Individual differences in fear learning: Specificity to trait-anxiety beyond other measures of negative affect, and mediation via amygdala activation

Running title: trait-anxiety predicts danger and safety learning

Rachel Sjouwerman, MSc[1], Robert Scharfenort, PhD[1], & Tina B. Lonsdorf, PhD[1]

[1] University Medical Center Hamburg-Eppendorf, Department of Systems Neuroscience, Hamburg, Germany.

Correspondence should be addressed to:

Dr. Tina B. Lonsdorf
Department of Systems Neuroscience
University Medical Center Hamburg-Eppendorf
20246 Hamburg
Germany
E-mail: t.lonsdorf@uke.de
Phone: +49 40 7410 55769
Fax: +49 40 7410 59955

Abstract

Identifying individual differences in the ability to discriminate signals of threat and safety holds great potential to elucidate etiological mechanisms of pathological anxiety and resilience and may ultimately foster the development of targeted prevention and clinical intervention programs. Constructs that can be subsumed under the umbrella term of negative affect such as trait-anxiety (STAI-T), neuroticism (N), and intolerance of uncertainty (IU) have been suggested to contribute to aberrant fear learning in different studies. However, collinearity between and individual contributions of these constructs in relation to fear learning, as well as the neurobiological mechanisms remain unclear. Here, we apply a multivariate and dimensional approach (structural equation modeling) across multiple units of analyses (ratings, skin conductance, fear potentiated startle, fMRI) in a differential fear conditioning paradigm in two independent samples ($N_{\text{behavioral study 1}}$=288; $N_{\text{fMRI study 2}}$=116). Trait-anxiety was identified as the unique facet of negative affect predicting differences in discriminating signals of threat and safety in skin conductance responses beyond other measures of negative affect (N, IU). This was replicated in a second independent sample and extended by showing that the association between trait-anxiety and skin conductance responding is mediated by differential amygdala activation. These findings elucidate an intriguing mechanism (discrimination deficits) by which the individual's disposition to experience anxiety-relevant emotions may confer a predisposition to the development of pathological anxiety and hence suggest a possible mechanistic target (i.e. discrimination training) for clinical intervention and prevention.

Introduction

Why do some individuals develop pathological anxiety in the aftermath of trauma while others are resilient[1]? It has been proposed that this differential vulnerability might hinge on individual differences in (associative) learning processes[2,3], representing a core mechanism of the development as well as the maintenance of pathological fear and anxiety. Importantly these processes can be captured experimentally in fear conditioning paradigms[4,5], which serve as translational models in fear and anxiety research[6,7]. Focusing on individual differences in fear conditioning research[3] is expected to provide critical insights into the mechanisms underlying individual risk and resilience for the development of anxiety and/or stress-related disorders[2,3]. Ultimately, this may move the field closer to the development of mechanism-based prevention and individualized intervention programs contributing to a personalized medicine approach[8,9]. To date however, the field has generated little clinically usable results as it is hampered by a number of major methodological and practical challenges[3].

A recent review[3] identified three constructs related to negative affect that have been most consistently linked to individual differences in fear conditioning performance and vulnerability to pathological fear and anxiety: Trait-anxiety, neuroticism and intolerance of uncertainty. Trait-anxiety (STAI-T), reflects the *general* tendency to react *anxiously* and to show cognitive as well as affective styles related to pathological anxiety to a wide range of events and contexts[10]. Neuroticism (N), a construct derived factor-analytically, reflects the tendency to *express* negative affect such as anger, envy, guilt, and depressed mood and assesses the tendency to be emotionally highly reactive and vulnerable to stress[11]. Finally, intolerance of uncertainty (IU) is defined as the dispositional cognitive bias to perceive and interpret ambiguous situations as threatening[12,13].

Problematically, despite profound conceptual overlap and empirical collinearity[12], the majority of results originate from studies investigating these singular *a-priori* defined 'risk' factors *in isolation*[3] (for few exceptions see [14–18]) - often by using singular outcome measures. A far-reaching problem arising from such isolated investigations in univariate approaches is that they produce separate lines of research, which may generate misleading results and leave the best, causal predictor of aberrant fear learning processes unidentified[3].

Shifting focus towards a more holistic approach necessarily calls for a multimodal approach in conjunction with specifically tailored multivariate methods beyond commonly applied group comparisons based on extreme group sampling or post-hoc dichotomization such as median split procedures– all of which have been subject to substantial criticism[19–22]. To tackle this problem, we here implement an approach that goes beyond the traditional focus on the investigation of singular *a-priori* defined 'risk' factors and outcome measures *in isolation*[3]: Dimensional analyses using multivariate structural equation modelling in a large sample ($N_{study\ 1}$:288) allow to account for shared variance between multiple 'risk' factors (i.e., STAI-T, N, IU) and outcome measures (i.e., skin conductance responding (SCR), fear potentiated startle (FPS), subjective ratings) in a single overarching model. As multiple outcome measures tap into different underlying processes, divergence between measures is expected to allow for additional mechanistic insights[23].

Surprisingly, the neurocognitive processes underlying the association between negative affect and fear conditioning remain largely unknown to date[3], in particular as studies integrating fMRI results with concurrently acquired psychophysiological measures are lacking[3]. Hence, in a second step, we address this fundamental gap and advance the findings from study 1 by exploring the neurocognitive processes underlying the association between fear learning and 'risk' factors related to negative affect[16,24–27] in a large sample ($N_{study\ 2}$: 116). This ties together hitherto parallel lines of research through simultaneous recordings of multiple outcome measures (fMRI, SCRs, subjective ratings).

In sum, the primary aim of this work is to identify a unique facet of negative affect related to differential fear learning through shifting focus from a univariate to a multi-variate, multimodal and dimensional approach and establish the neurofunctional mechanisms underlying this association.

Materials and methods

Participants and questionnaires

404 healthy participants were included (study $1_{behavioral}$: N=288, 206 female, mean age±SE: 24.97±0.23; age range: 18-40; study 2_{fMRI}: N=116, 44 female, mean age±SE: 25.13±0.32, age range: 19-34). Samples partially overlap with previously published results that that however focused on post-acquisition experimental phases[28–31] (see Supplementary Section 1.1 and 2.1 for details on sample characteristics and recruitment procedures). Trait-anxiety[10] (study 1 and 2), intolerance of uncertainty[13] and neuroticism[32] (study 1 only) were assessed.

Material and procedure

Fear acquisition protocols were identical for all participants within each study (see Supplementary Section 1.2 and 2.2 for details on materials, timings, and procedures). Fear extinction, reinstatement and return of fear test phases differed procedurally between both studies and participants[28–31] and were thus excluded for analyses with respect to individual differences (see Supplementary Section 5 for explorative extinction analyses). In brief, two black geometric shapes presented on colored backgrounds (study 1), and two white fractals on grey backgrounds (study 2) served as conditioned stimuli (CSs) during fear acquisition. One stimulus (CS+) was always followed by an individually adjusted electrotactile unconditioned stimulus (US) whereas the other (CS-) was never followed by the US (100% reinforcement-rate). A white fixation cross on a black (study 1) or grey (study 2) background served as ITI.

In both studies, the experiment consisted of US intensity calibration, explicitly US-free CS habituation (study 1: 2CS+/2CS-, study 2: 7CS+/7CS-), and uninstructed fear acquisition (delay conditioning; study 1: 9CS+/9CS-, study 2: 14CS+/14CS). A startle habituation phase (5 presentations) preceded CS habituation in study 1.

Dependent measures

SCRs and ratings of fear to the CSs were acquired in both studies. According to recommendations[33] SCRs were semi-manually scored within 0.9-4s after stimulus onset. Amplitudes were range and log corrected[33]. Ratings were provided on a visual analog scale (0-100) intermittently (study 1) or after each experimental phase (study 2). FMRI responses were only included in study 2. The amygdala, dorsal anterior cingulate cortex (dACC), hippocampus, insula, pallidum/putamen, ventromedial prefrontal cortex (vmPFC) and thalamus served as ROIs as they are key areas implicated in fear conditioning[34,35]. FPS was triggered by acoustic startle probes (95dB) and recorded using EMG-equipment in study 1, but not in study 2 due to technical restraints of combined EMG-fMRI acquisition at the time of data acquisition. FPS responses were semi-manually scored between 0.20-0.12s after startle probe onset. Amplitudes were t-transformed. CS-US contingency awareness was assessed after the experiment (i.e., after extinction and return of fear; study 1) or directly after fear acquisition (study 2). See Supplementary Section 1.3 and 2.3 for details on response registration and processing.

Data analysis

Statistical analyses were performed with IBM SPSS Statistics 22 and AMOS for Windows (Armonk, NY). P-values<0.05 were considered significant and Greenhouse-Geisser corrections were applied when appropriate. Partial eta^2 ($_p\eta^2$) was used as measure of effect size. FMRI data were preprocessed and analyzed in SPM8 (Welcome Trust Centre for Neuroimaging, UCL, London, UK) (see Supplementary

Section 2.3 for details on fMRI data acquisition, processing and analysis). In brief, the primary CS-discrimination contrasts (CS+>CS-; CS->CS+) were estimated on the first level and taken into the second level analysis employing voxel-wise regression analyses with the STAI-T. A ROI-based voxel-wise approach was employed, and small volume (SVC) family wise error (FWE) corrected at $p<0.05$.

Comparability to traditional analyses employed in the field

To allow comparability of results in study 1 with published studies, and for illustrative purposes, repeated measures ANOVAs (CS-type: mean CS+, mean CS- during fear acquisition) with dimensional scores of each construct (STAI-T, N, IU) as co-variate were conducted *separately* for the three dependent measures (SCR, ratings, FPS). Similarly, repeated measures ANOVAs (CS-type: mean CS+, mean CS- during fear acquisition) with categorical classifications (median-split and quartile-split groups) based on construct scores for all three questionnaires in isolation as between subject variable are provided for comparability (see Supplementary Table 1 for descriptives of categorical-groups). Significant effects with respect to CS-discrimination were followed-up by CS-specific (i.e., CS+ and CS- seperately) analyses.

Analyses of main interest: Path analyses for study 1 and 2

Importantly, structural equation modelling was performed to allow for multivariate analyses. For study 1, the full model included the three constructs (STAI-T, N, IU) and the three outcome measures of CS-discrimination (SCR, fear ratings, FPS; CS+>CS- contrast). For study 2, the full model included the STAI-T, SCRs and fear ratings as well as extracted peak parameter estimates from brain regions showing significant activation during fear acquisition (parameter estimates of CS+>CS- contrast derived from regression analyses with STAI-T) in fMRI analyses. All possible connections (i.e. direct and indirect paths between all variables) were allowed in full models. Subsequently, backward selection of non-significant paths converged into final path models. Trends ($p<0.1$) were included in interim models but not in final models. Significance levels were set at $p<0.05$. Significant effects with respect to CS-discrimination were followed-up by CS-specific path models (i.e., CS+ and CS- seperately). Two-sided model fit was assessed using root mean square error of approximation (RMSEA) with thresholds of <0.01,<0.05,<0.08,<0.10, and >0.10 indicating excellent, good, fair, mediocre or poor fit of the final model[36,37]. Reported regression coefficients reflect standardized betas. Indirect (i.e., mediation) paths were calculated using bootstrapping and the bias-corrected percentile method.

Results

Main effects of task (study 1 and 2)

Successful fear acquisition was demonstrated in both studies by significantly larger average CS+ than CS- responding (study 1: SCR, ratings, FPS, all p's<0.001; Supplementary Figure 2; study 2: SCRs and ratings, both p's<0.003, Supplementary Figure 4).

On a neuro-functional level (study 2) CS-discrimination (CS+>CS-) was reflected by enhanced activation of areas typically activated in fear acquisition[34,35] (i.e., thalamus, amygdala, dmPFC/dACC, insula/frontal operculum and putamen/pallidum; Supplementary Figure 4C, Supplementary Table 3). Stronger activation to the CS- than the CS+ was observed in the vmPFC (Supplementary Figure 4D, Supplementary Table 3).

Dimensional analyses for each construct and outcome measure in isolation (study 1)

SCRs: All three constructs (STAI-T, IU, N) were significantly negatively associated with CS-discrimination in SCRs (CS-type*construct interaction; all p's<0.045, Table 1A) indicating decreasing

CS-discrimination with increasing construct scores (Figure 1A-C). This interaction was primarily driven by enhanced CS+ responses in individuals scoring low on IU (p=0.03) and STAI-T (p=0.057), despite comparable CS- responding (Table 1A). The significant impact of N on CS-discrimination could however not be assigned to either CS+ or CS- responding alone (Table 1). Main effects of the constructs on general SCR responding (all p's>0.09, Table 1) or associations with unconditioned SCRs to the US (all F's<1.56, all p's>0.213) were absent.

Fear ratings: None of the three constructs was significantly associated with CS-discrimination in fear ratings (CS-type*construct; all p's>0.288, Table 1). However, significant or trend-wise main effects were observed (STAI-T: p=0.046, IU, p=0.092, N: p=0.002, Table 1A), indicative of generally heightened fear ratings with increasing construct scores.

FPS: Only IU was significantly linked to FPS CS-discrimination (CS-type*IU, p=0.022; for N and STAI-T: both p's>0.13, Table 1A, Supplementary Section 3.2) in absence of main effects of any construct on FPS responsivity (all F's<1). More precisely, higher IU scores were associated with low FPS CS-discrimination. Tentatively, this effect was driven by reduced CS+ responding in individuals scoring high on IU (p=0.07), whereas CS- responding did not differ depending on IU score (p=0.16).

Categorical analyses for each construct and outcome measure in isolation (study 1)

Analyses employing categorical operationalization by median-split or quartile-split groups (Table 1B-C provides statistics for all outcome measures, Figure 1D-F illustrates SCR results) are largely comparable to dimensional analyses for all three outcome measures despite the association between N and CS-discrimination not meeting statistical significance in categorical analyses.

Table 1. Statistical values from univariate repeated measures analyses in study 1 for the three different constructs related to negative affect: trait anxiety (STAI-T), neuroticism (N) and intolerance of uncertainty (IU) for **(A)** dimensional analyses as well as analyses based on **(B)** median split procedure or **(C)** quartile groups for the three outcome measures skin conductance (SCR), fear ratings, and fear potentiated startle (FPS) during fear acquisition training.

A. Dimensional analyses per construct

	SCR			Fear ratings			FPS		
	STAI-T	**N**	**IU**	**STAI-T**	**N**	**IU**	**STAI-T**	**N**	**IU**
Main effects									
Construct	F(1,269)<1	F(1,269)<1	F(1,269)=2.84, p=0.09	**F(1,266)=4.03, p=0.046, $_p\eta^2$=0.02**	**F(1,266)=7.22, p=0.008, $_p\eta^2$=0.03**	F(1,266)=2.87, p=0.092	F(244)<1	F(1,244)<1	F(244)<1
CS-type	F(1,269)=26.22, p<0.001, $_p\eta^2$=0.09	F(1,269)=22.50, p<0.001, $_p\eta^2$=0.08	F(1,269)=26.14, p<0.001, $_p\eta^2$=0.09	F(1,266)=18.63, p<0.001, $_p\eta^2$=0.07	F(1,266)=27.66, p=<0.001, $_p\eta^2$=0.09	F(1,266)=26.79, p<0.001, $_p\eta^2$=0.09	F(244)=6.64, p=0.011, $_p\eta^2$=0.03	F(244)=6.92, p=0.009, $_p\eta^2$=0.03	F(244)=13.06, p<0.001, $_p\eta^2$=0.05
Interaction effects									
CS-type * Construct	**F(1,269)=11.23, p=0.001, $_p\eta^2$=0.04**	**F(1,269)=4.05, p=0.045, $_p\eta^2$=0.02**	**F(1,269)=8.69, p=0.03, $_p\eta^2$=0.03**	F(1,266)<1	F(1,266)=1.13, p=0.288	F(1,266)<1	F(244)=2.26, p=0.13	F(244)<1	**F(244)=5.32, p=0.022, $_p\eta^2$=0.02**
CS+ * Construct	F(1,269)=3.66, p=0.057, $_p\eta^2$=0.01	F(1,269)<1	**F(1,269)=6.06, p=0.014, $_p\eta^2$=0.02**	--	--	--	--	--	F(244)=3.25, p=0.07
CS- * Construct	F(1,269)<1	F(1,254)=1.85, p=0.18	F(1,269)<1	--	--	--	--	--	F(244)=1.96, p=0.16

B. Categorical analyses (median-split)

	SCR			Fear ratings			FPS		
	STAI-T	**N**	**IU**	**STAI-T**	**N**	**IU**	**STAI-T**	**N**	**IU**
Main effects									
Construct-group	F(1,269)=1.85, p=0.18	F(1,269)<1	F(1,269)<1	F(1,266)=3.27, p=0.07	**F(1,266)=9.95, p=0.002, $_p\eta^2$=0.04**	F(1,266)=2.89, p=0.09	F(1,244)<1	F(1,244)=1.19, p=0.28	F(1,244)<1
CS-type	F(1,269)=62.70, p<0.001, $_p\eta^2$=0.19	F(1,269)=60.83, p<0.001, $_p\eta^2$=0.01	F(1,269)=60.45, p<0.001, $_p\eta^2$=0.18	F(1,266)=290.64, p<0.001, $_p\eta^2$=0.52	F(1,266)=291.28, p<0.001, $_p\eta^2$=0.52	F(1,266)=290.56, p<0.001, $_p\eta^2$=0.52	F(1,244)=23.78, p<0.001, $_p\eta^2$=0.09	F(1,244)=23.57 p<0.001, $_p\eta^2$=0.09	F(1,244)=22.78, p<0.001, $_p\eta^2$=0.09
Interaction effects									

CS-type * Construct-group	**F(1,269)=9.170, p=0.003, $_p\eta^2$=0.03**	F(1,269)<1	**F(1,269)=9.193 p=0.003, $_p\eta^2$=0.03**	F(1,266)=1.25, p=0.27	F(1,266)<1	F(1,266)<1	F(1,244)<1	F(1,244)<1	**F(1,244)=5.64, p=0.018, $_p\eta^2$=0.02**
CS+ * Construct-group	**F(1,269)=4.91, p=0.028, $_p\eta^2$=0.02**	--	F(1,269)=1.76, p=0.19	--	--	--	--	--	F(1,244)=3.79, p=0.053, $_p\eta^2$=0.02
CS- * Construct-group	F(1,269)<1	--	F(1,269)=1.14, p=0.29	--	--	--	--	--	F(1,245)=1.85, p=0.18

C. Categorical analyses (quartile-split)

	SCR			**Fear ratings**			**FPS**		
	STAI-T	**N**	**IU**	**STAI-T**	**N**	**IU**	**STAI-T**	**N**	**IU**
Main effects									
Construct-group	F(3,267)=1.67, p=0.17	F(3,267)<1	F(3,267)<1	F(3,264)=1.32, p=0.27	**F(3,264)=3.97, p=0.09, $_p\eta^2$=0.04**	F(3,264)=1.43, p=0.23	F(3,242)<1	F(3,242)=1.47, p=0.22	F(3,242)<1
CS-type	F(1,267)=61.76, p<0.001, $_p\eta^2$=0.19	F(1,267)=62.09, p<0.001, $_p\eta^2$=0.19	F(1,267)=62.85, p<0.001, $_p\eta^2$=0.19	F(1,264)=293.06, p<0.001, $_p\eta^2$=0.53	F(1,254)=272.23, p<0.001, $_p\eta^2$=0.52	F(1,264)=289.47, p<0.001, $_p\eta^2$=0.52	F(1,242)=24.00, p<0.001, $_p\eta^2$=0.09	F(1,242)=23.96, p<0.001, $_p\eta^2$=0.09	F(1,242)=23.36, p<0.001, $_p\eta^2$=0.09
Interaction effects									
CS-type * Construct-group	**F(3,267)=3.59, p=0.014, $_p\eta^2$=0.04**	F(1,267)=1.76, p=0.16	**F(3,267)=4.83, p=0.003, $_p\eta^2$=0.05**	F(3,264)=1.32, p=0.27	F(3,254)=1.32, p=0.27	F(3,264)=1.43, p=0.23	F(3,242)=1.75, p=0.16	F(3,242)=1.57, p=0.20	F(3,242)=2.53, p=0.06
CS+ * Construct-group	F(1,267)=2.16, p=0.093	--	F(1,267)=1.58, p=0.20	--	--	--	--	--	--
CS- * Construct-group	F(1,267)=1.67, p=0.17	--	F(1,267)=1.41, p=0.24	--	--	--	--	--	--

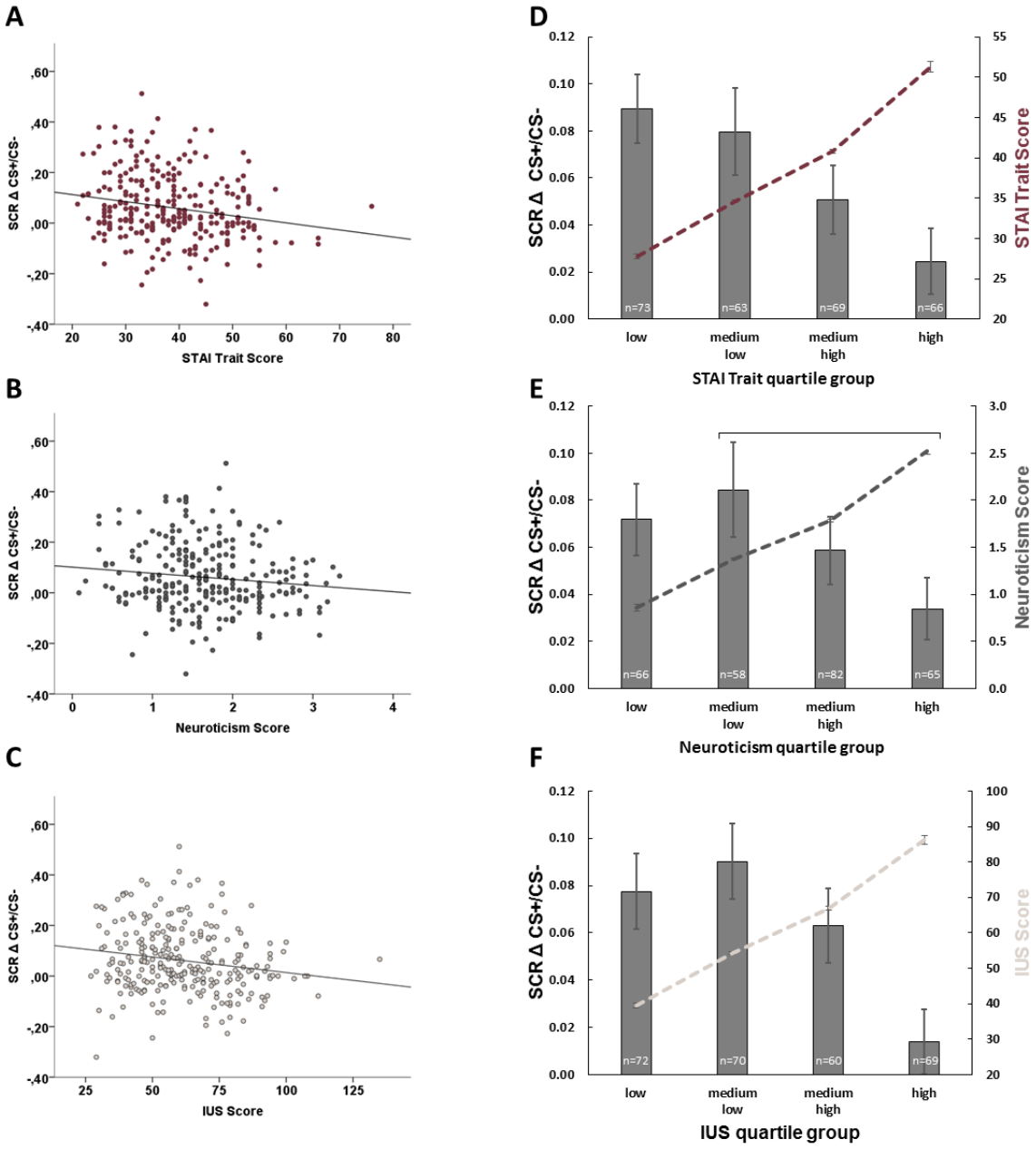

Figure 1. Dimensional and categorical display of the relation between SCR discrimination and negative affect constructs. Scatterplots display CS-discrimination during fear acquisition (Study 1) in SCRs (in µS, log, range-corrected) and its relation to STAI trait **(A)**, neuroticism **(B)** and intolerance of uncertainty **(C)** scores as well as bar charts displaying mean SCR CS-discrimination during fear acquisition (indicated by the bars) as well as number of individuals (n) for quartile groups (low, medium low, medium high, high) differing in STAI trait mean scores **(D)**, neuroticism mean scores **(E)** and IU mean scores **(F)**, which are indicated as mean scores per group by the dashed lines in each bar graph (see Supplementary Table 1 for descriptives and details on median-split and quartile groups). Error bars represent SEM. Note that the STAI is not a diagnostic tool and no clinical cut off score is available. Typical scores for patients diagnosed with anxiety disorders are however in the range of 47 and above [38], which corresponds to ~18.4% in this sample.

Integration of multiple constructs of negative affect and multiple outcome measures of fear learning in multivariate analyses (study 1)

A multivariate analysis (i.e., path model) accounting for shared variance between the three questionnaires shows the expected strong positive associations between constructs (STAI-T, IU, N) and outcome measures (SCRs, FPS, ratings), all p's <0.001, Figure 2. Importantly, the final model reveals a *unique* impact of STAI-T on CS-discrimination in SCRs (standardized path coefficient: -.19, p<0.001) in absence of significant associations with IU or N despite significant associations of all three constructs with SCRs CS-discrimination in univariate analyses (see above). This implies that the association of N and IU with differential fear acquisition is fully explained by shared variance with trait-anxiety.

Additionally, and congruent with univariate analyses, a *unique* impact of IU on CS-discrimination in FPS was observed (standardized path coefficient: -.14, p=0.024).

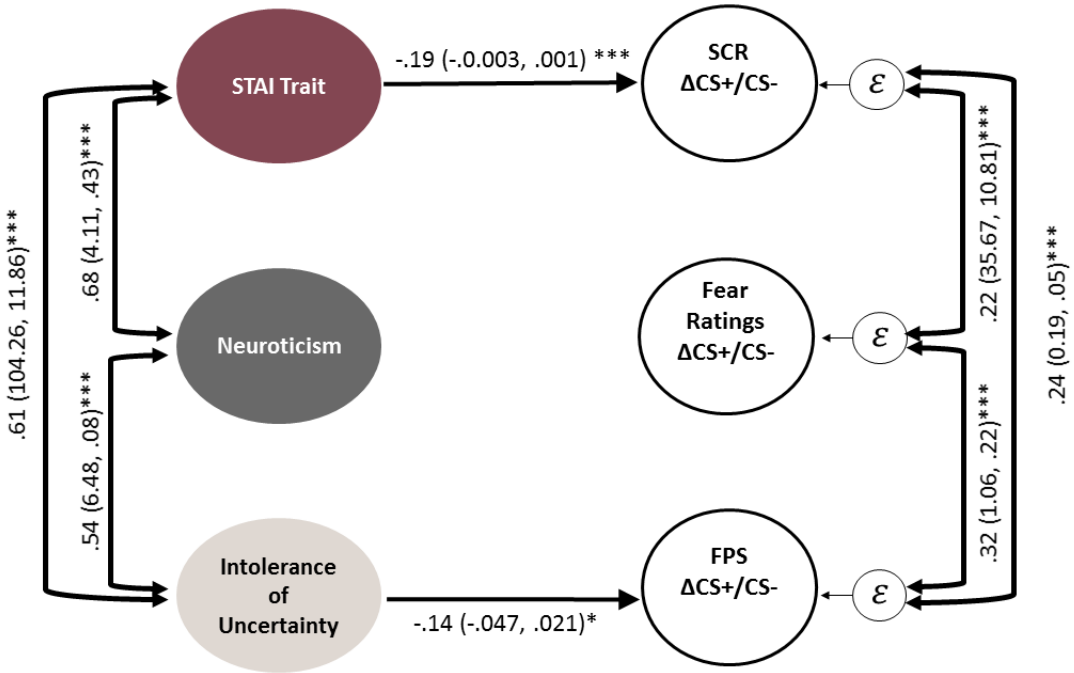

Figure 2. Final path model (study 1) showing the association between three different constructs related to negative affect (STAI trait, neuroticism and intolerance of uncertainty) and CS-discrimination during fear acquisition as assessed by three different outcome measures (skin conductance responses, SCRs, fear ratings and fear potentiated startle, FPS). The lines are labeled with standardized path coefficients. Regression weight estimates and standard errors are shown in parenthesis. Asterisks indicate statistical significance ***p<0.001, *p<0.05. Black bold lines indicate significant paths of the final model while any other connections between the variables not shown indicate that these connections, which were included in the saturated (i.e., initial) model, are excluded from the final model due to the lack of statistical significance for this path. Note, that we performed a backward selection of non-significant path starting from this saturated model (see methods). The final path model showed an excellent model fit (RMSEA<0.001).

Neural mechanism mediating the association between trait-anxiety and SCRs CS discrimination (study 2).

Higher STAI-T scores were associated with significantly stronger CS-discrimination related activation of the right amygdala ($p[SVC_{FWE}]$=0.006, Figure 3A,D), the right putamen ($p[SVC_{FWE}]$=0.005, Figure 3B,E) and the left thalamus ($p[SVC_{FWE}]$=0.040 and Figure 3C,F) during fear acquisition in regression

analyses (Table 2 and Supplementary Table 4 for an exploratory whole brain analysis). These areas are also significantly implicated in CS-discrimination irrespective of STAI-T in this sample (see above, main effects of task). Congruent with study 1, these effects are driven by positive associations between STAI-T scores and CS+ related, but not CS- related, neural activation (amygdala(R): x,y,z=22,-4,-16; k=5; T=3.58; p[SVC_{FWE}]=0.014; amygdala(L): x,y,z=-22,-12,-12; k=3; T=3.34; p[SVC_{FWE}]=0.023; putamen(R): x,y,z=22,20,-6; k=7; T=3.51; p[SVC_{FWE}]=0.043; thalamus(L): x,y,z=-10,-28,10; k=135; T=4.49; p[SVC_{FWE}]=0.003).

The final multivariate path model for study 2 (Figure 3G) also illustrates this significant positive association between STAI-T and parameters extracted from the above described regression analyses (i.e., CS-discrimination related amygdala, putamen and thalamus activation). Importantly, also significant positive associations (direct effects) between differential (CS+>CS) amygdala activation and SCR CS-discrimination (again driven by CS+ responses; not shown) was observed. Replicating results of study 1, STAI-T and differential SCRs correlated significantly negative (direct effect, Figure 3G) – however in a CS-unspecific manner. Importantly, also the indirect path between SCR CS-discrimination and STAI-T was significant, indicating partial mediation of STAI-T on SCR CS-discrimination through CS-discrimination in the amygdala (p=0.004; Figure 3G dashed line).

Table 2. Neural activation reflecting significant ROI-based results (p<0.05 SVC_{FWE}) for a regression of trait-anxiety on CS discrimination during fear acquisition training (study 2). Cluster size k and coordinates x, y and z of the respective cluster are reported. Note that CS-specific follow-up regression analyses (i.e. CS+ and CS- separately) are reported in the main text. Results of an exploratory whole-brain analysis at p<0.001 uncorrected (uc) is included in Supplementary Section 4.2 for completeness.

Contrast	Brain area	k	x	y	z	T	p(uc)	p(SVC_{FWE})
CS+>CS-	putamen (R)	98	22	18	-6	4.05	<0.001	**0.005**
			28	12	-2	3.97	<0.001	**0.006**
	amygdala (R)	6	26	-10	-12	3.50	<0.001	**0.011**
			28	-6	-14	3.29	0.001	**0.019**
	thalamus (L)	5	-2	-20	12	3.38	0.001	**0.040**
		10	-8	-10	8	3.36	0.001	**0.042**
CS->CS+	none							

Awareness and US intensity are not associated with trait-anxiety (study 1 and 2)

Neither awareness of CS contingencies nor US intensity was significantly associated with any of the trait constructs in study 1 and 2 (study 1/2: Supplementary Section 3.3-3.4/4.3-4.4), although individuals being unaware of CS-contingencies scored trend-wise higher on STAI-T and IU in study 1. Importantly, incorporating awareness in the path model did not cause changes in the final path model.

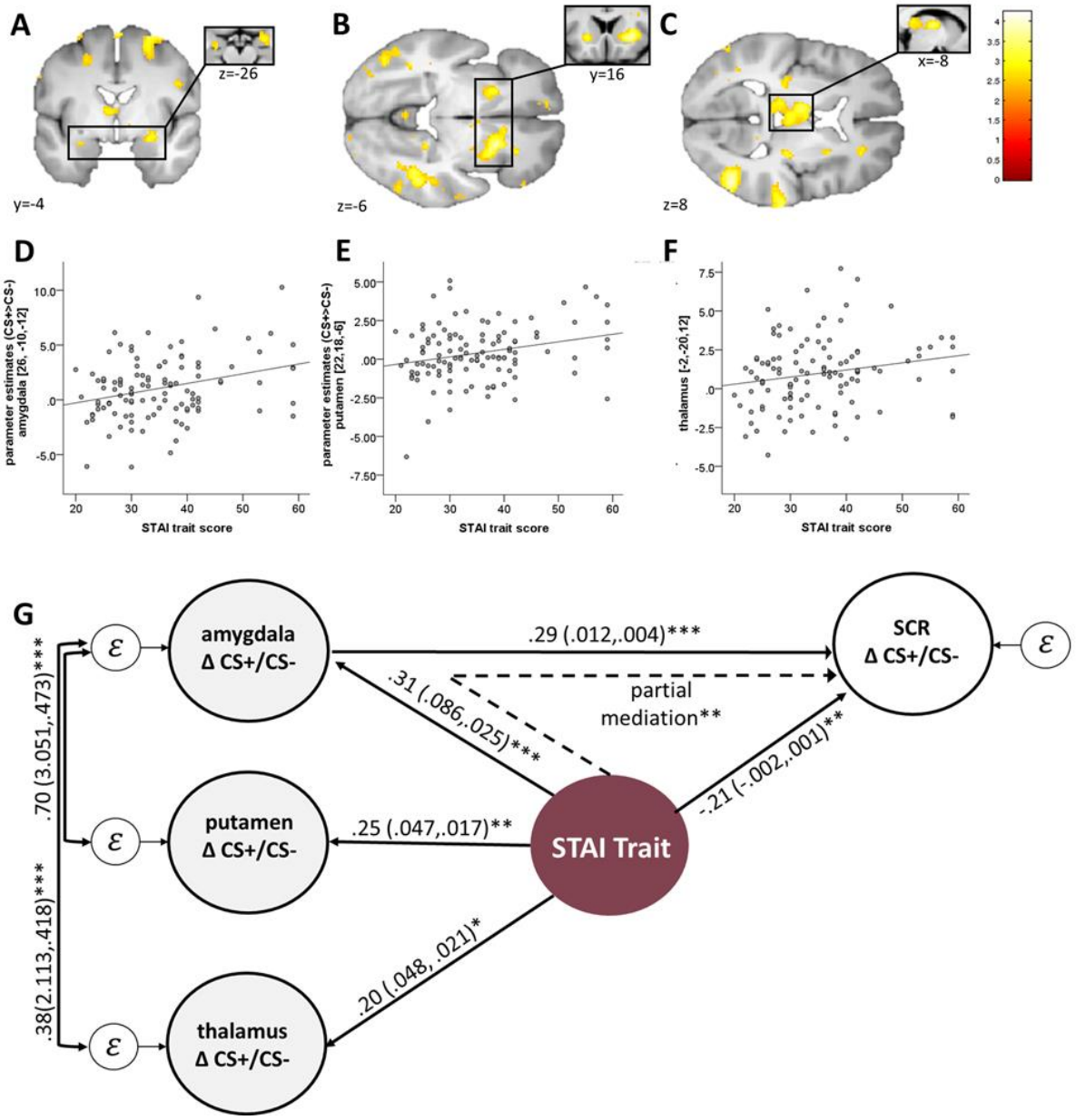

Figure 3. Neural activation reflecting a regression of trait-anxiety (STAI-T) on CS-discrimination during fear acquisition (study 2) in the (A) amygdala, (B) putamen and (C) thalamus as well as scatter plots presenting the association between trait-anxiety and extracted peak voxel parameter estimates (CS+>CS-) in the (D) amygdala, (E) putamen and the (F) thalamus, which are also fed into the path model displayed in (G). A display threshold of p<0.01uc was employed to illustrate the extent of peak activations but note that statistics are based on FWE-corrected values (see methods). Note that CS-specific follow-up analyses (i.e., separate analyses for the CS+ and the CS- are reported in the main text) indicate CS+-specific effects. (**G**) Final path model of the positive association (direct path indicated by solid lines) between trait-anxiety and CS-discrimination in the amygdala, thalamus and putamen as well as a positive association between CS-discrimination in the amygdala and CS-discrimination in autonomic (i.e. SCR) measures. The significant effect of a negative association of STAI-T on SCR CS-discrimination, replicating results observed in study 1, was complemented by a partial mediation of the impact of STAI-T on SCR CS-discrimination via CS-discrimination in the amygdala [indirect (i.e., mediation) path indicated by the dashed line].

Standardized path coefficients are displayed and regression weights as well as SEM are provided in parentheses. The final model shows a good fit of the data (RMSEA=0.047). Note, that we performed a

backward selection of non-significant path starting from a saturated model (see methods). Thus paths not included in the figure (i.e., all possible connections including CS-discrimination in subjective ratings and paths from putamen and thalamus to SCR CS-discrimination) were non-significant. Asterisks indicate statistical significance ***p<0.001, **p<0.01, *p<0.05.

Discussion

Our work identifies trait-anxiety as the key facet of negative affect associated with differential fear acquisition in SCRs beyond conceptually and empirically related constructs (i.e., neuroticism and intolerance of uncertainty) by employing a multivariate and multimodal approach in a large sample (N=288). Furthermore, we replicate and refine this association in an independent sample (N=116) by demonstrating that the ability to discriminate between danger and safety signals physiologically (i.e., SCRs) is partly *mediated* through differential (CS+>CS) amygdala activation– a core region implicated in fear processing[39–43]. Having identified trait-anxiety (STAI-T) as the unique facet of negative affect and having identified the neurobiological mechanisms underlying this association, brings together hitherto loose ends of research and provides insight into how individual differences may contribute to risk and resilience for pathological fear[3,44].

Notably, *not* accounting for conceptual and empirical collinearity between measures of negative affect revealed similar effects of STAI-T, N and IU on CS-discrimination. Hence, we argue that this commonly employed *isolated*, univariate approach can yield misleading findings[3], as results derived from the multivariate approach employed here imply that the association of N and IU with SCR CS-discrimination is fully explained by their shared variance with STAI-T. Yet, STAI-T has been criticized for representing a psychometrically inhomogeneous scale itself[45], capturing facets of anxiety and depression[45–48]. Hence, while selection of constructs for study 1 was based on the mere abundance of empirical work in fear conditioning[3], future studies may consider measures of depression to further narrow down the underlying causal facet(s).

Furthermore, we provide a mechanistic link between inter-individual differences in physiological and neural responding to learned threats. Importantly, simultaneous acquisition of these measures integrates hitherto unconnected reports of associations between STAI-T and differential amygdala activation[27,49] as well as differential amygdala activation and differential SCRs during fear acquisition[50,51 but see 25,26] or fear expression[27]. In addition, our work provides evidence for an involvement of the amygdala in individual differences underlying the *strength of fear learning* beyond the average (i.e., a general role in fear acquisition and expression). Interestingly, direct associations between STAI-T and CS-discrimination in SCRs were negative, while indirect associations through the amygdala were positive. This suggests that besides this indirect path over the amygdala other sources of variance must influence associations between STAI-T and CS-discrimination in SCRs[52]. In other domains of threat processing, i.e. facial threat processing[53], similar positive associations between STAI-T and amygdala reactivity have been observed[54], which again highlights the robustness of our results. Considering fear conditioning as a valid model for pathological fear acquisition[4,55], these results may translate into insights in the underlying mechanisms through which enhanced amygdala reactivity may predict the development pathological anxiety[56] or may provide a future intervention point.

Relatedly, the impact of STAI-T on CS-discrimination in both SCRs (study 1) and neural activation (study 2) exerted its influence primarily through differential CS+ (i.e., excitatory) but not CS-related responding[27,50] despite opposed directionality of direct effects. Importantly, in experimental designs employing a 100% reinforcement rate, STAI-T-related CS-discrimination has been attributed to differential responding to the CS+ (present results and one previous study on fear expression[27]). This high reinforcement rate can be assumed to generate an unambiguous (i.e., strong) experimental situation[3,57]. At first glance, these results seem to stand in contrast to previous reports on associations between STAI-T and deficits in *safety signal* (e.g., CS-) processing[58–61]. It is however noteworthy, that

the impact of individual difference factors on conditioned responding is likely impacted and moderated by seemingly subtle study design specifications such as the level of experimental ambiguity induced for instance through CS-US contingency instructions or variations in the reinforcement rate[3,23]. As such, it appears that studies linking STAI-T to *inhibitory processes* in fear conditioning might be characterized by relatively more ambiguous experimental situations through for instance lower reinforcement rates[58–61]. This speculation (for similar findings in decision making see[62]) has however not yet been addressed experimentally and mechanistic conclusions are hampered by the frequent unavailability of precise information on the nature of the observed CS-discrimination differences[3]. Hence, we urge authors to focus more on these underlying processes in future studies to facilitate mechanistic conclusions[3].

Our dimensional approach[63] in large samples allowed capturing the full range of STAI-T including scores falling well within the range observed in clinical populations[64,65] (10-18% of the samples). Of note, participants included in this study were free of any current or past neuropsychological disorder and in fact might represent highly resilient individuals able to maintain a high level of functioning despite being 'at risk' (i.e., scoring high on anxiety)[3]. Hence, future studies should focus on more heterogeneous populations including clinically diagnosed patient samples. Importantly, our work has major implications for the interpretation of past and future studies: We provide empirical evidence that the range of STAI-T scores in a given population critically influences the likelihood to observe a significant impact of STAI-T on CS-discrimination – a conclusion likely generalizing to other individual difference factors. Furthermore, our results imply that good characterization and reporting of study populations and experimental parameters is highly important especially in individual difference research[3].

Our multivariate approach across multiple units of analyses (i.e., outcome measures), revealed a rather specific association between STAI-T and responding to *danger signals* as assessed by SCRs or amygdala activation in two studies, whereas IU was specifically linked to CS-discrimination in FPS. Studies reporting associations of STAI-T with *safety signal* processing in turn have also reported findings based on FPS, and ratings of distress[59], US expectancy[60,61] or fear[61]. As SCRs to the CS- often consist of non-responses (i.e., zero responses), CS- responding can be less reliably assessed in SCRs as opposed to measures that rely on triggered responses and therefore ensure a certain response frequency (e.g., FPS, ratings)[23,30]. Consequently, this restricted variance in CS- responses might cause possible floor-effects that hamper valid interpretations concerning safety learning and the detection of individual differences[3,66]. Finally, null findings with respect to STAI-T and conditioned responding across outcome measures[14,26,67–72] are difficult to interpret as sample sizes for these studies fall well below the minimally required number of 64 participants (calculated for median-split analyses based on study 1) with one exception[72].

Importantly, the specific dissociations in outcome measures and constructs (i.e., specific association of STAI-T with CS-discrimination in SCRs, and IU with CS-discrimination in FPS) may provide mechanistic insights into the underlying processes. Different outcome measures capture and reflect diverse aspects of fear processing[23]: SCRs are thought to reflect general arousal which lines up with the STAI-T being a measure of general anxiety proneness. FPS in turn is considered a rather fear specific index[23] that per definition reflects an enhanced reflexive response towards an unexpected, and therewith uncertain, event. Hence, both results may carry complementary mechanistic information corresponding to multi-causal vulnerability in fear and anxiety. As it was technically not yet feasible to implement combined EMG-fMRI measurements at the time of data acquisition, future studies profiting from this novel option[73] are warranted to investigate the neurobiological mechanisms underlying the specific association between IU and FPS. Our results clearly highlight the value of multimodal work and multivariate analyses tools and suggest that 'compound profiles' that integrate multiple input and outcome measures and hence potentially capture multiple causal processes may prove useful from a 'personalized medicine' perspective.

Taken together, it is fundamental to uncover factors, and particularly their interaction contributing to individual risk and resilience to pathological fear in order to develop individually tailored prevention and intervention programs ('precision medicine') in the future. As such, improved understanding of (neurobiological) mechanisms underlying individual differences in experimental fear learning can be expected to translate into improved understanding on how adaptive responding to threats turns into maladaptive fear responding[74,75]. It will thus be important to extend the investigation of individual differences and the underlying neurobiological mechanisms beyond experimental fear acquisition to tests focusing on the long-term retention of fear and extinction memory (i.e., return of fear[69]), and ultimately to clinical populations. We provide a first step towards this overarching aim and provide mechanistic insights of inter-individual differences in fear processing.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary information

Supplementary information is provided as a separate file.