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Progress in Neuropsychopharmacology & Biological Psychiatry 89 (2019) 275–285

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Functional neuroimaging of associative learning and generalization in specific phobia

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ARTICLE INFO

Keywords:
Specific phobia
Fear learning
Fear generalization
Extinction learning
Extinction recall
fMRI

ABSTRACT

Background: Theoretical models have implicated classical fear conditioning, fear generalization, and extinction learning in the development of anxiety disorders. To date, it is largely unknown to what extent these mechanisms and the underlying neurobiology may be altered in specific phobia, a disorder characterized by focal fears. The current study systematically examined fear conditioning, fear generalization, extinction learning, and extinction recall in a sample of individuals with a specific phobia.

Methods: Participants with a specific phobia (SP) of spiders (n = 46) and healthy controls (HC) (n = 48) underwent a 3-day fMRI cue-conditioning protocol, including a fear acquisition and a fear generalization phase (day 1), an extinction learning phase (day 2), and an extinction recall phase (day 3). Stimuli were phobia-irrelevant, as geometrical shapes served as conditioned threat (CS+) and safety stimuli (CS-), and an electrical shock as the unconditioned stimulus (US). Self-reported fear, US expectancy, and blood-oxygen-level dependent responses were measured.

Results: Behavioral results only revealed enhanced CS+/CS-differentiation in fear scores during acquisition retention in SP. Some neural differences were observed during other task phases. During early fear acquisition, SP showed enhanced differential activation in the angular gyrus and lateral occipital cortex, and during extinction recall, more precuneus deactivation was found in SP compared to HC. There were no clear indications of altered neural fear generalization or extinction learning mechanisms in the SP group.

Conclusions: Results indicate that spider phobia may be characterized by enhanced differential fear retention and altered brain activation patterns during fear acquisition and extinction recall. The findings provide insight into the nature of fear learning alterations in specific phobia, and how these may differ from those found in disorders characterized by broad anxious distress.

1. Introduction

Anxiety disorders are characterized by excessive fear and/or anxiety and accompanying defensive states such as escape and avoidance behaviors (Association AP, 2013). Theoretical models have implicated alterations in fear acquisition, fear generalization, extinction learning, and extinction recall in the etiology of anxiety disorders (Duits et al., 2015; VanElzakker et al., 2014; Dymond et al., 2015). Laboratory studies have mostly provided evidence for alterations in these processes in individuals with high trait anxiety, and in those suffering from generalized anxiety disorder (GAD) or post-traumatic stress-disorder (PTSD) (Duits et al., 2015; Kaczkurkin et al., 2016; Lissek et al., 2014). Nevertheless, there is a shortcoming of studies looking at these processes in specific phobia.

The acquisition of clinically relevant fears has previously been modeled by fear conditioning or fear learning, a process by which a

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https://doi.org/10.1016/j.pnpbp.2018.09.008
Received 9 April 2018; Received in revised form 21 August 2018; Accepted 19 September 2018
Available online 25 September 2018
0278-5846/

neutral conditioned stimulus (CS+) acquires the ability to elicit fear responses by its pairing and association with an aversive outcome (unconditioned stimulus, US). However, fear responses are not often restricted to the initial CS+ − US association, but can be broadened to other stimuli and contexts that are similar or related to the CS, a process known as fear generalization. Furthermore, fear responses can be extinguished when the CS is repeatedly presented in the absence of the US, and thereby loses its predictive value. Through such extinction learning, a new, inhibitory CS+ − no US association is formed, that may suppress the original CS-US association. This extinction association is however often weaker than the CS-US association, and tests of extinction retention often show the recurrence of conditioned fear responses at a later moment (i.e. extinction recall). The neurobiology of these processes has been studied extensively in rodents and healthy humans, with studies showing that threat processing involves a network including the dorsal anterior cingulate, bilateral insula, and midbrain, while safety processing activates the ventromedial prefrontal cortex (vmPFC), hippocampi, and precuneus (Fullana et al., 2016; Milad & Quirk, 2012).

A recent meta-analysis found that anxiety disorder patients show dysregulated fear and extinction learning through enhanced fear responding (self-report and/or psychophysiological responses) to conditioned safety stimuli (CS-) that are not followed by a US, as well as smaller reductions in fear responses to the CS+ during extinction learning phases (Duits et al., 2015). Furthermore, a recent neuroimaging study has reported neurobiological alterations in fear acquisition and recall of the extinction memory, specifically demonstrating reduced activation in the vmPFC in individuals with anxiety disorders (Marin et al., 2017). However, no effort was made to differentiate between different types of anxiety disorders. Studies on fear generalization have also reported enhanced generalization in psychophysiology and/or neural activations in high trait anxious individuals, and patients suffering from PTSD or GAD (Kaczkurkin et al., 2016; Lissek et al., 2014; Greenberg et al., 2013; Haddad et al., 2012), while null findings have been reported (Torrents-Rodas et al., 2013; Tinoco-González et al., 2015). These dysregulations in safety learning, fear generalization, extinction learning and extinction recall may be reflective of general deficits in the inhibition of fear responses and inhibitory learning (Sijbrandij et al., 2013; Lissek, 2012; Graham & Milad, 2011).

Factor-analytic studies suggest that excessive fear and anxiety may form a spectrum of pathology, extending from diagnoses associated with more specific fears, such as specific phobia and discrete traumatization, to diagnoses associated with anxious-misery disorders such as GAD, major depressive disorder (MDD), and complex PTSD (Krueger, 1999; Clark & Watson, 2006; Watson, 2005). Such a spectrum can be observed in the startle potentiation response to threat, ranging from hyperresponsivity in phobic disorders to hypo-responsivity in anxious-misery disorders (Lang & McTeague, 2009; Lang et al., 2016; Lang et al., 2007; McTeague & Lang, 2012). Although OCD is separately classified, other disorders characterized by excessive fear or anxieties are grouped and studied together in the Diagnostic and Statistical Manual for Mental Disorders – 5th Edition (DSM-5) (Association AP, 2013) under the umbrella term 'anxiety disorders' and/or 'PTSD', and this may preclude finding specific pathological patterns across the fear-anxiety spectrum. Indeed, in fear learning, generalization, and extinction research, this fear-anxiety spectrum has received only limited attention.

Specifically, to date, it remains largely unknown if fear learning, fear generalization, extinction learning, and extinction recall are dysregulated in disorders on the fear-side of the fear-anxiety spectrum. Specific phobia (SP) is characterized by focal fear, and is therefore thought to represent a model fear disorder (Klahn et al., 2017). Remarkably, in specific phobia, fear learning has merely been investigated by three studies (Vriends et al., 2012; Schweckendiek et al., 2011), whereas threat-related generalization (Dymond et al., 2014) and extinction learning and recall (Mosig et al., 2014) were only examined by one study. Results of the fear learning studies suggest a different pattern of responses in SP compared to those reported in the meta-analytic study mentioned above (Duits et al., 2015). Specifically, the meta-analytic study showed indications of reduced CS+/CS- differentiation in subjective ratings and/or psychophysiology during fear conditioning in anxiety disorders and PTSD compared to healthy controls. In contrast, in SP, enhanced differential conditioning has been observed, as reflected by enhanced stimulus discrimination in valence ratings (Vriends et al., 2012; Mosig et al., 2014), higher psychophysiological hyper-responsitivity to phobia-relevant and phobia-irrelevant CS+ (Schweckendiek et al., 2011; Mosig et al., 2014), as well as increased fear circuitry activation to CS+ compared to CS- (Schweckendiek et al., 2011). However, enhanced differential conditioning was not reflected in all outcomes within each study. Furthermore, the one study on threat-related generalization reported higher threat-avoidance in response to phobia-relevant stimuli in spider phobic individuals compared to non-phobics (Dymond et al., 2014), while the study comparing SP and controls on extinction learning and recall study did not find any group differences (Mosig et al., 2014).

The current study builds on this previous work by systematically investigating whether excessive fear psychopathology (i.e. SP) is characterized by general alterations in behavioral and neural mechanisms of fear acquisition, acquisition retention, fear generalization, extinction learning, extinction retention. To minimize comorbidity and heterogeneity issues, we included individuals with spider phobia without comorbidities (SP; n = 46), thereby using the disorder as a model for fear-related symptoms to compare with healthy controls (HC; n = 48). All subjects underwent the same symptom measurements and a functional neuroimaging fear conditioning and generalization protocol, using abstract, phobia-irrelevant stimuli, in order to examine whether general deficits in fear-related processes could be observed beyond expected excessive fear responses to phobia-relevant stimuli. We hypothesized that individuals with SP would show increased self-reported fear and enhanced neural responding in threat-related regions to the CS + during fear acquisition, and reduced fear regulation-related activation in the vmPFC, reflecting enhanced fear generalization. It was also hypothesized that no alterations in extinction learning and extinction recall would be observed in those with SP relative to HCs.

2. Materials and methods

2.1. Participants

Data were acquired as part of a large randomized controlled trial investigating the effects of psychological therapy on psychopathology in emerging adulthood (Smartscan, Dutch Trial Register Number: NTR380). The current study included participants aged 16–25 years old with a specific phobia for spiders (SP), and healthy controls (HC). Participants were recruited via local advertisements. The SP group was required to meet the DSM-IV criteria for a specific phobia as confirmed by the MINI International Neuropsychiatric Interview (Lecrubier et al., 1997). Exclusion criteria for the SP group were any other current psychiatric diagnosis or current psychiatric treatment, and any past diagnosis other than an anxiety disorder. For HC, a current or past diagnosis or treatment were excluded. Exclusion criteria for both groups further existed of a history of neurological disease, use of psychotropic medication, left-handedness, and MRI contra-indications. The study was approved by the local medical ethics committee. All participants received full information about study procedures and provided informed consent before study onset. Parental consent was additionally obtained for minors (age < 18 years).

2.2. Psychopathology measures

Spider phobia severity was captured by two measures:

2.2.1. Fear of spiders questionnaire (FSQ)

A self-report questionnaire measuring current spider phobic symptomatology, with higher scores reflecting more phobia symptoms. It has 18 items, with scores between 18 and 126 (Szymanski & O'Donohue, 1995).

2.2.2. Behavioral approach test (BAT)

During the BAT (adapted from (Hood et al., 2010), participants were asked to complete 15 steps as far as they were willing to. The steps included entering a room with a tarantula in a see-through tank; approaching the tarantula from a 4.5-m distance; putting a hand in the tank, and touching the tarantula (asked but not allowed). A lower score on the BAT reflected lower approach behavior.

To check whether groups did not differ on other types of affective symptomatology, three additional measures were included:

2.2.3. Spielberger's trait anxiety scales

A self-report questionnaire that measures trait anxiety (Spielberger, 2010). The trait has 20 items with scores varying between 0 and 80.

2.2.4. Visual analogue scale (VAS) – state anxiety

A visual analogue scale ranging from 0 (no anxiety) to 100 (very anxious) that measures state anxiety (Davey et al., 2007).

2.2.5. Fear questionnaire (FQ)

A self-report questionnaire that measures phobic avoidance in agoraphobic situations and social situations, and avoidance of blood (Marks & Mathews, 1979). It consists of 15 items, with varying scores between 0 and 120.

2.2.6. Inventory of depressive symptomatology – self-report (IDS-SR)

A self-report questionnaire that measures depressive symptoms over the last week (Montgomery & Asberg, 1979). It has 30 items, with scores varying between 0 and 84.

2.3. fMRI task

2.3.1. Task procedure

The task consisted of a 3-day protocol: day 1) pre-conditioning, fear conditioning, and fear generalization; day 2) extinction learning; day 3) extinction recall. Participants were attached to the shock electrodes at the beginning of the task on each day. The fear conditioning phase and generalization phase has been previously validated ((Lange et al., 2017), adapted from (Lissek et al., 2013)).

2.3.2. Apparatus and stimuli

Stimuli were either seven circles or seven rectangles that parametrically increased in size (see Fig. 1). For half of the participants, conditioned threat stimulus (CS+) was the largest circle/rectangle, and the conditioned safety stimulus (CS-) was the smallest circle/rectangle. This was reversed for the other half of the participants. The remaining five circles/rectangles served as generalization stimuli (GS1-5). GS5 was the least similar to the CS+, while GS1 was most similar to the CS + A triangle served as an additional CS- (vCS-) not being subject to perceptual generalization. The duration of stimulus presentation was 4.4 s. The inter-stimulus-interval was either 2.2 or 4.4 s. A fixation cross was shown all times. The unconditioned stimulus (US) was a 200 millisecond (ms) electrical shock, which was applied to the left ankle via a Biopac STM100C stimulator module with a STIMSOC isolation unit (BioPac Systems, Goleta, CA). Before task onset, the intensity of the US (in milliampere) was calibrated to each participant to ensure that the shock was 'highly annoying but not painful'. The US co-terminated with the CS+. The task was presented via Presentation software package (Albany, CA, USA) on a mirror attached to the head coil, via a high-resolution screen placed at the rear of the magnet bore.

2.3.3. Behavioral ratings

US expectancy was assessed using a 4-point scale (1 = no risk; 2 = low risk; 3 = moderate risk; 4 = high risk of receiving a shock). Participants were required to rate US expectancy when the color of the fixation cross changed from white to red (for 880 ms). The fixation cross changed color four times per stimulus per task phase. Before and after each task phase, fear ratings for each stimulus were retrospectively reported on a VAS ranging from 0 (not fearful) to 100 (very fearful). All behavioral ratings were given via button responses.

2.3.4. Task instructions

Before task onset, button responses for the behavioral ratings were practiced. Participants received the following instructions: 'The stimuli may or may not be followed by a shock. Please try to learn to predict the shock.'

2.3.5. Day 1

During the pre-conditioning phase (Day 1), all stimuli were shown without the US. The subsequent fear conditioning phase started, showing all CS (CS+, CS-, and vCS-). The CS+ co-terminated with the US (66% reinforcement schedule. Next, a generalization phase started during which all stimuli were shown. The CS+ was followed by the US (50% reinforcement schedule). For all three phases, the expectancy of the US was measured during the task phase while fear ratings for each stimulus were measured after the task phase.

2.3.6. Day 2

The following day (Day 2), fear ratings for all CS were first obtained. Then the extinction learning phase started, showing the CS+, CS- and vCS-, without the US. US expectancy was again measured during the extinction learning phase, while fear ratings were also again obtained after the extinction learning phase.

2.3.7. Day 3

On day 3, self-reported fear ratings were again first obtained for all stimuli (CS and GS). During the extinction recall phase, all stimuli were shown, again without occurrence of the US. US expectancy and self-reported fear were again obtained during and after the extinction recall phase, respectively.

During each task phase, participants reported US expectancy four times for each stimulus. Stimuli were shown 12 times per task phase. The sequence of stimuli was quasi-random with no more than two consecutive presentations of the same stimulus. Each phase was divided into two blocks to establish an even stimulus distribution. A genetic algorithm for optimizing experimental task designs (Wager & Nichols, 2003) was used to establish the sequence. The duration of the pre-conditioning and extinction recall phases was 12.4 min, while the duration of the conditioning and extinction phases was 4.7 min.

2.4. Study procedure

Participants first received extensive information about the study and gave informed consent. Subsequently, all participants were screened with the MINI, and were asked to complete the BAT and all psychopathology questionnaires. Participants were then scheduled to come back for the 3-day fear learning, extinction learning, and extinction recall paradigm, and completed the VAS - state anxiety at the beginning at each scanning session.

2.5. MRI acquisition and preprocessing

MRI scans were acquired using a 3 T Siemens Magnetom Prisma system (Siemens Healthcare, Erlangen, Germany) equipped with a 64-channel head/neck coil. T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) images with a voxel size of 1 mm × 1 mmx1 mm were acquired to serve as anatomical reference

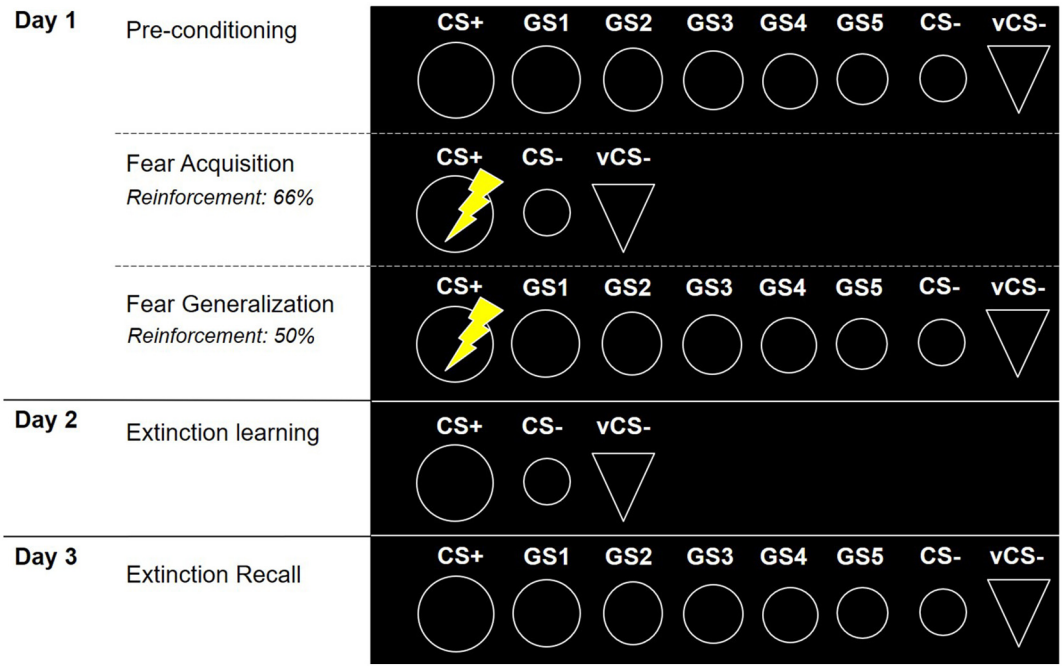

Fig. 1. Task stimuli per experimental phase. CS+ = conditioned threat stimulus, CS- = conditioned safety stimulus, GS = generalization stimulus.

(repetition time (TR) = 2250 msec, echo time (TE) = 2.21 msec, flip angle = 9°, field of view (FOV) = 256 × 256 × 192, sagittal slice orientation, GRAPPA = 2). Functional scans were acquired using a T2*-weighted echo-planar images (EPIs) sequence (TR = 2450 msec, TE = 28 msec, flip angle = 75°, interleaved, FOV = 216 mm, axial orientation, GRAPPA = 3) with a voxel size of 3 mm × 3 mm × 3 mm. During the pre-conditioning, generalization, and extinction recall phases, 303 volumes were acquired. During the acquisition and extinction learning phases, 114 volumes were acquired.

Functional magnetic resonance imaging data preprocessing and analyses were carried out using FEAT (FMRI Expert Analysis Tool) of FSL (FMRIB's Software Library) version 5.0.6 (Smith et al., 2004). Preprocessing included non-brain removal (BET) (Smith, 2000), motion correction using MCFLIRT with the middle volume as reference (Jenkinson et al., 2002), high-pass temporal filtering with a cut-off of 100 s, spatial smoothing with a Gaussian kernel of 6 mm FWHM, prewhitening (Woolrich et al., 2001), co-registration using FLIRT (Jenkinson et al., 2002), and normalization into Montreal Neurological Institute 152 stereotaxic space (MNI) using FNIRT for non-linear registration (Andersson et al., 2007). Because of excessive motion (cumulative motion translation > 3 mm) or motion-related artifacts, 3 participants (2 HC, 1 SP) were excluded for further analyses of the fear conditioning phase, and 5 participants (2 HC, 3 SP) were excluded from further analyses of the fear generalization phase.

2.6. Analyses

2.6.1. Demographics and questionnaires

Behavioral data were analyzed with SPSS version 23 (IBM SPSS Statistics, Armonk, NY: IBM Corp.) To test whether groups (SP, HC) differed in demographic characteristics (age, sex, education), measures of depression, anxiety, and spider phobia, and US intensity, independent samples *t*-tests for continuous variables and Chi-square tests for categorical variables were used. A p-value < .05 was considered statistically significant. In addition, it was checked whether groups did not differ on measures of depression and anxiety by independent samples t-tests.

2.6.2. Group analyses of behavioral experimental data

For each experimental phase, fear and US expectancy scores were used as main behavioral outcome measures.

2.6.2.1. Preconditioning. Analyses here checked whether fear and US expectancy scores did not differ across stimuli and between groups. Fear and the average US expectancy scores for each stimulus were entered into a mixed analysis of variance (ANOVA), with group as between-subjects factor and stimulus as within-subjects factor (8 levels: vCS-, CS-, GS5–1, CS+). Main effects of stimulus and group, as well as group x stimulus interactions were tested.

2.6.2.2. Acquisition. To investigate whether a) differential fear learning occurred during the fear acquisition phase and b) whether groups differed in fear learning, fear scores for each stimulus (3 levels: vCS-, CS-, CS+) and time point (2 levels; pre-acquisition, post-acquisition) were entered as within-subjects factors together with group (SP, HC) as between-subjects factor into a mixed ANOVA. Similarly, for the US expectancy scores, a group (SP, HC) x stimulus (vCS-, CS-, CS+) x time (4 levels; 4 time points) mixed ANOVA was conducted.

2.6.2.3. Generalization. To test whether groups differed in fear generalization, fear ratings and the average US expectancy ratings were entered separately in a mixed ANOVA, with group as within-subjects factor (SP, HC), and stimulus (8 levels; vCS-, CS-, GS5–1, CS+) as between-subjects factor. Furthermore, to test whether the shape of the generalization gradient differed between groups, quadratic polymonial tests were conducted. A more quadratic gradient reflects a lower tendency to generalize (Lissek et al., 2009).

2.6.2.4. Acquisition retention. Acquisition retention was examined within a group (SP, HC; between-subjects factor) x stimulus (3 levels; vCS-, CS-, CS+; within-subjects factor) mixed ANOVA, with fear scores obtained before the extinction learning phase, and with the first US expectancy ratings obtained during the extinction learning phase.

2.6.2.5. Extinction learning. To examine whether groups differed in extinction learning, fear scores for each stimulus over the extinction learning phase were entered with group into a group (SP, HC) x

Table 1
Demographics and psychopathology scores in the HC and SP groups.

	HC	SP
	n = 48	n = 46
	Mean (SD)	Mean (SD)
Age	20.94 (1.84)	20.57 (2.37)
Spider phobia (FSQ)	29.25 (13.06)	99.15 (15.24)***
Spider approach behavior (BAT)	14.02 (0.97)	5.96 (4.30)***
VAS - state Anxiety		
Day 1	7.04 (9.81)	7.68 (10.29)
Day 2	6.15 (8.67)	5.95 (10.05)
Day 3	7.10 (10.72)	7.02 (13.98)
Trait Anxiety	30.27 (8.17)	30.59 (8.01)
Depression (IDS-SR)	5.96 (4.16)	8.59 (9.93)
Avoidance (stimuli/situations other than spiders) (FQ)	8.08 (8.145)	11.37 (11.16)
US intensity	24.93 (16.68)	21.24 (15.35)
	n	n
Gender (male/female)	9/39	4/42
Educational level (low/mid/high)	1/3/44	0/5/41

SD = standard deviation. A low educational level was defined as lower vocational training or less; a high educational level was defined as Bachelor's level or higher. FSQ = Fear of Spiders Questionnaire. BAT = Behavioral Approach test, VAS = Visual Analogue Scale, IDS-SR = Inventory of Depressive Symptomatology – Self-Report (IDS-SR), FQ = Fear Questionnaire, US = unconditioned stimulus, mA = milliampere.
*** = $p < .001$.

stimulus (3 levels; vCS-, CS-, CS+) x time (2 levels; pre-extinction, post-extinction) mixed ANOVA, with stimulus and time as within-subject factors, and group as between-subject factor. Similarly, US expectancy scores over the extinction learning phase were entered with group into a group (SP, HC) x stimulus (3 levels; vCS-, CS-, CS+) x time (4 levels; 4 time points) mixed ANOVA.

2.6.2.6. Extinction retention. To compare extinction retention across groups, fear ratings and US expectancy ratings (first rating during recall phase) were entered into a group (SP, HC; between-subject factor) x stimulus (8 levels; vCS-, CS-, GS5–1, CS+; within-subject factor) mixed ANOVA. GS were added to the model as a measure of generalization of extinction recall.

Greenhouse-Geisser correction was applied in case the assumption of sphericity was violated. Post-hoc comparisons were corrected for the number of comparisons by a Bonferroni correction.

2.6.3. MRI analyses

2.6.3.1. First-level analyses. A general linear model was formed for each experimental task phase on an individual level. Covariates of non-interest, i.e. motion correction parameters and motion outliers, were added to each model. The pre-conditioning model included the eight stimuli levels as explanatory variables (CS+, CS-, vCS-, GS1–5). To check whether groups did not differ on pre-conditioning, all stimuli were contrasted with the vCS-. The acquisition model included six explanatory variables: the three stimuli (CS+, CS-, and vCS-) divided into an early phase (first six trials) and late phase (last six trials). Contrasts of interest were: 1. early CS+ > early vCS-, and 2. late CS+ > late vCS-. The generalization model included eight stimuli levels as explanatory variables (CS+, CS-, vCS-, GS1–5). The contrast CS+ > GS1–5 was used, similarly to our previous study (Lange et al., 2017), as this measure indexes broad generalization to all GS. To investigate acquisition retention, the extinction model was divided into early, middle, and late phase, with each phase containing four consecutive trials. As the model included the three stimuli (CS+, CS-, vCS-) in each phase, there were nine explanatory variables in total. The early phase was used to examine acquisition retention with the following contrast: CS+ > vCS-. To examine extinction learning, the following contrasts were formed for the extinction phase: 1. early CS+ > mid CS+; 2. early CS+ > late CS+; 3. late CS+ > vCS-. Extinction recall was examined by investigating only the first four trials from the recall phase, as in agreement with previous studies (Marin et al., 2017; Milad et al., 2009). The first-level models for the extinction recall therefore included 16 predictors: the eight stimuli (CS+, CS-, vCS-, GS1–5), divided into an early phase (first four consecutive trials) and a late phase (last eight consecutive trials). The following contrasts were of interest: 1. early CS+ > early vCS-, 2. early CS+ > early GS1–5, with the latter reflecting generalization of extinction recall.

2.6.3.2. Group-level analyses. To investigate main task effects and group differences, contrasts were entered into second-level mixed model analyses. Regions of interest (ROIs) were regions known to be involved in spider phobia and fear learning, including the ACC, insula, vmPFC, hippocampus, and amygdala (Fullana et al., 2016; Goossens et al., 2007). The probabilistic Harvard-Oxford atlases were used to define these anatomical ROIs (thresholded at 50%), which were then combined within one mask. Pre-threshold masking was used to examine neural activations within these ROIs only. Alongside to the ROI-analyses, whole-brain analyses were conducted for all contrasts. Both ROI-analyses and whole-brain analyses were corrected for multiple comparisons at the cluster level using Gaussian random field theory (GRF); ($Z > 2.3$, cluster-wise $p < .05$) using FSL's FMRIB's Local Analysis of Mixed Effects (FLAME1).

3. Results

3.1. Demographics and psychopathology

Table 1 provides an overview of demographics, psychopathology outcomes, and US intensity. The SP and HC groups did not differ in age ($t(1, 84.82) = 0.85$, $p = .40$), gender ($X^2(1,94) = 1.99$, $p = .23$), nor educational level ($X^2(2,94) = 1.56$, $p = .46$). As expected, the SP group reported higher spider phobia scores than the HC group ($t(1, 92) = -23.91$, $p < .001$), and less approach behavior towards a tarantula during the BAT ($t(1, 49.44) = 12.42$, $p < .001$). Groups did not differ in VAS - state anxiety scores (Day 1: $t(1, 92) = -0.30$, $p = .77$; Day 2: $t(1, 92) = 0.10$, $p = .92$; Day 3: $t(1, 92) = 0.03$, $p = .97$), trait anxiety scores ($t(1, 92) = -0.19$, $p = .85$), depression scores ($t(1, 59.69) = -1.66$, $p = .10$), nor avoidance behaviors over several other situations ($t(1, 92) = -1.64$, $p = .11$). Groups also did not differ on US intensity used during the experiment ($t(1, 92) = 1.06$, $p = .29$).

3.2. Manipulation check of task effects

The fMRI task evoked differential fear learning (CS+/CS- differentiation), fear generalization, and extinction learning as observed in the fear and US expectancy scores (see Figs. 2 and 3, and Supplemental Material for detailed results). In addition, expected activations in the ROIs occurred during all experimental task phases across the complete sample (see Supplemental Table S1 and Fig. S2).

3.3. Group differences across task phases

3.3.1. Pre-conditioning

Behavior: Fear ratings and US expectancy ratings of the pre-conditioning phase did not differ between groups, as no main effect of group (Fear: $F(1, 91) = 0.52$, $p = .47$); US expectancy: $F(1, 91) = 0.40$, $p = .62$) nor a group x stimulus interaction (Fear: $F(4.13, 375.41) = 0.71$, $p = .59$); US expectancy: $F(3.02, 271.84) = 0.90$, $p = .44$) were found.

Imaging: No group differences were observed during the pre-conditioning phase.

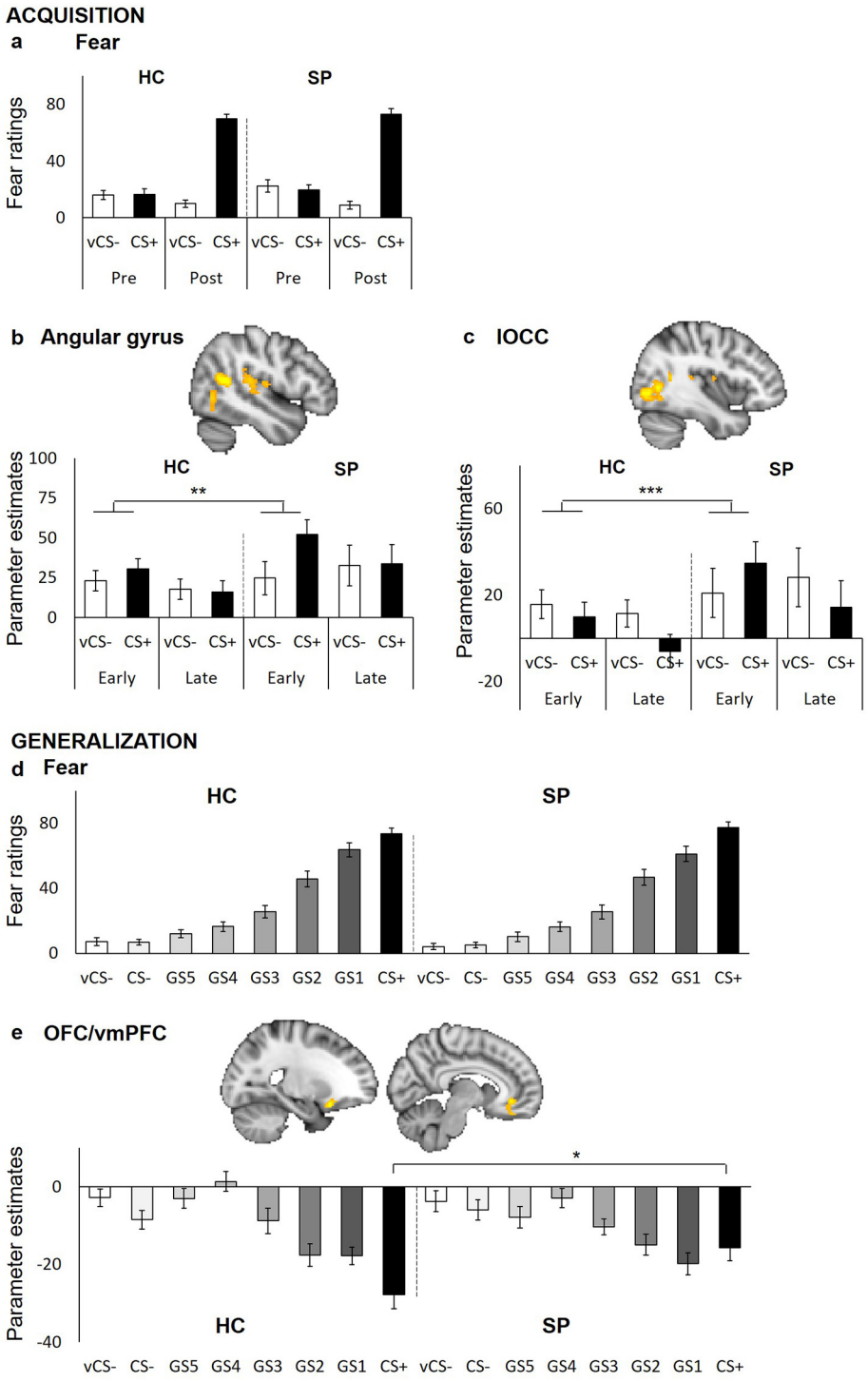

Fig. 2. Behavioral and imaging outcomes of the fear acquisition and fear generalization phases (Day 1). a. Fear ratings obtained before (Pre) and after (Post) the fear acquisition phase. b. Average parameter estimates of the conditioned stimuli during the early and late phase of fear acquisition in the right Angular gyrus cluster. c. Average parameter estimates of the conditioned stimuli during the early and late phase of fear acquisition in the right lateral occipital cortex (LOCC) cluster. d. Fear ratings obtained after the fear generalization phase. e. Average parameter estimates of all stimuli during the fear generalization phase in the orbitofrontal cortex (OFC)/ventromedial prefrontal cortex (vmPFC) cluster. *** = $p < .001$, **$p < .01$, * = $p < .05$, SP = specific phobia, HC = health controls.

3.3.2. *Fear acquisition*

Behavior: No group differences were found in the behavioral fear conditioning data, as reflected by non-significant group x stimulus x time interactions for the fear scores: $F(2, 182) = 0.42$, $p = .66$) (see Fig. 2a), or for the US expectancy scores ($F(4.80, 431.95 = 0.62$, $p = .68$) (see Supplemental Fig. S1). In addition, group x time and group x stimulus interactions, and the main effects of group analyses were also not significant (fear: all p's > 0.11; US expectancy: all p's > 0.24).

Imaging: The ROI-analysis did not reveal any group differences during early or late conditioning. The whole-brain analysis however showed enhanced activation in two clusters during early conditioning (CS+ > vCS-) in the SP-group compared to the HC: the right angular gyrus ([48–52 14], k = 813, Z = 4.32, p = .005) and the right lateral occipital cortex (LOCC) ([40–78 -4], k = 1074, Z = 4.08, $p < .001$ (see Fig. 2b and c).

3.3.3. *Fear generalization*

Behavior: Generalization gradients did not differ across groups, as group x stimulus interaction were non-significant (fear: $F(4.10, 377.05) = 0.29$, $p = .89$; US expectancy: $F(3.51, 319.53) = 0.71$, $p = .57$), as were the group differences in the quadratic trend across the stimuli (group x stimulus quadratic trends; fear: $F(1,92) = 0.30$, $p = .59$; US expectancy: $F(1,91) = 0.5$, $p = .83$) (see Fig. 2d and

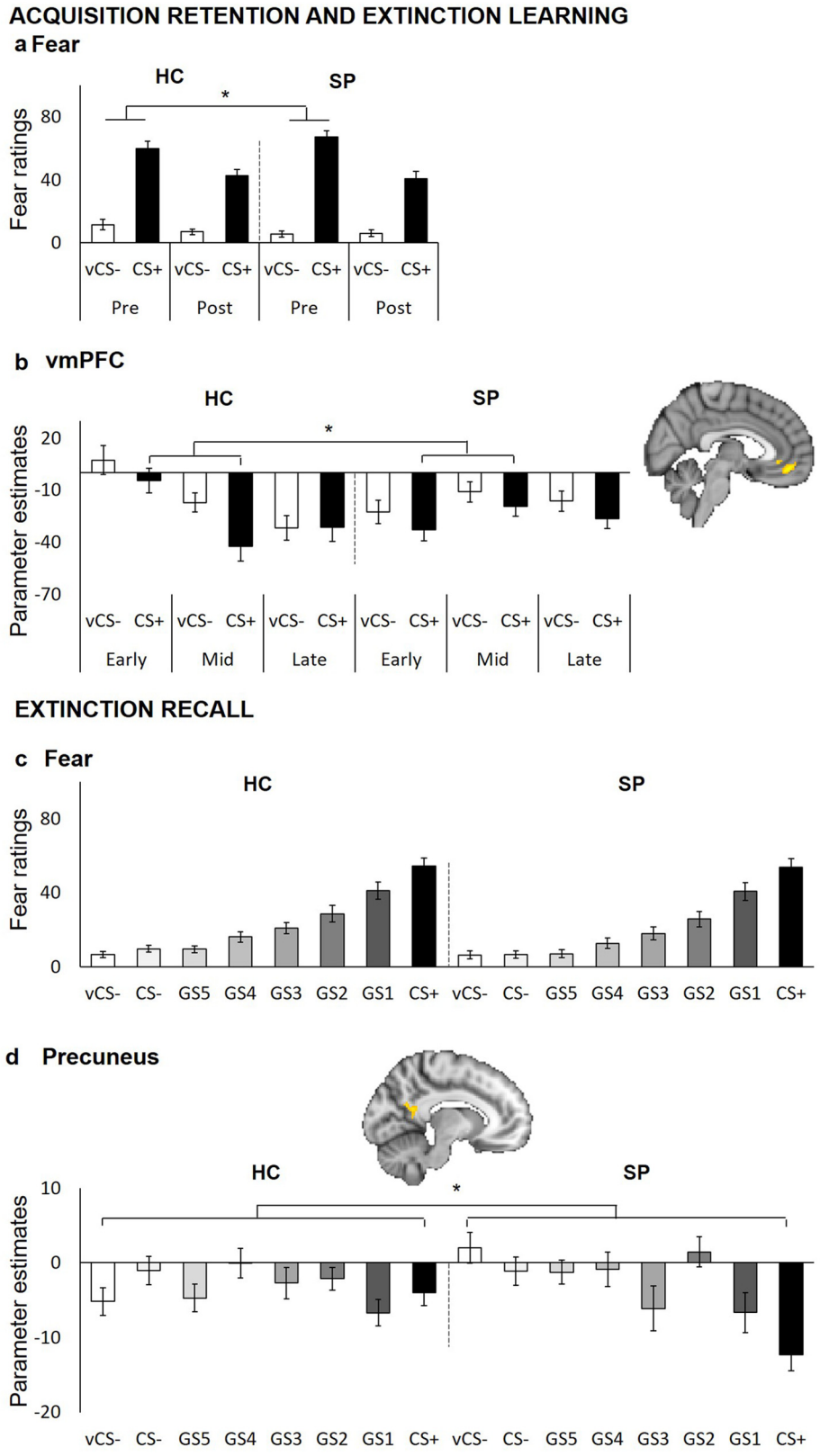

Fig. 3. Behavioral and imaging outcomes of acquisition retention, extinction learning (Day 2), and extinction retention (Day 3). a. Fear ratings for acquisition retention (Pre-Extinction) and after (Post) the extinction learning phase. b. Average parameter estimates of the conditioned stimuli during the early, mid, and late phase of extinction learning in the ventromedial prefrontal cortex (vmPFC). c. Fear ratings for extinction retention. d. Average parameter estimates of all stimuli during the extinction recall phase in the precuneus. * = $p < .05$, SP = specific phobia, HC = health controls.

Supplemental Fig. S1).

Imaging: The ROI-analysis revealed enhanced activation in the vmPFC in the HC group compared to the SP group ([10 38–8], k = 337, Z = 3.41 p = .04) for GS compared to the CS+. The whole-brain analysis further showed that this cluster extended into the OFC [−22 18–18], k = 870, Z = 3.73, p = .005). Post-hoc analyses on extracted parameters estimates revealed that the SP and HC group only significantly differed in response to the CS+, with the SP group showing less deactivation in response to this stimulus (t(1,87) = −2.34, p = .02; other p's > 0.21) (see Fig. 2e).

3.3.4. Acquisition retention

Behavior: Analyses including group revealed that the SP group reported higher fear for the CS+ than the CS- and vCS-, compared to the HC group, as reflected by a significant group x stimulus interaction scores (F(1.50, 134.77) = 3.76, p = .04) (see Fig. 3a). Post-hoc analyses showed that, in comparison with HC, SP reported higher differential fear for CS+ versus CS- (F(1, 90) = 4.76, p = .03), and for CS+ versus vCS- (F(1, 90) = 4.14, p = .04). No group differences were found in US expectancy scores, reflected by an absent group effect (F(1, 89) = 0.15, p = .70) and absent group x stimulus interaction (F(1.33, 118.01) = 0.27, p = .67) (see Supplemental Information). Also, no main effects of group were observed (fear: F(1,90) = 0.22, p = .64); US expectancy F(1,90) = 0.15, p = .70) (see Supplemental Fig. S1).

Imaging: Neither the ROI-analysis nor the whole-brain analysis revealed any group differences.

3.3.5. Extinction learning

Behavior: From pre-to-post extinction, the SP group tended to show a greater drop in fear to the CS+ than the (v)CS- as compared to the HC, as reflected by a trending group x stimulus x time interaction (F(1.68, 252.07) = 2.72, p = .08). At post-extinction, no group differences were observed in fear scores, as there was no significant group x stimulus interaction or main effect of group (F(1.28, 116.49) = 0.99, p = .34; (F1,91) = 0.95, p = .33) (see Fig. 3a). No group differences were observed in the US expectancy scores, as the group x stimulus x time interaction was non-significant (F(3.88, 345.34) = 0.76, p = .55), as well as the main group effect analysis and other interactions including group (all p's > 0.80) (see Supplemental Fig. S1).

Imaging: The ROI-analysis showed that the SP group had lower vmPFC activation during early extinction compared to mid extinction (early CS+ > mid CS+); ([2 48–10], k = 347, Z = 3.56, p = .02), compared to HCs. Further exploration of this result, by analyzing extracted parameter estimates within each group, revealed that vmPFC deactivation in HC became larger from early extinction to mid extinction (p = .001), while the deactivation in SP tended to decrease (p = .05). No further group differences were revealed for the other contrast (early CS+ > mid CS+) nor in the further whole-brain analyses (see Fig. 3b).

3.3.6. Extinction recall

Behavior: No significant group effect (F(1,89) = 0.37, p = .55), or group x stimulus interactions were observed in the fear ratings (F(3.22, 286.96) = 0.12, p = .96) (see Fig. 3c), nor in the US expectancy ratings (group: (F1, 91) = 0.21, p = .65; group x stimulus: F(3.31, 301.42) = 0.92, p = .44) (See Supplemental Fig. S1).

Imaging: The ROI analysis did not reveal any group differences. The whole-brain analyses revealed that the HCs showed higher activation in the left precuneus cortex (CS+ > vCS-) than the SP group ([−18–36 24], k = 489, Z = 3.32, p = .04) (see Fig. 3d).

4. Discussion

Theoretical accounts of fear learning, fear generalization, and extinction learning propose that these processes contribute to the development and maintenance of anxiety disorders, including specific phobia. The current study was the first to systematically examine fear learning and generalization, extinction learning, and recall in specific phobia compared to non-fearful healthy individuals. Behavioral results only revealed enhanced differential fear scores (CS+ vs. (v)CS-) during acquisition retention in the SP-group. Neuroimaging results revealed a few neural alterations in the SP-group during other task phase, however not in the expected regions of interest. Compared to HC, during early fear acquisition, the SP-group showed enhanced differential activation in the right angular gyrus and LOCC, regions known to become more activated during threat processing (Molapour et al., 2015; Pourtois et al., 2006). Neuroimaging data of extinction recall showed more precuneus deactivation (CS+ > vCS-) in SPs compared to HCs. During fear generalization and the early phases of extinction learning, the SP-group showed altered responding in the vmPFC, however, further analyses of neural responses per stimulus type revealed inconclusive patterns. Groups did not differ in the late phase of extinction learning. Collectively, no clear indications of altered neural fear generalization or extinction learning mechanisms were found in the SP group.

In the behavioral findings, groups only differed significantly in acquisition retention, with the SP group reporting larger differential fear (CS+ compared to (v)CS) than the HC group at day 2. This result may suggest that specific phobia is characterized by better fear memory specificity and fear discrimination. As the current study made use of phobia-irrelevant stimuli, this result may reflect a general risk factor in the development of phobias. It has been suggested that this may be a hallmark of fear-related disorders, including phobias (Norrholm et al., 2015), with further evidence coming from a previous study that found an exaggerated fear load in PTSD reflected by increased fear responses to threat at the onset of extinction (Norrholm et al., 2011). It has been suggested that this may be a hallmark of fear-related disorders, including phobias (Norrholm et al., 2015). However, the neuroimaging acquisition retention data did not differ between SP and HC. The acquisition retention data captured the first four trials of the extinction learning phase, and it is therefore possible that early extinction effects may have precluded to find group differences in acquisition retention on a neural level.

Although groups behaviorally did not differ on fear conditioning, the neuroimaging data seemed to reveal a similar pattern of enhanced discrimination, showing enhanced activation in response to the conditioned threat stimuli compared to the condition safety stimuli in the angular gyrus and the LOCC. These regions have been demonstrated to become more active during conditions of threat, possibly due to their role in attention and object recognition, respectively (Pourtois et al., 2006; Price et al., 2014). It is thought that the angular gyrus encodes and integrates several features of the stimulus as well as its context, including the neuronal representation of the stimulus in the lOCC (Ramanan et al., 2017; van der Linden et al., 2017). Enhanced activation in the SP-group might therefore reflect increased and more specific memory encoding of the conditioned threat stimulus. As there were no group differences in fear ratings or US expectancy during the fear conditioning phase, our results suggest that there is no direct linear relationship between angular gyrus and lOCC activation under threat, and cognitive and affective expressions of threat. However, activation in these regions may have contributed to the stronger retention of fear acquisition. Overall, our results may indicate that stronger discrimination during fear learning and retention could contribute to the etiology of specific phobia. Our study is partly in line with previous studies exploring fear conditioning in specific phobia. One study reported has increased fear circuitry activation and psychophysiological hyperresponsivity to phobia-relevant conditioned threat stimuli compared to safety stimuli (Schweckendiek et al., 2011), and others reported stronger conditioning in psychophysiology and self-reported valence scores in response to phobia-irrelevant conditioned threat stimuli (Vriends et al., 2012; Mosig et al., 2014). Similar to our findings, the latter two studies did not find indications of stronger differential conditioning when examining US expectancy or fear scores (Vriends

et al., 2012; Mosig et al., 2014).

Analyses of the fear generalization phase did not reveal any behavioral differences between SP and HC. The imaging data of the fear generalization phase however revealed that the SP group showed lower vmPFC activation to generalization stimuli relative to the conditioned threat stimulus, when compared to the HC group. This group difference in the generalization contrast (CS+ > GS1–5) seemed to be largely driven by the response to the CS+; the HC showed more vmPFC deactivation in response to this stimulus than the SP. The vmPFC has been demonstrated to be involved in fear regulation and safety processing (Sotres-Bayon & Quirk, 2010; Motzkin et al., 2015). Our results may therefore either reflect that the individuals with a SP may have processed the GS as less safe (relative to the CS+), or, alternatively, that they may have had to engage more fear regulation processes during the presentation of the CS+ to reach a similar behavioral output as the HC group. This latter explanation may be more in line with our fear acquisition imaging results which showed altered processing of the CS+ specifically, as well as with the behavioral fear generalization data, which do not show any indications of enhanced fear generalization in SPs compared to HCs. It must be noted that a previous study did provide evidence for enhanced generalization in SP (Dymond et al., 2014), yet this finding was based on symbolic relations between phobia-relevant stimuli.

Our behavioral results did also not show any significant indications of altered extinction learning or extinction retention in the SP-group. This result is in line with a previous study that in addition failed to show altered extinction learning and fear retrieval in specific phobia (Mosig et al., 2014); yet, it contrasts with findings in disorders that are characterized by general anxiety and broad distress (i.e. other anxiety disorders and PTSD) (Duits et al., 2015; Milad et al., 2009; Wicking et al., 2016; Rabinak et al., 2017). More specifically, these latter studies overall report reduced extinction learning and/or extinction recall, based on behavioral and/or psychophysiological outcomes (Duits et al., 2015; Milad et al., 2009; Wicking et al., 2016; Rabinak et al., 2017). Our imaging extinction learning results, however, did reveal some alterations, with SPs showing more vmPFC activation than HCs during later extinction compared to early extinction. This pattern observed in the SP group is consistent with other studies, showing increased vmPFC responses to the CS+ over the extinction learning phase (Phelps et al., 2004), which is thought to be reflective of increased fear inhibition. In HC, vmPFC activation in response to the CS+ reduced from early extinction to the mid-extinction-phase, while a trend for an increase in SP was found. The pattern in the HC may reflect an increased attempt to downregulate fear responses at early extinction, which may not be needed during later stages of extinction learning. Groups did not however differ at the end of the extinction learning phase, neither on a behavioral nor on a neural level, suggesting that specific phobia is not characterized by general alterations in extinction learning. Exposure therapy has been regarded as the clinical proxy of extinction learning (Craske et al., 2014). Our results may therefore indicate that individuals with a specific phobia may not show particular difficulties with extinction-based treatment protocols.

During extinction recall, precuneus activation during CS+ presentation was reduced in the SP group compared to HCs. The precuneus is one of the core regions of the default mode network and involved in awareness and self-related processing, and shows deactivations under conditions of threat (Fullana et al., 2016; Fransson & Marrelec, 2008; Marstaller et al., 2017). More deactivation of this region could therefore reflect that the SPs were less self-focused and engaged more attentional processes (for CS+ vs. vCS-) than HCs during extinction recall. Groups did, however, not differ in self-reported fear and US expectancy during extinction recall, which could speculatively be explained by the fact that these self-report measures do not capture aspects of self-focused or external attention. Previous studies in other anxiety disorders and PTSD have also demonstrated altered neural activations during extinction recall, but mainly in the vmPFC (Marin et al., 2017; Milad et al., 2009; Milad et al., 2007), which is thought to reflect reduced fear inhibition and altered contextual processing. Although the precuneus and vmPFC often in parallel show diminished involvement under conditions of threat (Lange et al., 2017; Harrison et al., 2017), we did not find altered vmPFC functioning during extinction recall, suggesting that specific phobia may not be characterized by reduced fear inhibition processes during extinction retention.

Our study showed some different patterns than usually seen across other anxiety disorders, in which mainly reduced differential fear learning has been reported (Duits et al., 2015). Our results therefore emphasize the importance of disentangling the nature and extent of fear conditionability and extinction learning across anxiety disorders by differentiating between disorders characterized by more focal fears versus broad distress. Alterations in fear acquisition and extinction learning may be disorder-specific and may follow a pattern along the fear-anxiety spectrum, with higher fear discrimination and fear load (or better acquisition retention) in fear-disorders, and reduced fear discrimination and delayed extinction in anxious-misery disorders as a consequence of a general dysfunction in fear inhibition and inhibitory learning (Duits et al., 2015). Results of psychophysiological studies further support the idea that conditioned responses may vary along a fear-anxiety spectrum, by showing enhanced fear-potentiated startle responses in disorders characterized by focal fears and attenuated defensive reactivity in disorders characterized by broad distress or negative affectivity (Lang et al., 2016; McTeague & Lang, 2012).

Our results suggest that Pavlovian fear learning and retention could contribute to the etiology of specific phobia. Yet, results are not as clear as would have been suggested by traditional theories that regard phobias as classically conditioned fears (Watson & Rayner, 1920). Indeed, later theories have emphasized the complexity of the development of phobias, stressing the additional contribution of operant conditioning, observational learning, and the transmission of negative information in the etiology of phobias (Rachman, 1977), as well as the notion that some phobias, such as spider phobia, may be more easily acquired when the feared stimuli are phylogenetically fear-relevant (i.e. preparedness; (Seligman, 1971; Öhman & Mineka, 2001)). Furthermore, in recent years, the influence of individual difference factors and contextual variables in the development of a specific phobia has also received more attention (Mineka & Oehlberg, 2008). Future research could therefore investigate the link between different forms of fear acquisition, contextual variables, and the origins of phobia.

5. Limitations

Limitations of the current study consisted of the absence of psychophysiological outcome measures, such as skin conductance responses of fear-potentiated startle (Lonsdorf et al., 2017), which would provide a more objective measure of fear learning. Secondly, we did not include measures of the aversiveness of the US, which could have affected the speed of fear learning and strength of the fear memory (Lonsdorf et al., 2017). Thirdly, our sample mainly consisted of female participants, which was expected based on female dominance in prevalence of spider phobia (Rakison, 2009), but nevertheless might mean that our results may not generalize to males with a SP. Fourthly, participants were emerging adults. Brain developmental effects in this age group can have possibly prevented the emergence of between-group differences. Lastly, we only used phobia-irrelevant stimuli, in an attempt to examine general deficits in fear and extinction learning processes beyond phobia-relevant processes, and to make the results more generalizable to other fear or anxiety disorders. It would be interesting to examine whether similar results would be obtained if phobia-relevant stimuli are used. In addition and for practical reasons, we only investigated spider phobia, and therefore it is unclear whether these results would extend to other phobia types.

6. Conclusions

In conclusion, our results showed some indications of stronger differential acquisition retention in specific phobia; no other behavioral deficits in fear acquisition, generalization, extinction learning, or extinction recall were observed. In addition, the imaging data revealed some minor alterations in the fear circuitry and safety-related activations in individuals with specific phobia during fear learning an extinction recall phases only. These dysregulations were primarily observed in response to the conditioned threat stimulus, with no clear indications of stimulus generalization. Future research would benefit from investigating these processes along the fear-anxiety continuum to gain more insight into pathophysiological processes across anxiety disorders.

Author contributions

Authors IL, LG, KS, MW, MM, TvA, JvO designed the experiments. IL, SM, JB collected the data. IL analyzed the data and wrote the paper. All authors contributed to the interpretation of the data and have approved the final manuscript.

Conflict of interest

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

Ethical statement

This material has not been published in whole or in part elsewhere; the manuscript is not currently being considered for publication in another journal; all authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

Acknowledgments

This study was funded by a research grant from Stichting De Weijerhorst and by a fellowship from the Hersenstichting granted to M. Wichers (Hersenstichting Nederland: 2012(1)-03). We thank Elizabeth Haris for her assistance in proofreading.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2018.09.008.

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