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Excessive generalisation of conditioned fear in trait anxious individuals under ambiguity

Alex H.K. Wong*, Peter F. Lovibond

School of Psychology, University of New South Wales, Sydney, Australia

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ABSTRACT

Trait anxiety has been widely accepted as a vulnerability factor for the development of anxiety disorders. However, few studies have examined how trait anxiety may affect fear generalisation, which is believed to be a core feature of anxiety disorders. Using a single-cue conditioning paradigm, the current study found a range of discrete generalisation gradients in both expectancy ratings and skin conductance, which were highly consistent with participants' reported abstract rules. Trait anxious participants showed the same level of threat expectancy to the conditioned cue as low anxious participants. However they showed over-generalisation to novel test stimuli, but only when they failed to identify a clear rule. This result suggests that over-generalisation of fear may be a special case of the more general principle that trait anxiety is associated with excessive threat appraisal under conditions of ambiguity.

1. Introduction

The etiology of anxiety disorders is thought to involve a range of contributing factors including traumatic experiences, pre-existing vulnerabilities, and excessive threat appraisal (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Mineka & Zinbarg, 2006). One way to examine the mechanisms involved is to conduct laboratory studies with anxious patients or non-clinical participants with a known vulnerability marker such as high trait anxiety (Watson & Clark, 1984). Fear conditioning has served as a well-controlled laboratory task to examine learning about both sources of danger and safety (e.g., Mineka & Zinbarg, 2006; Scheveneels, Boddez, Vervliet, & Hermans, 2016; Vervliet & Raes, 2013). Although traditionally interpreted as an automatic reflexive process, increasing evidence suggests that human conditioning is closely associated with symbolic cognitive processes such as language, reasoning and conscious beliefs (e.g., Mitchell, De Houwer & Lovibond, 2009; Weidemann & Lovibond, 2016). Therefore, fear learning serves as a promising paradigm for understanding the interplay between vulnerability, aversive experiences and cognitive appraisal in generating pathological anxiety.

Studies of fear conditioning in clinically anxious patients and healthy controls have found that anxious patients show higher level of psychophysiological responses to cues that signal an aversive outcome such as electric shock (e.g., Orr et al., 2000), especially when a single-cue conditioning paradigm is used (Lissek et al., 2005; but see; Duits et al., 2015). This suggests that anxious patients have heightened conditionability to stimuli that signal danger, potentially explaining the elevated, maladaptive fear to threat cues. Anxious patients also show increased fear responding to safety cues (e.g. Grillon & Davis, 1997), which may explain excessive fear responses to innocuous cues among anxious patients. Non-associative mechanisms have also been proposed to play a role in maladaptive fear acquisition, such as failure of physiological habituation or enhanced sensitization to cues (see Clemens & Selesnick, 1967; Lissek et al., 2005). Recently, evidence has been found for over-generalisation of fear in anxious patients. After differential training to a threat cue (CS+) and a safety cue (CS−), anxious patients show higher levels of fear responding to all test stimuli intermediate between CS+ and CS−, and also elevated responses to CS− (Lissek et al., 2008; 2010; 2014). These studies not only suggest excessive fear generalisation as a major feature of anxiety disorder, but also suggest elevated fear towards safety cues from over-generalisation of fear from CS+ (Grillon & Morgan III, 1999; Haddad, Pritchett, Lissek, & Lau, 2012; see also; Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015).

Despite this evidence for maladaptive fear learning in patients with current anxiety disorders, it cannot be distinguished whether maladaptive learning is a consequence of anxiety disorders, or whether it is a vulnerability factor for their development. In addition, clinical samples introduce a great deal of comorbidity as well as sequellae of their clinical condition. Hence, it is important to study individuals at risk of developing anxiety disorder, and examine if they show similar maladaptive patterns (Lonsdorf & Merz, 2017). Trait anxiety is a stable

* Corresponding author. School of Psychology, University of New South Wales, UNSW, Sydney, NSW 2052, Australia.
E-mail address: h.k.wong@psy.unsw.edu.au (A.H.K. Wong).

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predisposition to show negative emotional responses across situations, and has been widely proposed as a risk factor for developing anxiety disorders (e.g., Chambers, Power, & Durhama, 2004; Gershuny & Sher, 1998; Jorm et al., 2000). Despite the evidence highlighting over-generalisation of fear in anxiety disorders (Lissek et al., 2008; 2010; 2014), there is scarce evidence regarding fear generalisation in individuals high in trait anxiety, and mixed results have been found. Haddad et al. (2012) examined how trait anxiety affects fear responses to safety cues. Participants were presented with one CS+ and two CSs-, where one CS− was perceptually similar to CS+ (i.e., similar CS−) and the other one not (i.e., dissimilar CS−). A higher level of EMG eyeblink startle to the similar CS− was observed among highly anxious individuals, but not to the dissimilar CS−. The results provided some evidence that anxious individuals show greater fear generalisation from CS+ to a similar CS−. The authors argued that such results could not be explained by a general elevated fear response to safety cues, otherwise an increase in responding should have been observed to both safety cues.

However there have also been other studies that did not find any effect of trait anxiety on fear generalisation. After differential training, Torrents-Rodas et al. (2013) presented stimuli intermediate between both CSs along the stimulus dimension. Although the high anxious group showed higher risk ratings (i.e., shock expectancy ratings) to stimuli most similar to the safety cue compared to the low anxious control group, there were no significant differences in the shape of the generalisation gradients between anxiety groups, and all groups showed similar ratings to stimuli most similar to CS+. Furthermore, no differences were found in the psychophysiological responses to the generalisation cues. The authors therefore concluded not finding evidence that trait anxiety has any effect on fear generalisation. Using a similar paradigm, Arnaudova, Krypotos, Effting, Kindt, and Beckers (2017) also found no trait anxiety effect on fear generalisation.

One common feature of these studies was the use of a differential conditioning paradigm with CS+ and CS− located at the extreme ends of the stimulus dimension. All test stimuli were intermediate between the two CSs, with stimuli closest to CS− being most perceptually similar to CS−, and stimuli becoming more similar to CS+ in a linear fashion towards the direction of CS+ along the stimulus dimension. Two possible factors may explain the null effect of trait anxiety in this paradigm. First, it has been argued that the typical differential fear conditioning paradigm represents a 'strong situation', consisting of clear threat and safety cues (Lissek, Pine, & Grillon, 2006). In this case, most participants would show adaptive fear responses to the cues, making it difficult for any potential individual differences to be observed in fear acquisition (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013). In contrast, maladaptive fear responses may be more likely to occur in a 'weak situation' comprised of a more ambiguous experimental configuration (e.g., blocking, where the casual status of the blocked stimulus becomes ambiguous; Boddez et al., 2012). Secondly, the nature of the paradigm being used may contribute to the null effect for trait anxiety. Torrents-Rodas et al. (2013) argued that the test stimuli between CS+ and CS− had an unknown threat value, leaving them ambiguous. However, since the test stimuli differed from each other in a linear fashion along the dimension, it would be straightforward for participants to infer the threat value of each test stimulus based on their similarity to CS+ or CS−. This could arguably disambiguate the generalisation task and turn the experimental configuration into a 'strong situation', reducing the opportunity to detect any potential individual differences in fear generalisation.

The current study sought to examine if trait anxiety has any effect on fear generalisation, using a single-cue conditioning paradigm. Participants were trained with a single stimulus paired with shock (CS+), and were then tested on a range of stimuli that varied in their similarity to CS+ along a perceptual dimension. The major advantage of this paradigm is the ambiguity it provides compared to differential conditioning, as there is no reference cue and hence less information available to guide generalisation (see Homa, Sterling, & Trepel, 1981). This would create a 'weak situation', especially for novel stimuli that were dissimilar to CS+, thus providing an opportunity to examine the effect of trait anxiety on fear generalisation. The study also took advantage of recent developments in the literature to examine whether any interactions between trait anxiety and explicit reasoning processes may affect fear generalisation.

Previous studies have found that reasoning plays an important part in human fear generalisation (Ahmed & Lovibond, 2015; Boddez, Bennett, van Esch, & Beckers, 2016; Dunsmoor & Murphy, 2015; Vervliet, Kindt, Vansteenwegen, & Hermans, 2010). Furthermore, recent work in our laboratory has highlighted individual differences in inductive reasoning in fear generalisation. In these studies, participants were categorized into different subgroups according to the rules they reported inferring and using in test (Ahmed & Lovibond, 2018; Lee, Hayes, & Lovibond, 2018; Wong & Lovibond, 2017). The results showed a high level of consistency between the shape of generalisation gradients and the inferred rules. More interestingly, the gradients in each rule subgroup (e.g., linear or similarity-based) were distinctive from each other. These results not only suggest that abstract rules affect the shape of generalisation gradients, but also that the overall generalisation gradient in humans can be misleading, as it may comprise a combination of different gradients formed from different rules. Accordingly, in the current study we categorized participants into different subgroups according to the rules they reported in a post-experimental questionnaire. We examined the effect of trait anxiety on both overall generalisation gradients and gradients for individual rule subgroups in order to investigate whether trait anxiety may have different effect on generalisation in different rule subgroups.

2. Method

2.1. Participants

Undergraduate students were recruited as participants who received either course credit or AUD $15 for participation. Participants were prescreened by the DASS-21 (Lovibond & Lovibond, 1995). The DASS-21 is a short version of the original DASS (Depression Anxiety Stress Scales), designed to discriminate between three different constructs: anxiety, depression and tension/stress. Both the DASS and the DASS-21 have been shown to have good psychometric properties (Antony, Bieling, Cox, Enns, & Swinson, 1998; Brown, Chorpita, Korotitsch, & Barlow, 1997; Henry & Crawford, 2005; Lovibond, 1998). Participants with a DASS anxiety score of 4 or below were recruited to the low anxious (LA) control group, while those with a DASS anxiety score of 18 or above were assigned to the high anxious (HA) group. We followed the sample size in Torrents-Rodas et al.'s (2013) study, which was approximately 40 participants in each group. The recruitment strategy was to continue recruiting until there were 40 participants in each group who met inclusion criteria (e.g., acquisition of CS−US contingency; see Results for more detail). This led to a total recruitment of 113 participants, with 33 excluded. The final sample comprised 80 participants (43 females) with a mean age of 21.1 years (SD = 3.8).

2.2. Apparatus and materials

Participants were tested individually in an experimental room. A 64-cm computer monitor was used to present the experimental instructions and stimuli. A computer equipped with MatLab software (with Psychophysics Toolbox extensions; Brainard, 1997; The MathWorks Inc., 2014) was located outside the experimental room, which generated the stimuli presented to the participants and recorded the expectancy ratings, while another computer controlled AD instruments equipment to record the skin conductance data via GRASS® silver disc electrodes at a sampling rate of 1000/s throughout the experiment.

A symmetrical stimulus dimension was used to minimize any intensity biases (see Wong & Lovibond, 2017; Ahmed & Lovibond,

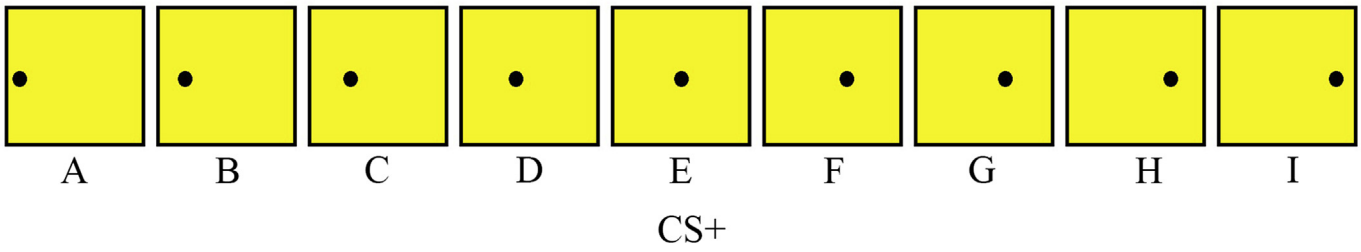

Fig. 1. Stimulus dimension; stimulus E served as the CS+. The stimulus labels (A–I) were not presented to participants.

submitted). The stimuli were yellow squares [5.5 × 5.5 cm] with a black outline containing a black dot varying horizontally from left to right (Fig. 1). Nine individual stimuli labelled A (the left-most stimulus) to I (the right-most stimulus) were defined by manipulating the dot location by 0.5 cm from one stimulus to the next. Stimulus E, with the dot in the center, served as the CS+. A red lightning bolt served as the symbolic shock. All stimuli and the symbolic shock were presented in the center of a white background on the computer screen.

The physical shock was a 0.5-s sinusoidal pulse electric shock (80 Hz) delivered through two stainless steel electrodes attached to the distal and middle segments of the index finger of participants' non-dominant hand. Skin conductance electrodes were attached to the distal and proximal segments of the third finger of the same hand. A semi-circular dial with a rotary pointer was attached to the table in front of the participants. The dial was labelled *Expectancy of SHOCK after figure*, with the left position labelled *Certain NO SHOCK* and the right position labelled *Certain SHOCK*.

2.3. Procedure

After signing the consent form, participants were asked to fill in the DASS-21. Shock electrodes and skin conductance electrodes were then attached to participants' fingers, and they were led through a work-up procedure in which they selected a level of shock that was 'definitely uncomfortable but not painful' (Ahmed & Lovibond, 2015; Vervliet et al., 2010). Participants were then taken into the experimental room. As shown in Table 1, the study consisted of an acquisition phase and a test phase, with each phase divided into two stages, similar to previous fear conditioning studies in our lab (Lee et al., 2018; Wong & Lovibond, 2017).

The acquisition phase consisted of two stages, Acquisition1 and Acquisition2. The shock electrodes were disconnected in the former and reconnected in the latter. The reason for administering electric shock only in Acquisition2 was to minimize habituation to the shock. The purpose of Acquisition1 was to increase the number of training trials, in order to facilitate learning of the CS-shock association.

Acquisition1 (shock electrodes disconnected). This stage consisted of 8 trials of CS+, reinforced at 75% (6 of the 8 trials were followed by the symbolic shock). CS+ was not fully reinforced to avoid a ceiling effect and hence allow room to examine if HA individuals would show elevated fear responding to CS+. It also allowed room for increased responding to stimuli beyond CS+ to be observed, for example a rule-based linear gradient (Wong & Lovibond, 2017; Ahmed & Lovibond, submitted). The presentation order was pseudo-randomized, so that non-reinforced CS+ trials did not occur twice in a row, and the first and last trials were always reinforced. Participants were informed that figures would be presented on the computer screen, which may or may not be followed by a shock; they were asked to learn the relationship between the figures and the shock. Participants were then instructed to use the dial to indicate their expectancy of shock whenever a figure appeared on the screen. They were also instructed not to focus on the order of stimulus presentation. This was to minimize superstitious learning arising from the partial reinforcement schedule. Participants were then informed that due to ethical restrictions, the number of shocks was limited, hence setting up the cover story for disconnecting the shock electrodes. They were told that when the shock electrodes were disconnected, only the symbolic shock would appear on the screen. The trial structure was made up by a 10-s baseline period, a 10-s stimulus presentation, followed by a 2-s period where feedback (symbolic shock) was either presented or not presented. The inter-trial interval (ITI) varied between 10 and 21s.

Table 1
Design of current study.

Phase	Acquisition1	Acquisition2	Test1	Test2
	CS+ (6)/CS* (2)	CS+ (3)/CS* (1)	CS* (1) TS- (8)	CS* (1) TS- (3)

Note. + indicates shock presentation; - indicates shock omission; * indicates non-reinforced CS+; TS refers to test stimuli; numbers in brackets indicate the number of trials of that type in each phase.

Acquisition2 (shock electrodes connected). After Acquisition1, the experimenter went into the experimental room to reconnect the shock electrodes. Participants were informed that they would now be receiving the real shock along with the symbolic shock. Acquisition2 consisted of 4 CS+ trials, which were identical to Acquisition1 in terms of trial structure, except that the electric shock was delivered in the last 0.5 s of the symbolic shock presentation. The 75% partial reinforcement schedule was maintained in Acquisition2 (3 of the 4 trials were shocked). The presentation order was again pseudo-randomized, so that the first and last trial were always reinforced.

Similar to acquisition, the test phase was divided into two stages, Test1 and Test2, with the shock electrodes disconnected in the former and reconnected in the latter (see Table 1).

Test1 (shock electrodes disconnected). Participants were informed that for ethical reasons, the shock electrodes would be disconnected again. They were also told that neither symbolic nor physical shock would be delivered, but they were asked to continue making their expectancy ratings, assuming hypothetically that it was still possible for them to receive a shock. This is conceptually equivalent to the 'missing data procedure', which is used to minimize the impact of extinction during testing in causal judgment and prediction tasks (e.g., Shanks & Darby, 1998). This procedure also avoids the confusion that participants may experience when stimuli they expect to be followed by shock are presented alone, potentially prompting them to modify their response strategy (see also Wong & Lovibond, 2017). In this stage, all 9 stimuli along the dimension were presented in a randomized order. In other words, CS+ and 8 test stimuli of varying degrees of similarity were presented in this phase.

Test2 (shock electrodes reconnected). The experimenter reconnected the shock electrodes and participants were told that it was again possible to receive physical shock (but not the symbolic shock) in this stage, so that skin conductance data for the test stimuli could be collected. In fact, no electric shocks were presented. In addition to CS+, only 3 selected test stimuli (C, G and I) were presented in Test2, in a randomized order, in order to minimize extinction. The right-most stimulus was included to maximize sensitivity to positively sloped linear gradients, since our previous research had shown that all participants

who reported a linear rule expected shock to follow stimuli to the right of CS+ (Wong & Lovibond, 2017).

When the conditioning task was completed, participants were asked to fill in a 2-page questionnaire. On the first page, the experimenter wrote down the expectancy ratings that the individual participant had made to the stimuli at opposite ends of the dimension (i.e., Stimuli A & I) during Test1. Participants were asked to explain why they made these ratings, and to write down in detail any rules/strategies of responding they used. The second page was administered only after the first page was completed, and consisted of 5 statements. Each statement described the relationship between stimuli and shock in terms of different rules (similarity, linear left, linear right, no rule and other). Participants were asked to indicate how much they considered the statement to be true on a 0 to 100 scale, with 0 being false and 100 being true. Participants were told that if none of the statements described their rule-based responding, they should write down their own description in the 'Other' section (see Appendix A for a copy of the questions).

2.4. Scoring and analysis

Although expectancy ratings were recorded in both Test1 and Test2, only those in Test1 were used for data analyses as they covered the whole stimulus dimension. Using a similar paradigm, our previous research has found that expectancy ratings made in Test2 were highly similar to those in Test1, supporting the validity of expectancy data in Test1 (see Wong & Lovibond, 2017).[1] For the skin conductance measure, analysis was based on the data collected when the shock electrodes were attached (Acquisition2 and Test2), since this was when physical shock could be delivered and anticipatory anxiety was expected to occur. A low-pass digital filter was applied to cut off any skin conductance activity higher than 50 Hz, in order to avoid aliasing. The raw skin conductance data were then log transformed to minimize individual differences. Skin conductance scores for each trial were calculated as the difference between the mean of log skin conductance level (SCL) during stimulus presentation and mean log SCL during the baseline period for that trial.

Planned contrasts were used to compare groups and to assess acquisition and generalisation gradients. For the acquisition data, learning of the CS-shock contingency across trials was analyzed with a linear trend repeated measures contrast. For the generalisation test data, a linear contrast was used to capture any linear gradients across the stimulus dimension, while a quadratic contrast was used to capture peaked, unimodal gradients. Group contrasts were used to compare HA with LA participants, and to compare pairs of rule subgroups identified in the post-experimental questionnaire. Finally, all interactions between the group and repeated measures contrasts were tested to evaluate group differences in trends.

3. Results

Statistical analyses were applied to participants who satisfied the acquisition criterion, that is, expectancy ratings to CS+ needed to be above 50 averaged over all 4 trials in Acquisition2. Eleven and eight participants in the HA and LA group were excluded respectively based on the acquisition criterion, suggesting no substantial group differences in fear learning. Participants who did not provide shock expectancy ratings for two or more stimuli during test were also excluded (2 in the HA group and 3 in the LA group). Furthermore, participants who reported responding based on the presentation order of stimuli in the post-experimental questionnaire and those who misinterpreted the instructions, were also excluded (4 in HA group and 5 in LA group). Altogether, 17 and 16 participants in the HA and LA group were excluded respectively, leaving 40 participants in each group.

3.1. Anxiety groups

The mean DASS anxiety scores were 20.0 and 2.2 for the HA and LA groups respectively. The mean shock intensities for both groups were 2.3 mA, indicating no group difference in the tolerance of electric shock, $F(1,78) = 0.004$, $p = 0.95$, n.s.

3.2. Acquisition

Fig. 2A shows the mean shock expectancy ratings during the acquisition phase for the HA and LA groups. Both HA and LA groups showed a steady increase in expectancy ratings to CS+, confirmed by a significant main effect of linear trend across groups, $F(1,78) = 101.6$, $p < 0.01$, $\eta_p^2 = 0.57$. There was no overall difference between groups, $F(1,78) = 0.03$, $p = 0.86$, n.s., and nor was there an interaction between linear trend and groups, $F(1,78) = 0.7$, $p = 0.41$ n.s., suggesting that there were no differences in acquisition between the anxiety groups.

Fig. 2B shows the mean change in log SCL during the last 4 acquisition trials (Acquisition2) in the HA and LA groups. Skin conductance responding to CS+ decreased over trials in both groups, resulting in a significant linear trend averaged across groups, $F(1,78) = 17.0$, $p < 0.01$, $\eta_p^2 = 0.17$. The skin conductance data did not directly align with the expectancy data, as the level of responding to CS+ decreased across trials for both groups. This pattern is consistent with previous studies in our lab (Lee et al., 2018; Wong & Lovibond, 2017), and may be due to several possible factors. First, by the beginning of Acquisition2, participants had already had the opportunity to learn the association between CS+ and shock, so there was limited scope for additional learning. In other words, the increase in SCL may have reached ceiling by the time of the first reinforced trial. Second, habituation of skin conductance to the CS and US could be responsible for the decrease in responding across trials. Finally, participants may have become anxious when they were told they were about to receive the first shock but quickly adapted to the shock, resulting in a heightened SCL on the first reinforced trial, and then a decrease in SCL across acquisition trials. Similar to the expectancy data, neither the main effect for group nor the interaction between linear trend and group were significant (highest $F = 0.03$, $p = 0.86$), suggesting that there was no difference in fear acquisition between groups as measured by skin conductance.

3.3. Test phase

Fig. 3A depicts the mean generalisation gradients for the shock expectancy ratings in the HA and LA groups. Both groups showed a peaked gradient with the highest ratings to CS+ and lower ratings to the test stimuli that were more dissimilar to CS+. This gradient shape resulted in a main effect of quadratic trend across the stimulus dimension, $F(1,78) = 49.0$, $p < 0.01$, $\eta_p^2 = 0.39$. The gradient in the HA group was relatively flatter than the one in the LA group, with greater generalisation to the test stimuli. This pattern was supported by two statistical effects. First, HA participants had higher ratings averaged across stimuli, as shown by a significant main effect for the contrast comparing the two groups, $F(1,78) = 11.0$, $p < 0.01$, $\eta_p^2 = 0.12$. Second, quadratic trend was stronger in the LA participants, leading to a significant interaction between quadratic trend and groups, $F(1,78) = 8.3$, $p < 0.01$, $\eta_p^2 = 0.10$. A significant main effect of linear trend across group was also observed, $F(1,78) = 23.8$, $p < 0.01$, $\eta_p^2 = 0.23$, presumably due to the slightly higher responding to stimuli right of CS+. However, the interaction between linear trend and group was non-significant, $F = 0.4$, $p = 0.53$ n.s., suggesting no group differences in the linear component of the generalisation gradients.

Interestingly, the HA and LA groups gave very similar shock expectancy ratings to CS+, which were both very close to the actual

[1] Similar analyses to Wong and Lovibond (2017) showed no substantial differences in expectancy ratings between the two test phases.

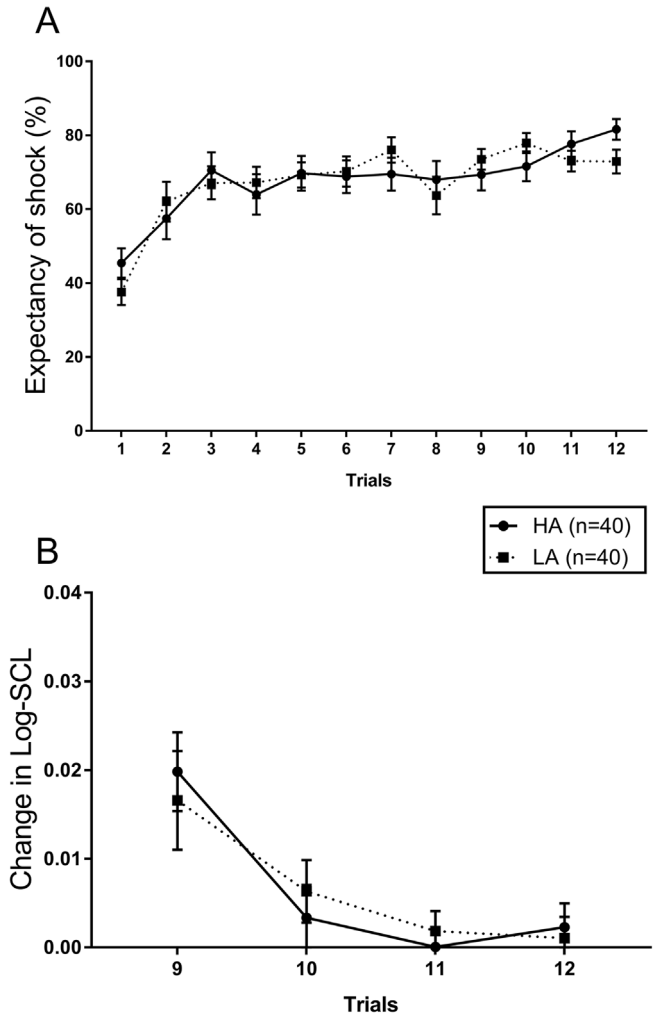

Fig. 2. Mean shock expectancy ratings (Top panel) and skin conductance level (SCL; Bottom panel) across acquisition trials. HA = High Anxious; LA = Low Anxious. The skin conductance data were collected during Acquisition2, when the shock electrodes were connected.

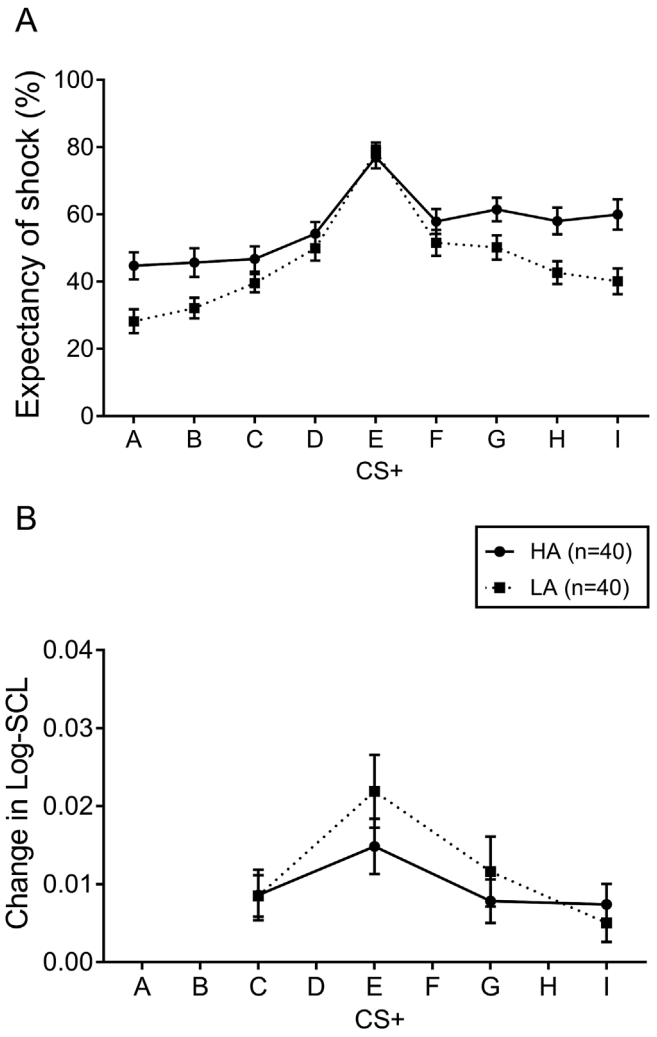

Fig. 3. Mean overall shock expectancy ratings (Top panel) and skin conductance level (Bottom panel) in the test phases. HA = High Anxious group; LA = Low Anxious group. The skin conductance data were collected during Test2, when the shock electrodes were connected.

reinforcement rate of 75%. To further explore the degree of generalisation in the two groups, an additional contrast was tested to directly compare CS+ to the test stimuli. Averaged across groups, shock expectancy to CS+ was significantly higher than to the average of the remaining stimuli, $F(1,78) = 119.4$, $p < 0.01$, $\eta_p^2 = 0.61$, confirming generalisation decrement to the test stimuli. This comparison also interacted with groups, $F(1,78) = 5.9$, $p = 0.02$, $\eta_p^2 = 0.07$, directly demonstrating greater generalisation decrement in the LA group – in other words, greater generalisation to the test stimuli in the HA group.

Fig. 3B shows the overall generalisation gradients for the skin conductance data in the HA and LA groups. The SCL gradients were broadly consistent with the expectancy data, showing a peaked gradient with the peak responding at CS+, supported by a significant main effect of quadratic trend across stimuli, $F(1,78) = 10.3$, $p < 0.01$, $\eta_p^2 = 0.13$. A significant main effect of linear trend across groups was also observed, $F(1,78) = 5.2$, $p = 0.03$, $\eta_p^2 = 0.06$, presumably due to the drop off in responding from CS+ to stimulus I. However, no interactions were found to be significant (highest F = 2.6, $p = 0.11$), suggesting there were no reliable differences in the SCL gradients between groups. The contrast comparing CS+ with the test stimuli was significant, $F(1,78) = 13.5$, $p < 0.01$, $\eta_p^2 = 0.14$, confirming generalisation decrement to the test stimuli. Although this decrement appeared to be greater in the LA participants, the interaction with group was not significant for the skin conductance measure, $F(1,78) = 1.4$, $p = 0.24$, n.s.

3.4. Post-experimental questionnaire

Since our previous work had shown that the rules inferred by participants strongly influence their generalisation gradients (Ahmed & Lovibond, 2018; Wong & Lovibond, 2017), we analyzed the questionnaire data to categorize participants into subgroups according to the rules they reported. Two raters, who were blind to the expectancy and skin conductance data, categorized participants into different subgroups based on their questionnaire responses. This was done by first classifying participants' self-reported rules from the open-ended question on the first page of the questionnaire. If the reported rule was ambiguous, that participant was categorized according to the rule they endorsed most strongly in the second forced choice section of the questionnaire. A high level of consensus was observed between the two raters using Cohen's Kappa (k = 0.82, $p < 0.01$). Discrepancies between raters' categorizations were resolved via discussion.

In the HA group, 10 participants stated that they adopted a similarity rule, whereby they expected that stimuli perceptually similar to CS+ would be more likely to predict shock, whereas those perceptually dissimilar to CS+ would be less likely to predict shock (Similarity subgroup). Eight participants reported inferring a linear rule that if the

Table 2
Examples of actual responses in questionnaires.

Rule subgroups	Examples
Similarity	'I expected the shock most when the black dot was in the middle. So I followed a strategy were the further away the black dot was from the middle, the less likely I would be shocked.'
Linear	'I just had the idea that maybe when the dots were towards the left, there was going to be no shock and when it was towards the right there was a high possibility of shock.'
No rule	'Honestly I could not deduce any pattern or correlation between shocks and the position of the dot in the square. Hence I played safe and just went with 50/50 on all displays.'

Table 3
Number of participants in each rule subgroup.

Rule subgroup/Anxiety group	Similarity	Linear	No rule
HA	10	8	22
LA	17	4	19

dot was more to the right, the more likely it would predict shock (Linear subgroup). The remaining 22 participants reported not identifying a clear rule (No rule subgroup). In the LA group, 17 participants were categorized into the Similarity subgroup, 4 participants reported a linear rule and the remaining 19 participants reported not identifying a clear rule. Please see Table 2 for examples of the actual responses in the questionnaires and Table 3 for an overview of the number of participants in each rule subgroup. Across the linear rule subgroups, no

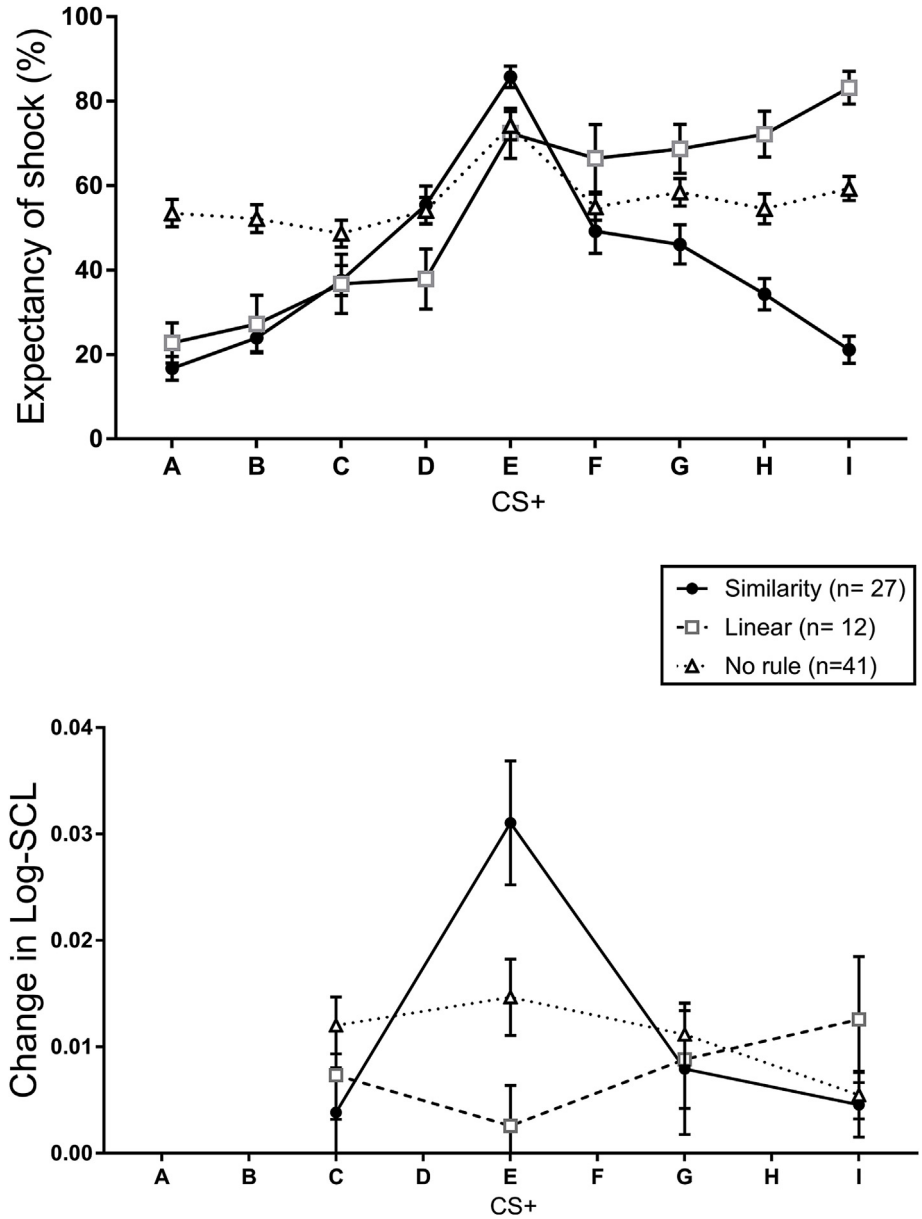

Fig. 4. Mean shock expectancy ratings (Top panel) and skin conductance level (Bottom panel) during test phases for each rule subgroup, collapsed across anxiety groups.

participant reported the alternative left-based linear rule, that is, the more the dot was to the left, the more likely it would predict shock. The number of participants in the HA group who came up with rules did not substantially differ from those in the LA group (χ^2 [1] = 0.45, p = 0.50, n.s.). Statistical analysis was first conducted to characterize the generalisation gradients in each rule subgroup, collapsed over anxiety (see Fig. 4). Subgroups were then compared across HA and LA participants to examine whether group differences in fear generalisation differed between subgroups (Fig. 5).

3.5. Interactions between rule subgroups

The expectancy gradient in the Linear subgroup showed a stronger positive linear trend (higher responding to stimuli on the right) than in the Similarity subgroup, leading to a significant interaction between linear trend and the Linear vs Similarity comparison, $F(1,37) = 58.7$, $p < 0.01$, $\eta_p^2 = 0.53$. Conversely, the expectancy gradient in the Similarity subgroup was more peaked than that in the Linear subgroup, leading to an interaction with quadratic trend, $F(1,37) = 58.7$, $p < 0.01$, $\eta_p^2 = 0.61$. A similar interaction was also observed in the skin conductance data, with the Similarity subgroup having a more peaked gradient than the Linear subgroup, $F(1,37) = 8.3$, $p < 0.01$, $\eta_p^2 = 0.18$. However the interaction with linear trend did not quite reach significance, $F(1,37) = 3.9$, $p = 0.056$, n.s.

The expectancy gradient in the Similarity subgroup was more peaked than that in the No rule subgroup, $F(1,66) = 89.3$, $p < 0.01$, $\eta_p^2 = 0.58$. A similar interaction was observed in the skin conductance data, $F(1,66) = 6.1$, $p < 0.02$, $\eta_p^2 = 0.08$. No other interaction effect was observed for these two subgroups (highest F = 0.1, $p = 0.75$).

The Linear subgroup had a stronger positive linear expectancy gradient than the No rule subgroup, confirmed by a significant interaction between linear trend and the Linear vs No rule comparison, $F(1,51) = 53.0$, $p < 0.01$, $\eta_p^2 = 0.51$. This interaction was also observed in the skin conductance data, $F(1,51) = 5.7$, $p = 0.02$, $\eta_p^2 = 0.11$. No other interactions were significant (highest F = 2.8, $p = 0.10$).

3.6. Comparison between high and low trait anxiety groups

The initial group comparisons indicated that there was broader generalisation in HA participants compared to LA participants, supported by significant interactions in the case of the expectancy measure. The rule subgroup analyses in turn suggested that generalisation was heavily modulated by the rules participants induced. Accordingly, we conducted further analysis of the HA and LA groups broken down by rules, in order to explore whether the overall group differences were modulated by differences in rule induction.

3.6.1. Rule subgroups

Fig. 5 shows the comparison of generalisation gradients between HA and LA participants within each rule subgroup. Fig. 5 A–D show the gradients for both measures in the Similarity and Linear rule subgroups. For these two rule subgroups, there were no significant interactions involving either the contrast comparing HA with LA or any of the stimulus contrasts (highest F = 3.1, $p = 0.087$). That is, there was no evidence to suggest trait anxiety had any effect on fear generalisation in the two rule subgroups (Linear and Similarity). In fact, HA and LA participants showed very similar gradients on both measures. These analyses suggest that trait anxiety had little effect on the degree of fear generalisation when participants came up with an abstract rule, regardless of whether it was similarity-based or linear.

3.6.2. No rule subgroups

Fig. 5E and F shows the expectancy and skin conductance gradients respectively for HA and LA participants in the No rule subgroup. The HA-LA differences here are similar to those seen in the analysis based on all participants, but more pronounced. For the expectancy data, the HA participants gave higher overall ratings than the LA participants, resulting in a main effect for anxiety group, $F(1,39) = 14.2$, $p < 0.01$, $\eta_p^2 = 0.27$. However, as in the full group data, the difference was isolated to the test stimuli as the HA and LA groups gave very similar shock expectancy ratings to CS+. This pattern is supported by a significant interaction between quadratic trend and anxiety group, $F(1,39) = 4.4$, $p = 0.04$, $\eta_p^2 = 0.44$. A significant interaction was also observed between anxiety groups and the comparison between CS+ and the test stimuli, $F(1,39) = 6.0$, $p = 0.02$, $\eta_p^2 = 0.13$, further confirming a specific pattern of greater generalisation in the HA group rather than an overall elevation in ratings to all stimuli including CS+. No other effects reached significance on the expectancy measure (highest F = 0.04, $p = 0.84$). Although the skin conductance measure showed a somewhat similar pattern to expectancy, no interactions involving anxiety group reached significance (highest F = 0.8, $p = 0.38$).

The above analyses indicate that the trait anxiety effect was only found in the No rule subgroups, not in the two subgroups who reported specific rules (Similarity and Linear). To verify this effect, analyses were carried out comparing the generalisation gradients as a function of anxiety groups and rule subgroups. For the expectancy data, a significant interaction was found between the anxiety groups and the contrast comparing the Similarity and No rule subgroups, averaged across the 9 test stimuli, $F(1,64) = 4.2$, $p = 0.04$, $\eta_p^2 = 0.06$, suggesting that the trait anxiety difference was greater in the No rule subgroup than in the Similarity subgroup. Although the interaction between the anxiety groups and the contrast comparing the Linear and No rule subgroups did not quite reach significance, $F(1,49) = 3.2$, $p = 0.08$, n.s., there was a significant 3-way interaction with the contrast comparing CS+ with the other 8 test stimuli, $F(1,49) = 4.9$, $p = 0.03$, $\eta_p^2 = 0.09$. This suggested that the HA and LA groups showed similar ratings to the CS+, but the HA group showed a higher degree of fear generalisation (higher ratings to all other test stimuli) in the No rule subgroup but not in the Linear subgroup. No significant interactions were observed in the skin conductance data (highest F = 1.1, $p = 0.30$).

4. Discussion

The current study aimed to investigate whether trait anxiety has any effect on fear generalisation, by using a single-cue conditioning paradigm. Looking first at the results collapsed across anxiety groups, we found that both expectancy and skin conductance showed a relatively flat but nonetheless peaked gradient, with the highest responding to CS+. However, qualitatively different generalisation patterns were observed when participants were categorized into subgroups according to their reported inferred rules. The Similarity subgroups showed a strongly peaked expectancy gradient, with the highest responding at CS+, and a decline in responding to stimuli more perceptually dissimilar to CS+ along the dimension. The expectancy gradient in the Linear subgroups revealed a gradual increase in responding from stimulus A, resulting in the highest responding at stimulus I. The No rule subgroups showed a flat expectancy gradient, except for a local peak at CS+. The SCL data in each subgroup were broadly similar to their corresponding expectancy gradients, except for the Linear subgroups, which showed a drop in responding to CS+. For both measures, the overall generalisation gradient could be understood as the composite of qualitatively distinct gradients shown by each rule subgroup.

The results replicated the distinctive generalisation patterns in subgroups of participants previously observed in our lab. The expectancy gradients were highly consistent with the reported rules, aligning with our previous work (Ahmed & Lovibond, 2018; Wong & Lovibond, 2017). Furthermore, the skin conductance data broadly aligned with the corresponding rules and expectancy data, consistent with the view that conditioned responses are the product of propositional knowledge of the CS-outcome contingency (De Houwer &

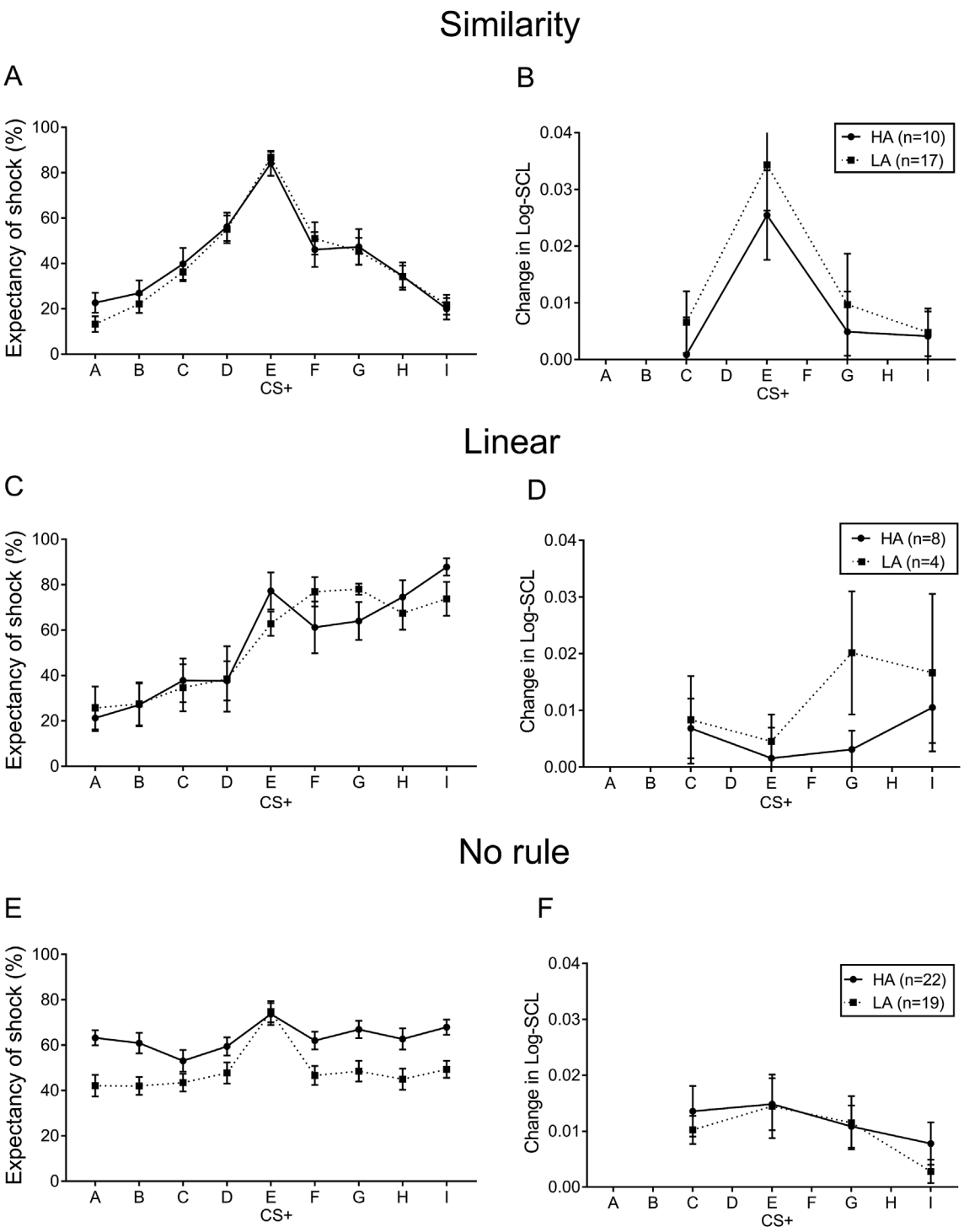

Fig. 5. Comparison of the High Anxious (HA) and Low Anxious (LA) participants within each rule subgroup for expectancy (Left panel) and the skin conductance (Right panel). Top row: Similarity subgroup; Middle row: Linear subgroup; Bottom row: No rule subgroup.

Beckers, 2002; Mitchell, DeHouwer, & Lovibond, 2009). The effect of trait anxiety on fear generalisation was observed in the expectancy ratings, but not in the skin conductance measures. One possible reason for this is the large variability that is characteristic of skin conductance data. In the following discussion, we will focus on the expectancy measures in our analysis of trait anxiety effects.

We found that both anxiety groups came up with similar generalisation rules. Therefore, to examine the effect of trait anxiety on fear generalisation, we made comparisons between HA and LA participants within rule subgroups, to avoid any confounding effect from rule formation. For those who endorsed a clear rule, either similarity or linear, there were no trait anxiety effects in the shape of generalisation gradient. Furthermore, HA participants did not show over-generalisation of fear, as they gave similar overall shock expectancy ratings and skin conductance responses to LA participants. However, by contrast to the rule subgroups, HA participants who reported not identifying a clear rule (No rule subgroup) showed higher expectancy ratings to all the test stimuli except CS+, suggesting that they were over-generalising their fear from CS+ to the test stimuli. In fact, the difference observed in the full group data was driven almost entirely by

the anxiety difference in the No rule subgroups.

Thus, trait anxiety had no effect on fear generalisation when a clear rule was identified, but induced excessive generalisation of fear when no rule was inferred. This finding is consistent with past research by our group and others, showing that high anxious individuals display expectancy bias to ambiguous threat. Using a conditioned inhibition paradigm, Chan and Lovibond (1996) found that high anxious individuals showed higher expectancy ratings to both danger and safety cues than low anxious individuals, but this effect was only observed among those unaware of the CS-shock contingency. Since the unaware participants did not know which cue predicted electric shock, all cues in the task effectively became ambiguous. We have recently observed a similar effect of ambiguous threat in individuals with high intolerance of uncertainty (Chen & Lovibond, 2016). Similarly, using a blocking paradigm, Boddez et al. (2012) found a positive correlation between trait anxiety and shock expectancy to the blocked stimulus. In other words, anxious individuals showed elevated threat appraisal to the blocked stimulus. Since the blocked stimulus had never been presented by itself, its predictiveness of shock was unknown, arguably rendering it ambiguous. In the current study, not being able to infer a clear rule during test can also be seen as making the task ambiguous, because participants had no basis to infer the threat value of the test stimuli. Conversely, identifying a clear rule may create a temporary schema to anchor threat assessment, allowing participants to accurately judge the threat value of the various stimuli.

The current results also provide a potential explanation for previous studies (Arnaudova et al., 2017; Torrents-Rodas et al., 2013) that did not find any trait anxiety effect in fear generalisation. These studies used a differential conditioning paradigm with the CS+ and CS− located at the extreme ends of the stimulus dimension, with all the test stimuli situated between the two CSs (see Lissek et al., 2008; 2010; 2014). As argued by Lissek et al. (2006) and Beckers et al. (2013), a weak situation is more likely to reveal any individual differences in fear learning. Differential conditioning paradigm arguably provides a 'strong situation' which is relatively unambiguous, since it provides extra information (i.e., CS−) to guide generalisation (see Homa et al., 1981). Our previous work also suggested that the differential conditioning paradigm is more likely to induce rule formation, compared to the single-cue conditioning paradigm (Wong & Lovibond, 2017). Hence, it is possible that most participants in the aforementioned studies (Arnaudova et al., 2017; Torrents-Rodas et al., 2013) came up with a rule, disambiguating the generalisation task and hence attenuating any effects of trait anxiety.

An alternative explanation for the apparent over-generalisation of fear in the HA No rule subgroup would be that anxious individuals fail to inhibit their fear responses (Davis, Falss, & Gewirtz, 2000; Gazendam, Kamphuis, & Kindt, 2013). However, this explanation would also predict higher responding to the CS+ among high anxious individuals, which was not observed in the current results. Given that the CS+ was partially reinforced, the null anxiety difference in responding to CS+ could not be explained by the presence of a ceiling effect. Furthermore, if the results obtained were due to failure of fear inhibition, HA individuals would have shown higher level of responding to all stimuli across the rule subgroups, which was not observed in the current study. Hence, the current results favor the interpretation of over-generalisation of fear in anxious individuals in the presence of ambiguity, rather than a failure to inhibit fear responses.

The current results are consistent with findings in the broader cognitive literature that trait anxious individuals show interpretation biases to ambiguity (Eysenck, MacLeod, & Mathews, 1987; MacLeod & Cohen, 1993) and estimation biases of negative events (e.g., Butler & Mathews, 1987), in paradigms other than fear conditioning. This research has shown that anxious individuals are more likely to interpret ambiguous cues in a negative or threatening way, and to overestimate the probability of negative events in the future. The finding of over-generalisation of shock expectancy under conditions of ambiguity in the current study is consistent with these biases, with anxious individuals interpreting ambiguous test stimuli in a negative way.

The fact that we found over-generalisation in trait anxious individuals suggests that over-generalisation of fear is a predispositional factor for the development of anxiety disorder, rather than a consequence of anxiety disorders. A study by Lenaert et al. (2014) provides some support for this interpretation. They found that participants who had displayed greater fear generalisation to stimuli resembling the safety cue in a laboratory task subsequently showed increased levels of anxiety in a six-month follow-up. One limitation of our study is that we did not assess the diagnostic status of our participants, so it is possible that some may have met criteria for an anxiety disorder. Another limitation was the fact that expectancy ratings and skin conductance were collected in two different test phases, that may render the comparison between expectancy and skin conductance measures difficult. However, given the high correspondence between expectancy ratings made in both test phases, it may otherwise suggest that the expectancy and skin conductance were highly comparable. The current literature on over-generalisation of fear in anxiety disorders and trait anxiety is somewhat mixed (Arnaudova et al., 2017; Haddad et al., 2012; Torrents-Rodas et al., 2013). Further research is needed to clarify which characteristics precede anxiety disorder and which are consequences of disorder. In this context we suggest that the present single-cue fear conditioning paradigm provides a useful tool for eliciting individual differences in generalisation and for investigating its cognitive and behavioral mechanisms.

Practically, our findings suggest that when clear rules are available for determining the level of threat, anxious individuals are able to generalise their fear adaptively like their low anxious counterparts. Conversely, ambiguous threat leads to elevated threat appraisal in anxious individuals. This conclusion is consistent with previous research showing that ambiguity can arise from different sources, including the unawareness of CS-outcome contingency (Chan & Lovibond, 1996), the unknown causal status of cues (e.g. Boddez et al., 2012), or cues that may predict threatening or neutral outcomes simultaneously (e.g., Eysenck et al., 1987). These findings suggest that anxious patients will benefit from therapeutic strategies that help them to disambiguate a potentially threatening situation, or generate rules to assess the situation more adaptively. As suggested by Chen and Lovibond (2016), explicit training in quantifying threat probability to an ambiguous, threatening situation may help patients more adaptively evaluate the probability of novel threatening events in the future. Along similar lines, other researchers have suggested that exposure therapy can be enhanced by interventions that encourage patients to identify a rule to help guide the generalisation of extinction memories (Treanor, Stapes-Bradley, & Craske, 2015; Vervliet, Baker, & Craske, 2012).

In conclusion, the present study confirmed that inferred rules shape the generalisation of fear learning in humans (cf. Wong & Lovibond, 2017). More importantly, the results showed that individuals with high trait anxiety displayed over-generalisation of fear, but only when they did not identify a clear rule, which essentially made the generalisation task ambiguous. The results are consistent with past findings that have shown increased threat appraisal to ambiguity among highly anxious individuals, and they provide preliminary evidence that over-generalisation of fear in the presence of ambiguity is a vulnerability marker for developing an anxiety disorder.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brat.2018.05.012.

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