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The malleability of memory:

The effect of intervention strength during reconsolidation

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Abstract

During the process of reconsolidation, memories become susceptible to change, by adding new information as a post-reactivation intervention. The current study examines the interaction effect between reactivation and new learning on an old strong memory. On day 1, participants learned a set of pictures three times to create a strong memory. On day 8, seven days after initial learning, participants reactivated the original pictures set an/or learned a second picture set once or thrice. A day later, participants engaged in a memory performance test in which they indicated which pictures belonged to the original set. Results show that reactivation prior to new learned had no effect on memory performance. New learning led to lower scores on memory performance. Interestingly, no difference was found between weak and strong new learning.

Introduction

The episodic memory holds representations that allow us to recall the context-bound and context-free information that form our view of the world (Nadel, Hupbach, Gomez & Newman-Smith, 2012). These learning experiences help us adequately adapt to our surroundings. Newly acquired information enters the short-term memory, where it is still active and labile, and thus vulnerable to disruption by amnesic agents (Lewis, 1979). Nader, Schafe and LeDoux (2000) show that if a person learns one task, and then a second task shortly afterwards, the memory of the first task can be compromised. However, if the second task is learned several hours after initial learning, then there is no interference. This suggests that there is a specific time window in which the newly acquired memory is vulnerable (Sara, 2000b; McGaugh, 2000), before being encoded and transformed into a more inactive and stabile state in the long-term memory (LTM; Artinian et al., 2008; Dudai & Eisenberg, 2004; McGaugh, 2000; Nader et al., 2000; Schwabe, Nader & Pruessner, 2014). This process is known as the consolidation hypotheses (Sara, 2000b; McGaugh, 2000).

For many years scientists believed that memory consolidation left long-term memories insensitive to disruption (McGaugh, 2000). More recent studies, however, show that memory formation and storage are not as rigid as suggested, but that memories enter a labile state, whenever they are being recalled (Alberini, 2011; Forcato et al., 2007; Schwabe et al., 2014). Nader et al. (2000) shows that a labile memory, after reactivation, needs to be stabilized again using novo protein syntheses to persist in long-term memory. The process in which a reactivated labile memory becomes stabile again, is called reconsolidation (Alberini, 2011; Nader et al., 2000; Sara, 2000a; Lee, 2009). As with consolidation, there appears to be a specific time window during which blocking of protein synthesis is effective (Alberini, 2011; Suzuki et al., 2004).

Researchers propose that the reconsolidation process is meant to update or integrate new information in the already existing memory (Alberini, 2011; Hupbach, Gomez, Hardt & Nadel, 2007; Nader et al., 2000; Sara, 2000a, 2000b; Suzuki et al., 2004; Wichert, Wolf & Schwabe, 2013a) allowing maintenance of the memory relevance to daily life (Exton-McGuinness, Lee, & Reichelt, 2015). A second theory proposes reconsolidation is meant for strengthening memories (Sara, 2000b, Wichert, Wolf & Schwabe, 2011). Research by Inda, Muravieva, and Alberini (2011) used inhibitory avoidance in rats and non-reinforced reminders. They found that reactivation of a young memory, accompanied by its reconsolidation, results in memory strengthening. An alternative function, however, might be to use the reconsolidation process to weaken traumatic memories (Lonergan & Pitman, 2013; Wichert et al., 2011).

Being able to weaken memories holds important advantages in changing unwanted and disruptive memories (Wichert et al., 2011), which is of great clinical relevance for people suffering from Post-Traumatic Stress Disorder (PTSD; American Psychiatric Association, 1994). In the case of PTSD these learning experiences produce dysfunctional thinking patterns and emotional responses that are impairing to everyday life (Alberini, 2011). Finding strategies to alter dysfunctional memories and thinking patterns are thus a crucial step in enabling successful treatments that aim at weakening pathogenic memories (Schwabe, Wolf & Oitzl, 2010; Schwabe et al., 2014).

In previous research, pharmacological interventions that target fearful memories show that the use of protein synthesis inhibitors on the reconsolidation process could be a promising treatment for traumatic memories (Dudai & Eisenberg, 2004; Lewis, 1979; Nader et al., 2000; Sara, 2000a; Suzuki et al. 2004). However, a meta-analysis by Lonergan and Pitman (2013) showed that the evidence of pharmacological interventions on reconsolidation are not robust due to the lack of heterogeneity across studies and the absence of clinical groups. Furthermore, A study by Wood et al. (2015) on pharmacological interventions for memory reconsolidation in PTSD, failed to replicate previous findings on the effects of pharmacological interventions, suggesting that there might be non-specific actions of the drug, that influence the results. Therefore, consistent evidence is lacking, on the use of amnesic agents to weaken strong emotional memories.

Another line of research relevant for the treatment of fear-related disorders is based on behavioral studies (Parsons & Ressler, 2013). There is first evidence that behavioral interventions during the reconsolidation window reduce subsequent responding to mental imagery of the traumatic event(s) in PTSD patients (Brunet et al., 2008). Research shows that presenting a subtle reminder just before additional learning takes place can modify episodic memory (Hupbach et al., 2007; Hupbach et al., 2008; Sara, 2000; Wichert et al., 2011, 2013a, 2013b). For example, in a study by Wichert et al (2013a), participants learned a set of pictures on day one, comprised of negative as well as neutral pictures. On day eight they recalled the pictures and learned a new set of pictures. The final testing was done 24 hours after day 2 by computer recognition test, indicating which pictures they had seen on day 1. Results showed that learning a second set of pictures after reactivation resulted in less remembered pictures from day 1. New Learning without previous recall did not show the same impairment in memory for pictures seen on day 1. In light of these results, it is proposed that brief reminders lead to reactivation, and can therefore be used to update memory (Hupbach et al., 2007; Hupbach et al., 2008; Wichert et al., 2011, 2013a).

Strength of memories appears to play a key role in the process of reconsolidation and the modification of apparently stable memories (Karpicke & Roediger, 2008; Suzuki et al., 2004; Wang, De Oliveira, Alvarez & Nader, 2009; Wichert et al., 2011, 2013a, 2013b). Research shows that strong new memories can significantly alter the original memories more than weak new memories can (Karpicke & Roediger, 2008; Wichert et al., 2013a, 2013b). For example, Wichert et al. (2013b) focused on the effect of strength of new learning on memory, after reactivation. Participants learned a set of 60 pictures on day 1 of the experiment. On Day 8 they reactivated the previously learned picture set by verbally describing the pictures they remembered from day 1, and/ or learned a new picture set either once (weak new learning) or thrice (strong new learning). On day 15, participants were asked to perform a recognition test, indicating which pictures they had seen on day 1. Results showed that reactivation in combination with weak learning had no effect on memory. Reactivation in combination with strong learning, however, led to participants falsely indicating pictures from day 2 as seen on day 1. These results show that strength of new learning plays an important role in the updating of memories.

Strength of the original memory also has an effect on the reconsolidation process. The original memories in PTSD are considered strong due to repeated strengthening as a result from spontaneous intrusions (Brunet et al., 2008), making them more resistant to modifications (Sara, 2000; Suzuki et al., 2004; Wichert et al., 2011). Thus far it is unclear whether such robust memories can be modified in a similar fashion as relatively weak memories.

The current research investigates the possibility of changing strong memories, by adding new information (Alberini, 2011; Dudai & Eisenberg, 2004; Hupbach et al., 2007; Nader et al., 2000b; Sara, 2000a; Sara 2000b; Suzuki et al., 2004; Wichert et al., 2013a). More specifically we investigate the benefit of using strong new learning over weak new learning in the capability of changing strong learned memories, as is the case in PTSD. To create a strong memory, participants learned a picture set three times on day 1 (Karpicke & Roediger, 2008; Wichert et al., 2013b). Seven days later, Participants were assigned to either an experimental group or the control group. In order to investigate the interaction effect of reactivation and new learning strength on memory performance, participants in the experimental groups learned a new picture set once (weak learning) of thrice (strong learning) after recalling the original picture set from day 1. To control for main effects of reactivation and new learning, additional groups either recalled the picture set from day 1 without new learning, or learned a new picture set without prior recall of the picture set from day 1. Another control group neither reactivated the initially learned pictures nor learned a new picture set. Twenty-four hours later, participants were asked to complete a recognition test, indicating which pictures they had seen on day 1. We hypothesized that the post reactivation integration of new information into the original memory depends on the strength of new encoding. We thus expected that new learning after reactivation affect subsequent memory more profoundly when the new pictures are learned three times than when they are learned only once. We also hypothesized to find a greater memory alteration for the new learning groups, in comparison to the no new learning groups, thus providing evidence for interference of new learned material on the original memory (Schwabe et al., 2014; Wichert et al., 2011, 2013a, 2013b). Furthermore we expected emotionality of the pictures to play a part in memory performance, meaning a better memory for emotionally negative pictures over neutral pictures (Wichert, et al., 2013b).

Finally, The dual- process theory of recognition memory holds that recognition decisions can be based on recollection or familiarity. The ‘Remember/Know’ procedure is widely used to investigate these processes (Wixted & Mickes, 2010). Familiarity is generally viewed as a continuous variable, whereas recollection is usually thought to be an either/or categorical variable. In other words, familiarity comes in degrees ranging from low to high, but recollection either does or does not occur (Mandler, 1980; Yonelinas, 1994). Due to an expected interference of reactivation in combination with new learning on memory performance, we hypothesized to find a less certain memory of pictures seen on day 1, thus meaning a lower score on ‘remember’ and a higher score on ‘know’ judgments.

Method

Participants and Design

One hundred forty four persons between the ages of 18 and 28 *(M* = 21.14, *SD* = 2.27) participated in the current research (72 men, 72 women). Each of the 6 experimental groups consisted of 12 men and 12 women, randomly assign to each group. Participants received either course credits or a moderate monetary compensation. Participants were excluded when they reported: current or chronic psychological disorders (including blood phobia), any medical condition, drug or alcohol abuse/ use within 24 hours prior to participating, current treatment with medication and prior participation in a similar study. This was in accordance with Wichert and colleagues (2013a, 2013b). A fully crossed between subjects design was used with the factors retrieval (reactivation vs. no reactivation) and new learning (strong vs. weak vs. none), resulting in six experimental groups: Reactivation + Strong Learning (Re + SL), Reactivation + Weak Learning (Re + WL), Reactivation (Re), Strong Learning (SL), Weak Learning (WL) and a control group (Control; see Figure 1).

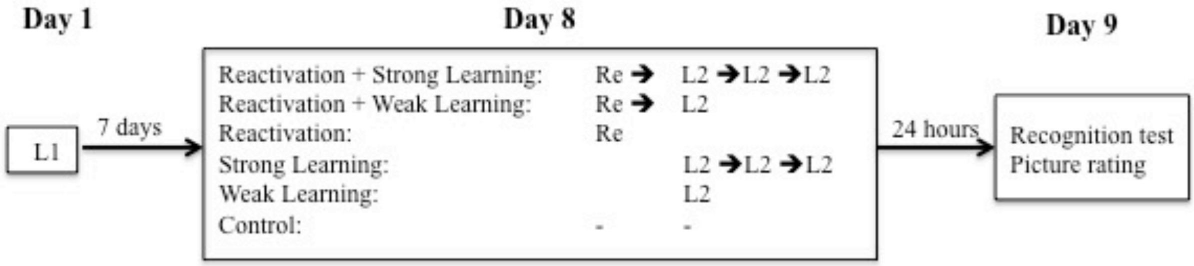

Figure 1. Experimental design. Day 1, initial Learning of pictures (L1); Day 8, reactivation of the initially learned pictures (Re) and/ or new learning (L2); Day 9, recognition test for the initially learned pictures, remember/know judgment, valence and arousal rating. Day 1 and 9 were identical for all participants.

Stimulus Materials

The stimulus materials consisted of three sets of 60 pictures taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1997). Each set consisted of 30 neutral and 30 negative pictures. The pictures of the three sets were matched with respect to emotional valence and emotional arousal, based on the IAPS standard scores for valence and arousal. The picture lists were identical to lists used in previous research (Wichert et al., 2013b), except for a few missing pictures that have been replaced by similar content ones with the same valence and arousal as the missing pictures. The picture sets were counterbalanced over the three days and groups. To ensure the emotional valence and arousal of the pictures as either neutral or negative/emotionally arousing or not arousing, participants rated each of the 60 pictures seen on day 1 on two scales, ranging 0 till 100; respectively negative-neutral and arousing-not arousing.

In retrospect, these ratings confirmed the classification of the 180 pictures with respect to emotional valence and arousal. As expected, participants rated negative pictures (M = 63.7, SD = 15.7) as significantly more negative than neutral pictures (M = 7.2, SD = 5.5), $t(143) = 46.1$, $p < .001$, $d = 3.84$. Participants also rated negative pictures (M = 53.6, SD = 16.5) as significantly more emotionally arousing than neutral pictures (M = 12.5, SD = 9.8), $t(143) = 29.4$, $p < .001$, $d = 2.45$.

Procedure

Testing took place on three experimental days. Day 1, initial learning; Day 8, reactivation (Re) and/ or new learning either once (WL) or thrice (SL); Day 9, recognition testing, remember/know judgment rating and arousal rating (see Figure 1).

On Day 1, all participants saw a set of 60 negative and neutral pictures (L1) in randomized order on a computer screen. Each picture was presented for 2 seconds, with a 1 second interval in between pictures. An immediate free recall test took place directly after the presentation of the picture set to control for possible group differences in encoding. Participants were asked to recall the presented pictures out loud and in detail. The experimenter scored the mentioned pictures, without giving feedback. In line with previous studies (Eisenberg, Kobilo, Berman & Dudai, 2003; Wichert et al., 2013a) this process was then repeated twice more, for a total of 3 repetitions to create a strong memory. There was no time limit for the free recall test.

The procedure on Day 8, 7 days after Day 1 (Wichert et al., 2013a, 2013b), depended on the experimental group. Participants who were assigned to one of the reactivation groups were placed in the same spatial context as used one day 1. They were asked to silently recall the pictures from Day 1, for two minutes, followed by a free recall test in which participants recalled the pictures out loud, with no time limit. The experimenter scored the mentioned pictures without giving feedback. Participants in the 'Reactivation + Strong Learning' and 'Reactivation + Weak Learning' -groups then were presented with Set 2 and learned this additional picture-set following the same procedure as used on Day 1. They either completed one (Weak Learning), or three repetitions (Strong Learning; Karpicke & Roediger, 2008). Finally, the new learning only groups followed a different path from the reception, by a different experimenter, to a different lab, to avoid spontaneous reactivation (Hupbach et al., 2007). Participants were, depending on the experimental group, asked to learn a new picture set respectively one (Weak Learning) or three (Strong Learning) times, following the same procedure as used on day 1. Participants belonging to the control group omitted Day 2.

On Day 9, 24 hours after the second experimental day (+/- 2 hours), participants were asked to perform a recognition task, whilst sitting in the same spatial context as on Day 1. The recognition task consisted of all the 180 pictures from the three picture sets combined. Pictures were shown one at a time. Participants were asked if they had seen the picture on day one, responding with pressing either yes (j-key) or no (n-key). If they answered yes, they were asked whether they "know" having seen the pictures on Day 1, or having "remembered" it. Remembering was explained as being in their memory in detail, while knowing meant having a faint picture of it, but without being able to excess details. If they answered no the following picture was shown After completing this task, participants were asked to perform a second computer task meant to rate the pictures on valence and arousal. The 60 pictures they had seen on Day 1were presented one at a time. Participants now rated the pictures from picture set 1 on two scales ranging from 1-100. The first scale was to rate valence with the end points neutral vs. negative. The second scale was to rate arousal with the endpoints not arousing vs. arousing. Participants rated the pictures by clicking on a point on the scale that best represented their idea of emotionality and arousal. This task was completed with follow up questions to check for any possible unexpected/ unwanted reactivation on a 5-point Likert-scale (* 1: Never, * 2: Rarely, * 3: Occasionally, * 4: A moderate amount, * 5: A great deal). Afterwards participants were debriefed about the intentions of the research.

Data-analysis

A total of 9 variables were non-normally distributed data and were thus transformed. Due to negative skewness, the data for Recognition Accuracy (total, negative and neutral) and Hits percentage (total, negative and neutral) were reflected and log transformed. The outcome of these variables needs to be interpreted in a reverse manner, due to reflection (Tabachnik & Fidell, 2013). A lower reflected score thus has to be interpreted as a higher score. Positively skewed data was found for False Alarm percentage; total, negative and neutral. These variables were thus log transformed. Finally, in line with previous research (Wichert et al., 2013a, 2013b) post hoc LSD tests were performed following significant ANOVA results.

Results

Randomization

An Age x Group ANOVA yielded no significant difference between the groups, F (5, 138) = 1.36, p = .24. The randomization was thus successful.

Initial Learning

On Day 1 of the experiment, participants learned a picture set three times and remembered a final recall average of 31.7 pictures (SD = 9.31). Negative pictures (M = 17.8, SD = 4.99) were recalled more than neutral pictures (M = 13.9, SD = 5.17); main effect of Emotionality, F (1, 138) = 127.32, p < .001, η_p^2 = .48. There was no significant difference between the groups in initial learning, F (5, 138) = .37, p = .87, which rules out any differences in encoding of picture set 1.

Table 1.

Initial learning performance.

Group	First Recall	Second Recall	Final Recall
Control	12.71 (4.29)	23.13 (6.96)	30.08 (8.72)
Reactivation	14.33 (4.16)	24.92 (6.67)	33.04 (10.02)
Weak learning	13.58 (5.13)	23.67 (8.27)	30.88 (9.53)
Strong Learning	14.08 (5.42)	24.46 (7.21)	32.79 (10.77)
Re + Weak Learning	14.54 (5.48)	25.71 (10.51)	32.13 (7.80)
Re + Strong Learning	13.33 (5.87)	23.54 (8.19)	31.12 (9.35)
Overall	13.76 (5.05)	24.24 (7.98)	31.67 (9.31)

Note. Data represents mean number of pictures recalled and standard deviations of initial learning

Memory Reactivation and New Learning on Day 8.

For the manipulation of reactivation to be successful, the reactivation groups (Re, Re+ WL, Re+ SL) should not differ in the number of pictures remembered. During memory reactivation on Day 8, participants in the three reactivation groups (Re, Re + WL en Re + SL) recalled on average 21.9 pictures (SD = 9.80). Negative pictures (*M* = 13.17, *SD* = 6.21) were remembered better than neutral pictures (*M* = 8.37, *SD* = 4.99); main effect of emotionality, F (1, 69) = 47.36, $p < .001$, $\eta_p^2 = .41$. There was no significant difference between the groups on recalled pictures, F (2, 69) = .041, $p = .96$. The manipulation of reactivation was thus successful.

A factorial repeated measures ANOVA was performed with the factor Emotionality (neutral vs. negative) on final recall after new learning on Day 8. Participants in the four new learning groups (WL, SL, Re+ WL, Re+ SL) remembered on average 23.44 pictures (*SD* = 11.18) on their final recall of picture set 2 (see Table 2). Negative pictures (*M* = 14.07, *SD* = 6.22) were again better remembered than neutral pictures (*M* = 9.36, *SD* = 5.67); main effect of emotionality, F (1, 92) = 128.70, $p < .001$, $\eta_p^2 = .58$.There was a significant difference in memory performance of participants in the strong and weak new learning groups; main effect of Group, F (3, 92) = 35.19, $p < .001$, $\eta_p^2 = .53$. Post hoc LSD tests showed that both the Re + SL and the SL group remembered more pictures than the Re + WL and the WL group, all significant at $p <$ .001 (See Table 2), indicating that learning the new pictures three times indeed resulted in stronger memories compared to learning them only once. Importantly, there was no difference found between the Re + SL group and the SL group, $p = .184$, There was also no difference found between the Re + WL and the WL group, $p =$ .941, indicating that reactivation prior to new learning had no effect on the memory encoding of picture set 2.

Table 2.

Learning performance on day 8.

Group	First recall	Second Recall	Final Recall
Weak Learning	-	-	15.29 (5.68)
Strong Learning	15.38 (5.40)	25.29 (8.20)	33.00 (8.42) *
Re + Weak learning	-	-	15.46 (4.86)
Re + Strong Learning	14.67 (5.02)	23.63 (7.73)	30.00 (10.67) *
Overall	-	-	23.44 (11.18)

Note. There are no data for the control group and the Reactivation only group. These groups omitted new learning. Data represents means and standard deviations. * Significant at $p < .001$ compared to WL groups.

Memory performance on day 9

Recognition accuracy. In order to assess the impact of new learning after reactivation on subsequent memory performance, participants completed a recognition test, 24 hours after reactivation and/ or new learning. Memory performance is expressed as *Recognition Accuracy*, the difference between percentage of correctly identified pictures as seen on day 1 (Hits) and the percentage of incorrectly identified pictures (False Alarm).

An Emotionality x Group ANOVA on Recognition Accuracy revealed a significant main effect of Group, F (5, 138) = 12.10, p < .001, η_p^2 = .31. Learning new pictures on Day 8 reduced memory performance on Day 9; the groups that learned new pictures on Day 8 scored lower on Recognition Accuracy compared to the groups that did not learn new pictures (LSD post hoc tests, all *p*s <. 001; see Table 3). In contrast with expectations, the memory impairment due to new learning was not influenced by the strength of new learning, that is, both the strong learning groups did not differ significantly on recognition accuracy to both the weak learning groups (LSD post hoc tests, all *p*s >.111; see Table 3). Interestingly, reactivation of the initially learned picture set prior to learning a new picture set, also had no significant effect on Recognition Accuracy for both the Weak learning groups (Re + WL vs. WL, LSD post hoc test, *p* = .775) and the Strong Learning Groups (Re + SL vs. SL, LSD post hoc test, *p* = 733; see Tale 3). Additionally, a Reactivation (yes vs. no) x New Learning (none, weak, strong) x Emotionality ANOVA revealed, apart from a main effect of New Learning strength, *F* (2, 138) = 29,74, *p* < .001, η_p^2 = .30, no significant main effect of Reactivation, *F* (1, 138) = .244, *p* = .622, nor a Reactivation x New Learning strength interaction effect, *F* (2, 138) = .393, *p* = .676. In contrast to expectations, these results indicate that Reactivation prior to New Learning does not influence the encoding of newly learned information.

Memory performance was not modulated by picture emotionality, for both ANOVA's, *p* > .208.

In order to determine whether the memory impairment in participants who learned a new picture set on day 8 was owing to a decrease in the number of correctly identified pictures from set 1 (hits) or to an increase in number of pictures incorrectly identified from set 2 or 3 (false alarms), a separate analyses for hits and false alarms was conducted.

Correctly identified pictures (hits). An Emotionality x Group ANOVA on the percentage of hits revealed a significant main effect of Group, *F* (15, 414) = 5,15, *p* < .001, η_p^2 = .16. Learning new pictures on Day 8 reduced the memory accuracy on Day 9, as expressed in percentage of hits; the groups that learned new pictures on Day 8 scored lower on percentage of hits compared to the groups that did not learn new pictures, irrespective of reactivation prior to new learning (LSD post hoc tests, all *p*s < .003). Similarly, a Reactivation (yes vs. no) x New learning (none, weak, strong) x Emotionality ANOVA showed a significant main effect of New Learning strength, *F* (2, 138) = 23.46, *p* < .001, η_p^2 = .25. Both the WL en SL groups identified less pictures correctly as seen from set 1, than did the no new learning groups (LSD post hoc tests, all *p*s < .001; see Table 3). However, there was no difference in correctly identified pictures as seen on Day 1 between the WL and SL groups (Post hoc LSD, *p* = .296). Additionally, there was no significant effect of Reactivation, *F* (1, 138) = .45, *p* = .503, nor was there an interaction effect of Reactivation x New Learning, *F* (2, 138) = .94, *p* = .393. In contrast to expectations, these results indicate that Reactivation prior to New Learning does not influence the encoding of newly learned information.

Memory performance as expressed in hits was not modulated by picture emotionality, for both ANOVA's, all *p*s > .376.

Incorrectly identified pictures (false alarms). An Emotionality x Group ANOVA on the percentage of false alarms revealed a significant effect of Group, F (5, 138) = 8.13, p < .001, η_p^2 = .23. Participants who learned new pictures on Day 8 showed significantly more false alarms compared with participants who did not learn new pictures (LSD post hoc tests, all *p*s < .013; see Table 3). In contrast with expectations, false alarms occurred most often in groups who learned the new pictures only once (WL vs. all other groups, LSD post hoc tests, all *p*s < .05; see Table 3). Moreover, reactivation before new learning lowered the false alarm rate when pictures were learned once (Re + WL vs. WL, LSD post hoc test, p = .036), but not when they were learned thrice (Re + SL vs. SL, LSD post hoc test, p = .853), indicating that reactivation prior to new learning only influences memory performance for weak new learning, and opposite to expectations. Accordingly, a Reactivation (yes vs. no) x New Learning strength (none, weak, strong) x Emotionality ANOVA yielded a main effect of New Learning strength, F (2, 138) = 18.04, p < .001, η_p^2 = .21. Both the WL and SL groups identified more pictures incorrectly as seen on Day 1, then did the no new learning groups (LSD post hoc tests, all *p*s< .001). There was no difference in incorrectly identified pictures as seen on day 1 between the WL and SL groups (Post hoc LSD, p = .092). Additionally, there was no significant main effect of Reactivation, F (1, 138) = .47, p = .49, nor was there an interaction effect of Reactivation x New Learning, F (2, 138) = 2.06, p = .132.

Memory performance as expressed in false alarms was not modulated by picture emotionality, for both ANOVA's, all *p*s > .187.

Table 3.

Memory performance on day 9, as expressed in Recognition Accuracy, hits and False Alarms.

Group	Recognition Acc.	Hits perc.	False Alarm perc.
Control	.881 (.435)*	.754 (.447)*	.329 (.342)**
Reactivation	.832 (.402)*	.638 (.456)*	.397 (.347)**
Weak Learning	1.356 (.197)	1.140 (.226)	.917 (.291) ***
Strong Learning	1.219 (.274)	1.072 (.255)	.676 (.402)
Re + Weak Learning	1.338 (.261)	1.175 (.350)	.703 (.370)
Re + Strong Learning	1.268 (.344)	1.127 (.350)	.702 (.368)

Note. Data represents means and standard deviations. * *p*s < .001 compared with the New Learning groups. ***p*s < .05 compared with the New Learning groups. ****p*s < .05compared with all other groups.

Remember vs. Know

Tests were performed to examine the effects of Reactivation and New Learning on judging a picture as 'remembering' or 'knowing' from Day 1. On average, participants said to remember a percentage of 59.86 pictures to be from Day 1 (*SD* = 19.26). They said to know a percentage of 40,14 pictures to be from Day 1 (*SD* = 19.26). An Emotionality x Group ANOVA on 'remember-know percentage' revealed a main effect of Emotionality, *F* (1, 138) = 48.72, $p < .001$, $\eta_p^2 = .26$, indicating that negative pictures (*M* = 65.26, *SD* = 20.28) were indicated as 'remembered' more than neutral pictures (*M* = 53,91, *SD* = 23.05) and indicated as 'known' less than neutral pictures (*M* = 34.74, *SD* = 1.68 vs. *M* = 46.10, *SD* = 1.87). No main effect of Group was found, *F* (5, 138) = 2.16, *p* = .062. An additional Reactivation (yes vs. no) x New Learning ANOVA on 'remember-know' percentage showed no significant effect for either Reactivation, *F* (1, 138) = .634, *p* = .427, or New Learning, *F* (2, 138) = 2.79, *p* = .065, nor was there an interaction effect, *F* (2, 138) = 2.12, *p* = .113.

Spontaneous vs. deliberate reactivation/strengthening

The assumption was made that participants who did not have reactivation in their program on day 2 did not reactivate the previously learned material. However, such assumptions, when incorrect, can influence the results in a major fashion. Therefor an assumption check was performed on reactivation; deliberately and spontaneous, and on strengthening deliberate and spontaneous. We expect no difference between the groups on deliberate and spontaneous reactivation and deliberate and spontaneous strengthening.

A Reactivation prior to Day 8 x Group ANOVA showed no significant effect on both deliberate, *F* (4,112) = .947, *p* = .440, and spontaneous reactivation, *F* (4,112) = .361, *p* = .836 (see Table 4). A Strengthening prior to Day 8 x Group ANOVA showed no significant effect on both deliberate, *F* (5,136) = 2.05, *p* = .076, and spontaneous strengthening, *F*(5,136) = .89, *p* = .491 (see Table 4).

Table 4.

Means and standard deviations on post experiment question

	Reactivation		Strengthening	
Group	Deliberate	Spontaneous	Deliberate	Spontaneous
Control	-	-	1.62 (.65)	2.63 (.65)
Reactivation Only	2.00 (1.13)	2.57 (1.20)	1.74 (1.05)	2.43 (.79)
Weak Learning	1.87 (1.06)	2.35 (1.11)	2.33 (1.17)	2.50 (1.22)
Strong Learning	1.83 (1.24)	2.58 (1.32)	2.25 (.79)	2.83 (.92)
Re + Weak learning	2.42 (1.25)	2.75 (1.11)	2.13 (1.19)	2.87 (.90)
Re + Strong Learning	1.96 (1.19)	2.52 (1.04)	1.87 (.92)	2.61 (.89)
Overall	2.02 (1.17)	2.56 (1.15)	1.99 (1.00)	2.65 (.91)

Note. Data was collected on a 5-point likert scale (1-Never, 2-Rarely, 3-Occasionally, 4-A Moderate Amount, 5-A great Deal).

Discussion

The present study examined whether the updating of consolidated memories after reactivation depends on the strength of new learning. Therefore, participants learned new pictures either once or thrice (Wichert et al., 2013a, 2013b) after the reactivation of initially learned pictures. In line with previous research (Wichert et al., 2013b), the current study expected a greater interference of the original memory for a strong learned memory (learned three times) following reactivation, compared to all other groups. Interestingly, our results showed no interference for newly learned pictures after reactivation, regardless of new learning strength (once vs. thrice). The current research, however, did show that learning new pictures impaired memory performance without prior reactivation, as shown by lower scores on recognition accuracy and percentage of correctly identified pictures (hits) as seen on day 1, and higher scores on percentage of incorrectly identified pictures (false alarms) as seen on day 1. Moreover, the results on recognition accuracy and hits percentage were not influenced by strength of new learning. On percentage of false alarms, the current study found a modulating effect for memory strength. Surprisingly, the results show a greater impairment of memory by account of false alarm percentage for weak learning over strong learning. In contrast with expectations, reactivation prior to new learning affected the weak learning group, by lowering the false alarm percentage. These results, however, were only found when comparing groups in general. Statistical analysis on the interaction effect of reactivation and new learning and main effects did not yield the same results, indicating that the results of strength of new learning on false alarm percentage were not robust. Furthermore examination of the remember/know paradigm did not show any effect of reactivation, new learning, or their interaction. Finally, emotionally negative pictures were overall better remembered than emotionally neutral pictures, but yielded no modulating effects on any of the outcome variables. On the remember/know paradigm, in line with expectations, emotionally negative pictures were more judged as remembered from day 1 than neutral pictures, whereas neutral pictures were more often judged as known from day 1 than negative pictures.

Despite the promising findings in previous research that show that a consolidated episodic memory may be weakened (Wichert et al., 2011) or updated by the incorporation of new information during reconsolidation (Forcato et al., 2007; Hupbach et al., 2007; Wichert et al., 2011, 2013a, 2013b), the current research found no effects for reactivation nor interaction effects between reactivation and new learning. Explanations for the lack of interaction effect in the current study can be found on either of the two key processes necessary in the successful alteration of memory during this process of reconsolidation: reactivation and new learning (Wichert et al., 2011, 2013a, 2013b).

One possible explanation for the lack of effect in the current study might be the strength of the initial learning experience. In contrast to Wichert et al. (2013b), participants learned the initial picture set thrice instead of once, in order to create a strong memory (Suzuki et al., 2004), as would be the case in PTSD. Suzuki et al. (2004) showed that these strong memories are less susceptible to reconsolidation than weaker memories, which can account for the difference found in results between the current study and Wichert et al. (2013b). Different to Wichert et al. (2013b), the current study aimed at examining the interaction effect of reactivation and new learning on a strong original memory, to investigate possible intervention techniques for PTSD. It is important to examine if the technique as used in the current study is sufficient or not to use on stronger memories. However, previous studies revealed that stronger memories could undergo reconsolidation under some conditions (Lee, 2009; Robinson & Franklin, 2010; Suzuki et al., 2004). In their study with rats, Robinson & Franklin (2010) demonstrated that although strong memories (more intensive training) are less susceptible to disruption of reconsolidation, a combination of more reminder trials with an amnesic agent (propranolol) and a longer delay between training and reactivation can render strong memories susceptible to disruption. Other studies found that strong memories, although initially resistant to reactivation, eventually undergo reconsolidation with the passage of time (Wang, De Oliveira Alvares & Nader, 2009), suggesting that the strength of initial learning is transient as boundary condition. It is therefore possible that the strong initial memory might have become susceptible to reconsolidation if the post-reactivation intervention was applied after a longer period of time, than was the case in the current study.

A second explanation for the lack of effect could be the duration of the reminder trial. Studies suggest that the reminder trial must be of specific duration; if the reminder is too short, reconsolidation is not initiated, whereas if the reminder is too long; extinction learning rather than reconsolidation can be engaged (Pedreira & Maldonado, 2003). For extinction to take place, the reactivation groups would perform less on the memory performance test than the other groups. This was not the case, therefor we rule out extinction training as possible cause for the lack of effect. Furthermore, research shows that even the shortest reminder can activate an old memory. For example, Hupbach et al. (2007) asked participants to remember and describe some details about the original learning experience. This self-generated reminder was sufficient to induce memory lability enabling reconsolidation of the original learning experience. Even though the current research used a similar process of reactivation as mentioned in Hupbach et al. (2007), we can't just rule out that the reminder trial was too short. In contrast to other studies, the current study examined the effects on a strong original memory. Since it is suggested that stronger memories are more resilient to reactivation and disruption (Suzuki et al., 2004; Wang et al., 2009), it is possible that the current reminder trial was too brief to yield an actual reactivation. For reactivation to have taken place, it can be argued that there would be an effect of reactivation, showing that reactivation only leads to a better recollection of the original memory (reconsolidation theory; Inda et al., 2011; Sara, 2000b, Wichert et al., 2011). The results show that this was not the case. It is therefore unclear whether reactivation was successful.

In addition, repeated (unwanted) mental rehearsal of the original memory may strengthen the underlying memory and pose an obstacle to reconsolidation disruption (Lee, 2009; Wang et al., 2009). The study of Wichert et al. (2013a) showed that both one and three additional reactivations in the week prior to new learning have an impairing effect on memory performance. However, the current research forestalled problems with unwanted reactivations, by using a different researcher and spatial context for the second day of testing, in line with research by Hupbach et al. (2007). Also, participants answered post-experiment questions about the level of both deliberate and spontaneous reactivation for days as well as hours before new learning, with ‘rarely’. It is possible that participants (unknowingly) misrepresented the amount of reactivation. The building itself, conversations participants had and other cues, can have subconsciously affected the results. However, considering this could have happened for all groups in an equal fashion and the answers to the post-experiment questions were marked ‘rarely’, it seems unlikely that these self-induced reactivations influenced the research process.

The second process examined is the disruption of the reconsolidation process by adding new information. Previous research shows that strength of the post reactivation intervention is key to disrupting the reconsolidation process (Forcato et al., 2010; Hupbach et al., 2007; Wichert et al., 2013a, 2013b). New information that is learned three times after reactivation can be considered as being of higher relevance than new information that is learned only once and is therefore more likely to be incorporated into the original memory (Lee, 2009). The current research did find an effect for new learning in general, stating that learning new information (regardless of prior reactivation) led to lower memory performance scores. There was however, no difference found between strong and weak new learning on memory performance, which is against expectations (Forcato et al., 2010; Hupbach et al., 2007; Wichert et al., 2013). It is possible that stronger memories (and strong new learning) take more time to reconsolidate, resulting in no difference between weak and strong new learning, because there was not enough time to fully set (Suzuki et al., 2004). In prevous study by Wichert et al. (2013a), memory testing was done 7 days after new learning occurred. The current research only took 24 hours between new learning and memory testing. That said, we find this explanation unlikely, given that in other research (Hupbach et al., 2007; Wichert et al., 2011) testing 24 hours after new learning did not impair results. Also, Reconsolidation theory states that reconsolidation is finished after 4 to 6 hours post intervention.

Emotionality is another factor that determines relevance of information. Emotionally arousing information is deemed more relevant for survival than neutral information. It is therefore not surprising that negative pictures were generally better remembered than neutral pictures in the present study. Emotionality however had no modulating effect on the results.

In conclusion, the present study examines whether reactivating a strong memory, and adding new information into the existing memory, can alter the original memory. It was hypothesized that interference would be biggest for the reactivation and new learning groups. There was, however, no effect found for reactivation, nor an interaction effect for reactivation with new learning. Reactivation did lead to a bigger confidence of having seen pictures on day 1. New learning both once and thrice did have an (equal) effect on the old memory. It is unclear why the current research was unable to find an interaction effect. It is possible that strong initial memories act in a different way when reactivating and/ or reconsolidating. Given the need for behavioural interventions for disorders like PTSD, future research should further investigate the possibility to alter strong memories. By combining behavioural interventions with the use of amnesic agents, a longer delay between training and reactivation, longer reminder trials, and a longer delay between intervention and testing, new information will be added to the boundary conditions of reconsolidation and its use in altering strong memories.

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