Avoidance: From threat encounter to action execution
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Chapter 4

Fearing shades of gray: Individual differences in fear responding towards generalization stimuli

A version of this chapter is submitted as:

Fear can generalize to innocuous stimuli that resemble genuine threat cues. Individual differences in such fear generalization have been proposed as a factor in the etiology and/or maintenance of anxiety disorders. In recent years, research has documented generalization of both peripheral (e.g., startle potentiation) and central physiological (e.g., activation of the brain’s limbic system) fear responses, and individual differences therein. Considerably less research effort has been devoted to the generalization of avoidance, the behavioral component of fear. In two experiments, we evaluated how neuroticism, a known vulnerability factor for clinical anxiety, modulates fear responses, including avoidance tendencies, toward generalization stimuli (GS). Participants underwent differential fear conditioning, in which one conditioned stimulus (CS+) was repeatedly paired with an aversive outcome (shock; unconditioned stimulus, US), whereas another was not (CS-). In Experiment 1, we measured US expectancies, CS recognition and conditioned avoidance tendencies toward CSs and perceptually similar GSs. In Experiment 2, we additionally included psychophysiological responses and safety behaviors. Fear generalization was observed across measures in both experiments, with the highest responding to the CS+, the lowest to the CS- and intermediate responding to the GSs. In Experiment 1, we found that neuroticism modulated the recognition accuracy for CSs and GSs. In Experiment 2, we found generalization of avoidance tendencies only in participants low and high in neuroticism, not in a middle group. No other differences related to neuroticism were found. A limitation to the studies is that we had to exclude a number of participants, which reduced the power of the experiments and may have obstructed the emergence of further inter-individual differences in generalization. In conclusion, fear generalization seems to be a robust process, largely unaffected by neuroticism.
Both excessive fear generalization and excessive threat avoidance are increasingly receiving attention as potential vulnerability factors for anxiety disorders. However, little attention has been paid to individual differences in the overgeneralization of avoidance (van Meurs et al., 2014), or the excessive tendency to avoid stimuli resembling danger cues (generalization stimuli, GS), as a pathway to pathological anxiety. In two experiments, we examined the effects of neuroticism (N), a known predisposing factor for clinical anxiety (e.g., D. Watson et al., 2005), on avoidance and other fear responses towards GSs.

Fear tends to generalize from threat stimuli towards stimuli that are conceptually (e.g., Dunsmoor, White, & LaBar, 2011) or perceptually similar (e.g., Lissek et al., 2008). In a prototypical generalization experiment, individuals are exposed to circles of different sizes (see Lissek et al., 2008). During conditioning, one circle (e.g., the smallest) is repeatedly followed by an aversive consequence (e.g., shock; unconditioned stimulus, US) and becomes a conditioned threat stimulus (CS+), eliciting a fear response. Another circle (e.g., the largest; CS-), on the contrary, is never followed by the US. At test, fear responses typically generalize to a different extent to the various circles, in accordance with their position on the continuum between the CS+ and the CS-. Relative to controls, overgeneralization (increased fear responding towards stimuli more dissimilar to the CS+) is observed in individuals with panic disorder (Lissek et al., 2010) and generalized anxiety disorder (GAD, Lissek et al., 2014; but see Tinoco-González et al., 2014), indicating that excessive generalization is a potential marker for (some) anxiety disorders. However, establishing differences between anxiety patients and controls does not allow discerning whether overgeneralization predisposes individuals to pathology or rather is a consequence of pathology (Beckers et al., 2013). Research in people at risk for the development of pathological anxiety, such as those high on neuroticism, can help to establish whether individual differences in generalization are present even before the onset of anxiety pathology. Neuroticism is a trait disposition characterized by a general tendency to react with increased negative emotions to a variety of situations (Clark, Watson, & Mineka, 1994; D. Watson & Clark, 1984) and might therefore be particularly relevant to overgeneralization.

To our knowledge, only a few generalization studies have examined people at risk, with varying results. Torrents-Rodas et al. (2013) found differences between high and low trait-anxious participants on certain self-report measures of fear, but not in fear-potentiated startle (FPS) or skin conductance responses (SCR) to GSs. Another study (Kaczkurkin & Lissek, 2013) found increased generalization only on FPS in participants who scored high on the Threat Estimation scale of the Obsessive Beliefs Questionnaire (Steketee et al., 2005). Using a different paradigm, Haddad, Xu, Raeder, and Lau (2013) found differences in generalization between high and low trait anxious individuals on FPS, but not on SCR. Given the mixed results so far, more research in at-risk individuals seems warranted.

Most studies on fear generalization have disregarded a critical component of fear responding, avoidance. Yet excessive avoidance is a purported risk factor for anxiety pathology (Beckers et al., 2013). A notable exception is a study by Lom-
men et al. (2010), who found that individuals high on neuroticism performed more (button press) avoidance responses to GSs than individuals low on neuroticism (at least under some conditions). In another experiment (van Meurs et al., 2014), participants could guide a symbolic “farmer” to his “garden” through either a short or longer route on a computer screen in the presence of GSs. Results indicated that individuals who were low on distress endurance showed more avoidance (choosing the longer route when GSs were presented) than those high on distress endurance. Taken together, these studies suggest that individual differences might play an important role in moderating the degree of avoidance generalization.

According to dual-process models, overt (avoidance) behavior results from the interplay of automatic action tendencies and controlled decision-making processes guided by two response systems (e.g., Strack & Deutsch, 2004). Automatic avoidance tendencies can be measured in the laboratory through symbolic approach-avoidance reaction time tasks (AAT; De Houwer et al., 2001). In the literature, faster initiation of away than toward responses (avoidance tendencies) has been observed for a variety of negative stimuli (Phaf et al., 2014) and and individuals with anxiety show increased avoidance tendencies towards objects related to their fear (e.g., spiders in spider fear; Klein et al., 2011; Rinck & Becker, 2007). In addition to overgeneralization, such increased avoidance tendencies might contribute to the development of clinically severe anxiety (e.g., Klein et al., 2011; Rinck & Becker, 2007). However, we are not aware of any studies that examined the effect of neuroticism on these avoidance tendencies specifically, despite their purported role in overt avoidance and fear. In addition, a recent study showed that participants also show automatic avoidance tendencies to (previously neutral) fear-conditioned CSs (Krypotos et al., 2014), but it is yet unclear whether they generalize to perceptually similar GSs.

In two experiments, we tested the generalization of fear responding towards perceptually similar GSs following conditioning. No individual differences during conditioning were expected, but we hypothesized that individual differences would emerge in responding to the more ambiguous GSs (Lissek et al., 2006). We measured controlled (US expectancy ratings) as well as more automatic (speeded responding in an approach/avoidance reaction time task, AAT) indices of fear for the CSs and GSs, as it has been argued that individual difference are more likely to occur in less controlled response systems (Beckers et al., 2013). So, we hypothesized that conditioned avoidance tendencies would generalize more widely in individuals high than low on neuroticism. In Experiment 2, we additionally measured FPS and SCR responses to the CSs and GSs, to evaluate whether automatic psychophysiological fear responses would be similarly affected. Finally, in order to examine whether overt behavior mirrors differences in avoidance tendencies and to conceptually replicate the findings of Lommen et al. (2010), we also included a (safety) behavior test in Experiment 2, in which participants could prevent an expected US in the presence of a stimulus by pressing a button.
4.2 Experiment 1

4.2.1 Materials and methods

Participants

Participants were pre-screened for the following exclusion criteria: 1) age under 18; 2) a history of psychiatric disorders, heart problems or epilepsy; 3) use of medication affecting memory, attention or reaction times; 4) current pregnancy; and 5) color blindness (Lommen et al., 2010). Seventy participants took part and received financial compensation (€10) or research credits. One participant was excluded due to technical problems, five participants for having participated in similar experiments before, one participant for not following experimental instructions and three participants for having used illegal substances in the 24 hours preceding participation\(^1\). Two participants terminated the experiment prematurely. We used the criteria from Lommen et al. (2010) to divide the remaining sample (\(N = 58\); 19 male; \(M_{AGE} = 21.91, SD_{AGE} = 2.66\)) into Low (score \(\leq 4, n = 18\)), Moderate (score \(> 4 \text{ and } < 11, n = 23\)) and High (score \(\geq 11, n = 17\)) neuroticism (N) groups, based on their total scores on the neuroticism scale of the Eysenck Personality Questionnaire (EPQ; Eysenck & Eysenck, 1975). For more information about the sample, see the supplementary material (Section A.1.1).

Materials

Two pairs of stimuli at both ends of the black-white continuum were used as CSs with stimulus assignment counterbalanced across participants (Figure 1). Six intermediate gray stimuli served as GSs; for data analysis, stimuli were grouped in classes of two. For more information about the stimuli, see supplementary material (Section A.1.2). Circles were superimposed on white square frames and presented against a black background. White rectangular frames with horizontal or vertical orientation were used for the AAT.

The US, a 2-ms electric stimulus, was administered 7.5 s after CS+ onset to the dorsal side of the wrist of the participant’s non-dominant hand (Eftting and Kindt, 2007). The US was delivered through two Ag electrodes, covered with conductive gel (Signa Gel, Parker Laboratories Inc., Fairfield, NJ) and connected to a DS7A Constant Current Stimulator (Digitimer Ltd., Hertfordshire, UK).

Subjective measures

*Online US expectancies* were measured on an 11-point computerized Likert scale, ranging from -5 (certainly not expecting an electric stimulus) to 5 (certainly expecting an electric stimulus). A cursor was located at 0 (uncertain) at the beginning of each trial. Participants were given 5 s to move the cursor and confirm their response with a mouse click. Otherwise, the cursor’s last position was recorded.

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\(^1\)Technically, some of the substances used by these participants are not in fact illegal in the Netherlands (i.e., marihuana).
Figure 4.1: Conditioned and generalization stimuli
Evaluative ratings of CSs, GSs and the US were collected on an 11-point Likert scale ranging from -5 (unpleasant) to 5 (pleasant). The US was also evaluated on intensity (light, moderate, intense, enormous, unbearable) and startlingness (not, light, moderate, strong, very strong).

During a forced-choice recognition test participants had to report on each trial whether or not a specific stimulus had been presented during conditioning, while electrodes were attached. They did this by pressing a keyboard button (1 = yes, it was presented, 2 = no, it was not presented) upon the presentation of each stimulus. Participants also reported either their retrospective (for the CSs: how much they would have expected an electric stimulus, had they seen the stimulus when electrodes were attached) and hypothetical (for the GSs: how much they would have expected an electric stimulus, had the stimulus been presented when electrodes were attached) US expectancies on the same scale as their online US expectancies.

Questionnaires

Neuroticism and extraversion were measured using the respective subscales (EPQ-N and EPQ-E) of the Eysenck Personality Questionnaire (Dutch translation by Sanderman, Arrindell, Ranchor, Eysenck, & Eysenck, 2012). Negative affects were examined with the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995; Dutch version by de Beurs et al., 2001). Trait worry was assessed with the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990; Dutch translation by van Rijsoort, Emmelkamp, & Vervaeke, 1999). Other than EPQ-N, these questionnaires were included in the experiment for exploratory purposes. More details about the scores of the sample can be found in the supplementary material (Section A.1.1).

Procedure

After reading an information brochure and signing for informed consent, participants were pre-screened and a color blindness test (Ishihara & Ishihara, 1970) was administered. Participants then underwent an electric stimulus work-up procedure where the level of the electric stimulus was increased incrementally to an uncomfortable, but non-painful level (Orr et al., 2000).

At the start of acquisition, participants were instructed that objects presented on the screen would either be followed by a US or not and that their task was to predict the US and report online US expectancies. Participants received 10 CS+ and 10 CS- trials (five of each CS circle) during this acquisition phase. CS pictures were presented centered on the screen, with the US expectancy scale centered underneath. Presentation order was block-randomized so that no more than two consecutive trials of the same type could occur. Each CS trial had an 8-s duration and all CS+ trials ended with US presentation. Trials were separated by an inter-trial interval (ITI) with an average duration of 20 s (15, 20 or 25 s). During ITIs, the US expectancy scale was inactive.

After a three-minute pause, the electric stimulation electrodes were removed afterwards. Participants were then introduced to the AAT.

The AAT consisted of two blocks of 10 practice trials and 40 target trials. On each AAT trial, participants were requested to move a small stick figure that
Chapter 4 appeared centered at the bottom or top half of the screen either toward or away from a stimulus picture presented 1500 ms later on the other half of the screen. Before each practice or target AAT block, participants were instructed to make their response by pressing a key on the keyboard (B marked as ↓ or Y marked as ↑) based on the orientation of the stimulus picture’s frame (horizontal or vertical). Instructions to approach or avoid horizontal or vertical pictures were reversed before the second training block; the starting instructions were counterbalanced across participants. Reaction time (RT) was recorded.

During the practice trials, pictures of every stimulus were presented once, so that one stimulus of each CS or GS class was presented superimposed on a vertical and one superimposed on a horizontal white frame. During the target trials, each stimulus was presented four times, twice in a horizontal and twice in a vertical frame, so that each pictures had to be approached and avoided twice in each block. Trials were separated by 2000 ms ITIs and semi-randomized, so that no more than two consecutive trials of the same type could occur. Incorrect responses were marked by the 500-ms presentation of a red cross at the manikin’s starting position. After correct responses, the manikin remained at its end location for 500 ms. The AAT trial set-up has been described in more detail elsewhere (Krypotos et al., 2014).

The experiment was concluded after participants gave their evaluative ratings, performed the forced-choice recognition test, reported their retrospective or hypothetical US expectancies, and filled in the computerized EPQ, DASS and PSWQ. The University of Amsterdam Ethics Committee approved the experiment.

### 4.2.2 Data analysis

Demographic and US evaluation comparisons between the Neuroticism groups were analyzed with one-way Analyses of Variance (ANOVA). Mixed repeated-measures ANOVA was used for the US-expectancy data (mean US-expectancy ratings across 10 acquisition trials, averaged across the two stimuli in each class) with Stimulus Class (CS+, CS-) as a within-subjects variable and N Group (Low, Moderate, High) as between-subjects variable. When the assumption of sphericity was violated, Greenhouse-Geisser correction was applied.

For the AAT, median RTs (RTmd) were calculated per stimulus class and response (approach and avoid). All practice trials as well as all trials with incorrect responses and trials with RTs exceeding 3000 ms were removed. Mean RTmd were analyzed with a mixed ANOVA, focusing on the Response (Approach, Avoid) × Stimulus Class (CS+, CS- or GS1, GS3) interaction and the Response (Approach, Avoid) × Stimulus Class (CS+, CS- or GS1, GS3) × N Group (Low, Moderate, High) interaction. The GS2 class was omitted from the analyses for lack of a comparison class.

Evaluative ratings and retrospective/hypothetical US expectancies were analyzed in the same way as the online US-expectancy data, but with all five Stimulus Classes as within-subject variables. Alpha levels for post-hoc multiple pairwise comparisons per Stimulus Class were Bonferroni-corrected.

In order to maximize between-group differences, the analyses were repeated including the Low and High Neuroticism groups only. For the latter analysis, only significant effects that did not emerge in the analyses including all three groups...
are reported.

4.2.3 Results

US evaluation

No significant differences between the N groups were observed in actual US intensity or US evaluation (all $ps > .30$). As a whole, participants found the US unpleasant ($M = -3.19, SD = 1.13$), intense ($M = 2.79, SD = 0.49$) and startling ($M = 3.24, SD = .84$).

Acquisition

Acquisition was successful, with higher US expectancies for the CS+ ($M = 3.93, SD = .56$) than the CS- stimulus class ($M = -3.84, SD = .81$), yielding a main effect of Stimulus Class, $F(1, 55) = 2399.96, p < .001, \hat{\eta}^2 = .98$. There was no significant Stimulus Class $\times$ N Group interaction ($F < 1$).

Recognition

Participants indicated on average for 3.24 circles ($SD = 1.48$) that they had seen them during acquisition. No significant difference between the three N groups was observed, $F(2, 55) = 1.67, p = .20$.

Visual examination of the data (Figure 4.2A) suggested that recognition patterns might be predicted from the perceptual distance of the GS to the CSs and N group membership. In order to validate this impression, we fitted a logistic regression model to the data with Circle, N group and their interaction as independent variables and the individual binary response to each circle as a dependent variable. The model was a good fit to the data (see Table 4.1) and showed that the High N group differed significantly in their recognition pattern from both the Low and Moderate N group. So, surprisingly, the high neuroticism group showed better recognition accuracy (higher correct recognition rates for the actual CS+ and CS- stimuli and lower false recognition rates for the GSs most dissimilar to the actual CS+ and CS- stimuli) than the other groups.

Retrospective/Hypothetical US expectancies

Retrospective/hypothetical US expectancies differed as a function of Stimulus Class, $F(3.37, 185.30) = 215.16, p < .001, \hat{\eta}^2 = .80$ (all pairwise comparisons were highly significant, $p < .001$), but the interaction with N group did not reach significance, $F(6.74, 185.30) = 1.30, p = .25, \hat{\eta}^2 = .05$. This suggests that all groups showed similar generalization patterns (Figure 4.2B).
**Table 4.1:** Logistic regression analysis model summary with recognition (yes/no) as outcome and Circle (1-10, 2-9, 3-8, 4-7, 5-6) and N group (Low, Moderate, High) and their interaction (Circle × N group) as predictor variables

<table>
<thead>
<tr>
<th>Included</th>
<th>β(SE)</th>
<th>95% CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Constant</td>
<td>2.32 (0.38)***</td>
<td>5.06</td>
</tr>
<tr>
<td>Circle (1-10/2-9/3-8/4-7/5-6)</td>
<td>0.78 (0.14)***</td>
<td>0.34</td>
</tr>
<tr>
<td>N group (Low/Moderate)</td>
<td>0.37 (0.54)</td>
<td>0.50</td>
</tr>
<tr>
<td>N group (Low/High)</td>
<td>1.72 (0.72)*</td>
<td>1.45</td>
</tr>
<tr>
<td>Circle × N group (Low/Moderate)</td>
<td>0.05 (0.19)</td>
<td>0.72</td>
</tr>
<tr>
<td>Circle × N group (Low/High)</td>
<td>-0.59 (0.25)*</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Note:** $R^2 = 0.22$ (Hosmer-Lemeshow), 0.93 (Cox - Snell), 0.93 (Negelkerke). Model $\chi^2(5) = 157.65$, $p < .001$. *** $p = .001$ * $p = .05$
Figure 4.2: Experiment 1: Percentage of participants who reported to have seen a particular circle during acquisition (A), (retrospective/hypothetical) US expectancies (B) and pleasantness ratings (C), by N group and stimulus.

**Evaluative Ratings**

All stimuli were evaluated differently in terms of pleasantness, $F(2.17, 119.48) = 120.86, p < .001, \eta^2 = .69$, with no significant differences between the N groups (Stimulus Class × Group interaction, $F < 1$). Pairwise comparisons of the stimuli were also significant (all $ps < .01$, Figure 4.2C).

**AAT**

AAT results for the CSs showed that overall, no conditioned avoidance tendencies were present, Response × Stimulus Class interaction, $F < 1$, with no significant interaction with N groups, $F(2, 55) = 1.75, p = .18, \eta^2 = .06^2$ (Figure 4.3A). Planned analyses per N group showed that a trend for significant conditioned avoidance tendencies was present in the High N group only, Response × Stimulus Class interaction, $F(1, 16) = 3.83, p = .07, \eta^2 = .19^3$.

The Response × Stimulus Class interaction was not significant for the GS stimuli either, $F < 1$, with no significant difference between groups, $F < 1$ (Figure 4.3B). Planned analyses showed that the pattern was significant for none of the groups (largest $F = 1.76$ for Low N Group).

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2Two participants had very long mean RTs (more than 2SD away from the sample mean) across AAT trials. When they were excluded from the analyses, the Response × Stimulus Class × N Group interaction showed a trend towards significance, $F(2, 53) = 2.82, p = .07, \eta^2 = .10$.

3When the two participants with very long mean RTs were excluded from the analyses, the pattern for the High N group became significant ($p = .02$).
Figure 4.3: Experiment 1: Mean median RTs in the AAT for CSs (A) and GSs (B), by N group.

<table>
<thead>
<tr>
<th></th>
<th>Low N</th>
<th>Moderate N</th>
<th>High N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CS+</td>
<td>CS−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GS1</td>
<td>GS3</td>
</tr>
</tbody>
</table>

Note: + p < .10

4.2.4 Discussion

Following successful acquisition, clear downward generalization gradients for stimuli on the continuum between the CS+ and CS− were observed in retrospective/hypothetical US expectancies. There was no evidence for an effect of individual differences in neuroticism on this generalization. We did find that high N individuals had more accurate recognition responses than those low or moderate on N. This might suggest that high N individuals are more vigilant during the experiment and more aware of perceptual differences between stimuli.

We were unable to show conditioned avoidance tendencies toward the CS+ as in Krypotos et al. (2014), but we found some indication for such conditioned avoidance tendencies in individuals high in neuroticism. Our AAT included five stimulus classes, while so far such tasks have included only two (Krieglmeyer 82...
Fearing shades of gray

& Deutsch, 2010; Krypotos et al., 2014). This difference in task characteristics might potentially explain our inability to find robust acquisition of conditioned avoidance tendencies or their generalization. Thus, in Experiment 2, we split the AAT task in two tasks that each included only two stimulus classes, to make the procedure more similar to the original cue-irrelevant version of the AAT used in Krypotos et al. (2014).

Additionally, we included physiological measurements (FPS and SCR) and a test phase to evaluate differences between neuroticism groups on other fear measures. This would allow us to examine whether neuroticism affects all fear responses similarly or not (Beckers et al., 2013). We also included a variation on the avoidance task used by Lommen et al. (2010) to measure overt avoidance responses. In this safety behaviors task (SBT), participants could press a response button (safety behavior) to prevent the occurrence of an US whenever they expected one. We expected more overt avoidance responses to GSs more dissimilar from the CS+ in High N individuals relative to Moderate or Low N individuals, in replication of Lommen et al. (2010).

4.3 Experiment 2

4.3.1 Materials and methods

Participants

Participants in Experiment 2 were compensated financially (20 euro) or through research credits. Additional exclusion criteria to those of Experiment 1 were age above 50 and hearing problems. Three participants were excluded due to technical problems and five participants for having used drugs within 24 hours before participation. The remaining 58 participants (19 male, \(M_{AGE} = 21.95, SD_{AGE} = 4.02\)) were divided into Low (n = 15), Moderate (n = 21) and High (n = 22) N groups, as in Experiment 1. For more information about the sample, see the supplementary material (Section A.2.1).

Materials

The same CSs, GSs and US were used as in Experiment 1. The computer screen (ASUS VW222U, 22", 1680 \(\times\) 1050) was calibrated to a linear gamma of 2.2 with a maximum stimulus luminance of 40cd/m\(^2\) (CalMan 5, C3 Colorimeter, Spectracal, Shoreline, WA).

FPS was assessed by measuring the strength of the reflexive eye blink response to an acoustic startle probe with two electrodes (BME-175 6-mm sintered Ag/AgCl, BioMed Products Inc., Fair Oaks, CA) filled with conductive gel (Signa Gel, Parker Laboratories Inc., Fairfield, NJ) and attached to the participant’s left orbicularis oculi muscle and a ground electrode placed below the hairline in the center of the forehead (Blumenthal et al., 2005). The 40-ms startle probe (104 dB) was delivered at the 7\(^{th}\) second after stimulus onset, binaurally, through headphones (Senheiser HD 280 pro 64Ω, Wedemark, Germany).
**Table 4.2:** Schematic representation of the procedure of Experiment 2

<table>
<thead>
<tr>
<th>Habitation 1</th>
<th>Acquisition</th>
<th>AAT</th>
<th>SBT</th>
<th>Habituation 2</th>
<th>Reminder</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 x NA</td>
<td>8 x CS+</td>
<td>CS+/CS-</td>
<td></td>
<td></td>
<td>2 x CS+</td>
<td>4 x CS+</td>
</tr>
<tr>
<td></td>
<td>8 x CS-</td>
<td></td>
<td></td>
<td>10 x NA</td>
<td>2 x CS-</td>
<td>4 x GS1-</td>
</tr>
<tr>
<td></td>
<td>8 x NA</td>
<td>GS1/GS3 circles</td>
<td></td>
<td></td>
<td>2 x NA</td>
<td>4 x GS2-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 x GS3-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 x NA</td>
</tr>
</tbody>
</table>

*Note:* Letters represent types of trials; - represents no US was administered; + represents US was administered.
SCR was assessed by measuring the electrodermal activity on the skin of the index and ring finger of participants’ non-dominant hand with two curved Ag/AgCl electrodes (20 × 16 mm). For more technical details on FPS and SCR, see the supplementary material (Section A.2.2).

Procedure

After reading an information brochure and signing for informed consent, participants directly underwent the electric stimulus work-up procedure (Orr et al., 2000). The electrodes for the physiological measurements were attached thereafter.

A schematic representation of the experimental procedure can be found in Table 4.2. Trial set-up during all phases was the same as in Experiment 1.

Following 10 habituation trials, where the startle probe was presented alone (noise alone trials, NA), participants received 8 CS+, 8 CS- and 8 NA trials during the acquisition stage. NA trials had the duration of the startle probe (40ms), separated by ITIs.

After a three-minute pause, all electrodes were removed and participants were introduced to the AAT. In this experiment, participants received four blocks of four practice trials and 16 target trials. In the first two blocks, pictures of one stimulus from the CS+ class (circle 10 or 1) and one stimulus from the CS- class (circle 1 or 10) were presented centered to the top or bottom half of the screen. In the last two blocks, pictures of one stimulus from the GS1 class (circle 7 or 4) and one from the GS3 class (circle 4 or 7) were used.

Before starting the SBT, electric stimulus electrodes were reattached. Participants were instructed that a written message presented underneath a stimulus picture would indicate the possibility to prevent the US by pressing the space bar on the keyboard and were encouraged to press the button only when they expected a US. All CSs and GSs were presented centered on the computer screen, in random order, and the message appeared underneath all GS and CS- presentations. CS+ presentations were always followed by the US and no message was present. However, participants could still press the button at that time. Stimulus duration was 8 s and ITI was 1 s. Total button presses were recorded for all stimuli.

FPS and SCR electrodes were then reattached and participants were instructed to use the knowledge gained during acquisition to predict the US. Following a second habituation phase, participants were presented six reminder trials (2 CS+, 2 CS-, 2 NA). During acquisition and reminder, trials were semi-randomized as in Experiment 1. The following test phase was semi-randomized into two blocks of 12 trials, so that every half of each block included one CS+, GS1, GS2, GS3, CS- and NA trial. Thus, each circle of every stimulus class was presented twice during the test phase.

At the end of the experiment, participants reported their evaluative ratings, filled in the computerized EPQ, DASS and PSWQ4, and provided demographic information. The University of Amsterdam Ethics Committee approved the experiment.

4Results from questionnaires other than the EPQ-N can be found in the supplementary materials (Section S2.1.)
4.3.2 Data Analysis

Data were analyzed as for Experiment 1. Absolute raw SCR were square-root transformed as in Milad et al. (2006) with the negative sign re-applied where the raw SCR was lower than 0. Raw FPS and SCR were analyzed in the same way as US expectancies in Experiment 1. Mean values per stimulus class across trials were used for the analyses of fear responses on all measures during all phases.

Data from the SBT were analyzed with one-way ANOVAs, using number of GSs and maximum color of GS to which a safety behavior was executed as dependent variables. Since participants were not informed about the possibility to perform safety behaviors during the presentation of the CS+ and some did not perform a SB to any GSs, we set the value for the maximum color for these participants at 2, similar to Lommen et al. (2010). Maximum color values thus ranged from 2 to 10 (when the response key was pressed to the CS-).

4.3.3 Results

US evaluation

Unlike in Experiment 1, there was a trend for a difference in the actual strength of the US ($p = .07$), with the Low N group selecting a stronger electric stimulus than the Moderate N group. However, US ratings did not differ between groups and participants rated the US as unpleasant ($M = -3.33$, $SD = 1.33$), intense ($M = 2.91$, $SD = 0.43$) and startling ($M = 3.52$, $SD = .80$).

Acquisition

Participants reported higher US expectancies for the CS+ ($M = 3.72$, $SD = .78$) than the CS- ($M = -3.54$, $SD = .86$), yielding a main effect of Stimulus Class, $F(1, 55) = 1317.05$, $p < .001$, $\eta^2 = .96$, which indicates that acquisition was successful. The Stimulus Class $\times$ N group interaction did not reach significance, $F(2, 55) = 1.39$, $p = .26$, $\eta^2 = .05$.

Results were similar for physiological measures, with higher responding for the CS+ (FPS, $M = 203.01$, $SD = 97.42$; and SCR, $M = 0.62$, $SD = 0.54$) than the CS- (FPS, $M = 185.35$, $SD = 103.18$; and SCR, $M = 0.35$, $SD = 0.35$). The analysis yielded a main effect of Stimulus Class for FPS, $F(1, 55) = 8.55$, $p = .005$, $\eta^2 = .14$, and SCR, $F(1, 55) = 34.44$, $p < .001$, $\eta^2 = .39$, in the absence of Stimulus Class $\times$ N group interactions (FPS: $F(2, 55) = 1.00$, $p = .37$, $\eta^2 = .04$; SCR: $F(2, 55) = 1.29$, $p = .28$, $\eta^2 = .05$).

Reminder

Conditioning effects remained significant on the reminder trials: main effect of Stimulus Class for US expectancies, $F(1, 55) = 2267.23$, $p < .001$, $\eta^2 = .98$, for FPS, $F(1, 55) = 11.58$, $p = .001$, $\eta^2 = .17$, and for SCR, $F(1, 55) = 34.44$, $p < .001$, $\eta^2 = .39$. This suggests that being exposed to the CSs during the AAT and SBT did not produce extinction. No between-groups differences in fear responding were observed.
**Figure 4.4:** Experiment 2: US expectancies (A), FPS (B) and SCR (C) during the test phase; evaluative ratings for all stimuli (D); number (E) and maximum color (F) of GSs responded to during the SBT test.

<table>
<thead>
<tr>
<th>Stimulus Class</th>
<th>US Expectancy</th>
<th>FPS (mV)</th>
<th>SCR (μS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS+</td>
<td>5</td>
<td>200</td>
<td>0.25</td>
</tr>
<tr>
<td>GS1</td>
<td>0</td>
<td>150</td>
<td>0.00</td>
</tr>
<tr>
<td>GS2</td>
<td>-5</td>
<td>100</td>
<td>0.50</td>
</tr>
<tr>
<td>GS3</td>
<td>-10</td>
<td>50</td>
<td>0.75</td>
</tr>
<tr>
<td>CS-</td>
<td>-15</td>
<td>0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulus Class</th>
<th>Pleasantness</th>
<th>Number GSs</th>
<th>Max Color GSs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS+</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>GS1</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>GS2</td>
<td>-5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>GS3</td>
<td>-10</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>CS-</td>
<td>-15</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N Group</th>
<th>Safety Behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
</tr>
<tr>
<td>High</td>
<td>6</td>
</tr>
</tbody>
</table>

Generalization was observed across all measures, with clear downward gradients from CS+ to CS-. There was a significant main effect of Stimulus Class for US expectancies, $F(1.70, 93.71) = 428.31$, $p < .001$, $\eta^2 = .89$, with all classes being different from each other (all $ps \leq .001$, Figure 4.4A); for FPS, $F(4, 220) = 10.63$, $p < .001$, $\eta^2 = .16$, with CS+ and GS1 significantly different from GS2, GS3 and CS- (Figure 4.4B); and for SCR, $F(3.21, 176.74) = 2.99$, $p = .03$, $\eta^2 = .05$, with only GS1 differing from GS3 (Figure 4.4C).

No significant differences in the effect of Stimulus Class were observed between the three N groups (all $ps > .6$) for FPS and US expectancies, which suggests that generalization was similar across groups. The main effect of N group on SCR responding, however, approached significance, $F(2, 55) = 2.88$, $p = .07$, $\eta^2 = .10$. This finding was driven by heightened responding for the Moderate N group as compared to the High N group (main effect of group in pairwise comparison, $p = .03$). One-way ANOVAs showed significant or trend-like differences in SCR.
between the three groups for GS2 \((p = .02)\), GS3 \((p = .07)\) and CS- \((p = .08)\) only.

**Evaluative Ratings**

Similar to Experiment 1, stimuli were evaluated differently, \(F(2.71, 149.12) = 143.47, p < .001, \eta^2 = .72\) (Figure 4.4D), without effect of N group \((p = .71)\). All pairwise comparisons were highly significant \((all ps \leq .001)\). Low and High N groups differed in their evaluative ratings for the CS+ only, \(F(1, 35) = 4.23, p = .05\.

**Figure 4.5:** Experiment 2: Mean median RTs in AAT for CSs (A) and GSs (B), by N group

**AAT**

No significant difference between the three N groups was observed on the Response × Stimulus interaction for the CSs, \(F < 1\) (Figure 4.5A). When N group was removed from the model, a trend for significance was observed for the Response 88
Fearing shades of gray

$\times$ Stimulus interaction, $F(1, 57) = 2.85, p = .10, \eta^2 = .05^5$, which suggests that the sample as a whole showed conditioned avoidance tendencies.

When data for the GSs were analyzed, the Response $\times$ Stimulus interaction was not significant, $F(1, 55) = 1.13, p = .29, \eta^2 = .02$, and there was no significant difference between the three N groups, $F(2, 55) = 1.89, p = .16, \eta^2 = .06$ (Figure 4.5B). However, when only the Low and High N groups were included, a significant Response $\times$ Stimulus interaction was obtained, $F(1, 35) = 4.87, p = .03, \eta^2 = .12^6$, with no significant interaction with N group. Thus, in Experiment 2, we observed a trend towards conditioned avoidance tendencies towards the CSs. Further, we observed generalization of avoidance tendencies towards GSs, but those seemed to be affected by individual differences.

**SBT**

No significant difference was observed in the number of GSs or in the maximum color of the GS to which individuals from the three groups executed safety behaviors, both $F$s < 1 (Figure 4.4E and 4.4F, respectively). We failed to replicate the finding reported by Lommen et al. (2010) even when Low and High N groups were compared separately.

We performed a similar logistic regression analysis as for the recognition data of Experiment 1 on the SBT data, to examine if the safety behavior pattern differed as a function of stimulus class and N group. We used a binary dependent variable for this analysis, which represented whether or not individuals pressed the button at least once to a stimulus from a given class. We found a significant main effect of Stimulus Class, but not of N group, with no significant interaction between the two (Table 4.3, Figure 4.6).

### 4.4 General discussion

In two experiments, we examined the effect of neuroticism on gradients of perceptual fear generalization. Following successful fear acquisition, clear downward generalization gradients were observed across automatic and controlled measures of fear responding. Very limited evidence was found for an influence of individual differences in neuroticism on those gradients. Differences were neither found in terms of US expectancies or evaluative ratings (both experiments), nor in terms of physiological responding (SCR and FPS) or safety behaviors (Experiment 2). However, in Experiment 1, individuals high on neuroticism showed more accurate recognition for stimuli than other individuals; this was also the only group in which some indication for conditioned avoidance tendencies was found in Experiment 1. In Experiment 2, a trend for conditioned avoidance tendencies toward the CS+ was present across the whole sample, while conditioned avoidance tendencies towards the GSs were observed for some individuals only (i.e., those high and low on N).

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$^5$One participant had very long mean RTs (more than 2SD away from the sample mean) across AAT trials. When he was excluded from the analyses, the interaction showed a trend for significance also when N group was included in the model ($p = .10$).

$^6$This interaction remained significant ($p = .04$) when the one participant with long RTs was removed from the sample.
Table 4.3: Logistic regression analysis model summary with safety behavior (yes/no) as outcome and Stimulus Class (CS+, GS1, GS2, GS3, CS-) and N group (1 - Low, 2 - Moderate, 3 - High) and their interaction (Stimulus Class × N group) as predictor variables

<table>
<thead>
<tr>
<th></th>
<th>β(SE)</th>
<th>95% CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td><strong>Included</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.31 (0.30)</td>
<td>0.76</td>
</tr>
<tr>
<td>Stimulus Class (CS+, GS1, GS2, GS3, CS-)</td>
<td>-0.59 (0.14)***</td>
<td>0.41</td>
</tr>
<tr>
<td>N group (Low/Moderate)</td>
<td>- 0.57 (0.39)</td>
<td>0.26</td>
</tr>
<tr>
<td>N group (Low/High)</td>
<td>- 0.39 (0.38)</td>
<td>0.32</td>
</tr>
<tr>
<td>Stimulus Class × N group (Low/Moderate)</td>
<td>0.18 (0.19)</td>
<td>0.83</td>
</tr>
<tr>
<td>Stimulus Class × N group (Low/High)</td>
<td>0.15 (0.18)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Note: $R^2 = 0.07$ (Hosmer-Lemeshow), 0.58 (Cox - Snell), 0.58 (Negelkerke). Model $\chi^2(5) = 50.03, p < .001$. *** p = .001
In both experiments, downward generalization gradients were observed, with highest fear responding towards the CS+, lowest towards the CS-, and responses to the generalization stimuli in between. Using this experimental paradigm, generalization occurs across both automatic (SCR, FPS) and controlled (US expectancies, evaluative ratings, safety behaviors) response systems. Our failure to find individual differences in generalization contradicts some earlier reports (Kaczkurkin & Lissek, 2013; Lommen et al., 2010), but not others (e.g., Torrents-Rodas et al., 2013). The findings presented here suggest that fear generalization is often a robust phenomenon (Torrents-Rodas et al., 2013) that occurs across measures and across participants.

A notable finding from Experiment 1 is that individuals high in neuroticism seemed to have more accurate recognition memory for the different circles. This resulted in a steeper downward gradient in the recognition task in Experiment 1 for individuals high on neuroticism. This might reflect the ability of high neuroticism individuals to differentiate better between the stimuli and remember the perceptual characteristics of the stimulus material present during the fear-conditioning phase, which might in turn protect from overgeneralization. This recognition task measures a component of emotional memory that might not be covered by other measures that are typically used in fear conditioning research. It is unclear whether this recognition pattern is based on the perceived familiarity of the objects, better recollection or better recall of the stimulus material (Yonelinas & Ritchey, 2015). Future research should examine which of these processes is enhanced for emotional events in individuals high in neuroticism. Importantly, the findings of the recognition task in Experiment 1 show that the generalization gradients observed are not the result of a failure to perceptually discriminate between the different GSs, but rather suggests that they result from a non-perceptual deci-
sion process that derives US expectations from the degree of perceptual similarity between each GS and the CS+ (Wiech, Ploner, & Tracey, 2008).

The current experiments are the first to examine whether conditioned avoidance tendencies generalize along a perceptual dimension. These automatic tendencies represent a level of conditioned responding often overlooked in fear-conditioning research (Beckers et al., 2013). We replicated the finding of conditioned avoidance tendencies towards the CS+ (Krypotos et al., 2014) in Experiment 2 across the whole sample and in Experiment 1, a trend toward conditioned avoidance tendencies was observed for individuals high in neuroticism. The inability to find conditioned avoidance tendencies in Experiment 1 for the whole sample can be attributed to the modifications we made to the task compared to Krypotos et al. (2014). In Experiment 1, we included all ten CSs and GSs, which might have obstructed the emergence of clear avoidance tendencies due to the increased difficulty of the task.

Crucially, in Experiment 2, we observed generalization of avoidance tendencies in some individuals. This is important because it suggests that generalization of avoidance can occur on an automatic level, rather than a controlled level (unlike what has been suggested by Lommen et al., 2010). Contrary to expectations, however, avoidance tendencies towards GSs perceptually similar to the CS+ were not only observed in the group high in neuroticism, but also in the group low in neuroticism. Possibly, rather than neuroticism, another individual difference factor governs this generalization. We calculated an approach-avoidance index for the GSs (congruent trials: approach GS3 and avoid GS1, minus incongruent: avoid GS3 and approach GS1) and exploratorily correlated it with the other personality measures assessed in Experiment 2. However, no significant correlations were observed. Another possibility is that the generalization of avoidance tendencies is not a sufficiently robust phenomenon to allow for the observation of group differences using the sample sizes included here. We had to exclude a number of participants from the data analysis in both experiments (see participants sections), which might have made it more difficult to observe differences between the three neuroticism groups especially in the RT tasks, where high power is needed to detect effects. Differences in sample size and tasks between Lommen et al. (2010) and the present study might also explain our inability to replicate their overt avoidance behavior findings. Our failure to find differences between high and low neuroticism groups on safety behaviors/overt avoidance cannot be attributed to the sample characteristics, given that in the present experiments we used exactly the same criteria to compose the neuroticism groups as Lommen and colleagues.

In conclusion, fear generalization seems to be a robust process. Using a gradient of colors from black and white as generalization stimuli, we obtained generalization gradients of conditioned fear across controlled and automatic measures from subjective, physiological and behavioral response systems, including generalization of avoidance tendencies. Fear generalization seems largely unaffected by neuroticism levels, unlike we anticipated. Despite having included measures of many levels of fear responding, we found little evidence for overgeneralization in individuals at risk for developing clinically severe anxiety. Even though the experiments presented here have limitations (e.g., small sample size), taken together, the results suggest that differences in fear generalization may be a consequence of anxiety pathology rather than a vulnerability factor.