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Research report

Gone for 60 seconds: Reactivation length determines motor memory degradation during reconsolidation

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ABSTRACT

When a stable memory is reactivated it becomes transiently labile and requires restabilization, a process known as reconsolidation. Animal studies have convincingly demonstrated that during reconsolidation memories are modifiable and can be erased when reactivation is followed by an interfering intervention. Few studies have been conducted in humans, however, and results are inconsistent regarding the extent to which a memory can be degraded. We used a motor sequence learning paradigm to show that the length of reactivation constitutes a crucial boundary condition determining whether human motor memories can be degraded. In our first experiment, we found that a short reactivation (less than 60 sec) renders the memory labile and susceptible to degradation through interference, while a longer reactivation does not. In our second experiment, we reproduce these results and show a significant linear relationship between the length of memory reactivation and the detrimental effect of the interfering task performed afterwards, i.e., the longer the reactivation, the smaller the memory loss due to interference. Our data suggest that reactivation via motor execution activates a time-dependent process that initially destabilizes the memory, which is then followed by restabilization during further practice.

1. Introduction

Animal research has convincingly demonstrated that reactivated stable memories are rendered transiently labile. These memories require subsequent restabilization, a process known as reconsolidation. In a landmark study, Nader, Schafe, and Le Doux (2000) showed that fear memories can be erased when protein synthesis is inhibited shortly after memory reactivation. Since then similar interference effects have been demonstrated across various memory domains indicating that reconsolidation is a universal process contributing to long-term memory formation (Besnard,

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Caboche, & Laroche, 2012; Nader & Hardt, 2009; Nader & Einarsson, 2010).

Although reconsolidation has also been demonstrated in humans there are far fewer studies than in animals (Schiller & Phelps, 2011). Unlike in animal models where reconsolidation is blocked by injecting protein synthesis inhibitors directly into specific brain areas, human memories are interfered with either by acquiring a competing task (Chan & LaPaglia, 2013; Forcato et al., 2007; Hupbach, Gomez, Hardt, & Nadel, 2007; Walker, Brakefield, Hobson, & Stickgold, 2003) or by orally administered drugs like propranolol (Brunet et al., 2008; Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2011). In particular, the opportunity to erase pathological memories holds considerable clinical potential, e.g., in the treatment of chronically relapsing disorders caused by post-traumatic stress (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Parsons & Ressler, 2013) or for weakening erroneous movement representations hampering the development of more efficient movement patterns during recovery from brain injury. However, previous studies in both humans and animals are inconsistent regarding whether a memory is truly degraded (e.g., in humans Kindt et al., 2009; Walker et al., 2003) or not (e.g., in humans Censor, Dimyan, & Cohen, 2010, Censor, Horovitz, & Cohen, 2014; Hupbach et al., 2007). These inconsistent findings are captured by two competing accounts (Lee, 2009): First, *the destabilization theory* posits that in order to add new information to an existing memory it is first destabilized, then modified, and finally restabilized generating a modified memory trace for future recall (Fig. 1A). Importantly, this hypothesis predicts that causing interference during the destabilization phase results in memory *loss* (Fig. 1B) (Chan & LaPaglia, 2013; Kindt et al., 2009; Nader et al., 2000; Walker et al., 2003). By contrast, *the updating theory* postulates that reactivating a stable memory opens a time window where the memory is modifiable, but importantly, no destabilization phase occurs (Fig. 1C). It predicts that interference blocks performance gains that would have been observed during uninterrupted memory formation (Fig. 1D), but it does *not* induce performance decrements (Censor, Dimyan, et al., 2010, Censor, Horovitz, et al., 2014; Rodriguez-Ortiz & Bermúdez-Rattoni, 2007).

One explanation for the divergent findings is that subtle boundary conditions constrain whether a memory can be experimentally interfered with upon reactivation (Rodriguez-Ortiz & Bermúdez-Rattoni, 2007). Whilst specific determinants of reconsolidation have been identified for animal models in the past (Auber et al., 2013; Bustos, Maldonado, & Molina, 2009; Suzuki et al., 2004), they are currently not well understood in humans (Auber et al., 2013; Schiller & Phelps, 2011; Sevenster, Beckers, & Kindt, 2013).

Here we used a motor sequence learning paradigm to show that the length of reactivation constitutes a crucial boundary condition determining whether a motor memory can be degraded. We show that reconsolidation is a dynamic time-dependent process that is initiated by memory reactivation and characterized by an initial destabilization phase followed by restabilization when reactivation is prolonged.

2. Materials and methods

In total 84 right-handed healthy volunteers participated in this study ($n = 12$ per group, 38 men, 46 women; mean age 22.9 years; range 18–31 years). None had musical training or extensive gaming experience. All subjects were naïve to the purpose of the experiment which was approved by the local

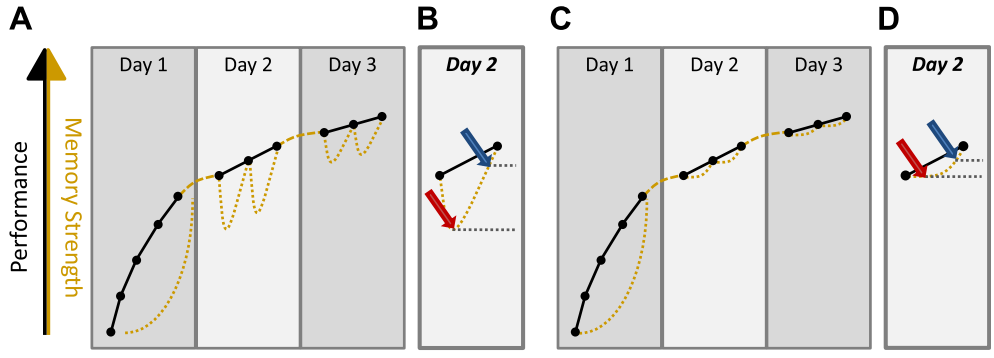

Fig. 1 – Schematic overview of the two hypotheses on the functional role of reconsolidation. The black dots depict the behavioral measurements representing the current level of performance. The solid black line connecting these dots represents the task learning curve. The yellow dotted lines indicate the evolution of the strength of the recalled memory in between the behavioral trials, and the dashed yellow lines indicate this evolution overnight. The red and blue arrows represent the administration of an interfering influence (e.g., learning a new motor sequence) and the grey dotted lines show the level of memory strength that is retained. A) Destabilization theory: A destabilization process is needed to update a motor memory, which is then followed by a restabilization phase to strengthen the new unitary memory. The destabilization phase enables new information to be added to the existing engram. B) When interference is administered during the destabilization phase (red arrow), a loss of memory strength will occur since the restabilization phase has been disrupted. When this interference is administered during the restabilization phase, no such loss will be observed (blue arrow). C) Updating theory: In this view a full destabilization process is not needed to update the memory. D) The integration of interfering information (arrows) will therefore not have any deleterious effect on the existing memory trace but will only result in partial amnesia for the newly formed memory. Adapted from Lee (2009).

Ethics Committee for Biomedical Research at KU Leuven and conformed to the Declaration of Helsinki. All participants gave written informed consent prior to participation.

2.1. General setup

The subjects were seated comfortably in front of a laptop in a quiet room free of visual distractors. Subjects performed the sequence tapping task with their left (non-dominant) hand in order to reduce the likelihood of a ceiling effect on learning (Censor, Dimyan, et al., 2010; Censor, Horovitz, et al., 2014; Karni et al., 1998; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002, 2003). Key presses were registered by four neighboring keys marked by 1, 2, 3 and 4, which corresponded to the little, ring, middle and index finger, respectively. The experiment was conducted on 3 consecutive days. For each subject all test sessions were performed at approximately the same time of day to ensure sessions were separated by 24hrs. There were no restrictions between subjects whether the sessions were held in the morning or afternoon, since it was previously shown that this does not influence motor sequence learning (Brawn, Fenn, Nusbaum, & Margoliash, 2010; Walker et al., 2002). It was verified that subjects slept at least 6 h per night (self report) before every experimental session to ensure overnight consolidation and to reduce the influence of general fatigue.

2.2. Motor task

The sequence tapping task was adapted from Karni et al. (1998) and has previously been used in motor reconsolidation research (Censor, Dimyan, et al., 2010; Censor, Horovitz, et al., 2014; Walker et al., 2003) (Fig. 2A). Two different five element sequences (A: 4-1-3-2-4 and B: 2-3-1-4-2) were used interchangeably throughout the experiment; one being the learning sequence (*SeqLearn*) and the other the interfering

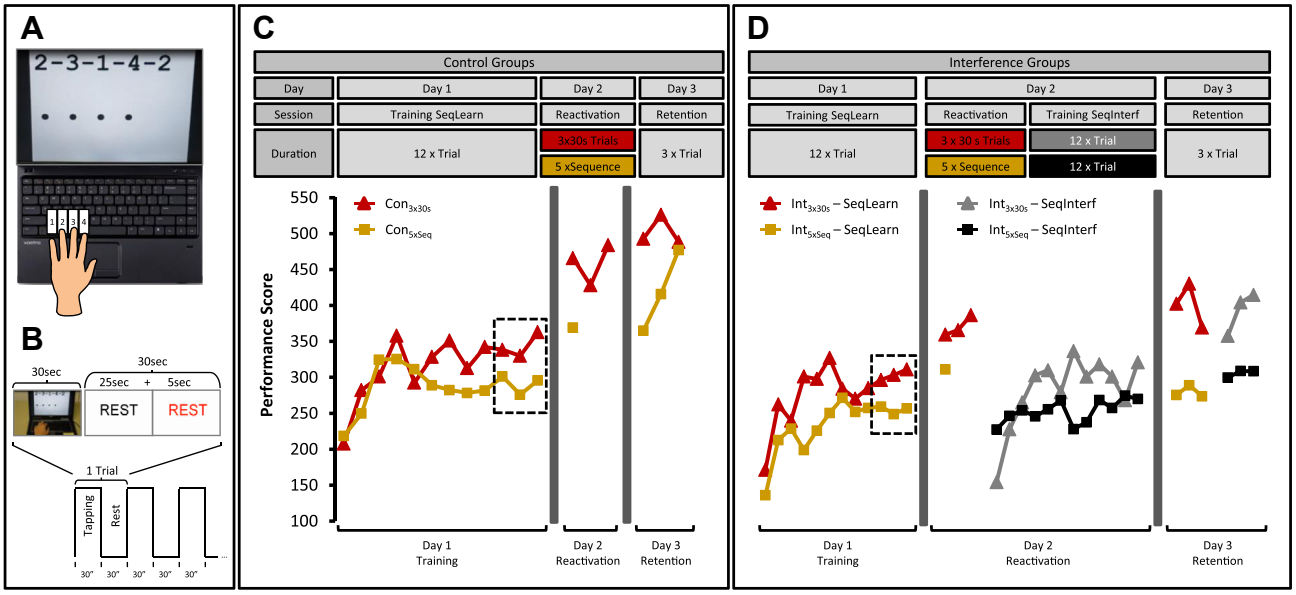

Fig. 2 – Schematic representation of the finger tapping task and experimental protocol. Individual subject data are presented in the temporal order of the testing protocol. A) The motor task was performed with the left non-dominant hand on a laptop keyboard. Two different five element sequences were used throughout the experiment, both consisting of four numeric keys (A: 4-1-3-2-4 and B: 2-3-1-4-2). Each number represented a finger; with '1' being the little finger, '2' the ring finger, etc. Assignment of sequences to the learning and interference conditions was counter-balanced. The sequence to be executed was shown on the computer screen using the same numbering system to reduce the likelihood of the task including a working memory component. While performing the task a black dot appeared on the screen indicating that a key had been pressed. Key presses were recorded and no feedback was provided regarding task accuracy. B) An experimental trial consisted of 30 sec of sequence tapping followed by a rest period of 30 sec to prevent fatigue. Participants were instructed to type the sequences as quickly and as accurately as possible and were motivated continuously throughout the experiment. C + D) The experiment was conducted on 3 consecutive days and all test sessions were performed at approximately the same time of day to exclude circadian effects on learning. During the first day of the experiment (*training session*) subjects were trained on the same sequence (SeqLearn) for 12 trials, with the average of the last 3 trials used as an indicator of final performance (black dashed box). On the second day, SeqLearn was reactivated (*reactivation session*) and was followed by rest (C, control groups) or by learning a new sequence (SeqInterf) (D, interference groups). Depending on which group the subject was in, the reactivation lasted for 3 × 30 sec (red graphs; $Con_{3 \times 30\ sec}$ and $Int_{3 \times 30\ sec}$) or 5 complete SeqLearn sequences (yellow graphs; $Con_{5 \times Seq}$ and $Int_{5 \times Seq}$). On the third and final day of the experiment (*retention session*) we measured the final performance level of SeqLearn (3 × 30 sec) and SeqInterf (3 × 30 sec).

sequence (*SeqInterf*). Sequences were counter-balanced across subjects. The required sequence was displayed on the computer screen and each number represented a finger tap as specified above.

While performing the task each key press produced a black dot on the screen indicating that a response was registered. Feedback regarding whether or not the response was correct was not provided. An experimental trial consisted of 30 sec sequence tapping followed by 30 sec of rest to prevent fatigue (Fig. 2B). Participants were motivated throughout the experiment to type the sequences as quickly and accurately as possible.

During the first day of the experiment (*training session*) subjects practiced the sequence for 12 trials. The second day started by reactivating SeqLearn which was learned the day before (*reactivation session*), with the duration of reactivation varying across the experimental groups. In the interference (INT) groups reactivation was followed by the acquisition of a new interfering sequence. On the third and final day of the experiment (*retention session*) subjects performed three SeqLearn trials, which were followed by three SeqInterf trials in the INT groups, to provide an indication of the final level of performance (Fig. 2C,D).

First, we tested four experimental groups that differed only with respect to the reactivation session on the second day: two were control (CON) groups in which either a short or a long reactivation was followed by rest, and two were INT groups in which either a short or long reactivation was immediately followed by the acquisition of an interfering sequence. The long reactivation condition consisted of three experimental trials of 30 sec each (3 × 30 sec), while the short reactivation condition consisted of five complete sequences (5 × Seq) lasting on average less than 10 sec. Reactivation lengths were chosen on the basis of previous research in human and animals, in addition to pilot testing. While the length of our long reactivation condition is similar to other reconsolidation studies in humans (Censor, Dimyan, et al., 2010; Censor, Horovitz, et al., 2014; Walker et al., 2003), animal research often uses a very brief reminder, for example, one repetition of the conditioned stimulus or simply re-exposure to the context in which the task was learned. In our case, during the short reactivation condition we still wanted to obtain a reliable measure to determine whether or not learning was consolidated. Pilot testing indicated five complete sequences was optimal for this purpose. In summary, our first analysis comprised (i) a control group that performed a long reactivation phase followed by rest ($Con_{3 \times 30\ sec}$); (ii) a control group that performed a short reactivation phase followed by rest ($Con_{5 \times Seq}$); (iii) an interference group that performed a long reactivation phase followed by acquiring an interfering sequence ($Int_{3 \times 30\ sec}$); and (iv) an interference group that performed a short reactivation phase followed by acquiring an interfering sequence ($Int_{5 \times Seq}$).

Next we aimed to replicate and extend on our first experiment by testing three additional INT groups who followed the exact same protocol as described above except that they experienced intermediate reactivation lengths: (v) 10 × SeqLearn ($Int_{10 \times Seq}$); (vi) one trial of 30 sec ($Int_{1 \times 30\ sec}$); and (vii) one trial of 60 sec ($Int_{1 \times 60\ sec}$).

2.3. Data analysis and statistics

Key presses were recorded (E-Prime Psychology Software Tools, inc – Shapsburg, USA) and accuracy was calculated as the percentage of correct sequences completed during each 30 sec trial. Performance speed was measured as the time (sec) between key presses, i.e., the inter-tap interval (ITI). It is well known that most motor tasks are characterized by the so-called "speed-accuracy trade off", meaning that for a given skill level, accuracy diminishes when speed is increased. True skill improvement is therefore indicated by a shift of the speed-accuracy function (Reis et al., 2009; Shmuelof, Krakauer, & Mazzoni, 2012). A pilot experiment ($n = 10$) revealed that accuracy decreased when sequence tapping speed was increased (paced by a metronome), and that this speed-accuracy function is well approximated by a linear relationship between the % accuracy and the ITI ($r = .94$, see Supplementary material). Based on these results, we quantified performance by a score derived from this speed-accuracy function. The performance score was calculated for each subject and trial by dividing the % of accurately typed sequences by the ITI, hence higher scores indicate better performances. This measure better reflects the skill level of the performer compared to assessing changes in speed and accuracy separately.

Performance scores of the last 3 training trials on day 1 were averaged and represented the baseline performance for subsequent comparisons to the second day. The data available for the reactivation session differed between experimental groups due to the wide range of reactivation lengths tested. To keep the analysis consistent across all groups performance scores calculated for day 2 and day 3 were based on the first 5 sequences only. This approach is also advantageous since it is typical for performance on this task to deteriorate after the first 10 sec, most likely due to fatigue (Brawn et al., 2010).

To visualize performance increases or decreases between two consecutive days, a ratio was calculated by dividing the performance scores. A negative ratio indicated memory loss while a positive ratio indicated further memory improvement overnight, or so called off-line gains.

For the first analysis, performance scores of the $Con_{3 \times 30\ sec}$, $Con_{5 \times Seq}$, $Int_{3 \times 30\ sec}$, and $Int_{5 \times Seq}$ groups were subjected to an Analysis of Variance for repeated measurements (ANOVA, Statistica 8, StatSoft, USA). Separate models were estimated according to the specific research question. We first performed three control analyses to investigate the learning and consolidation of SeqLearn: (i) Initial learning was assessed by testing for a significant increase in performance over the first 9 trials of the training session on day 1. This model included the between subjects factor *group* ($Con_{3 \times 30\ sec}$; $Con_{5 \times Seq}$; $Int_{3 \times 30\ sec}$; and $Int_{5 \times Seq}$) and the within subjects factor *trial* (1–9). (ii) We tested whether there were differences between these four experimental groups in the final performance level obtained on the last 3 training trials of day 1. For this model we included the between subjects factor *group* ($Con_{3 \times 30\ sec}$; $Con_{5 \times Seq}$; $Int_{3 \times 30\ sec}$; and $Int_{5 \times Seq}$) and the within subjects factor *trial* (10–12). (iii) Our final control analysis tested if the task acquired during the learning session on day 1 was successfully consolidated by

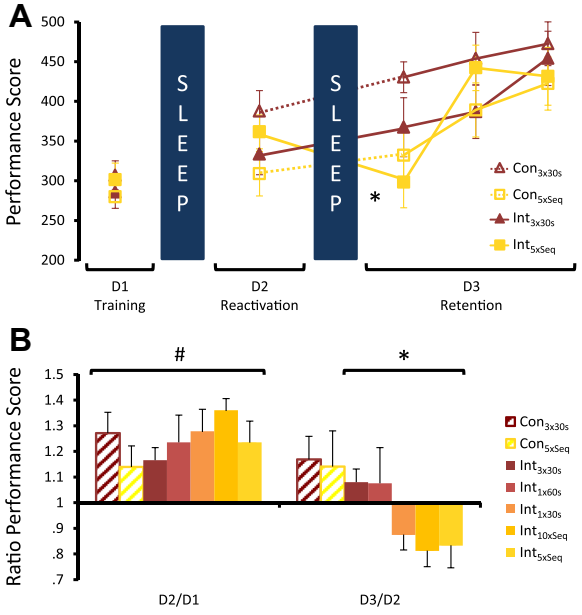

Fig. 3 – Evolution of performance on the finger tapping task over 3 consecutive days. A) The performance scores for the two control and two interference groups with either a long ($Con_{3 \times 30\ sec}$ and $Int_{3 \times 30\ sec}$) or short reactivation ($Con_{5 \times Seq}$ and $Int_{5 \times Seq}$). On day 1, the final performance level is characterized by the average of the last three training trials (black dashed boxes in Fig. 2C,D). The data representing day 2 performance are the first 5 complete SeqLearn sequences (for all experimental groups). The data representing performance on the third and final day are the first 5 complete SeqLearn sequences of each trial. * represents a significant *day* × *group* interaction [$F(3,44) = 2.88$, $p = .046$] that is driven by the significant drop in performance from day 2 to day 3 for $Int_{5 \times Seq}$ (LSD Posthoc test: $p = .034$). On day 3 a quick recovery is observed for $Int_{5 \times Seq}$ indicating that the effect of the interfering sequence is only short-lived. B) The performance ratio between day 2 (first 5 tapped sequences) and day 1 (average of last 3 training trials of 30 sec) (*D2/D1*), and between day 3 (first 5 tapped sequences) and day 2 (first 5 tapped sequences) (*D3/D2*), is shown for all experimental groups. An ANOVA revealed similar overnight gains for all groups indicated by a main effect of *day* ($p < .00001$) (#) and no *day* × *group* interaction ($p > .05$). The change in performance between day 2 and day 3 on the other hand is different between interference groups, indicated by a significant *day* × *group* interaction ($p \leq .047$) (*). Moreover a preplanned comparison shows a linear effect of the reactivation length, with shorter reactivation periods preceding the interference corresponding to greater motor memory degradation [$F = 7.81$, $p = .0071$]. # represents a main effect and * an interaction effect ($p < .05$). Vertical bars indicate SEs.

examining performance changes between day 1 and day 2 in these four experimental groups. For this model we included the between subjects factor *group* ($Con_{3 \times 30\ sec}$; $Con_{5 \times Seq}$; $Int_{3 \times 30\ sec}$; and $Int_{5 \times Seq}$) and the within subjects factor *day* (baseline at the end of the learning session – first five sequences of the reactivation session).

We then tested the influence of reactivation length on reconsolidation by examining performance changes between day 2 and day 3. First we determined whether a different outcome was apparent when comparing the shortest (5 × Seq) and longest (3 × 30 sec) reactivation length. To test this, our model included the between subjects factor *group* ($Con_{3 \times 30\ sec}$; $Con_{5 \times Seq}$; $Int_{3 \times 30\ sec}$; and $Int_{5 \times Seq}$) and the within subjects factor *day* (first 5 tapped sequence of the reactivation session – first 5 tapped sequenced of the retention session).

On the basis that the long reactivation length resulted in less memory degradation compared to the short reactivation length, we then included intermediate reactivation lengths to test if a similar pattern was observed across a range of reactivations lengths, and if the relationship between reactivation length and memory degradation is linear. This was tested with a model including the between subjects factor *group* ($Int_{3 \times 30\ sec}$; $Int_{1 \times 60\ sec}$; $Int_{1 \times 30\ sec}$; $Int_{10 \times Seq}$; and $Int_{5 \times Seq}$) and the within subjects factor *day* (first 5 tapped sequence of the reactivation session – first 5 tapped sequenced of the retention session). Linear effects of the reactivation length were tested with a preplanned comparison using the following contrast vector [2, 1, 0, −1, −2] for the factor *group*. We reproduced the three control analyses as described above (i, ii, iii) with the inclusion of the $Int_{1 \times 60\ sec}$, $Int_{1 \times 30\ sec}$ and $Int_{10 \times Seq}$ groups to test whether all groups learned and consolidated SeqLearn. The alpha level for all statistical tests was set to .05. Post hoc comparisons were performed with Fisher's LSD test.

3. Results

Four groups of subjects practiced the finger tapping task (Fig. 2) and all groups significantly improved performance of SeqLearn over the course of training on day 1 (*trial* main effect $F(8,352) = 60.51$, $p < .001$; no *trial* × *group* interaction $p = .654$) (see Supplementary Fig. 2). The final level of performance, quantified by the average of the last three training trials, was not significantly different between groups (no *group* main effect or *trial* × *group* interaction $p \geq .703$). Reactivating the motor memory on day 2 revealed further over-night improvements that ranged between 13.9% ± .28 and 27.1% ± .28 across groups (so called "offline gains" Fig. 3A, left panel of Fig. 3B; *day* main effect $F(1,44) = 24.44$, $p < .001$; no *day* × *group* interaction $p = .417$). These results confirm that all groups learned and consolidated the task in a manner consistent with previous findings (Censor, Dimyan, et al., 2010; Censor, Horovitz, et al., 2014; Walker et al., 2002, 2003).

If reactivating the motor memory on day 2 was followed by rest (control condition), further offline gains were observed during retention on day 3. This effect was present irrespective of whether the reactivation period was long, requiring subjects to tap the sequence for 3 blocks of 30 sec ($Con_{3 \times 30\ sec}$), or short, requiring subjects to tap only 5 sequences lasting on average 7.52 sec ± 1.55 ($Con_{5 \times Seq}$) (Fig. 3A,B open symbols). However, if reactivating the motor memory was immediately followed by acquiring an interfering sequence, the length of

the reactivation period had a significant influence on retention performance on day 3. If the reactivation period was long ($Int_{3 \times 30\ sec}$), only minor interference was observed with off-line gains from day 2 to day 3 smaller than for the $Con_{3 \times 30\ sec}$ group but still present (8.0% ± .18 *vs* 16.9% ± .31). By contrast, when the reactivation period was short ($Int_{5 \times Seq}$), acquiring an interfering sequence significantly degraded the motor memory and corresponded with a performance drop of −16.8% ± .30 from day 2 to day 3 (Fig. 3A, solid yellow square; Fig. 3B right most yellow bars). An ANOVA revealed a significant *day* × *group* interaction [$F(3,44) = 2.88$, $p = .046$] and post-hoc tests confirmed that the performance drop of the $Int_{5 \times Seq}$ group differed significantly ($p = .034$) from each of the other groups [$F \geq 4.49$, $p \leq .04$]. Performance decrements in the $Int_{5 \times Seq}$ group were only present in the first trial of day 3 but recovered quickly during the subsequent two trials. Hence, our data support the destabilization theory since interference after a short memory reactivation induced a temporary memory loss.

One unexpected result was that when the long reactivation was followed by learning the interfering sequence ($Int_{3 \times 30\ sec}$), off-line gains from day 2 to day 3 were reduced but no performance decrease was observed. We hypothesized that even though reactivation destabilized the motor memory initially, prolonged execution of the SeqLearn would induce new learning and trigger memory restabilization. To test the hypothesis that new learning occurred in the long reactivation groups, reactivation data of $Con_{3 \times 30\ sec}$ and $Int_{3 \times 30\ sec}$ were subjected to a repeated measures ANOVA. We observed a significant *trial* main effect [$F(2,44) = 4.09$, $p < .05$] but no *trial* × *group* interaction ($p = .44$), indicating that there were performance gains during these 3 reactivation trials and that a learning effect was present in both groups.

Next we tested whether a gradual increase in reactivation length would reduce the probability of memory degradation due to interference. Therefore, three additional INT groups performed the task with varying lengths of reactivation periods requiring subjects to tap either until 10 sequences were completed (corresponding to 15.64 sec ± 3.72, $Int_{10 \times Seq}$), for one block lasting 30 sec ($Int_{1 \times 30\ sec}$), or for one block lasting 60 sec ($Int_{1 \times 60\ sec}$). Performing the control analyses with the inclusion of the three additional INT groups confirmed learning during the first 9 trials (*trial* main effect $F(8, 616) = 121.25$, $p < .001$; no *trial* × *group* interaction $p = .16$) and similar performance levels between groups during the last three trials of day 1 (no *group* main effect or *trial* × *group* interaction $p \geq .254$). All groups also showed offline gains from day 1–2 (*day* main effect $F(1, 77) = 58.477$, $p < .001$; no *day* × *group* interaction $p = .45$) indicating that the task was well consolidated (Fig. 3B, left panel). Interestingly, interference after memory reactivation induced a drop in performance from day 2 to day 3 for the $Int_{10 \times Seq}$ (−18.8% ± .21) and $Int_{1 \times 30\ sec}$ (−12.6% ± .2) groups, whereas offline gains were observed for the $Int_{1 \times 60\ sec}$ group (+7.6% ± .48). Furthermore, in the $Int_{1 \times 60\ sec}$ group individual differences were particularly large (Fig. 3B, right panel): one third of the subjects showed clear memory degradation, one third exhibited clear offline gains, and one third exhibited only minor changes (i.e., performance from day 2 *vs* day 3 differed by less than 10%). Including all INT groups in one statistical model confirmed that there was a significant linear relationship between the extent to which motor memory changed from day 2 to day 3 and the length of the reactivation period, with a shorter reactivation resulting in a larger memory loss when followed by interference [$F = 7.81$, $p = .0071$].

Here we quantified tapping performance via a performance score based on a linear speed-accuracy function, whereas previous motor reconsolidation studies reported speed and accuracy measurements separately (Censor, Dimyan, et al., 2010; Censor, Horovitz, et al., 2014; Walker et al., 2003). We therefore repeated our final analysis independently for speed and accuracy measures. The linear relationship between reactivation length and memory degradation due to interference was confirmed for both variables (Fig. 4 A,B), however, significance was only reached for speed ($p < .05$), but not accuracy ($p = .21$). This indicates that interference was manifested as reduced speed (longer ITI), and to a lesser extent, reduced accuracy.

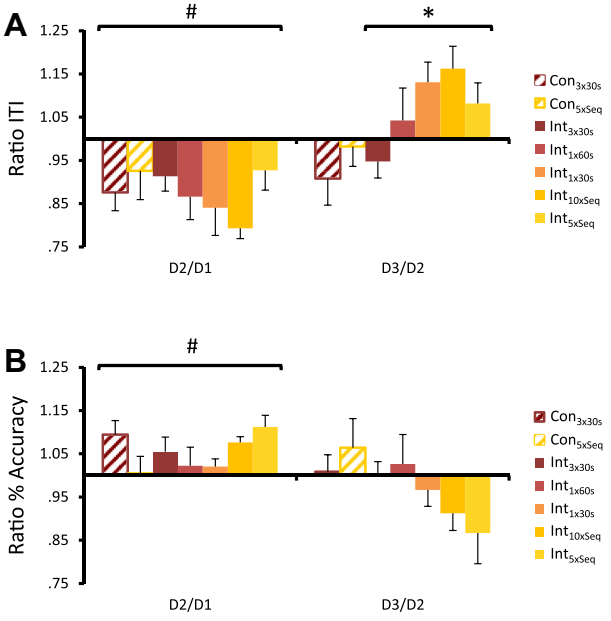

Fig. 4 – Task performance represented by speed (A) and accuracy (B) measures. The speed and accuracy data are presented in an identical manner to the performance score shown in Fig. 3B. A) Overnight gains in speed were obtained for all groups, indicated by a main effect of *day* [$F(1, 77) = 51.742$, $p < .001$] (#) and no *day* × *group* interaction [$F(6,77) = 1.2092$, $p = .31072$]. A shorter reactivation period preceding the interference corresponds to greater motor memory degradation resulting in slower execution speeds ($p < .05$) (*). B) Overnight gains in accuracy were obtained for all groups, indicated by a main effect of *day* [$F(1, 77) = 21.424$, $p < .001$] (#) and no *day* × *group* interaction [$F(6,77) = 1.7066$, $p = .13073$]. A trend toward reduced accuracy during shorter reactivations was also observed, but this effect was not significant ($p = .21$). # represents a main effect and * an interaction effect ($p < .05$). Vertical bars indicate SEs.

4. Discussion

Our results demonstrate that the length of the reactivation period constitutes a crucial boundary condition for interfering with the reconsolidation of human motor memories. Our findings are consistent with previous work in rodents reporting that the length of memory reactivation and extinction training sessions is a critical parameter that determines whether amnestic treatment will block reconsolidation (Eisenberg, Kobilo, Berman, & Dudai, 2003; Lee, Milton, & Everitt, 2006; Pedreira & Maldonado, 2003; Rodriguez-Ortiz & Bermúdez-Rattoni, 2007; Suzuki et al., 2004). We measured the strength of the motor memory via a performance score reflecting changes in the speed-accuracy function. This analysis proved to be more sensitive than using speed or accuracy alone, even though a similar pattern of results was observed for both parameters and statistical significance was reached when ITI was used as an estimate of motor performance.

The observed pattern of changes in performance score from day 2 to day 3 clearly support the *destabilization hypothesis* (Fig. 1A), an assertion based on our finding that a motor memory can be *degraded* when a short reactivation is followed by interference. We propose that within the motor memory domain, reactivation via motor execution activates multiple time dependent processes such that a previously stable memory is destabilized initially but restabilization occurs when practice commences, with a transition occurring after approximately 60 sec. It is important to note, however, that this estimate is only suitable for the present paradigm since the exact reactivation length needed to optimally interfere with memory reconsolidation might additionally depend on the intensity and remoteness of initial learning (Rodriguez-Ortiz & Bermúdez-Rattoni, 2007; Tronson & Taylor, 2007).

Even though our data suggest that the transition from memory destabilization to memory restabilization occurs after approximately 60 sec at the group level, we observed substantial inter-subject variability. Not surprisingly, interference effects were more variable for long than for short reactivation periods suggesting that in some subjects the transition to memory restabilization occurred earlier than in others. Our finding might reconcile much of the inconsistencies regarding previous studies using reconsolidation interference paradigms: Walker et al. (2003), Censor, Dimyan, et al. (2010) and Censor, Horovitz, et al. (2014) used the same motor task and design as we did, but only Walker et al. (2003) reported memory loss after interfering with the reactivated memory. Censor, Dimyan, et al. (2010) and Censor, Horovitz, et al. (2014) found reduced offline gains and concluded that their interfering intervention, i.e., disruptive brain stimulation of the primary motor cortex, was less effective in perturbing memory relevant circuits than acquiring a new motor sequence. Our results offer a potential alternative explanation since both previous studies used long reactivation periods of 3 times 30 sec, which our finding suggests does not lead to robust destabilization. This raises the possibility that the results of previous reconsolidation studies using relatively long reactivation periods might have been susceptible to individual differences within the investigated sample.

The fast recovery of performance on day 3 when practice continued indicates that the detrimental effects of the interfering intervention were only temporary, a finding that is consistent with previous motor learning studies in animals (Peng & Li, 2009) and humans (Censor, Horovitz, et al., 2014). Censor, Horovitz, et al. (2014) found that corticostriatal functional connectivity in an interference group measured at the end of a retention session recovered after additional execution of the initially learned task. Currently, it is unclear whether interference diminished initial performance on day 3 because of a retrieval failure (retrieval theory) or because the memory was partly erased (storage theory) (Tronson & Taylor, 2007), but whatever process is perturbed appears to recover quickly when re-exposed to the task.

In conclusion, our study shows that the length of reactivation is a crucial boundary condition determining whether human motor memories can be partly disrupted. Our results clearly indicate that reconsolidation initiated by memory reactivation is a dynamic time-dependent process that is characterized by an initial destabilization phase followed by restabilization when reactivation is prolonged. This finding has important implications for optimizing reconsolidation-based treatments and future experiments investigating reconsolidation.

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Supplementary data

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