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# Mechanisms, genes and treatment: Experimental fear conditioning, the serotonin transporter gene, and the outcome of a highly standardized exposure-based fear treatment

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ABSTRACT

There is considerable interindividual variation in response to psychotherapeutical intervention. In order to realize the long-term goal of personalised treatment approaches, it is important to identify behavioural and biological moderators and mediators of treatment responses. Here, we tested the predictive value of experimental fear extinction efficacy as well as the role of genetic variation of the serotonin transporter gene for the outcome of a fear-exposure treatment. A discriminative fear conditioning paradigm was conducted in 159 adults highly fearful of spiders, dental surgeries or blood, injuries and injections. Participants were genotyped for the long (L) and short (S) allelic variant of the serotonin transporter gene linked polymorphic region (5HTTLPR) and treated with a highly standardized exposure-based one-session treatment. Participants' subjective fear was assessed during experimental fear conditioning and extinction. Furthermore, subjective phobic fear was assessed at pre-, post and at 7 months follow-up treatment assessment. A threat-biased contingency learning pattern characterized by exaggerated fear responses to the CS− was associated with larger initial subjective fear reduction immediately following the large-group treatment, $p = .03$. There were no learning pattern-associated differences in subjective fear at 7-month follow-up. The odds of homozygous s-allele carriers to display a threat-biased contingency learning pattern were 3.85 times larger compared to l-allele carriers, $p = .01$. Fear-recovery in homozygous S-allele carriers at follow-up assessment, $p = .01$, emerged regardless of the experimental fear acquisition pattern. Our results suggest the homozygous S-allele carriers are biologically biased towards ignoring safety signals in threat-related situations. Short-term, this response pattern might be positively related to the outcome of exposure treatments, potentially due to increased responding to safe context conditions or a stronger violation of threat expectancies. However, alterations in inhibiting the response to cues formerly signalling threat evidenced for S-allele carriers can have negative impact on exposure success.

## 1. Introduction

Most theories concerning the pathogenesis of clinically relevant fears and anxiety disorders address abnormalities in the acquisition and extinction of learned fear responses [see Lissek et al., 2005 for an overview]. Discriminative fear conditioning paradigms offer a possibility to test for alterations of such processes. In such paradigms, a formerly neutral stimulus (CS+) is paired with an aversive stimulus (UCS) such as a shock or an aversive tone stimulus. As a consequence of pairings, the CS+ acquires the same fear eliciting properties as the UCS and evokes a fear response (conditioned response, CR) also when presented alone. A second neutral stimulus, the CS−, is never paired with the UCS and is likely to acquire the function of a safety signal throughout the acquisition phase, since it signals that the UCS will not follow. During extinction, both CS are repeatedly presented in the absence of the UCS and the ability of the CS+ to elicit a fear response gradually decreases, since it no longer signals threat. Inhibitory learning is considered the critical process underlying fear extinction (Bouton, 1993; Miller & Matzel, 1988). It suggests that extinction learning does not erase the original conditioned CS−UCS association but rather that a new acquisition of a fear inhibitory CS−nonUCS association is formed which competes with the original CS−UCS

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association, rendering it less accessible (see Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014 for a detailed description).

Regarding experimental fear acquisition and extinction of learned fear responses, numerous comparisons between patients suffering from anxiety disorders and healthy controls so far mainly yielded three deficits in patients: Their responses to a CS+ were elevated during acquisition as well as during extinction trials (e.g. Peri, Ben-Shakhar, Orr, & Shalev, 2000), they showed impaired safety signal learning, i.e. elevated fear response to a CS− during acquisition and extinction (e.g. Craske et al., 2008; Waters, Henry, & Neumann, 2009) and they expressed elevated fear responses to a CS+ in delayed retests (e.g. Milad et al., 2009, see Duits et al., 2015 and Lissek et al., 2005 for a detailed review of findings).

Albeit most likely encompassing more than the acquisition of an inhibitory association (see Scheveneels, Boddez, Vervliet, & Hermans, 2016 for a detailed consideration), exposure treatments are considered the clinical analogue to extinction learning in laboratory fear studies (Hermans, Craske, Mineka, & Lovibond, 2006; Vervliet, Craske, & Hermans, 2013). Surprisingly, research addressing the question as to whether interindividual differences in experimental fear acquisition and extinction directly predict the outcome of exposure-based treatments is very sparse, so far consisting of only one report in children (Waters & Pine, 2016) and one in adults (Kircher et al., 2013). In the first study, children whose response patterns during fear acquisition and extinction resembled that of healthy children were more likely to benefit from a cognitive-behavioural treatment. The second study by Kircher et al. (2013) demonstrated an effect of successful exposure treatment on neuronal activation during experimental fear conditioning in individuals with panic disorder. Considering the high relevance attributed to laboratory fear acquisition and extinction processes, it seems warranted to investigate its predictive power concerning the outcome of real exposure treatments in greater depth.

In addition to behavioural markers such as learning patterns displayed by patients during experimental fear conditioning paradigms, other types of data, including genetic, epigenetic, stress-associated hormonal, or brain-imaging data might serve as useful predictors of treatment response. Analogous to pharmacogenetic studies, the field of therapygenetics aims to identify genetic variants which predict differential response to psychological interventions to eventually use this information for individual tailoring of treatments. Despite concerns surrounding the candidate gene approach, the serotonin transporter gene – given converging evidence of its role in emotion regulation, stress sensitivity and fear learning (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; McGuffin, Alsabban, & Uher, 2011) – might represent a viable biomarker of variation and mechanisms of treatment responses. For instance, reports on experimental fear learning and fear expression using neurophysiological and peripheral physiological parameters showed that both fear learning and expression were facilitated in carriers of the short (S)-allele of the serotonin transporter linked polymorphic region (5-hydroxytryptamine transporter, 5-HTTLPR) (Crişan et al., 2009; Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001; Klucken, Alexander, Schweckendiek, Merz, & Kagerer, 2012; Lonsdorf et al., 2009). On a neurophysiological level, homozygous S-allele carriers displayed hyper-reactivity of the amygdala during fear conditioning (Klucken et al., 2012). Their fear potentiated startle response to a CS+ was larger (Lonsdorf et al., 2009) compared to L-allele carriers and they expressed larger CS+/CS− discrimination concerning their skin conductance response (SCR) (Garpenstrand et al., 2001). Further, compared to L-allele carriers they showed elevated SCR-responses when observing another person being exposed to a CS + or UCS but not when exposed to a CS− (Crişan et al., 2009). Some of the effects mentioned also carried over into subsequent extinction trials (see Lonsdorf & Kalisch, 2011 for an overview). Enhanced parallel activity of the inhibitory vmPFC and excitatory amygdala was observed during extinction retention, possibly indicating an overcompensation of exaggerated amygdala activity in S-allele carriers (Heinz et al., 2004). In line with these findings, S-allele carriers reporting low social support (Kilpatrick et al., 2007) or living in high-risk environments (Koenen et al., 2009) were reported to have a higher risk of developing a post-traumatic stress disorder, where fear conditioning is considered a key factor in aetiology.

The present study aimed to test whether - and in which direction - experimental fear- and extinction learning might predict the outcome of a highly standardized exposure treatment. We hypothesized exaggerated fear learning, delayed fear extinction and attenuated safety signal learning to be associated with poor exposure outcome. The second aim was to replicate the finding of sensitized fear learning in homozygous S-allele carriers compared to L-allele carriers and to explore whether the homozygous S-allele phenotype also expresses altered extinction learning. Third, we asked whether the previously reported genotype-related differences in long-term outcome following the large-group exposure-treatment (Wannemueller, Moser, Kumsta, Joehren, & Margraf, 2018) might be explained by alterations in fear or extinction learning.

## 2. Methods

### 2.1. Participants

Participants aged between 18 and 70 years requested fear treatment at the Mental Health Research and Treatment Centre in Bochum, Germany. They received detailed information on the treatment program for their respective fears on websites established for the project and registered for participation. Inclusion criteria were subjective high and impairing fear of spiders, dental surgeries or blood, injuries, injections (BII). Mean-scores of specific fear questionnaires applied in all cohorts were comparable to those reported in individuals diagnosed with Specific Phobia (see Wannemueller et al., 2015, 2017, submitted for publication for more detailed information). All participants gave written consent before participation after adequate explanation. The study was approved by the local Ethics Committee of the Faculty of Psychology at Ruhr-University Bochum.

### 2.2. Discriminative aversive conditioning paradigm

The experiment took place prior to the large group treatments and was adjusted for the use in a large-group setting. Altogether 159 individuals participated in the conditioning paradigm which we conducted prior to the treatments of spider fear (n = 77), BII fear (n = 42) and dental fear (n = 40). Setup was equal for all three groups and consisted of three phases: pre-acquisition, acquisition and extinction phase. An 85 dB [A] scratching noise (fork-scratch over slate) with a length of 3 s as introduced by Neumann and Waters (2006) served as unconditioned stimulus (UCS). The UCS was presented via speakers positioned in the front and the middle of the lecture hall. To warrant equal UCS-intensity in all room positions and in all cohorts, we assessed the sound intensity prior conducting the experiment using a digital sound level meter (Brüel & Kjaer® Type 2240) and adjusted the speakers accordingly. At pre-acquisition, the UCS was once presented for the sake of demonstration and rated for adversity. Two Rorschach-Figures served as CSs and were projected on a large screen. During the pre-acquisition phase they were alternately projected three times each for 6 s each. Between CS-presentations, blank screens were randomly presented with inter-trial-intervals ranging between 3 s (if the CS+ was followed by a UCS) and 6 s. During acquisition, both CS were presented ten times each. The CS+ was instantaneously followed by the UCS, applying a contingency rate of 80% throughout the acquisition phase. CS− was never paired with the UCS. Subsequent to a 40 min delay in which the participants completed questionnaires, the extinction phase started. During extinction both CS were presented again ten times each and both were never followed by a UCS, see Fig. 1 for an overview.