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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.

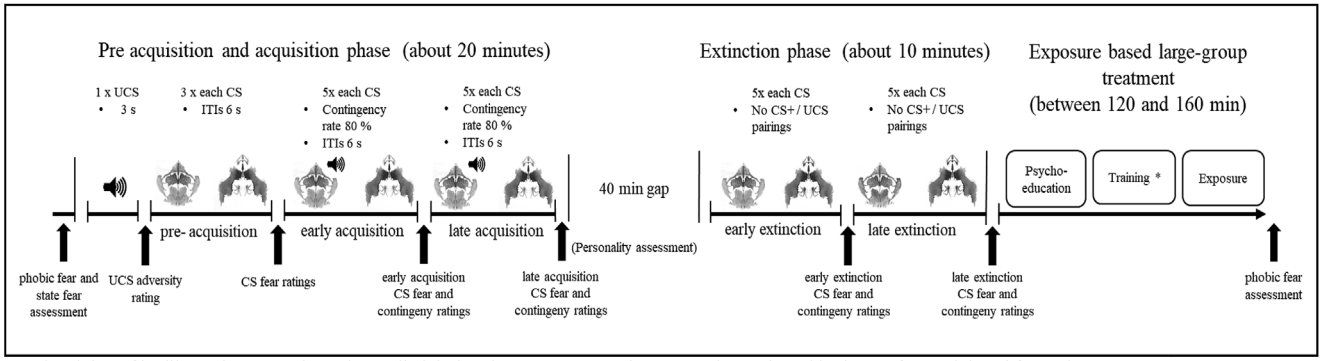
2. Methods

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.



Note: * training of bodily coping strategies to be applied during the exposure exercises was only conducted in the BII fear and dental fear cohort

Fig. 1. Scheme of the large-group dicriminative fear conditioning paradigm and subsequent large-group treatment.
Note: * training of bodily coping strategies to be applied during the exposure exercises was only conducted in the BII fear and dental fear cohort.

2.3. Treatment

Treatment was based on the recommendations for exposure-based one-session treatments by Öst (1989) and modified for the analogue use in large-group settings. Treatment was provided in three large-group settings, where participants were gathered in a lecture hall and treated simultaneously. Treatment consisted of a psychoeducation phase and various video-, pictoral and life-exposure exercises adjusted for the respective fear treated. In the dental- and BII-fear cohorts we additionally imparted bodily coping strategies to applying them during subsequent exposure exercises. Treatment duration ranged between 120 min (spider-fear) and 160 (BII-fear). Seventy-seven spider fearful individuals, 43 dental fearful participants and 40 BII fearful individuals participated in the large-group fear treatments (for a detailed description of treatment contents see Wannemueller et al., 2015, 2017, submitted for publication).

2.4. Applied measures

2.4.1. Fear acquisition and fear extinction

We assessed the level of *state fear* prior to pre-conditioning on an 11-point Likert-scale: *How fearful are you at this point?* (0 = not at all fearful; 10 = extremely fearful). UCS − adversity was assessed on a 9-point Likert-scale ranging from −4 (very unpleasant) to 4 (very pleasant). After pre-acquisition and five (early acquisition) and ten (late acquisition) presentations during acquisition and extinction, both CS were presented again and participants were asked to rate *subjective fear*, by answering the following question. *"How fearful are you, when watching this picture?"* (0 = not at all – 10 = extremely). Additionally, at early and late acquisition and extinction contingency learning was assessed via the question: *"Do you think this picture is followed by the noise?"* Participants should mark '*yes*' or '*no*' and should give a percentage expectancy rating (0–100). Due to the large-group format the experiment was conducted in, data collection only encompasses subjective measures.

As a proof-of-principle test for our large group discriminative conditioning paradigm, we conducted a 2 (CS) x 3 (Phase) repeated measures ANOVA containing the pre-acquisition fear and averaged acquisition and extinction fear ratings (for means and *SD*s see Table 1). Analysis yielded highly significant main effects of CS, $F(1, 156) = 36.59$, $p < .0001$, $\eta^2 = .19$ and phase, $F(2, 312) = 37.69$, $p < .0001$, $\eta^2 = .20$, and a CS × phase interaction effect, $F(2,312) = 52.32$, $p < .0001$, $\eta^2 = .25$. Post-hoc trend analyses yielded a highly significant quadratic trend extension for the CS+, $F(1, 156) = 95.42$, $p < .0001$, $\eta^2 = .38$, with highest fear rates during acquisition. In contrast, CS− followed a linear decreasing trend, $F(1, 156) = 12.40$, $p = .001$, $\eta^2 = .07$. As intended, results show successful fear acquisition and extinction to the CS+ as well as correct safety signal learning to the CS−.

2.4.2. Treatment outcome

We assessed individuals' subjective fear at pre-post and FU on an 11-point scale (0 = not at all 10 = extremely) in response to the following question, adapted for their respective fear: *"Please imagine … to see a spider … to get a dental surgery … your personal worst situation regarding blood, injuries or injections. How fearful are you?"* As described in Wannemüller, Moser, Kumsta, Joehren, and Margraf (2018), pre-treatment fear levels assessed with the 11-point scale were highly significantly correlated with those assessed with specific fear questionnaires applied as the main outcome measures regarding subjective fear within each cohort. In a dataset that in addition to the here presented large group data contained 67 individually treated individuals we found a correlation of $r = 0.78$ with the German Spider Fear Screening (Rinck et al., 2002) and a correlation of $r = 0.78$ with the Dental Anxiety Scale (Corah, 1969) in the dental cohort. In injection fearful participants of the BII-cohort the score was highly correlated [$r = .61$] with the injection-fear subscale of the German 'Blood Injury Injection Questionnaire' (Voßbeck-Elsebusch, Schroers, & Gerlach, 2012) and in those fearful of blood or injuries the correlation with the 'blood/injury self' subscale was $r = .70$. Furthermore, fear reduction from pre to post, $r = .68$, as well as from post to follow up, $r = .65$, assessed with the 11-point scale was highly significantly correlated with fear reduction assessed with those questionnaires. This suggests that our 11-point measure validly assesses subjective fear evoked by the anticipation of the feared stimulus and sensitively reflects short- and long-term changes following the respective intervention in all three fear cohorts (see supplementary data for pre- to post-treatment changes assessed via specific fear questionnaires and their associations with learning patterns and 5-HTTLPR allelic variants).

2.4.3. 5-HTTLPR related personality traits and trait fear

To assess genotype-related personality traits we used the NEO-FFI (Costa & McCrae, 1992), consisting of 60 items. The instrument is well evaluated and showed sufficient reliability and validity. In our sample the internal consistency scores (Cronbach's α) ranged between α = 0.73 (agreeableness) and α = 0.87 (neuroticism). The Trait-subscale of the State-Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970) served as a measure of trait-anxiety. Scores range from 20 (no anxiety) to 80 (high anxiety). The trait-scale has very good reliability, *r*tt = 0.96, and proven validity. We found an internal consistency (Cronbach's α) of $r = 0.92$ within our sample.

2.4.4. DNA extraction and genotyping

DNA was extracted from buccal swabs using the Masterpure DNA purification kit (Epicentre) following the protocol provided with the kit. The 43-base pair insertion/deletion 5-HTTLPR polymorphism was

Table 1
Sociodemographic variables, subjective fear acquisition- and extinction data, and treatment data for the whole sample and split by 5-HTTLPR allelic variant.

		Participated in fear conditioning paradigm (n = 159)	L-allele carriers (n = 129)	Homozygous S-allele carriers (n = 28)	Group Comparisons
Age		36.89 ± 14.72	37.44 ± 14.71	35.43 ± 15.99	$F(1, 154) = 0.43$, $p = .51$
Sex[a]	Male	25 (15.9%)	20 (15.7%)	4 (14.3%) 24 (85.7%)	$\chi^2(1) = 0.85$, $p = .56$
	Female	132 (84.1%)	107 (84.3%)		
Treated for	spider fear	77 (48.4%)	64 (49.6%)	12 (42.9%)	
	dental fear	40 (25.2%)	30 (23.3%)	9 (32.1%)	$\chi^2(2) = 0.99$, $p = .61$
	BII-fear	42 (26.4%)	35 (27.1%)	7 (25.0%)	
Returned for follow-up assessment		76 (47.8%)	64 (49.6%)	12 (42.9%)	$\chi^2(1) = 0.42$, $p = .54$
Post-follow-up interval (months)		6.84 ± 2.81	6.69 ± 2.76	7.67 ± 3.08	$F(1, 75) = 1.22$, $p = .23$
Personality traits (NEO-FFI)					
Neuroticism		1.92 ± 0.71	1.87 ± 0.71	2.14 ± 0.70	$F(1, 153) = 3.21$, $p = .075^{\#}$
Extraversion		2.43 ± 0.58	2.45 ± 0.50	2.32 ± 0.69	$F(1, 153) = 0.45$, $p = .30$
Openness		2.47 ± 0.59	2.46 ± 0.59	2.47 ± 0.59	$F(1, 153) = 1.15$, $p = .94$
Agreeableness		2.75 ± 0.47	2.64 ± 0.44	2.70 ± 0.58	$F(1, 153) = 0.31$, $p = .28$
Conscientiousness		2.78 ± 0.64	2.79 ± 0.61	2.75 ± 0.74	$F(1, 153) = 0.99$, $p = .79$
Trait fear (STAI-Trait)[b]		41.52 ± 10.49	40.60 ± 10.41	45.14 ± 10.68	$F(1, 112) = 3.23$, $p = .075^{\#}$
Data related to fear conditioning					
State fear prior conditioning (0–10)		3.32 ± 2.76	3.52 ± 2.83	2.57 ± 2.32	$F(2, 155) = 2.73$, $p < .10$
UCS-adversity (-4–4)		−2.43 ± 1.55	−2.38 ± 1.50	−2.79 ± 1.60	$F(2, 154) = 1.63$, $p < .20$
Fear rating CS+ (z-score)					
At pre-acquisition		−0.42 ± 0.84	−0.33 ± 0.90	−0.80 ± 0.32	$F(1, 153) = 7.38$, $p = .007^{**}$
At early acquisition		0.31 ± 1.35	0.36 ± 1.38	0.21 ± 1.24	$F(1, 153) = 0.28$, $p = .60$
At late acquisition		0.50 ± 1.48	0.52 ± 1.47	0.50 ± 1.53	$F(1, 153) = 0.01$, $p = .94$
At early extinction		−0.06 ± 1.16	−0.04 ± 1.13	−0.07 ± 1.32	$F(1, 153) = 0.03$, $p = .96$
At late extinction		−0.33 ± 0.95	−0.32 ± 0.97	−0.35 ± 0.90	$F(1, 153) = 0.18$, $p = .89$
Fear rating CS− (z-score)					
At pre-acquisition		0.14 ± 1.43	0.27 ± 1.52 2.03	−0.41 ± 0.78	$F(1, 153) = 5.29$, $p = .023^{*}$
At early acquisition		0.24 ± 1.33	0.30 ± 1.38	0.00 ± 1.06	$F(1, 153) = 1.18$, $p = .28$
At late acquisition		0.08 ± 1.24	0.12 ± 1.28	−0.04 ± 1.11	$F(1, 153) = 0.40$, $p = .53$
At early extinction		−0.13 ± 1.16	−0.07 ± 1.21	−0.34 ± 0.95	$F(1, 153) = 1.16$, $p = .28$
At late extinction		−0.34 ± 0.95	−0.32 ± 0.95	−0.41 ± 0.99	$F(1, 153) = 0.19$, $p = .67$
Is CS + followed by UCS (yes/no)?					
At early acquisition		124/33	98/29	24/4	$\chi^2(1) = 1.00$, $p = .23$
At late acquisition		133/23	106/20	25/3	$\chi^2(1) = 0.77$, $p = .36$
At early extinction		74/81	57/68	17/11	$\chi^2(1) = 2.09$, $p = .11$
At late extinction		36/121	27/100	8/20	$\chi^2(1) = 0.70$, $p = .27$
Is CS− followed by UCS (yes/no)?					
At early acquisition		34/123	23/104	10/18	$\chi^2(1) = 4.24$, $p = .039^{*}$
At late acquisition		20/136	13/113	6/22	$\chi^2(1) = 2.62$, $p = .11$
At early extinction		25/130	17/108	8/20	$\chi^2(1) = 3.75$, $p = .053^{\#}$
At late extinction		11/145	6/120	4/24	$\chi^2(1) = 3.42$, $p = .064^{\#}$
Contingency expectation CS+ (%)					
At early acquisition		64.80 ± 22.56	65.59 ± 22.78	62.04 ± 22.31	$F(1, 153) = 0.56$, $p = .45$
At late acquisition		75.83 ± 24.89	76.04 ± 25.38	75.29 ± 23.34	$F(1, 153) = 0.21$, $p = .89$
At early extinction		42.40 ± 27.99	42.64 ± 28.02	43.29 ± 28.41	$F(1, 153) = 0.12$, $p = .91$
At late extinction		28.10 ± 27.98	28.50 ± 28.47	25.39 ± 25.10	$F(1, 153) = 0.36$, $p = .85$
Contingency expectation CS− (%)					
At early acquisition		31.96 ± 26.78	31.29 ± 26.86	34.93 ± 27.44	$F(1, 153) = 0.42$, $p = .52$
At late acquisition		22.85 ± 27.48	21.41 ± 26.69	28.00 ± 29.89	$F(1, 153) = 1.34$, $p = .25$
At early extinction		23.39 ± 27.49	22.78 ± 27.34	27.29 ± 28.93	$F(1, 153) = 0.61$, $p = .44$
At late extinction		17.19 ± 24.98	16.70 ± 24.54	17.68 ± 25.27	$F(1, 153) = 0.36$, $p = .85$
Data related to large-group exposure treatment					
Fear reduction (pre to post, z-score)		0.72 ± 0.92	0.69 ± 0.95	0.79 ± 0.78	$F(1, 147) = 0.25$, $p = .62$
Fear reduction (pre to FU [z-score)[c]		1.04 ± 1.09	1.16 ± 1.08	0.40 ± 0.98	$F(1, 75) = 5.17$, $p = .026^{*}$
Fear reduction (post to FU, z-score)[c]		0.34 ± 1.08	0.48 ± 1.06	−0.39 ± 0.90	$F(1, 75) = 7.67$, $p = .009^{***}$

Note. BII, Blood-Injury-Injection fear; [a] missing information on sex for two participants [b]Trait anxiety was not assessed in the BII cohort; [c]Completer-analyses; ***p < .001. *p < .05. # < .10. Values presented are M ± SD or N (%).

genotyped using primers and PCR-conditions as previously described (Wendland, Martin, Kruse, Lesch, & Murphy, 2006).

2.4.5. Data reduction and statistical analysis

To eliminate between-cohort differences with regard to fear conditioning, we z-standardized [$(x - M_{tot}/SD_{tot}$] every response to the CS+ and CS− separately in the spider-, dental- and BII-cohort and did the same z-transformation for the treatment outcome data. To analyse differences between homozygous S-allele carriers and L-allele carriers in fear acquisition, we calculated difference scores by subtracting the pre-acquisition scores from early and late acquisition scores. To analyse differential extinction we subtracted early or late extinction scores from late acquisition scores. We used univariate ANCOVAs controlled for age and state fear levels to analyse fear learning and extinction learning either containing genotype or learning patterns as a group factor. Contingency learning was calculated using chi^2-tests and appropriate post-hoc procedure for contingency tables (Beasley & Schumacker, 1995). With regard to treatment outcome we used age-controlled repeated measures ANCOVAs either containing allele (L- vs. homozygous S) or learning pattern (*'poor'*, *'accurate'*, *'threat-biased'*, see results section for an exact definition) as post-hoc group factor. We conducted Pearson correlation analyses to assess the association between

experimental fear acquisition and extinction and exposure treatment success. All analyses were conducted using the IBM Statistics SPSS 23 software package.

3. Results

3.1. Sample description

Due to low DNA-quality two participants could not be genotyped for the 5-HTTLPR. The L-allele carrier group (n = 129) consisted of 80 heterozygous and 49 homozygous L-allele carriers and the homozygous S-allele carrier group consisted of n = 28 participants. We observed no deviation from Hardy-Weinberg equilibrium, $\chi^2 = 0.22$, $p = .89$.

There were no differences between groups regarding age, sex or the type of situational fear treated. Likewise, allele-groups did not differ concerning the distribution of individuals who returned for follow-up assessment and the lengths of post-follow up interval. Compared to L-allele carriers homozygous S-allele carriers expressed enhanced neuroticism- and trait-fear levels both significant on a trend-level, see Table 1.

3.2. UCS-expectancy patterns and differences in fear learning and fear extinction

At early acquisition, we identified three UCS expectancy patterns with regard to dichotomous forced choice (expected/not expected) UCS-expectancy ratings: 1) '*poor*' (UCS neither expected to follow on CS + nor CS− or UCS not expected to follow on CS+ but expected to follow on CS−); 2) '*accurate*' (UCS expected to follow on CS + but not expected to follow on CS−); and 3) '*threat-biased*' contingency learning (UCS expected to follow on CS+ and CS−).

The three UCS-expectancy patterns were associated with large differences in experimental fear learning. The '*poor*' contingency learning pattern was associated with less fear acquisition to the CS+, both at early, $F(2, 151) = 4.40$, $p = .014$, $\eta^2 = .06$, and late acquisition, $F(2, 151) = 6.20$, $p = .003$, $\eta^2 = .08$, compared to the '*accurate*' contingency learning pattern, see Fig. 2. Likewise, responding to the CS− differed between groups, $F(2, 151) = 3.99$, $p = .02$, $\eta^2 = .05$. A '*threat-biased*' contingency expectation was associated with fear increase to the CS− whereas accurate contingency learning was associated with a fear decrease to the CS−, see Fig. 2.

Consequently, UCS-expectancy patterns were also associated with differences in contingency awareness, i.e. the ability to explicitly discriminate between the CS that was followed by the UCS, and the CS that was not. As depicted inFig. 2 in the early acquisition phase only in individuals who displayed '*accurate*' UCS-expectancy a difference in the percentage UCS-expectancy rating between CS+ and CS− was observed, $F(1, 96) = 342.54$, $p < .0001$, $\eta^2 = .78$. Only at late acquisition, also '*poor*', $F(1, 32) = 11.88$, $p = .002$, $\eta^2 = .27$, and '*threat-biased*' contingency learners, $F(1, 25) = 6.92$, $p = .014$, $\eta^2 = .22$, were able to discriminate between the CS+ and CS− and showed contingency awareness above chance level.

Regarding extinction, we found the relative amount of '*threat-biased*' contingency learners who expected the UCS to follow the CS− at early extinction [33.3%] was threefold as high compared to '*accurate*' [11.5%] and twice as high compared to '*poor*' [15.6%] contingency learners, representing a highly significant difference, $\chi^2(2) = 7.46$, $p = .024$. Post-hoc tests yielded a highly significant difference between '*accurate*' and '*threat-biased*' contingency learners ($p = .010$). At late extinction this difference was still significant $\chi^2(2) = 6.68$, $p = .035$. At that stage 18.5% of threat-biased contingency learners still assumed the UCS would follow the CS− whereas only 6.1% of '*poor*' and 4.2% of '*accurate*' contingency-learners assumed this.

3.3. Does fear and extinction learning predict treatment success?

To assess treatment success in relation to contingency learning patterns, we conducted a 3 (learning pattern at early acquisition) x 2 (time) repeated measures ANCOVA. Analysis yielded a significant group × time interaction effect, $F(2, 145) = 3.44$, $p = .035$, $\eta^2 = .05$. Post-hoc analyses showed that pre-to post fear reduction following treatment in '*threat-biased*' contingency learners was larger compared to '*accurate*' learners, $p = .03$. Fear reductions between '*threat-biased*' and '*poor*' contingency learners did not significantly differ, $p = .22$. In order to investigate whether this learning pattern effect on treatment outcome emerged independently from genetic make-up, we additionally conducted a 3 (learning pattern) x 2 (allelic group) x 2 (time) repeated measures ANCOVA, containing the allelic variants as an additional between-subject factor. Again, the analysis yielded a significant learning pattern × time interaction effect, $F(2, 140) = 4.71$, $p = .01$, $\eta^2 = .06$. No other interaction effect reached the level of significance.

At follow-up assessment we no longer observed a significant learning pattern × time interaction effect, $F(4, 144) = 1.07$, $p = .37$, see Fig. 3.

Furthermore, we did not find any significant correlations between any other indices of experimental fear acquisition or fear extinction and subjective fear reduction following treatment at immediate or delayed post-treatment assessment, all p`s $> .10$.

3.4. Differences in fear learning and fear extinction between homozygous S- and L-allele carriers

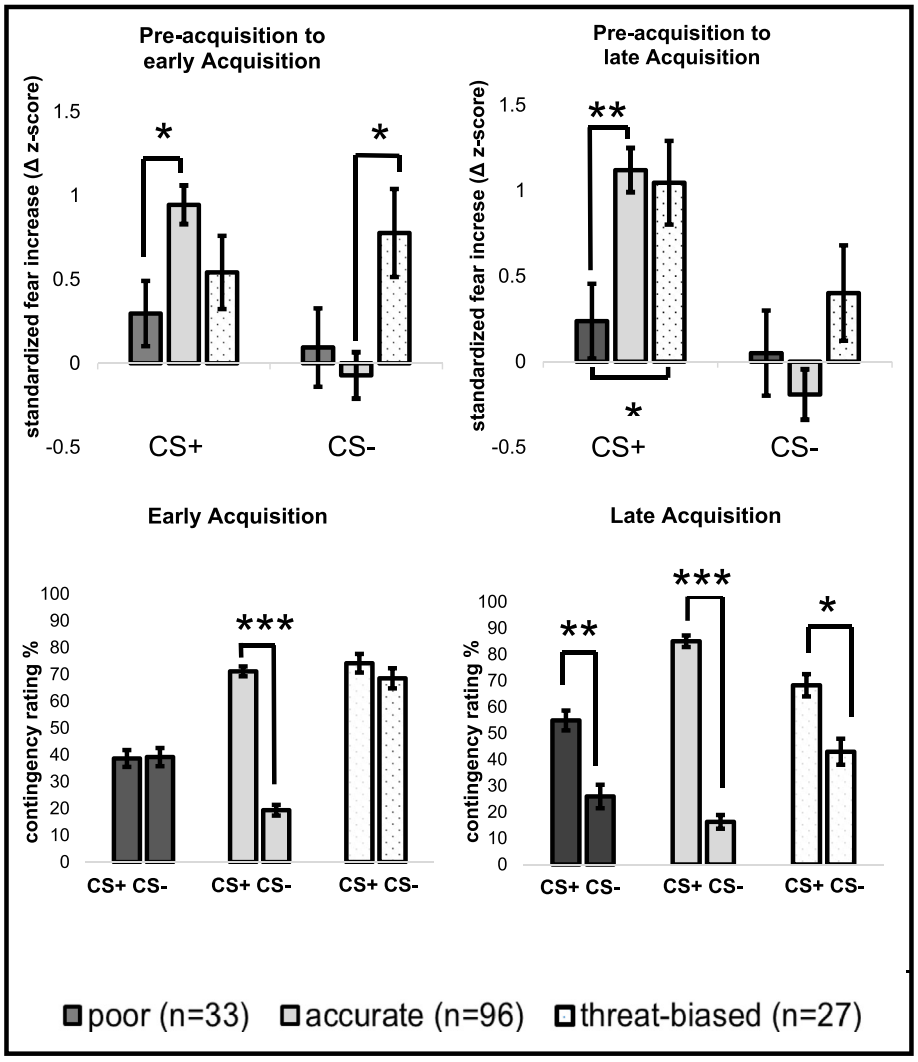
There was a trend-level difference between L- and homozygous S-allele carriers regarding the increase of verbalized fear to the CS+ during acquisition, $F(1, 150) = 2.94$, $p = .09$, $\eta^2 = .02$, with homozygous S-allele carriers expressing a higher fear increase from pre-acquisition to late acquisition compared to L-allele carriers. In contrast to L-allele carriers whose fear to the CS− decreased during acquisition, homozygous S-allele carriers verbalized a fear increase to the CS−, see Fig. 4. However the between-group difference only was significant on a trend level, $F(1, 150) = 2.66$, $p = .10$, $\eta^2 = .02$.

With regard to fear extinction, we observed no allele-related differential fear decrease to the CS+ neither at early, $p = .87$, nor late extinction, $p = .87$. The same was true for the CS−. Homozygous S-allele carriers neither differed at early, $p = .66$ nor late extinction, $p = .74$ from L-allele carriers concerning their fear decrease to the CS−.

3.5. Differences in US-expectancy and contingency awareness between homozygous S- and L-allele carriers

With regard to dichotomous forced choices (expected/not expected) UCS-expectancy, homozygous S-allele carriers were significantly more likely to expect the UCS would follow on the CS− at early acquisition compared to L-allele carriers (see Table 1). A highly significant allele-related effect emerged when comparing the distribution of individuals between the contingency learning patterns, $\chi^2 = 8.82$, $p = .01$, see Fig. 4 , with post-hoc procedure for contingency tables showing that this difference was due to the '*threat-biased*' category, $p = .003$. The odds for homozygous S-allele carriers to display a threat-biased contingency learning pattern were 3.85, 95% CI: [1.52–9.81], times larger, compared to L-allele carriers. At late acquisition phase, we no longer observed a differential distribution concerning UCS-expectancy, $\chi^2 = 0.73$.

There were no allele-related effects concerning contingency assumptions towards the CS + throughout extinction. However, a trend level-difference existed regarding the allele-related distributions of UCS-expectancy at early, $p = .053$, and late extinction, $p = .064$, with relatively more homozygous S-allele carriers expected the CS− being followed by the UCS (see Table 1 for χ^2 results).



Note. Error bars show SEMs; * p < .05; **p < .01; ***p < .001

Fig. 2. *Top.* Age and state fear controlled standardized fear increase from pre-acquisition to early *(left)* and late *(right)* acquisition to the CS+ and CS− in individuals showing *'poor'* (dark grey bars), *'accurate'* (grey bars) and *'threat-biased'* (spotted bars) contingency ratings at early acquisition phase. *Bottom.* Mean contingency ratings at early *(left)* and late acquisition *(right)* of individuals showing *'poor'* (dark grey bars), *'accurate'* (grey bars) and *'threat-biased'* (spotted bars) contingency learning at early acquisition.
Note. Error bars show SEMs; * p < .05; **p < .01; ***p < .001.

3.6. Do allelic differences predict treatment success?

As depicted in Fig. 5, homozygous S-allele carriers did not differ from L-allele carriers in regard to post-treatment fear reduction following the group treatment. This was the case when conducting a repeated measures ANCOVA with allelic-group being the sole between-subject factor, $F(1, 147) = 0.22$, $p = .64$, or when using learning pattern as an additional between-subject factor, $F(1, 140) = 0.37$, $p = .85$.

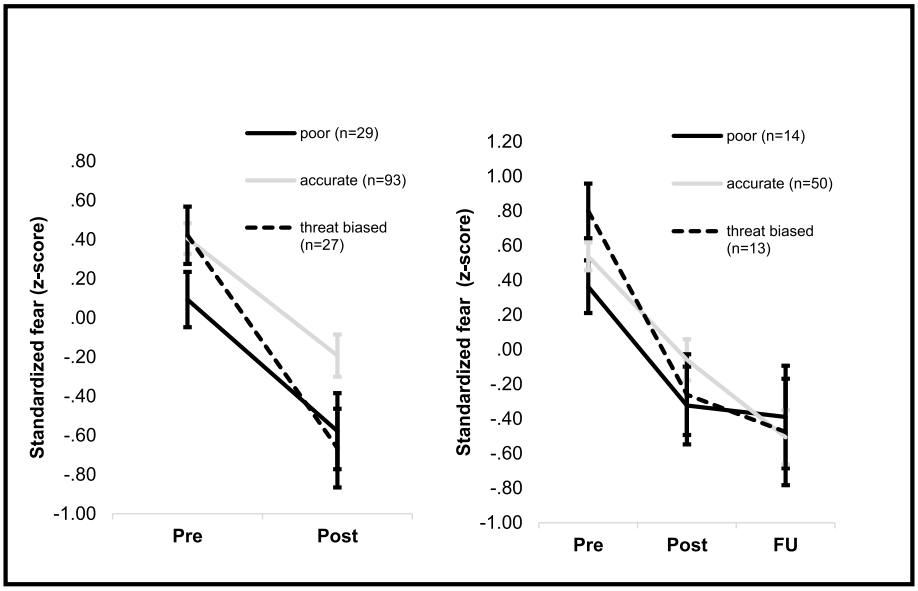
However, at follow-up assessment a strong allelic-variant x time interaction effect emerged, $F(2, 148) = 4.76$, $p = .014$, $\eta^2 = .06$, with homozygous S-allele carriers showing a strong fear-reinstatement, see Fig. 5. As already mentioned, there was no significant learning-pattern x time effect at that point. Because statistical power was too week, we did not conduct a repeated measures follow-up ANCOVA containing both, learning pattern and allelic variant as between subject factors in the same analysis.

4. Discussion

In this study we investigated the influence of 5-HTTLPR allelic variants on fear learning and fear extinction. Furthermore, we assessed whether individual functioning and potential gene-related alterations within these mechanisms are predictive concerning the outcome of a highly standardized one-session exposure large-group fear treatment.

Interestingly and quite inconsistent to prior research reports (Kircher et al., 2013; Waters & Pine, 2016), we observed the largest post-treatment fear reductions following our large-group exposure treatment in individuals who expressed a threat-biased UCS-expectancy pattern, associated with impaired discriminative and safety signal learning during experimental fear conditioning. In contrast, individuals whose early contingency learning was accurate and in which the CS− acquired the function of a safety signal throughout the acquisition phase showed the poorest post-treatment outcome. Their phobic fear reduction due to treatment was comparable to that of individuals who displayed poor contingency learning characterized by low fear acquisition to the CS+ throughout the acquisition phase and delayed discriminative fear learning during experimental fear conditioning.

As mentioned, inhibitory learning is considered a critical process underlying successful exposure-based treatments. Modern approaches postulate that inhibitory learning depends on the extent expectancies



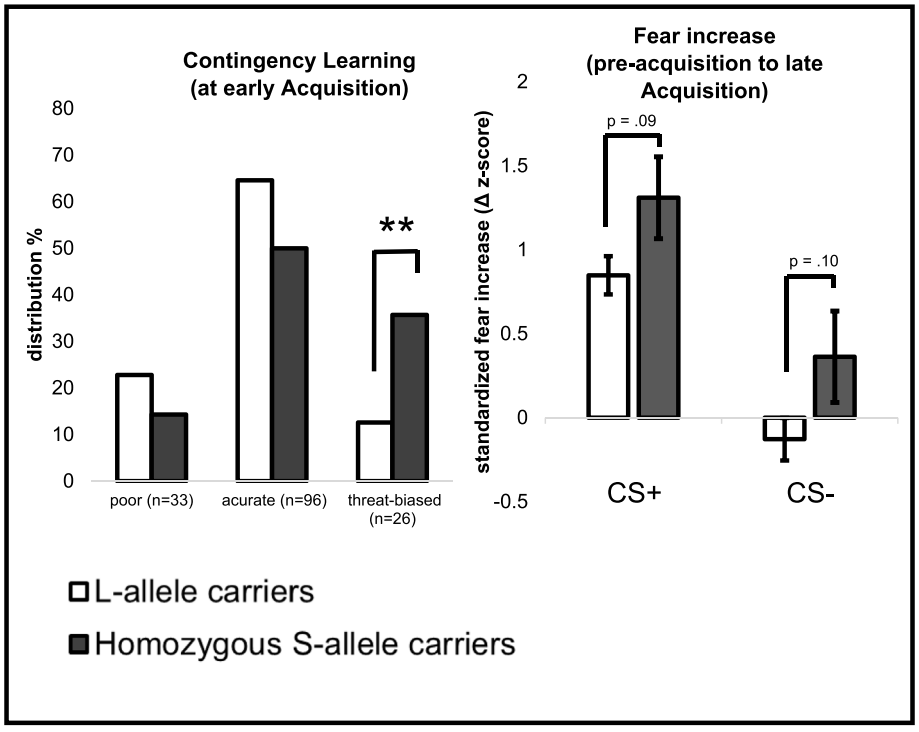
Note. Error bars show SEMs

Fig. 3. Pre- to post-treatment *(left)* and pre-treatment to follow-up *(right)* age-controlled standardized fear change following group exposure treatment in individuals who showed *'poor'*, *'accurate'* and *'threat-biased'* contingency learning at early acquisition.
Note. Error bars show SEMs.

regarding the intensity or rate an aversive outcome occurs are violated throughout exposure treatment (Craske et al., 2014). Potentially, in individuals ready to expect a wider range of aversive outcomes under uncertain conditions, such expectancies are more likely to be violated in the treatment context or these individuals simply feel more relieved under safe conditions, as warranted by the treatment context.

According to our expectations and in line with previous reports (Lonsdorf & Kalisch, 2011; Lonsdorf et al., 2009), we observed a tendency towards biologically gated fear learning in homozygous S-allele carriers indicated by trend level differences in verbalized fear increase to the CS+. In contrast to L-allele carriers whose subjective fear to the CS− decreased throughout the acquisition phase, homozygous S-allele carriers' fear to the CS− increased. Besides displaying this pattern of sensitized fear expression we observed enhanced neuroticism and trait anxiety levels in homozygous S-allele carriers. Both, sensitized fear expression and enhanced anxiety-related personality traits have been evidenced to be closely linked to enhanced amygdala activity (Hariri et al., 2002; Klucken et al., 2012; see Munafò, Brown, & Hariri, 2008 for a meta-analysis).

However, the differential effects concerning verbal fear reports were



Note. Error bars show SEMs; **p < .01

Fig. 4. *Left.* Percental distribution of L-allele carriers (white bars) and homozygous S-allele carriers (grey bars) showing *'poor'*- (CS+ not followed by UCS), *'accurate'*- (only CS+ followed by UCS), and *'threat-biased'* (CS+ and CS− both followed by UCS) contingency learning at early acquisition. *Right.* Age- and state fear-controlled mean fear increase to the CS+ and CS− during acquisition from pre-acquisition to late acquisition in L-allele carriers (white bars) and homozygous S-allele carriers (grey bars).
Note. Error bars show SEMs; **p < .01.

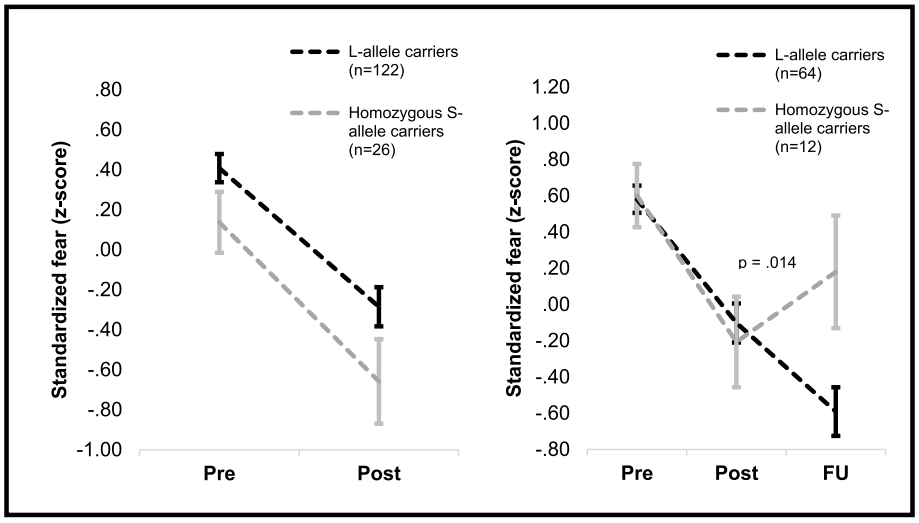


Fig. 5. *Top.* Pre- to post-treatment *(left)* and pre-treatment to follow-up *(right)* age-controlled standardized fear change following group exposure treatment in individuals who showed *'poor'*, *'accurate'* and *'threat-biased'* contingency learning at early acquisition. *Bottom.* Pre- to post-treatment *(left)* and pre-treatment to follow-up *(right)* age-controlled standardized fear change in homozygous S- and L-allele carriers following group exposure treatment. *Note.* Error bars show SEMs.

Note. Error bars show SEMs

rather small, might at least partially be due to differences existing at pre-acquisition assessment and neither the extent of experimental fear increase during acquisition nor the extent of fear decrease during extinction directly predicted the outcome of the large-group exposure treatments. Rather, our data suggest the effects of the homozygous S-allele variant on fear expression to both CS and, in a second step, also concerning the immediate outcome of exposure treatment were strongly mediated by UCS expectancy patterns. We found the homozygous S-allele phenotype to be specifically likely to display a threat-biased contingency learning pattern defined by the expectation that both CS are followed by the UCS when contingencies between cues and negative outcomes were still quite weak, as being the case during the early acquisition phase. Hence, our results suggest that the influence of 5-HTTLPR allelic variants on subjective fear learning and fear expression mainly is mediated by contingency learning.

Associative fear learning and contingency learning are related processes, e.g. because contingency judgements tend to become more accurate as the number of cue-outcome presentations increases (Van Overwalle & Van Rooy, 2001), as also was the case in the participants of the present study. However, causal models of contingency learning emphasize the role of cognitions i.e. a person's beliefs and assumptions about the nature of the cues and outcomes and the nature of the relation between these two (Waldmann, 2000). Especially when associative information about CS-UCS pairings is still sparse, these cognitive appraisals proved to be highly relevant for an individual when being asked to make contingency judgements (see Houwer & Beckers, 2002 for an overview). In our study the importance of cognitive factors might even have increased, given that colloquial German is fairly ambiguous in terms of the use of present and future tense. Therefore, (some of) our participants could have understand the question the way '*Do you think this picture will be followed by the noise?*' instead of ' … *is followed*'. If an individual will rely on a rule about the relation between a stimulus and the (non-) occurrence of a negative outcome and how certain it has to be about the correctness of such a rule when being asked to make future predictions depends on more than simple rule learning. Rather, it will imply estimations concerning possible consequences and costs a miscalculation would cause in a specific situation or past experiences and convictions about the general predictability of aversive events and safety, processes all known to vary with trait anxiety (Mitte, 2007; Stöber, 1997; see Grupe & Nitschke, 2013 for an overview). Besides cognitive appraisals that might have influenced participants' UCS expectancy ratings, it is also possible that their capacity to detect the CS – as a safety signal differed due to a biased attention towards threat related cues, that is known to impede an exact discriminative analysis of environmental cues under highly uncertain conditions and associated with enhanced trait and clinical anxiety (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & Van Ijzendoorn, 2007). Given that the detection of and trust in safety signals relieves the individual from an otherwise permanent state of anticipatory anxiety in threat-related or ambiguous situations, it is not surprising that heightened reactivity to objectively safe conditions has been observed across a wide range of anxiety disorders and is considered as a vulnerability factor for the development of anxiety-related psychopathology (Lissek et al., 2005).

Albeit so far surprisingly little is known concerning the neural mechanisms underlying safety signal learning (see Christianson et al., 2012 for a review), the same prefrontal-amygdala circuits involved in the recall of fear extinction memories (Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk, Russo, Barron, & Lebron, 2000) revealed also to play an important role in learning and responding to cues that predict safety (Christianson et al., 2012). In the context of safety learning, the role of the vmPFC is suggested to consist of signalling to the amygdala which particular stimuli in the environment are safe to ignore (Schiller, Levy, LeDoux, Niv, & Phelps, 2008). As mentioned, evidence resulting from animal as well as human studies showed a strong link between serotonergic availability and altered vmPFC morphology (Wellman et al., 2007) and function (Narayanan et al., 2011) as well as concerning the quality of the amygdala-vmPFC interplay (Heinz et al., 2004; Pezawas et al., 2005).

Our conditioning results demonstrate potential phenotypical consequences of these neuronal alterations. At early acquisition, where contingencies were still quite uncertain for our participants, homozygous S-allele carriers were substantially more likely to show a biased threat prediction associated with impaired safety signal learning. This pattern can be interpreted as a state of being 'ready to (defensively) respond', that also reflects enhanced plasticity to contextual conditions associated with the S-allele as conceptualized within the framework of differential susceptibility (see Belsky & Hartman, 2014; Belsky et al., 2009). The correct identification of environmental contingencies is adaptive for an individual and bears a clear survival benefit, since it allows the individual to explain past events and prepare for the future (see Alloy & Tabachnik, 1984). However, under dangerous environmental conditions a biologically altered capability to ignore threat-irrelevant stimuli certainly increases the individuals' chance to survive, because relaying on a false safety signal might potentially be fatal under these conditions. A possible drawback for the homozygous S-allelic phenotype might consist in an enhanced risk to perceive the world as a quite unsafe and unpredictable place, consequently enhancing the risk to develop anxiety-related psychopathology at least if often exposed to

or grown up under uncertain environmental conditions.

In contrast to immediate post-treatment assessment, we observed a strong effect of 5-HTTLPR allelic variants on long-term phobic fear reduction, so large that it seemingly even superimposed the impact of learning patterns on follow-up results. Independently from experimental responding, homozygous S-allele carriers showed a large relapse. As already mentioned, a huge body of evidence deriving from animal as well as human studies has demonstrated strong effects of serotonergic availability on the recall of extinction memories. Therefore, extinction retention even has been suggested to be an endophenotype, mediating the effects of genetic variability in 5-HTT function on anxiety disorders and depression (Hartley et al., 2012). In an earlier study exclusively focussing on genotype-related effects on treatment outcome, which in addition to the treatment data reported here also contains data of individually treated individuals (Wannemüller et al., 2018), we describe that all processes known to affect extinction retention have been evidenced to be context sensitive and discuss possible reasons for the observed relapse in homozygous S-allele carriers. The present results suggest that genotype-related long-term effects are not mediated by differential experimental fear acquisition or fear extinction. However, given that retention encompasses a conglomerate of fear and extinction memory, future research should shed light on whether extinction retention itself is impaired in homozygous S-allele carriers or whether they differ in fear memory reconsolidation processes, as postulated by Agren, Furmark, Eriksson, and Fredrikson (2012).

Some limitations to our study need to be mentioned. We only assessed subjective fear levels throughout fear conditioning and interrelated them with changes of subjectively rated fear levels resulting from our highly standardized exposure treatment. Due to the lack of assessing psychophysiological markers of fear learning such as skin conductance response or fear potentiated startle response, generalization of our results on other fear levels is restricted. Furthermore, we treated individuals who, albeit expressing phobia-typical subjective fear levels, were not explicitly diagnosed for specific phobia. Probably other allelic *SLC6A4* genetic variants might have contributed to the phenotypes described here, though were not analysed. Albeit the reported findings strongly suggest a specific threat-bias in homozygous S-allele carriers' fear-related contingency learning we did not perform a non-fear based learning task. Thus, the extent to which this finding is specific to fear learning or possibly results from broader cognitive differences remains unclear. Moreover, with 84.1% the amount of female participants in our large-group trials was overrepresented. Hence, validity of findings in regard with men needs to be closer investigated. Finally, because only 12 homozygous S-allele carriers returned for follow-up assessment the reported follow-up effects need to be interpreted very cautiously (but see also Wannemüller et al., 2018, for a larger sample additionally containing treatment results of an individually treated group). These factors clearly restrict generalization of our results and necessitates the replication of findings.

To summarize, our findings suggest that the influence of 5-HTTLPR genotype-length on the short-term outcome of a highly standardized exposure-based fear treatment is strongly mediated by fear learning patterns. There was no direct genotype-related effect on treatment outcome. However, individuals who displayed a biased prediction towards threat expectancy associated with impaired discriminative fear learning and fear expression to a signal that actually predicts safety expressed larger initial fear decrease following treatment. Homozygous S-allele carriers were significantly more likely to show this specific pattern, considered as quite adverse concerning the development of anxiety-related psychopathology. This might represent a biologically triggered weakness to ignore threat irrelevant stimuli under uncertain conditions and enhanced plasticity to threat-related context conditions in homozygous S-allele carriers. We discuss the positive effect on treatment-outcome might be due to unrealistic threat expectancies in these individuals that potentially are easier to violate. However, alternatively this simply might reflect a larger response to safe context conditions warranted by the treatment context. In contrast, we observed a direct genotype-related effect on long-term treatment outcome emerging independently from the response pattern shown throughout the fear conditioning paradigm and the level of post-treatment fear reduction. Homozygous S-allele carriers expressed a large relapse concerning their phobic fear at follow-up assessment. We suggest this might reflect their biologically biased capability to inhibit fear to former fear cues that actually no longer signal threat.

Conflicts of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brat.2018.06.003.

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