Preventing the Return of Fear Using Reconsolidation Update
Mechanisms Depends on the Met-allele of the Brain Derived Neurotrophic Factor Val66Met Polymorphism

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ABSTRACT

Background: Memory reconsolidation is the direct effect of memory reactivation followed by stabilization of newly synthesized proteins. It has been well proven that neural encoding of both newly and reactivated memories requires synaptic plasticity. Brain derived Neurotrophic Factor (BDNF) has been extensively investigated regarding its role in the formation of synaptic plasticity and in the alteration of fear memories. However, its role in fear reconsolidation is still unclear; hence, the current study has been designed to investigate the role of the BDNF val66met polymorphism (rs6256) in fear memory reconsolidation in humans.

Methods: An auditory fear-conditioning paradigm was conducted, which comprised of three stages (acquisition, reactivation, and spontaneous recovery). One day after fear acquisition, the experimental group underwent reactivation of fear memory followed by the extinction training (reminder group), whereas the control group (non-reminder group) underwent only extinction training. On day three, both groups were subjected to spontaneous recovery of earlier learned fearful memories. The treat-elicited defensive response due to conditioned threat was measured by assessing the skin conductance response (SCR) to the conditioned stimulus (CS). All participants were genotyped for rs6265.

Results: The results indicated a diminishing effect of reminder on the persistence of fear memory only in the Met-allele carriers, suggesting a moderating effect of the BDNF polymorphism in fear memory reconsolidation.

Conclusions: Our findings suggest a new role for BDNF gene variation in fear memory reconsolidation in humans.

Keywords: brain derived neurotrophic factor (BDNF); memory reconsolidation; fear conditioning; genetics
INTRODUCTION

Overestimation of threatening situations is one major aspect for the development of pathological fear memories (Maren, 2011). Effective treatment of anxiety disorders depends on understanding how fear is memorized and regulated (Johansen et al., 2011). The most effective treatment used in the treatment of pathological fear is behavioural therapy based on exposure-based methods. In this form of therapy, threatening stimuli are presented repeatedly to the patients until the fear response is reduced or diminished (Myers and Davis, 2007). Much of our understanding emerges from the traditional Pavlovian fear-conditioning paradigm where a neutral stimulus [conditioned stimulus (CS)] is coupled with an unpleasant stimulus [unconditioned stimulus (UCS)]. After a few CS-UCS couplings, the CS becomes effective in eliciting a fear-based conditioned response (CR) and thereby establishes a fear memory. The defensive response to the reinforced CS can be reduced or diminished through extinction training (i.e. repeated presentation of non-reinforced CS), thereby showing that the CS no longer predicts threat (Myers and Davis, 2007; Johansen et al., 2011).

Nevertheless, a return of fear has been reported even after successful extinction training (Rescorla, 2004). Hence, much effort has been put into the development of effective treatment to prevent fear from returning. The efficacy of using pharmacological treatment to prevent of pathological memories from returning has been well established; yet its effect in humans remains unpredictable and unverified (Rieder, 1994). Recent studies in both humans (Schiller et al., 2010; Agren et al., 2012b; Oyarzun et al., 2012) as well as animal models (Monfils et al., 2009) show that a single brief exposure of a non-reinforced CS used for memory reactivation just before extinction training, can attenu-
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ate pathological memories. This attenuation could be due to the fact that memory becomes labile upon reactivation and undergoes reconsolidation. Nevertheless, some studies also failed to affect reconsolidation (Chan et al., 2010; Golkar et al., 2012; Kindt and Soeter, 2013; Wood et al., 2015). An important issue previously not addressed in detail is whether the failure to update old information i.e. memory reconsolidation might result from inter-individual differences, e.g. conferred by genetic variation as reported earlier (Agren et al., 2012a). The maintenance of reactivated fear memories (i.e. human fear reconsolidation) might be suppressed by certain allelic differences, and enhanced by others. For example, individuals with the short-allele of the serotonin-transporter (5-HTTLPR) and val/val homozygotes of the functional val158met polymorphism of the catechol O-methyltransferase (COMT) exhibited the reacquisition of fear within the reconsolidation window (Agren et al., 2012a).

In addition to 5-HTTLPR and COMT polymorphism, other polymorphisms have been shown to influence processes of learning and memory. For example, Met-allele carriers of brain-derived neurotrophic factor polymorphism (BDNF val66met) have been associated with alteration of hippocampal plasticity and weak episodic memory (Egan et al., 2003). In addition, the met-allele has been associated with the attenuation of hippocampal activation during encoding and retrieval of a declarative memory task (Hariri et al., 2003), which suggests a role in memory processes (Bekinschtein et al., 2007). Moreover, a recent animal study (Radiske et al., 2015) demonstrated, a putative role of BDNF in reconsolidation of extinction memory. It was shown that the direct infusion of BDNF into the hippocampus impaired reconsolidation of extinction memory both 6 hours or immediately after reactivation of learned fear in rats. This highlights an important role of BDNF in the maintenance of reactivated fear memories, and hence in the fear reconsolidation update mechanism as well.
Furthermore, $BDNF$ \textit{(val66met)} polymorphism is closely associated with memory dysfunction (Monfils et al., 2007). The \textit{met}-allele is associated with lower levels of activity-dependent secretion and distribution of BDNF protein in neurons (Egan et al., 2003) and with lower activity of BDNF secretion in general (Rybakowski, 2008), which leads to an impairment in learning and memory processes (Li et al., 2011). In accordance with the neurobiological effects of BDNF; $BDNF$ \textit{val66met} polymorphism has been shown to modulate hippocampus-related memory processes (Chen et al., 2004). For example, animal studies have consistently affirmed the relevance of BDNF/neutrophic tyrosine kinase receptor B (TrkB) signalling in learning and memory processes (Minichiello, 2009; Li et al., 2011). In humans, $BDNF$ \textit{met}-allele carriers display differences in hippocampal morphology, which leads to selective memory dysfunction (Mukherjee et al., 2011).

Prior investigations (Egan et al., 2003; Cunha et al., 2010) have shown that increased expression and secretion of BDNF protein is strongly associated with learning and memory. This, it is possible that $BDNF$ polymorphism may modulate reactivated fear memories targeting reconsolidation mechanism. The involvement of BDNF in memory stabilization (Bekinschtein et al., 2007), resembles the reconsolidation mechanism which acts to stabilizes a reactivated memory (Nadel and Land, 2000; Nadel et al., 2012). Such an interaction would suggest a failure to target reconsolidation and could be associated with inter-individual differences. Alternatively, the combined effect of reactivated memory and allelic differences might moderate the reconsolidation mechanism.

Therefore, we tested a large sample of healthy volunteers to investigate whether functional $BDNF$ \textit{val66met} polymorphism affects the return of fear using reconsolidation update mechanisms (by applying reminder presentation followed by
We hypothesized that *BDNF* met-allele carriers would show less persistence of fear memory.

**MATERIALS AND METHOD**

**Participants**

One hundred and forty-three healthy participants of Caucasian descent were recruited via online advertisements to participate in the present study. The study was described briefly to the interested participants, with the information that the study was conducted on three consecutive days and included collection of blood sample; 24 Euros were given as compensation. Participants were eligible for inclusion if they met the following criteria: (i) age 18-40 years; (ii) German native speaker. Participants were excluded from the trial if they met the following criteria: (i) any neurological or psychiatric illnesses; (ii) pregnancy; (iii) students with psychology as major. Information about these criteria was obtained through questionnaires (see below). Participants were randomly assigned into two groups: the reminder and non-reminder group. After data analysis (see below), five participants were excluded due to artefacts (i.e. origin of response to stimuli before a baseline in more than eight numbers of trials resulting in less than three artefact-free trials for each condition) and 26 participants were excluded because they did not show a conditioned response [that means: conditioned stimuli CS+ > CS- during acquisition and also CS+ during acquisition > CS+ during habituation]. Four participants did not volunteer to participate in extinction training (24 hours later: day 2). Sixteen participants either did not donate blood or their blood samples could not be genotyped correctly, and one participant dropped due to data corruption. The demographical data of the remaining 91 participants is shown in Table 1. According to recent literature (Hajcak et al., 2009; Lonsdorf et al., 2010; Torrents-Rodas et al., 2012), participants were grouped
into two groups: *met*-allele carriers (*met66met* and *val66met*) and *val*-allele homozygotes (*val66val*). Genotype groups did not differ with respect to sex and age (see Table 1). The study was in accordance with the declaration of Helsinki in its latest version from 2008 and has been approved by the local ethical board. All participants gave their written informed consent.

Psychometric characterization

To characterize the participants of this study and to ensure that the different genotype groups did not differ with respect to relevant anxiety traits, the Anxiety Sensitivity Index (ASI 3, (Taylor et al., 2007)), the Fear Questionnaire (FQ, (Marks and Mathews, 1979)), the Positive And Negative Affect Scale (PANAS, (Watson et al., 1988)), the Penn State Worry Questionnaire (PSWQ, (Meyer et al., 1990)), the Spielberg State-Trait Anxiety Inventory (STAI-T, (Spielberger and Gorsuch, 1983)), the “Allgemeine Depressions Skala”, a screening instrument for depressive symptoms, in its short version (ADS-k, (M. and M., 1993)), and the Behavioral Approach System / Behavioral Inhibition System Scale (BISBAS, (Carver and White, 1994)) were completed by each participant. In Table 2, psychometric characteristics are given for the two experimental groups (reminder vs non-reminder) and the two *BDNF* genotype groups (*met66met* and *val66met* vs. *val66val*).

The main effect for BIS can be explained by higher values in *met*-allele carriers compared to *val66val* homozygotes. The main effect for PANAS1 (positive affect) can be explained by higher values in *val66val* homozygotes. The significant interaction effect *BDNF* x group for PANAS2 (negative affect) can be explained by lower negative affect
in the no reminder group for met-allele carriers compared to val66val homozygotes (t[48]=−2.2, p < 0.05). However, none of these main or interaction effects survive a strict Bonferroni correction with p < 0.004.

**Skin conductance response (SCR) recording and analyses**

SCRs were recorded from the volar surfaces on medial phalanges of the participants’ non-dominant palm (Dawson et al., 2000) as a means to measure the psychophysiological effects of fear learning. SCRs were recorded with the help of a V-Amp 16 (Brain Products GmbH, Gilching, Germany), a 16-channel DC amplifier system using the BrainVision Recorder Software (V-Amp Edition 1.10, Brain Products GmbH, Gilching, Germany), at a sampling rate of 1000Hz. All data was filtered with a 50Hz notch filter during recording. SCRs were recorded by using two Ag/AgCl electrodes (diameter = 13 mm) filled with non-hydrating gel.

SCRs data was filtered offline with a 1Hz low pass filter and segmented into different phases (e.g. habituation, acquisition, extinction, and reextinction) as well as single CS+ and CS- trials. Thereafter, each segment was baseline corrected 1000ms prior to the onset of the stimuli and characterized by taking the maximum of the SCR deflection in the 1-4.5 seconds (s) interval after stimulus onset. Consistent with the previous study (Asthana et al., 2013). All SCRs scores were square root transformed to normalize distributions and were then range-corrected (Vansteenwegen et al., 2005; Schiller et al., 2010) by using the maximum (unconditioned) deflection (peak between 4.5 and 7.5 s during fear acquisition) elicited by the UCS as the maximum range for each individual.

**Procedure**

The present study was conducted on three consecutive days, 20 hours to 26 hours apart. The first session (day 1) consisted of a habituation and acquisition period, in which par-
Participants learned the association of conditioned (CS) and unconditioned stimuli (UCS). During these two phases, blue and yellow colored squares (presented at a 16 degree visual angle) were presented to the participants in a randomized order on a PC monitor for 4 s with an inter-stimulus-interval (ISI) of 10-12 s and counterbalanced as CS+ and CS− to each participant, so both squares were equally often selected as CS+ and CS−. Stimuli were presented in a pseudo randomized order (i.e. not more than two-consecutive trials of the same CS in a row were repeated) using Presentation® Version 13.0 software (Neurobehavioral Systems, Inc., Albany, Calif., USA).

During the habituation phase, 8 trials of each square (i.e. blue and yellow) were presented to reach a stable response to the stimuli. In the acquisition phase, 16 trials of squares of each color were presented. Along with one of the colored squares, a women’s scream (code number 276 adapted from the International Affective Digital Sounds (IADS) for 2s of 102-db loudness) was presented 2 s after CS+ onset, while the other square served as CS− (color was randomized between both conditions). Sixteen CS+ and CS− were presented with an 80% reinforcement rate i.e., 80% CS+ trials were paired with UCS.

During the second day (day 2), participants of the reminder group first underwent reactivation, which was followed by extinction training (16 CS+, 16 CS−). In the reactivation phase, a single presentation of the CS+ for 4 s without the UCS served as a reminder trial. The experimental group received the reminder trial 10 minutes before extinction training. In contrast, the reminder trial was never presented in the control group, but extinction training also started after a 10 minutes break. Extinction training consisted of repeated presentation of CS+ and CS− in absence of the UCS.

A day later (day 3), both groups (control and experimental group) underwent extinction training (16 CS+, 16 CS−) for a second time in order to trace the spontaneous recovery
of previously learned fear memory contents. On all days, headphones and skin conductance electrodes were connected to all of the participants and the SCR were recorded from the start until the end (Asthana et al., 2013).

**Genotyping**

Participants were genotyped for the functional *BDNF* *val66met* polymorphism (rs6265) according to previously published protocols (Hunnerkopf et al., 2007). Genotyping was performed by standard polymerase chain reaction applying the following primers, Forward: 5’-AAA GAA GCA AAC ATC CGA GGA CAA G and reverse: 5’-ATT CCT CCA GCA GAA AGA GAA GAG G. The distribution of genotypes was in Hardy-Weinberg equilibrium (G/G: 54 [59.3%], G/A: 31 [34.1%], A/A: 6 [6.6 %], $\chi^2 = 0.29$, p = 0.87). The allele distribution did not differ between both experimental groups ($X^2 =1.31$; n.s; see Table 3).

<Please insert Table 3 here>

**Subjective Ratings**

In the current study, individual learning was also assessed using ratings of subjective experience. As reported earlier, subjective experience is particularly sensitive to emotional valence and arousal. Moreover, in a fear conditioning paradigm physiological recordings (e.g. skin conductance response) are sensitive to emotional arousal (Beckers et al., 2013). Subjective CS+ and CS- ratings were assessed at different experimental phases using self-assessment manikins (SAM; (Bradley and Lang, 1994)) for both valence and arousal. Subjects were asked to report whether the blue/yellow squares were perceived as pleasant or unpleasant and whether they induced arousal or calmness on a 9-point Likert scale. On the ratings of valence and arousal, 1 was labelled “very unpleasant” / “calm”, and 9 was labelled “very pleasant” / “arousing”, respectively. In
addition, all participants were verbally questioned after each experimental phase about the perceived likelihood that the UCS occurred during the presentation of each CS+ (contingency awareness), although this response was not recorded.

Statistical Analysis

Preprocessing and statistical analysis were performed by using Vision Analyzer 2.0 (Brain Products GmbH) and SPSS version 21.0 (IBM SPSS Statistics, Munich, Germany). Demographic data such as gender (male, female) and genotype (met66met/met66val, val66val) were compared between groups (reminder, no-reminder) by using Chi-square Test (see Table 1 & 3). Psychometric data (Arousal, Valence, ASI, FQ, PANAS, PSWQ, and STAI-T, ADS-k, BIS-BAS scores) were analyzed by using the Univariate Analysis of Variance (see Table 2). The ratings for the arousal and valence are reported in the Supplementary Material (see Supplementary Table 1).

To evaluate fear conditioning, extinction, and spontaneous recovery, differential SCRs values (CS+ minus CS-) were calculated for each experimental phase (acquisition, extinction and re-extinction (Agren et al., 2012a)). Furthermore, the two trials of both CS+ and CS- were averaged across the total number of trials (i.e. 16 trials in each). Each single trial block is comprised of two-trials. Hence, in total we received 4-trial blocks during habituation and 8-trial blocks for each CS+ and CS- during acquisition, extinction, and re-extinction. For the statistical analysis, the first 4-trial blocks (trials 1-8) during acquisition, the last 2-trial blocks (trials 13-16) during extinction and the first 1-trial block (trials 1-2) were taken into consideration and statistically evaluated using repeated measures analysis of variance (ANOVA) with phase mean as within-subject factors and group (reminder, no-reminder) as between-subject factor. To test the spontaneous recovery last 2-trial blocks of extinction and first 1-trial block of re-extinction were con-
considered. Significant interaction effects were further elucidated by post-hoc Student’s t tests at a significance level of $P < 0.05$ (two-tailed). Non-sphericity was considered by applying the Greenhouse-Geisser correction.

RESULTS

Effect of reactivation on reconsolidation

The reminder group was given a single brief exposure of non-reinforced CS+ (without presentation of the UCS). The effect of reactivation was evaluated by assessing whether there was an increase in $\Delta$ SCR (CS+ minus CS-) from end of extinction (last two trials of day 2) to re-extinction (first two trials of day 3). This analysis revealed no effect of time ($F[1, 89] = 0.18; P = 0.67$), group ($F[1,89] = 2.60; P = 0.11$) or time x group ($F[1, 89] = 0.30; P = 0.59$), suggesting that reactivation of learned content did not affect memory reconsolidation.

Effect of genotype on reconsolidation

The effect of the reminder in relation to the BDNF genotype on fear reconsolidation (Figure 1(B) and 2(A, B)) was assessed using a 3-way ANOVA with a 2 x 2 x 2 (group x genotype x time) design, for $\Delta$ SCRs (CS+ minus CS-) during extinction (again the last two trials of day 2) and re-extinction (first two trials of day 3). This showed a significant interaction effect of time x group x genotype ($F[1, 87] = 11.89; P < 0.001$). No significant main effect of time ($F[1,87] = 0; P = 0.99$), group ($F[1,87] = 2.3; P = 0.13$), genotype ($F[1,87] = 1.66; P = 0.20$) or further interaction effects were found (group x genotype ($F[1,87] = 1.12; P = 0.29$); group x time ($F[1,87] = 1.56; P = 0.22$) genotype x
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time (F[1,87] = 0.003; P = 0.96). Post-Hoc test revealed a tendency for reduced amplitudes during re-extinction for the reminder group (t[13] = 1.83; P < 0.10), and increased amplitudes during re-extinction for the no-reminder group (t[22] = -2.88; P < 0.01), but only for met allele carriers. For Non-met allele carriers, no significant differences between groups were found. Comparing reminder with no-reminder group revealed significant higher amplitudes during re-extinction for Non-met allele carriers (t[52] = 2.42; P < 0.05).

To investigate the interaction effect of time x group x genotype, we calculated the differences between re-extinction and extinction (thereby eliminating the factor time), with negative values indicating a decrease from day 2 to day 3 in SCR for the contrast (CS+ minus CS- condition; see Figure 2(B)).

Comparison of the reminder to the no-reminder groups revealed a significantly larger decrease in ΔSCRs (CS+ minus CS-) for the met-allele carriers from extinction to re-extinction (t[35] = -3.3; P < 0.003) in the reminder group. Additionally, we found that the met-allele carriers show a significantly larger decrease in the reminder group as compared to the non-met allele carriers (t[39] = -2.3; P < 0.05). Furthermore, we found in the no-reminder group a significantly larger decrease in the non-met carriers as compared to the met-allele carriers (t[35] = 2.6; P < 0.05).

DISCUSSION

Blocking fear memory consolidation after reactivation moderates the return of fear (Monfils et al., 2009; Schiller et al., 2010), yet significant number of studies failed to target reconsolidation mechanism and prevent fear from returning (Chan et al., 2010; Golkar et al., 2012; Kindt and Soeter, 2013; Wood et al., 2015). Schiller and colleagues
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(2010) developed the first drug-free paradigm that prevented fear memory from returning by using a reconsolidation mechanism in humans. Specifically, extinction training (i.e. repeated presentation of non-reinforced CSs) followed by memory reactivation has been shown to reduce the spontaneous recovery of fear the day after extinction training. It has been argued that the labile nature of memory after reactivation is necessary for memory maintenance or memory updating (Alberini, 2011). However, some studies failed to target the reconsolidation of memory in the human differential fear-conditioning paradigm. Therefore our finding that memory reactivation followed by extinction training is consistent with earlier investigations (Chan et al., 2010; Golkar et al., 2012; Kindt and Soeter, 2013) as it has been suggested that memory reactivation is not the sufficient condition for memory modification (Sevenster et al., 2012; Kindt and Soeter, 2013). The authors found evidence that a prediction error (i.e. the disparity between learned contingencies during fear acquisition and missing consequences during extinction training) is highly relevant for fear memory modification (Sevenster et al., 2013). Discrepant findings with regard to fear reconsolidation led to the proposed hypothesis that allelic differences can moderate the fear reconsolidation mechanism. BDNF has a role in the stabilization of memory in an associative learning paradigm (Bekinschtein et al., 2007), and thus may be potential polymorphism for moderation of the fear reconsolidation mechanism BDNF up- or down-regulation influences fear acquisition, extinction and reacquisition. Rasmusson and co-workers (2002) showed in their study that the down-regulation of BDNF mRNA in the dentate gyrus of hippocampus in rats influences learning and memory. They also argued that reduction of BDNF mRNA in the dentate gyrus depends upon several factors such as: (i) intensity, (ii) duration, and (iii) type of stress exposure. They explained that exposure to stressful cues such as UCS or sensory cues associated with stressful situations down-regulates the
level of BDNF mRNA and reaches a normal level after 48 hours. Similarly, Bekinschtein and colleagues (2007) demonstrated in their study that BDNF protein signalling is a necessary prerequisite for the stabilization of memory at an interval of 12 hours post-learning. This suggests roles for BDNF protein in (i) dynamic regulation of BDNF protein in memory formation and (ii) that delayed regulation of BDNF is important in the persistence of memory. Hence, we hypothesized that BDNF is necessary for memory stabilization via a process of memory reconsolidation. Consistent with this prediction, our results showed an attenuation of differential SCRs reflecting memory reconsolidation as modulated by BDNF polymorphism. Furthermore, only met-carriers demonstrated reacquisition by presenting a reminder followed by extinction within reconsolidation period, but not BDNF val66val allele homozygotes. These results suggest a hypostable pathological memory state in met-carriers, in accordance with previous studies (Chen et al., 2004) showing memory dysfunctions in BDNF met-allele carriers. The current results are aligned with the consistent findings that the met-allele of the BDNF polymorphism is responsible for impairment in learning and memory (Chen et al., 2006; Soliman et al., 2010; Papaleo et al., 2011). Moreover, neuroimaging evidence indicates that the met-allele carriers have reduced hippocampal and prefrontal cortex volumes compared to the Non-met allele carriers (Pezawas et al., 2004).

Our results confirm those of a recent study (Radiske et al., 2015) showing that the intrahippocampal infusion of BDNF antibodies immediately or 6 h after extinction memory reactivation impaired the reconsolidation of extinction memory in rats. This recent study demonstrated the significance of BDNF in the persistence of extinction memory after reactivation. Along the same line, we also observed a lower persistence of fear memory after reactivation of the BDNF val66met genotype in met-allele carriers.
One of the questions that arises from our study is how allelic differences influence human fear reconsolidation. To our knowledge only one study in humans has addressed this issue. Agren and co-workers (2012) reported the genetic modulation of fear memory persistence in humans, selected for 5-HTTLPR and COMT genotypes. Specifically, the S-carrier of 5-HTTLPR and the Val-carrier of COMT displayed significantly higher SCRs for the reactivated-memory group outside the reconsolidation-window (i.e. 6-hours) compared to the group within reconsolidation-window (i.e. 10 min). The moderating effect of the BDNF polymorphism that we report here shares similar findings like previous investigation (Agren et al., 2012a).

Our results are also interesting in the light of genetic predictors in post-traumatic stress disorder (PTSD). There has been considerable speculation that the BDNF genotype plays a significant role in the pathogenesis of PTSD. This speculation arises from the fact that the variance of PTSD is naturally heritable (Domschke, 2012). Recent preliminary evidence has affirmed the association of BDNF genotype with PTSD symptoms (Felmingham et al., 2013). The authors concluded that met-alleles showed behavioral resistance towards exposure therapy in PTSD compared to val66val. They also suggested met-allele as a genetic predictor of exposure therapy in PTSD (Felmingham et al., 2013). The present finding suggests that the coupling of memory reconsolidation and exposure therapy in healthy subjects with the met-allele may be responsible for diminishing spontaneous recovery, further accounting for the discrepant research findings in memory reconsolidation (Chan et al., 2010; Golkar et al., 2012; Kindt and Soeter, 2013; Wood et al., 2015). It has been well established that BDNF plays a critical role in the facilitation of neuronal differentiation and survival. Moreover, BDNF signalling is associated with abnormal fear memory and extinction (Autry and Monteggia, 2012). A common single-nucleotide in the BDNF polymorphism (rs6265), which results in a
valine to methionine substitution (val66met), leading into impaired fear memory (Soliman et al., 2010). Specifically, Solimon et al., (2010) demonstrated the joint combination of heightened anxiety and impaired fear extinction in val66met compared to val66val allele carriers.

The clinical relevance of memory modification through targeting the reconsolidation of pathological memory has recently gained much attention (Johansen et al., 2011; McKenzie and Eichenbaum, 2011). A recent study tested for the first time, whether reactivation of fear memory prior to exposure therapy reduces relapse in a randomized clinical sample of arachnophobes (Shiban et al., 2015). However, both the control and experimental group benefited significantly from treatment and a return of fear was not observed for either group. Therefore, more research on the mechanisms of memory reconsolidation and the effects of fear reactivation on memory is necessary, in order to enhance the effects of exposure therapy in the context of persistent pathological fears.

Based on the results of our study we would suggest taking inter-individual differences into account, for example with respect to the BDNF genotype. Additionally, several other factors have been shown to play a significant role in memory modulation. Factors such as memory strength, age of memory, and specificity of the CS, are known as boundary conditions of memory (Kwak et al., 2012). For example, strong memories are susceptible to disruption and do not undergo reconsolidation (Kindt and Soeter, 2013). Specifically, the rate of reinforcement level (i.e. CS-UCS associations) defines the strength of the memory, which directly affects memory reconsolidation after reactivation (Oyarzun et al., 2012).

We approached this study with the prior hypothesis that BDNF polymorphism partially prevents fear from returning by targeting reconsolidation mechanism. However, BDNF val66met polymorphism is unlikely to be the only genetic factor moderating the effects
of reactivated fear memory on fear reconsolidation. It is likely that many other allelic differences are relevant in the persistence of fear memory in humans as previously discussed. Although the current study demonstrated an effect of BDNF polymorphism on fear memory reconsolidation and spontaneous recovery, some limitations of our finding must be taken into account. First, the small sample sizes of the groups may have affected the validity of the results. Second, we did not measure other threat-based physiological response such as startle response, heart rate variability, and blood pressure. We also did not assess threat expectancies and contingency ratings for the CS-UCS association. The inclusion of other physiological and scaling data may have deepened our understanding of the threat expectancy and prediction error for different BDNF genotype groups. Finally, several undetected factors could also provide deeper insights into these measures such as stress-level, and early-undetected trauma. Despite these limitations, our study hints at the significant role of BDNF in fear memory reconsolidation after memory reactivation. In sum, our results show a moderation of a threat-based response after memory reactivation is significantly facilitated in BDNF met-carriers compared to BDNF val66val homozygotes.

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REFERENCES

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1. **Table 1**: Descriptive data of final sample (N=91) with respect to reminder, sex and *BDNF* genotype subgroups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Gender</th>
<th>Met66Met/Val66Met</th>
<th>Val66Val</th>
<th>$X^2$</th>
<th>P-value</th>
<th>d’</th>
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</thead>
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<tr>
<td>Reminder</td>
<td>Females</td>
<td>14.6 (6)</td>
<td>29.2 (12)</td>
<td>.01</td>
<td>.92</td>
<td>-.02</td>
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<tr>
<td></td>
<td>Males</td>
<td>19.5 (8)</td>
<td>36.5 (15)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-reminder</td>
<td>Females</td>
<td>24.0 (12)</td>
<td>24.0 (12)</td>
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<td>.59</td>
<td>.08</td>
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<tr>
<td></td>
<td>Males</td>
<td>22.0 (11)</td>
<td>30.0 (15)</td>
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<td></td>
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</tbody>
</table>

Results are expressed as percentage; number of subjects in brackets.
Table 2: Univariate analysis of variance results are presented for reported anxiety sensitivity, fear questionnaire, PSWQ, PANAS, ADSk, STAI, and BISBAS

<table>
<thead>
<tr>
<th>Reminder</th>
<th>Non-reminder</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met66Met/ Val66Val</td>
<td>Met66Met/ Val66Val</td>
<td>R</td>
</tr>
<tr>
<td>Val66Met</td>
<td>Val66Met</td>
<td></td>
</tr>
<tr>
<td>FQ</td>
<td>1.47 (.82)</td>
<td>1.27 (.71)</td>
</tr>
<tr>
<td>PANAS₁</td>
<td>2.27 (.28)</td>
<td>2.41 (.41)</td>
</tr>
<tr>
<td>PANAS₂</td>
<td>2.62 (.38)</td>
<td>2.51 (.39)</td>
</tr>
<tr>
<td>PSWQ</td>
<td>2.30 (.41)</td>
<td>2.35 (.38)</td>
</tr>
<tr>
<td>STAI</td>
<td>1.00 (.47)</td>
<td>.99 (.50)</td>
</tr>
<tr>
<td>ADSk</td>
<td>.75 (.25)</td>
<td>.74 (.31)</td>
</tr>
<tr>
<td>BIS</td>
<td>2.25 (.27)</td>
<td>2.09 (.30)</td>
</tr>
<tr>
<td>BAS drive</td>
<td>2.01 (.56)</td>
<td>2.05 (.47)</td>
</tr>
<tr>
<td>BAS fun seeking</td>
<td>1.98 (.57)</td>
<td>1.94 (.45)</td>
</tr>
<tr>
<td>BAS reward resp</td>
<td>1.70 (.44)</td>
<td>1.60 (.40)</td>
</tr>
<tr>
<td>ASI3</td>
<td>1.00 (.47)</td>
<td>.99 (.49)</td>
</tr>
</tbody>
</table>

df = 89, displayed is the mean (with the standard deviation in brackets) for the four groups; PANAS₁ = Positive affect and PANAS₂ = Negative affect; R Main effects Reminder Group, B main effects BDNF Genotype group effects, R*B Interaction effects (Note:⁺ p < 0.10, * p <0.05, ** p <0.01).
Table 3: Percentage (number of subjects in brackets) and statistics (Chi-square and Significant-value (P)) of the participants’ distribution over BDNF polymorphisms and experimental group are shown.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Met66Met</th>
<th>Val66Met</th>
<th>Val66Val</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reminder</td>
<td>7.3 (3)</td>
<td>26.8 (11)</td>
<td>65.9 (27)</td>
<td>1.31</td>
<td>.25</td>
</tr>
<tr>
<td>Non-reminder</td>
<td>6.0 (3)</td>
<td>40.0 (20)</td>
<td>54.0 (27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure legends:

**Figure 1:** A brief single CS display (i.e. memory reactivation) followed by extinction training within reconsolidation window prevents spontaneous recovery in met-allele carriers of BDNF genotype in humans. (A) Schematic representation of experimental procedure and chronology. (B) Mean differential skin conductance response (SCR) [CS+ minus CS-] during fear acquisition (early phase), extinction (last 4 trials), and test for spontaneous recovery (first two-trials compared to last 4 trials of extinction) were observed in the 20-26 hours for each experimental group (reminder and no-reminder). Met-allele group (black bars), Non-met group (grey bars), each bar show the ΔSCRs (CS+ minus CS-), error bars reflect SEM. Both genetic group (Met-allele and Non-met allele) showed equivalent fear acquisition and extinction. Statistical test scores comparing different phases for both groups (reminder and no-reminder) w.r.t. allelic differences (met and non-met allele) are as follows: Fear acquisition (early phase (trials 1-8)) versus fear extinction (trials 13-16) over both allele groups in reminder group [t\text{met}(13)=3.16; p=0.008; t\text{non-met}(26)=4.87; p=0.000] and no-reminder group [t\text{met}(22)=6.78; p=0.000; t\text{non-met}(26)=7.96; p=0.000]. Further, Spontaneous recovery was considered by using the ΔSCRs during the extinction (trials 13-16) and reextinction (trials 1-2). T-test revealed the spontaneous recovery in met-carrier for reminder group [t\text{met}(13)=3.34; p=0.005] and no-reminder group [t\text{met}(22)=-2.27; p=0.03]. While, non-met allele carrier showed non-significant of spontaneous recovery in reminder [t\text{non-met}(26)=-0.66; p=0.51] and no-reminder [t\text{non-met}(26)=-0.27; p=0.79] group. (Note: + p < 0.10, *p < 0.05, **p < 0.01).

**Figure 2:** (A) Differential (CS+ minus CS-) SCRs mean during extinction and re-extinction minus for both experimental groups and for BDNF genotype. Error bars reflect standard error of the mean. (B) Differential (CS+ minus CS-) SCRs mean (re-extinction minus extinction) for both experimental groups and for BDNF genotype. Error bars reflect standard error of the mean (Note: + p < 0.10, *p < 0.05, **p < 0.01).
Figure 2

(A) SCR: CS+ minus CS-

μS

-0.20

-0.15

-0.10

-0.05

0.00

0.05

0.10

0.15

0.20

0.25

0.30

+ **

Reminder No-reminder Reminder No-reminder

Met allele Non-Met allele

(B) SCR: Reextinction minus Extinction for (CS+ minus CS-)

μS

-0.20

-0.15

-0.10

-0.05

0.00

0.05

0.10

0.15

0.20

Reminder No-reminder

Met allele Non-Met allele