all trials ('Window size 5' condition), participants could see five digits on the screen at a time, cueing the next five keypresses. In the other half of all trials, they saw only a single digit at a time, and could therefore not plan more than the single next movement ('Window size 1' condition), i.e., once a key was pressed, the next number was revealed. Conditions alternated every four trials for a total of 40 trials. During rest periods, no advance information was provided, and 'XXXXX' was displayed.

We observed MOG in both conditions ($t(34)=10.049$, $p<.001$, $d=1.699$, $BF_{10}=8.822x10e8$, 'Window size 5' condition; $t(34)=7.823$, $p<.001$, $d=1.322$, $BF_{10}=2.949x10e6$, 'Window size 1' condition; **Figure 3C**). Importantly, however, MOG were larger in the 'Window size 5' condition, compared to the 'Window size 1' condition ($t(34) = 2.228$, $p=0.033$, $d=0.377$, $BF_{10}=1.593$). In addition, consistent with pre-planning, reaction times for initiating the first movement after a break were slower in the 'Window size 5' condition, due to more pre-planning, compared to the 'Window size 1' condition ($t(34)=4.343$, $p<.001$, $d=0.734$, $BF_{10}=212.874$; **Figure 3B**). Given that participants in the 'Window size 1' condition could not only pre-plan, but also plan online during the 10-second practice periods, we tested whether planning specifically improved performance at the onset of practice. A 2x2 ANOVA, comparing the time needed to complete the first vs. last correct sequence of a practice period in the 'Window size 5' and 'Window size 1' conditions, provided evidence that a larger window size improved performance at the onset of practice. There was a significant condition x position interaction effect ($F(1,34)=4.252$, $p=0.047$, $\eta^2_{partial}=0.111$), due to trend towards faster performance of the first ($t(34)= 2.157$, $p_{corr}=.072$, $d=0.365$, $BF_{10}=1.401$), but not the last ($t(34)= 1.085$, $p_{corr}=.285$, $d=0.183$, $BF_{10}=0.312$), sequence in the 'Window size 5' condition, compared to the 'Window size 1' condition (**Figure 3D**).

In addition, participants who showed more pronounced reaction time costs in the 'Window size 5' condition, i.e., took longer to initiate the first response compared to the 'Window size 1' condition,

also displayed a larger benefit in execution time of the first sequence, compared to the 'Window size 1' condition (rho=0.4181, p=.0124; considering only trials in which the first five keypresses were correct; **Figure 3E**). This indicates that participants took advantage of the opportunity to pre-plan when allowed to do so, and hints towards a significant contribution of pre-planning in modulating the magnitude of MOG.

Discussion

Our results challenge the current view that MOG are due to offline learning[1,2,6], mediated by sequence-specific replay[3]. We found that training with breaks gives rise to MOG, but conveys no lasting learning benefit over training without breaks, casting doubt on the idea that MOG result from additional, offline 'learning' during breaks. Furthermore, MOG persisted when we prevented sequence-specific effects of replay on performance. Instead, our data indicate that MOG represent short-lived performance benefits that are partly mediated by motor pre-planning.

Introducing breaks between practice sessions (spaced training) can enhance long-term learning success in many tasks, compared to massed training. However, while there is extensive research on spacing effects on declarative knowledge[11], from vocabulary learning[12] to mathematics[13] (for a review, see Smolen et al.[14]), the effects of spacing on motor skill acquisition[15–19], particularly on motor sequence learning[18], has remained largely unexplored. While spacing improved performance during training in our study, this benefit rapidly dissipated in test sessions that followed within seconds after training. These test sessions allowed for an unbiased comparison of skill levels, and revealed that people acquire similar skill levels whether, or not, they train with breaks. Our findings, therefore, do not support a spacing effect in motor sequence learning on a short-term scale. This highlights a crucial distinction in motor skill learning, as noted by Kantak et al.[20], between motor performance, i.e., observable changes during training, and motor learning, i.e., the long-term retention and stability of skills developed through training[20–22].

Consolidation of declarative and spatial memories is known to involve hippocampus[23–26] . Buch et al.[3] found that MOG correlated with rapid replay of the trained sequence during rest periods in MEG data source-localised to hippocampus (see also[27]). If neural replay of the trained sequence were driving MOG, MOG should vanish when sequences never repeat during training, because this would prevent any (sequence-specific) effect of replay on subsequent performance. Yet Experiments 3 and 4 demonstrated that MOG persisted even for sequences that never repeat. While our findings do not rule out neural replay during rest, they challenge the necessity of sequence-specific replay for MOG. Consequently, interpretations of MOG as a behavioural index of replay-mediated micro-consolidation – as extensively discussed in the literature[1–8,28] - should be considered with caution, as other mechanisms likely influence MOG.

Blood oxygenation level dependent (BOLD) signals in the hippocampus and precuneus during rest has been shown to correlate with MOG[6]. Additionally, multivariate patterns in functional MRI persist from training into short inter-practice rests[8]. While these findings have been interpreted as indicative of a memory reactivation mechanism, they may alternatively reflect motor sequence planning. Kornysheva et al.[29] identified an MEG signature of motor sequence planning that involved medial temporal lobe, and that linked practice-related information with the preceding planning stage, broadly consistent with the abovementioned fMRI studies. They found that several finger movements in an upcoming sequence could be decoded in parallel during preparation. This parallel planning signature source-localized to the parahippocampus and cerebellum, and predicted motor sequence performance[29,30].

Rest interspersed with training could thus provide an opportunity to plan the first elements of the upcoming sequence before re-starting training. In that regard, the disruptive impact of theta burst stimulation of the dorsolateral prefrontal cortex (DLPFC) on the degree of MOG[7] may potentially be attributed to the DLPFC's critical role in sequential event planning[31,32] . Building on this, in Experiment 5 we observed diminished MOG when the rest period, as well as the time before first movement

initiation, could not be used for planning several upcoming finger movements, compared to conditions allowing for pre-planning. Thus, pre-planning appears to contribute to MOG.

Previous studies have suggested that early learning predominantly occurs offline, with no evidence of learning during 10-second practice periods in these studies[1,3,4,6] (but see [5]). However, the absence of performance improvements across these practice periods may be obscured by gradual slowing of motor performance. Such slowing could arise due to a switch from fast pre-planned movements to slower online planning[9], fatigue or reactive inhibition[33], occurring concurrently or in isolation. The slowing trend might result in no net performance change from the beginning to the end of a practice session. Subsequently, the latent effects of learning during practice would then become evident after the rest period, after which pre-planning can occur again, or after fatigue and reactive inhibition dissipated[33,34]. Indeed, our finding of “negative micro-online gains” for the Non-repeating group and close-to-zero micro-online gains for the Repeating group in Experiment 3 suggest that the sequence-specific learning actually occurred during the practice periods, which then became visible after the rest periods, providing a temporary performance boost (equivalent for Repeating and Non-repeating groups).

In that regard, the method to asses MOG, i.e., comparing the speed of the first correct sequence performed after the rest to that of the last sequence before the rest, likely introduce a bias, as it assumes stable performance levels throughout the practice period, overlooking a potential decline over time. Indeed, “negative online gains”, i.e. a decrease in performance from the beginning to the end of practice periods, have been reported in several[27,33] previous studies (but see [1–3]). Furthermore, in a conceptual replication of Bönstrup et al.’s original paradigm, we observed significant micro-offline gains, coupled with substantial and significant micro-online decreases in performance, or "online forgetting" (see **SI, Figure S4**). In addition, micro-offline and micro-online gains were highly correlated (see **SI, Figure S4**). Attributing MOG solely to processes that take place during the rest may thus overlook the role of the dynamics of learning and performance during practice.

In conclusion, we propose that the improved performance observed after a short break are short-lived and do not depend on sequence-specific neural replay. The cause of the post-rest performance improvements are likely multifactorial, with the ability to pre-plan movements playing an important role. Other contributing factors could include the dissipation of reactive inhibition or fatigue during rest. Moreover, attentional recovery facilitated by context switching[35], in conjunction with pre-planning, may explain why a pause of only 3 seconds is sufficient to unveil true performance levels.

Methods:

Across the five experiments, a total of 631 participants (270 females) took part in this study. 89 (31 females, age mean = 25.9 years, SD = 3.2 years) took part in Experiment 1 and 413 (175 females, age mean = 27.9 years, SD = 7.7 years) took part in Experiment 2, 49 (29 females, age mean = 39.9 years, SD = 15.8 years) in Experiment 3, 37 (20 females, age mean = 39 years, SD = 13.5 years) in Experiment 4 and 43 (15 females, age mean = 27.3 years, SD = 3.2 years) took part in Experiment 5. For Experiment 1, we calculated the sample size sufficient to detect a difference in the number of correct keypresses between two groups of participants (with and without breaks) that is at least medium-sized (Cohen's d≥0.6) with at least 80% power (independent-samples t test; the pooled standard deviation was estimated from a previous study with 62 participants (see **SI Figure S1**)). In Experiment 2, our goal was to achieve ≥80% power to observe an effect of group on number of correct keypresses of d≥0.3. Experiments 3 and 4 were crowdsourcing studies during a science communication event at the Leibniz Institute for Neurobiology (the Magdeburg Long Night of Science), during which we collected data from as many volunteers during the six hours of the event as possible (total of 86 adult datasets collected). Cohort size for Experiment 5 was determined by our goal to observe any effect of size of d≥0.5 with ≥80% power for the effect of pre-planning on 'micro-offline gains'.

Participants of all experiments, except the crowdsourced ones (Experiments 2 , 3 and 4), were recruited based on the following exclusion criteria: they had to be right-handed, between 18 and 40 years of age, could not be professional typists or skilled musicians (i.e., recruited participants did not

play any musical instrument requiring skilled finger movements for consecutive 4 years at any point in their life), did not have any prior or existing neurological or psychiatric conditions, and were naïve to the task. Participants for Experiment 2 were recruited via the following criteria. They had to be right-handed, between 18-40 years of age, and without prior neurological or psychiatric condition. There were no criteria applied for participation in Experiments 3 and 4, but we performed analysis only on the data of participants who were right-handed and 18 years of age and above. Participants for the in-lab studies were recruited via local participant databases (Sona systems, https://magdeburg.sona-systems.com/) whose members are mostly students and staff of Otto-von-Guericke University in Magdeburg, whereas participants for the online study (Experiment 2) were recruited via the Prolific (www.prolific.com) database. Participants for Experiments 3 and 4 were volunteers during a science communication event, as described above. Handedness was determined by the Edinburgh Handedness Inventory assessment (Oldfield et al[36]). The study was approved by the ethics committee of the Otto-von-Guericke University Magdeburg, Germany, and conducted in accordance with the Declaration of Helsinki. All participants, except for visitors of the Long Night of Science taking part in Experiments 3 & 4, received financial reimbursement for their time of participation, and were additionally rewarded with bonus money based on their performance in the task.

Apparatus:

All experiments except the online crowdsourced study were conducted in a behavioral laboratory (a room with four computers on desks, and chairs arranged in the form of cubicles, for participants to sit and perform the task in privacy without distraction) using LCD displays, PC keyboards and headphones. Stimuli for Experiments 1 and 3-5 were presented using MATLAB (R2021b, The MathWorks, Inc., Natick, Massachusetts, United States) and Psychtoolbox (Brainard 1997[37]; Pelli 1997; Kleiner et al. 2007), whereas Experiment 2 (the online study) was programmed in PscyhoPy[38] and run on Prolific (www.prolific.com). In the laboratory, we tested either single participants, or up to four participants at a time simultaneously.

General task design:

Participants were asked to sit in front of an LCD monitor (60 Hz) and place the little finger, ring finger, middle finger, and index finger of their left hand on four keys of the keyboard. Throughout all experiments except Experiment 2, we used the keys F, T, Z and J (on a German-layout QWERTZ keyboard). In Experiment 2 (online) participants were instructed to use the numbered keys 1, 2, 3, 4 located on top of the keyboard. These keys were uniquely associated with the numbers 1 to 4 displayed on the monitor, so that the number 1 (or key F) corresponded to the little finger, 2 (or key T) corresponded to the ring finger, 3 (or key Z) corresponded to the middle finger, 4 (or key J) corresponded to the index finger. In Experiments 1 and 2, the screen displayed a static string of five numbers (4-1-3-2-4), for a certain duration depending on the experiment design. The task was to produce that sequence using the corresponding fingers as fast and accurately and as many times as possible throughout the practice period, i.e., as long as the sequence stayed on the screen. The string of numbers was displayed in white on a black background, surrounded by the outline of a rectangle, whose color changed with every keypress, from white to grey, or back from grey to white, as feedback that the keypresses were being registered. In Experiments 3, 4 and 5, only the first number of the sequence was surrounded by a white square and the display of the five numbers changed with every keypress, replacing the first number with the second, the second with the third, and so on, and adding a new number as the fifth. This was done to accommodate non-repeating finger sequences, as described in detail in the corresponding section below. In the different experiments, the durations of the practice periods were altered depending on the experimental manipulation, but the task instruction for practice periods remained the same throughout.

The practice periods were interleaved with rest periods of 10 seconds in some experiments. In Experiments 1, 2, 4 and 5, the screen displayed a static string consisting of five times the letter X (i.e, 'XXXXX') during rest periods, replacing the numbers. Participants were asked to fixate on the X's while resting, and not move their fingers or any other body part. In the rest periods of Experiment 3,

participants were shown the first sequence of the upcoming practice period, in red font (see details below).

Experiment 1 & 2 (with versus without breaks, in-lab and online):

In order to test if offline periods during training resulted in additional learning, i.e., overall more skill acquisition, we used a between-subject design with two groups of participants: one group trained with interspersed breaks ('With Breaks') whereas another group trained continuously for the same duration without interspersed breaks ('No Breaks'). We ran Experiment 1 with a total of 85 participants tested in-lab and Experiment 2 with a total of 358 participants who participated online. During the training, participants in the 'With Breaks' group had to learn the sequence '4-1-3-2-4' via trials that consisted of 10 s practice and 10 s rest. Participants in the other group ('No Breaks') had to learn the same sequence via continuous practice without breaks. Following the training, both groups took a break of 5 minutes, which enabled washout of fatigue. Identical tests of continuous 20 s practice were introduced at the beginning of training, end of training, and end of washout (**Figure 1A**). In order to explore potential group differences across longer training, a second block of training, washout, and tests was added. Both training blocks for both groups lasted 30 s – including 10 s practice and 10 s interspersed rest for the group 'with breaks', and a continuous 30 s practice period for the 'No Breaks' group. In Experiment 2 (online), with identical design, two attention checks were introduced during each of the 5-minute washout periods, in order to ensure that the participants did not move away from the keyboard. Each attention check required participants to press a key within the number of seconds shown on the screen. Participants were motivated to perform to the best of their capacity by rewarding them bonus money based on the total number of correct sequences they performed throughout the experiment.

Experiments 3 & 4 (Repeating versus non-repeating sequences, with and without advance information):

Experiments 3 and 4 tested whether the performance improvement across rest requires replay. To this end, one group ('Non-repeating') each in Experiments 3 and 4 performed sequences of finger movements which never repeated, neither within a given practice period, nor across the entire experiment. If MOG are due to replay, they should vanish when sequences never repeat. This is because replay of any sequences that were previously encountered could only improve future performance of that same sequence, i.e., benefits mediated by this type of specific replay effects would require that sequences repeat across practice periods. To accommodate never-repeating sequences, we modified the practice periods to closely mimic a DSP[9] (discrete sequence production) task. Every practice period was 10 s in duration. During that time, participants always saw five numbers at a time on the screen (in white on a black background). The first number was surrounded by a square frame and cued the next required movement. Participants were instructed to a press the key corresponding to that number as accurately and fast as possible. As soon as they pressed, the sequence of numbers moved to the left by one position, such that the number within the square disappeared, and the second number from the left moved into its position, while a new number appeared in the fifth position. Practice periods lasted 10 seconds, so that the participants paced the rate of cue presentation. The 'Repeating' group of participants performed the same 5-element sequence of numbers (4-1-3-2-4) repeatedly, whereas the 'Non-repeating' group of participants were presented with a set of 5-element sequences that never repeated. Both groups completed 10 trials of alternating practice and rest periods. To mimic the 4-1-3-2-4 structure of the repeating sequence, the non-repeating sequences were generated with some constraints: all four possible numbers (1-4) had to be present, with any one of these numbers presented twice in a sequence of 5 elements, and consecutive repetitions of a single number or of a pair of numbers within the sequence were not allowed. These unique 5-element sequences were organised into a long string such that the last number of a sequence was the same as the first number of the next sequence (e.g., '2-4-2-3-1-1-2-4-1-3...'), in order to match the Repeating group ('4-1-3-2-4-4-1-3-2-4...'). Participants of neither group were explicitly aware of the sequence demarcations.

In Experiment 3, both groups received advance information about the first sequence of an upcoming practice period, keeping it similar to the original studies[1,3], in which participants knew throughout rest periods which sequence they would have to perform next. Therefore, the rest periods displayed the first five cues of the upcoming practice period, in the form of a 5-element sequence displayed in red. For the 'Repeating' group, this sequence was always 4-1-3-2-4, and for the 'Non-repeating' group, it was the first sequence of the upcoming practice period.

In Experiment 4, on the other hand, the rest period preceding every practice period displayed 'XXXXX' in red at the centre of the black screen. This restricted participants from pre-planning the first sequence during rest.

Experiment 5 (pre-planning):

In this within-subject study, we tested if preventing planning of more than the next finger movement diminishes MOG. All the sequences in all trials were non-repeating. There were 40 trials, each having a 10-second rest period and a 10-second practice period, with two interleaved conditions: 'Window size 5' and 'Window size 1', for operationalising scope to pre-plan up to 5 upcoming movements and 1 upcoming movement, respectively. The two conditions switched every four trials, which was not pre-disclosed to the participants. In the 'Window size 5' condition, the practice period showed a sequence of five numbers in white, with the leftmost number being surrounded by a white square. Thus, participants were able to know the full sequence and pre-plan their finger movements before initiating the first finger movement at the start of each practice period, as well as plan online. Participants were instructed to respond as correctly and as quickly as they could, by pressing the key corresponding to the number within the square, using their left hand's corresponding finger. As soon as they pressed, the sequence shifted one number to the left, as described for Experiments 3 and 4. Participants were instructed to respond to the number within the square at all times. In the 'Window size 1' condition, the practice period showed a single number within the white square, followed by four 'X's to its right (for example, '3 X X X X'), thus removing the possibility for planning any movement

beyond the single next movement. Participants were instructed to respond as correctly and immediately as possible. As soon as they pressed a key, the white square showed the next number to be pressed, while the Xs stayed in the same position. All presented numbers in both conditions belonged to 5-element sequences, unknown to participants. All practice periods were timed to stay on the screen for 10 seconds, so the number of sequences that each participant encountered was dependent on their speed of responses. Sequences were generated in a way that there were no single number or pair of numbers that appeared consecutively within the same sequence. We also ensured that all four fingers were represented in each sequence at least once and any one finger number appeared twice in the combination in order to obtain a 5-element sequence. Each practice period was preceded by a rest period. On those periods, participants saw 'XXXXX' in white on a black background, and were asked to fixate on the 'XXXXX' without making any movement, and rest their fingers on the keys. Thus, any pre-planning in the 'Window size 5' condition could only be done once the practice period began and the five numbers showed up.

Data analysis:

Dataset exclusion criteria

Out of the complete datasets collected for each experiment, the following number of datasets were discarded from analysis for the respective experiments (see Exclusion Criteria table below): 4 from Experiment 1, 55 from Experiment 2, 8 from Experiments 3 & 4, and 8 from Experiment 5.

In Experiment 2, all participants' data were screened to check for completion, attention tests and time of initiation after the washout periods. Fully complete datasets which passed both attention tests for both washout periods, and showed initiation times less than 2.5 s from start of the post-washout test, were included for analysis.

Experiment	Reason for exclusion (No. of participants excluded)

Experiment 1 (breaks vs no breaks): in-lab study	Anomalous performance - Zero correct keypresses after baseline and 1st training (1) Anomalous performance - Zero correct keypresses in the last test T5 although they performed well throughout (1) Problems during data acquisition - incorrect keyboard, failure to follow instructions (2)
Experiment 2 (breaks vs no breaks): online study	Failed attention checks during 5-min breaks (5) Poor task commitment - took more than 2.5 s to make first response after task started post-5min breaks (38) Unable to follow task instructions – pressed wrong keys in a pattern throughout the experiment (12)
Experiments 3 & 4 (Repeating vs Non-repeating sequences, with & without advance information)	Due to poor commitment to the task – zero correct sequence performed in multiple trials (6 & 2)
Experiment 5 (pre-planning)	Due to technical issues during data acquisition leading to invalid datasets (7) Due to poor performance, pressing wrong keys on the keyboard (1)

All recorded data were stored anonymously and with the consent of participants under GDPR regulations. Information about identity and timings of the keypresses were recorded, for every practice and rest period in all the experiments. Most of the data analyses were done with the same methods as used by previous studies[1–3,6,28].

Number of correct keypresses:

While the task required producing as many fully correct sequences as possible during each practice period, errors likely resulted in sequences that were only partially correct (for example, four of the five required keypresses correct). Quantifying performance purely as the number of fully correct sequences would therefore disregard part of the task-appropriate performance, i.e., sequences that were only partially correct. To avoid this, we computed the number of correct keypresses per practice period, rather than the number of fully correct sequences[1]. Our approach to calculate the number of correct keypresses differed between Experiments 1 and 2 on the one hand, and Experiments 3-5 on the other hand. In Experiments 1 and 2, the same sequence was required throughout the entire experiment, and we did not cue single keypresses, one at a time. As a result, when they made an error, participants were free to complete the current sequence despite the error, or restart. To accommodate this, we used a sliding window approach. We first calculated keypresses that were part of fully correct sequences. For calculating the number of keypresses which were not part of a fully correct sequence, the positions of all keypresses in fully correct sequences were replaced by NaN (as a placeholder), and we then moved a 5-element sliding window to match keypresses in the current window with the required sequence position-wise, according to the following criterion. If at least three keypresses in the current 5-element window matched the required sequence by position, these keypresses were counted as correct keypresses, and they were replaced by NaN before the sliding window moved on. The reason why we set the threshold to at least three correct keypresses was to only count correct attempts to perform a fully correct sequence, and disregard instances when participants repeated, e.g., the first one or two elements of the sequence. In the case of Experiments 3, 4 and 5, in which keypresses were

cued individually, one at a time, all correct responses to the currently valid cue were counted as correct keypresses.

In Experiments 1 and 2, the continuous training durations of the 'No Breaks' group were binned into three 10-second bins, for comparison with the other group ('With Breaks'). Due to this artificial binning, there were instances where continuous correct sequences were interrupted and that is why our metric of number of correct keypresses was a better estimation of performance within 10-second bins. For consistency, each of the five 20-second test periods were also binned, and the number of correct keypresses in the two resulting 10-second bins were averaged to represent a single value for the number of correct keypresses during tests.

Tapping speed of a sequence:

Tapping speed of a completely correct sequence was evaluated as the inverse of the mean of the four inter-press intervals separating the five keypresses that constituted a fully correct sequence (keypresses per second).

Online and offline gains:

Using the same method as Bönstrup et al.[1,2], the difference in tapping speed between the first and the last correct sequence within a practice period was evaluated as 'micro-online gains', and the difference in tapping speed between the last correct sequence of a practice period and the first correct sequence of the next practice period was evaluated as 'micro-offline gains', i.e., MOG. The sum of these performance changes across all trials, results in the 'online gain' or 'offline gain' for each participant.

In Experiments 3, 4 and 5, the time between four consecutive responses in the task, corresponding to a sequence (pre-defined by us as described above, but unknown to the participants) were used to evaluate tapping speed for a correct sequence. The last five correct keypresses which were a part of a complete sequence were considered to be the last correct sequence of a practice period. For evaluation of the sum of offline performance improvements in both conditions of Experiment 5 ("Window size 5" or "Window size 1"), no MOG were computed for the rest periods for

which the condition changed (i.e., every fourth rest period) were omitted. Initiation times (**Figure 3E**) of practice periods were considered only for the trials in which the first response was part of a fully correct sequence.

Statistical analysis:

For statistical testing of online and offline performance changes, i.e. MOG, we computed one-sample t tests against zero in all experiments. In Experiments 1 and 2, we compared learning between groups via 2x5 repeated measures ANOVA (two groups, five test sessions). For the two training blocks, we computed two 2x4 repeated measures ANOVA (two groups, four time points: baseline test and three training bins), one for each training block. The baseline for each training block was the average of performance in two 10-second bins which were a part of the test that preceded the respective training blocks (i.e., first test period for the first training block, and third test period for the second training block). The Greenhouse Geisser correction was used for all instances where the sphericity assumption was violated. Interaction effects were followed up by t tests. We corrected all p values in these cases for multiple comparisons using the Holm-Bonferroni method. For statistical comparison of MOG between the 'Non-repeating' groups of Experiments 3 and 4, a Mann-Whitney U test was used to account for unequal variances between the groups of the two experiments, whereas an independent samples student's t test was used to compare between the two groups in each experiment. In Experiment 5, we used Pearson's correlation to correlate differences in sequence durations between conditions ("Window size 5" and "Window size 1") with the differences in their respective initiation times. All statistical analyses were conducted in JASP (Version 0.17.1.0, jasp-stats.org) and MatLab (The Mathworks).

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