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Development of a Protocol for Studying Premature Onset of Fear as a Feature of
Pathological Fear: The Effects of Conditional Stimulus Duration and Counting Behavior

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Abstract

We propose that the premature onset of fear responding is a potentially important feature of pathological fear. A behavioral protocol to study the temporal regulation of fear in humans is, however, lacking. The present study aims at developing such a protocol for healthy individuals. To this end, we investigated the effect of conditional stimulus duration (50 seconds or 90 seconds) and the effect of verbal counting during presentation of the conditional stimulus (counting or no counting) in an aversive conditioning paradigm. Results favored training conditions in which participants were asked to count for the duration of the conditional stimulus, regardless of conditional stimulus duration. Under these training conditions, we observed optimal temporal control over the onset of fear. Furthermore, it was shown that intervals followed by an aversive stimulus were retrospectively estimated to have lasted longer than those that were not followed by an aversive stimulus. We argue that the developed protocol may serve as a powerful method to study premature onset of fear responding in future studies.

1. Introduction

Fear is an adaptive reaction that helps organisms to cope with various types of threats. It motivates to escape or fight danger through activation of defensive behaviors (Fanselow & Lester, 1988; Frijda, 1986; Öhman & Mineka, 2001). However, when initially adaptive fear reactions become overly intense or trait-like, they become pathological. The diagnostic nomenclature groups such pathologies under the umbrella category of anxiety disorders (American Psychiatric Association, 2013). Literature states lifetime prevalences of up to 29% (Baxter, Scott, Vos, & Whiteford, 2012; Kessler, Berglund, Demler, Jin, & Walters, 2005; Somers, Goldner, Waraich, & Hsu, 2006), making it the most prevalent class of mental disorders in the general population (Kessler et al., 2009). Anxiety disorders are associated with high economic burden (Kessler et al., 2009; Lépine, 2002) and substantial impairments in the quality of life of patients and their families (Angermeyer, Kilian, Wilms, & Wittmund, 2006; Weiller, Bisslerbe, Maier, & Lecrubier, 1998). Significant impairments can also be found in individuals with subclinical forms of anxiety disorders (Mendlowicz & Stein, 2000).

Notwithstanding the great progress in our understanding of fear over the past decades, we still know disappointingly little about the factors that govern the transition from adaptive to pathological fear. The current article focuses on one of the mechanisms that might be involved in this transition: We propose that the temporal dysregulation of fear responding is an important feature of anxiety disorders. Three independent, but converging lines of argumentation — coming from different research domains and discussed below — suggest that the premature onset of fear responding is a potentially important feature of pathological fear.

(1) Clinical psychology — Relying on face validity, it certainly seems that (a subgroup of) anxiety patients display a premature onset of preparatory fear responding. The social anxiety disorder patient, for example, does not only display elevated fear responding during or

right before public speaking, but already displays this pattern while waiting to deliver a speech at a distant time in the future (Davidson, Marshall, Tomarken, & Henriques, 2000). Lengthened negative preoccupation, sometimes expressed with high levels of worry, is indeed characteristic of a variety of anxiety disorders and is also predictive of the onset of anxious symptomatology (Calmes & Roberts, 2007). Such extended anticipation time is not trivial as it produces a series of negative emotional effects: Stress research has for example demonstrated that the effects of chronic anticipation are far more damaging than the effects of acute stress (Gottlieb, 1997).

(2) Experimental psychology (time perception) — The time perception literature also suggests that pathological fear may involve a premature onset of fear responding. There is a large amount of evidence demonstrating a strong effect of emotion on temporal judgments. Healthy participants overestimate the duration until a fearsome event when asked to judge this duration retrospectively in a verbal or behavioral task (Droit-Volet & Meck, 2007). The pacemaker-accumulator time-keeping model — a widespread theory of undeniable predictive and heuristic value (for a review see Droit-Volet & Meck, 2007) — states that retrospective overestimation is due to the fact that the anticipation of a fearsome event (say, the anticipation of public speaking) increases the level of arousal which in turn increases the speed of an internal clock system. Because the internal clock speeds up, more time seems to have passed than is actually the case (see Panels b and c of Figure 1 for a detailed explanation). Particularly interesting for our present purposes is that such an acceleration of the internal clock should also result in a premature onset of preparatory fear responding: Since time is experienced to go faster in a state of heightened arousal, the fearsome event (say, public speaking) is expected to arrive sooner, so one will start to prepare sooner (see Panels a and b of Figure 1 for a detailed explanation). Until now, this hypothesis has not been investigated in the laboratory, but it is clear that the time perception literature is in line with our proposal that

the premature onset of fear responding is a plausible and potentially important feature of pathological fear.

(3) Learning psychology (fear conditioning) — One of the dominant models for the study of (pathological) fear and its treatment is the Pavlovian fear conditioning procedure. The fear conditioning paradigm entails the pairing of a neutral stimulus (conditional stimulus or CS) with an intrinsically aversive stimulus (unconditional stimulus or US), resulting in fear activation by the initially neutral CS. In an inhibition of delay procedure, a long CS of fixed duration is followed by deliverance of a US. The normative inhibition of delay effect is that over trials the temporal onset of fear responding shifts away from CS onset, with its maximum intensity toward the end of the CS, just before onset of the US (see the exponential function in Figure 2). In other words, the portion of the CS that is close in time to the US acquires threat value, whereas the portion of the CS further away in time from the US is treated as safe. There is evidence of inhibition of delay in a variety species including goldfish (Drew, Zupan, Cooke, Couvillon, & Balsam, 2005), rats (Rosas & Alonzo, 1997), rabbits (Millenson, Kehoe, & Gormezano, 1977), dogs (Rescorla, 1967), and humans (Molet, Leconte, & Rosas, 2006). What is important for our present purposes is that learning theory holds that such temporal regulation is dependent upon an inhibitory learning mechanism (Pavlov, 1927). Crucially, anxiety patients are known to show a deficit in fear inhibition mechanisms (Jovanovic et al., 2009; Jovanovic et al., 2010). Although not yet tested, it follows that anxiety patients would show a deficit in inhibiting fear in the face of a temporally distant threat, again illustrating the potentially crucial role of premature fear responding in pathological fear. However, it should be noted that an alternative explanation for inhibition of delay has been proposed. In this alternative interpretation, the temporal onset of responding is about learning when to respond rather than about learning when not to respond (Drew et al., 2005), leaving no role for inhibition and therefore qualifying the importance of this third line

of argumentation.

One of the aims of experimental psychopathology is to model psychopathology acquisition mechanisms in the laboratory. Despite the fact that temporal dysregulation of fear responding is a potentially important feature of anxiety disorders, a behavioral protocol to study the temporal regulation of fear in humans is currently lacking. The present study aims at developing such a protocol for healthy individuals. This may then prove useful for future research into the temporal regulation of fear responding of anxiety patients and at-risk individuals.

In view of this aim, we have systematically examined a number of parameters. First, CS duration was manipulated: All participants were presented with both short (50 seconds) and long (90 seconds) CS durations. Second, the effect of counting was investigated since research shows that counting improves temporal sensitivity (Clément & Droit-Volet, 2006). Counting was manipulated between subjects in order to avoid carry-over of the experimental treatment, so in order to avoid participants from counting when they were not supposed to. The dependent variable was the degree of fear subjects reported during the CS presentations.

In order to verify that the counting manipulation produced temporal sensitivity, a manipulation-check measure was used. At the end of the experiment, subjects were asked to complete a time reproduction task in which they were instructed to estimate how long they thought each CS had been presented by pressing a button for the same length of time (Fraisse, 1984). In line with earlier studies on the effect of counting, we assume that counting will improve performance in this task (e.g., Clément & Droit-Volet, 2006; Grondin, Meilleur-Wells, & Lachance, 1999). In addition, this task allows us to check whether the duration of CSs followed by a fearsome event will be estimated to last longer than control durations (as suggested by the time perception literature discussed above).

2. Method

2.1 Participants

Fifty students participated to earn course credits or a monetary reward. Participants were randomly assigned to two experimental conditions (non-counting condition: $N = 24$, mean age = 24.08, $SD = 5.69$; counting condition: $N = 24$, mean age = 25.46, $SD = 13.12$). They all gave written informed consent and were informed that they could decline to participate at any time.

2.2 Apparatus and stimuli

Participants were tested in individual sessions on a desktop computer. All testing was done in a sound-attenuated experimental room, adjacent to the experimenter's room. Four geometrical figures, presented in the centre of the computer screen, served as the conditional stimuli (CSs). The figures in question were a black moon, a blue cloud, a yellow star and a red planet (all approximately 4 cm in diameter). The presentation of the stimuli, the stimulus sequence, and the inter-trial intervals were controlled by Affect 4.0 software (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010). A 2 ms electrocutaneous stimulus, delivered to the wrist of the hand of choice, served as the unconditional stimulus (US). It was delivered by a Digitimer DS7A constant current stimulator (Hertfordshire, UK) through surface sensormedics electrodes (1 cm in diameter) filled with KY gel.

2.3 Measures

Subjective fear. Participants indicated their subjective fear on a semi-continuous scale from 0 to 100, with 0 meaning “*this stimulus currently elicits no fear at all*” and 100 meaning “*this stimulus currently elicits fear to a very high degree*”. The rating scale appeared at the bottom of the screen at the time of CS onset and receded from view at CS offset. Participants used a computer mouse in order to select a value between 0 and 100. During CS presentations, the subjective fear-score of each participant was registered at every 5-second interval, resulting in 10 values for the short CSs and 18 values for the long CSs. Registration

of responses started when participants first selected a value. At intervals for which participants did not respond yet, an empty cell would appear in the data.

Time reproduction. Participants had to press the 'x' button on the keyboard for the same amount of time each of the CSs had been presented during the conditioning phase.

2.4 Procedure

Following completion of the informed consent, electrodes were attached and participants were given a series of shocks with increasing intensity. They were asked to select a 'definitely uncomfortable, but not painful' shock intensity. Subsequently, experimental instructions appeared on the computer screen and were verbally recapitulated by the experimenter. Participants were informed that on each trial one out of four geometrical figures would be presented on the computer screen and that some of these figures would always be followed by an electric shock, while others would never be followed by an electric shock.

Next, the use of the subjective fear scale was explained. It was explained to the participants that this scale would appear at the bottom of the computer screen during each stimulus presentation. It was made clear that fear could increase, decrease, fluctuate or remain stable during one single stimulus presentation. Participants were told that they could adjust their score at every moment during each stimulus presentation.

Importantly, participants were randomly assigned to a counting or non-counting condition. In the counting condition, participants were required to count aloud during each CS presentation and to adopt the counting rhythm that they felt comfortable with. In the non-counting condition, participants were instructed to produce aloud repetitive, nonsense speech ("blablabla ...") as fast as possible during each CS presentation, in order to suppress vocal or subvocal counting (Droit-Volet, Clément, & Fayol, 2003; Gallistel & Gelman, 1992).

Thereafter, the actual experiment started. The experiment consisted of 20 trials and four types of CSs were used. The first type of CS (short CS+) was presented for 50 seconds,

immediately followed by an electrocutaneous stimulus. The second type of CS (short CS-) was also presented for 50 seconds, but was never followed by an electrocutaneous stimulus. In addition, the third type of CS (long CS+) was presented for 90 seconds, immediately followed by an electrocutaneous stimulus. Finally, the fourth type of CS (long CS-) was also presented for 90 seconds, and was never followed by an electrocutaneous stimulus. The assignment of each of the four geometrical figures to each type of CS was counterbalanced, resulting in 24 counterbalancing conditions. During the experiment, each CS was presented five times, in a randomized order. The inter-trial interval was 50 seconds.

Afterwards, participants were asked to complete a time reproduction task: They had to press the 'x' button on the keyboard for the same amount of time each CS type had been presented during the experiment. Instructions included written descriptions of the CSs (e.g., "press x for the same amount of time *the picture of the red planet* was shown earlier") rather than a depiction of the stimuli themselves.

3. Results

3.1 Subjective fear ratings

As mentioned earlier, the present study aims to set the training parameters that produce adequate temporal regulation of fear responding, meaning that there is a clear increase in subjective fear with time such that fear is low for early time portions of the CS+ and exponentially higher for late time portions (see the exponential function in Figure 2). We therefore tested whether the fear trajectories that were observed under the different training conditions (for the short CS+ with counting, for the short CS+ without counting, for the long CS+ with counting, and for the long CS+ without counting; see Figure 3) followed a linear or exponential function.

The exponential function that we used was as follows:

$$Fear = \alpha + \beta \exp(\gamma Time),$$

where $\exp(\gamma)$ is a growth factor, which contributes to the “exponential” increase as a function of *Time*. Both α and β control the starting value (i.e., intercept) of the function, but β is also related to steepness of the increase. For linear assimilation, we used an ordinal two-parameter linear function:

$$Fear = \alpha + \beta Time,$$

where α denotes an intercept, and β is a linear change.

In order to determine which training conditions produced adequate temporal regulation of fear responding, we estimated the three models described in Table 1, once for the short CS+ and once for the long CS+. We decided to examine the fear trajectories at the beginning and end of fear acquisition (at Trial 1 and Trial 5), because the change from beginning to end gives the clearest information on the acquisition of temporal control over the onset of fear. Adding to this decision was the observation that fear trajectories at Trials 2, 3, and 4 nicely fall in between the fear trajectories at Trial 1 and 5 (Figure 3). The fear trajectory in Trial 1 was fitted linearly in all three models, based on visual inspection of the fear trajectories (Figure 3) and on theoretical considerations about inhibition of delay (temporal regulation develops over trials; Rosas & Alonso, 1997). The three models, however, differed with respect to the fitting of Trial 5: (a) in model A, this fear trajectory was fitted linearly for both the counting and the non-counting condition; (b) in model B, this fear trajectory was fitted exponentially for both the counting and the non-counting condition; (c) and in model C this fear trajectory was fitted exponentially for the counting condition, but linearly for the non-counting condition. If model A shows the better fit, we can conclude that none of the current training conditions result in acquisition of an exponential increase in fear responding. If, however, model B or model C shows the better fit, we can respectively conclude that the exponential pattern of fear responding is acquired either in the counting and the non-counting condition or only in the counting condition. In order to avoid scaling problems, we rescaled

time and subjective fear by dividing them, respectively, by 50 and 100 (so the time variable ranged from 0.1 to 1 for the short CS+ and from 0.1 to 1.8 for the long CS+, and the fear score ranged from 0 to 1). Model estimations were performed by SAS (v.9) PROC NLMIXE. We did not include any random effects for the α , β , γ coefficients, because of convergence problem and unstable estimation in the multilevel modeling approach. Thus, in the present analysis, all observations were treated as independent.

Table 1 shows the model fit indices (-2 log likelihood or -2LL; Akaike Information Criterion or AIC; Bayesian Information Criterion or BIC) for the three models. AIC and BIC (the smaller the better) indicated that model C was better than models A and B, although model B had an almost similar log-likelihood score as model C. This is because model selection based on information criteria results in a preference for more parsimonious models. So the fear trajectory in Trial 5 of the non-counting condition could be assimilated by an exponential function, but this exponential curve fitting did not provide meaningful additional information over the linear assimilation for the extra parameter (i.e., γ).

The fitted functions for the short CS+ are visualized in Figure 4 and individual parameter estimates are shown in Table 2. The significant growth (γ) parameter in Trial 5 of the counting condition indicates that the fear trajectory exponentially increased over time. In contrast, Trial 5 of the non-counting condition had a significant linear (β) parameter. The linear parameter for Trial 1 in both the counting and non-counting conditions was not significant, which suggests that fear levels remained constant in these trials. The differences between Trial 1 and Trial 5 demonstrate that the course of fear responding changed in both conditions, although the development of an exponential increase in fear was only seen in the counting condition.

These results were replicated for the long CS+ (Figure 5; Table 2, below): Only in Trial 5 of the counting condition, subjective fear showed an exponential increase as a function of

time, whereas in other trials, fear increased linearly (Trial 5, Non-counting) or remained unchanged over time (Trial 1, Counting, and Trial 5, Non-counting).

In summary, results favored the protocols in which participants were asked to count for the duration of the CS presentation, regardless of the length of the CS+. Under these training conditions, an exponential increase in subjective fear developed over trials.

3.2 Time reproduction task

To verify that the counting manipulation improved temporal sensitivity, the results of the counting and non-counting conditions on the time reproduction task were compared. We performed a repeated-measures ANOVA with two within-subjects variables (Reinforcement and CS duration) and one between-subjects variable (Condition; see Figure 6). The first within-subjects variable refers to whether or not the CS was followed by an electrocutaneous stimulus (2 levels: CS+ or CS-). The second one refers to the fact that participants were presented with short (50 seconds) and long (90 seconds) CS durations.

In this ANOVA, the three-way interaction of Condition x CS duration x Reinforcement did not reach significance, $F(1, 46) = 1.22, p = .275, \eta^2_p = .03$. There were significant main effects of Condition, $F(1, 46) = 24.30, p < .001, \eta^2_p = .35$, and of CS duration, $F(1, 46) = 78.54, p < .001, \eta^2_p = .63$, which were qualified by a significant Condition x CS duration interaction, $F(1, 46) = 40.61, p < .001, \eta^2_p = .47$. Figure 6 illustrates that there was considerable underestimation in both conditions, but this underestimation was larger in the non-counting condition. In the counting condition, further analysis revealed a significant main effect of CS duration, $F(1, 23) = 97.47, p < .001, \eta^2_p = .81$: The long CSs were estimated to have lasted longer than the short CSs (see Figure 6, top panel). In the non-counting condition, the difference between long and short CSs was only marginally significant, $F(1, 23) = 3.83, p = .063, \eta^2_p = .14$ (see Figure 6, bottom panel). Hence, counting did indeed improve temporal sensitivity. There was also a main effect of Reinforcement, $F(1, 46) = 5.27, p = .025, \eta^2_p = .10$,

which did not differ between conditions, $F(1, 46) = 1.77, p = .189, \eta^2_p = .04$. As can be seen in Figure 6, reinforced CSs were estimated to have lasted longer than non-reinforced CSs.

4. Discussion

The aim of the present study was to set the training parameters to obtain optimal temporal control over the onset of fear in healthy individuals. To this end, we investigated the effect of CS duration (50 seconds or 90 seconds) and the effect of verbal counting during CS presentation (counting or no counting) in an aversive conditioning paradigm. Statistical analysis indicated that an exponential increase in fear responding only developed when participants were asked to count, regardless of CS duration. Thus, the protocols in which participants were asked to count may serve as a powerful method to study deviations from optimal temporal control over the onset of fear responding in future studies.

The results of the time reproduction task showed that the counting manipulation was effective in improving temporal sensitivity. This is in line with earlier studies on the effect of counting (e.g., Clément & Droit-Volet, 2006; Grondin et al., 1999). Furthermore, it was shown that intervals followed by an aversive stimulus (CS+) were estimated to have lasted longer than those that were not followed by an aversive stimulus (CS-). This finding is consistent with other studies investigating the influence of fear on time perception (e.g., Droit-Volet, Mermillod, Gil, & Cocenas-Silva, 2010). As mentioned earlier, retrospective temporal overestimation of the CS+ compared to the CS- can be explained in terms of a speeding-up of the pacemaker mechanism (internal clock) in response to the anticipation of a threatening event and the related increase in arousal. Since the internal clock speeds up, more time seems to have passed than is actually the case (see Figure 1, Panels b and c for a detailed explanation).

The development of behavioral methodology to study psychopathology acquisition mechanisms is an important step in experimental psychopathology research. As mentioned

earlier, we propose that the temporal dysregulation of fear responding is an important feature of anxiety disorders: Presumably, (a subgroup of) anxiety patients display a premature onset of fear responding. Therefore, we predict that (sub)clinical individuals will display an earlier onset of fear responding than controls in a task such as the one developed here. Future research can put this hypothesis to test. In future studies, it would also be interesting to involve physiological measures to investigate temporal (dys)regulation of fear responding. In the present study, only a subjective measure of fear was used. Even though it has been shown that rating data provide a valid proxy for fear, physiological measures would provide an additional index of arousal (Boddez et al., 2013).

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Table 1

Model fit of hypothesized and control models

Model Specification (Condition; Trial)	Model A	Model B	Model C
Non-counting; Trial 1	Linear	Linear	Linear
Non-counting; Trial 5	Linear	Exponential	Linear
Counting; Trial 1	Linear	Linear	Linear
Counting; Trial 5	Linear	Exponential	Exponential
Fit Statistics			
Short CS+ (50 sec)			
-2LL	-2.4	-11.9	-11.8
AIC	15.6	10.1	8.2
BIC	58.8	62.9	56.2
Long CS+ (90 sec)			
-2LL	449.7	441.5	441.5
AIC	467.7	463.5	461.5
BIC	516.4	523.1	515.7

Note. -2LL = -2 Log Likelihood; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion.

Table 2

Parameter estimates of model C for the short CS+ without counting, the short CS+ with counting, the long CS+ without counting, and the long CS+ with counting

Parameter	Estimate	SE	<i>t</i>	<i>p</i>
Short CS+ (50 sec)				
Non-counting; Trial 1				
α (Intercept)	0.12	0.04	3.19	.00
β (Linear)	-0.01	0.06	-0.20	.85
Non-counting; Trial 5				
α	0.34	0.03	9.74	.00
β	0.12	0.06	2.10	.04
Counting; Trial 1				
α	0.17	0.04	4.31	.00
β	-0.05	0.06	-0.78	.44
Counting; Trial 5				
α	0.23	0.06	3.90	.00
β	0.02	0.03	0.87	.38
γ (Exponential growth)	2.98	1.07	2.78	.01
Long SC+ (90 sec)				
Non-counting; Trial 1				
α (Intercept)	0.20	0.03	6.65	.00
β (Linear)	-0.02	0.03	-0.80	.43
Non-counting; Trial 5				
α	0.37	0.03	12.79	.00
β	0.10	0.03	3.94	.00
Counting; Trial 1				
α	0.19	0.03	6.20	.00
β	0.05	0.03	1.78	.08
Counting; Trial 5				
α	0.16	0.11	1.46	.14
β	0.10	0.08	1.24	.22
γ (Exponential growth)	1.04	0.38	2.76	.01

Figure 1. The internal clock functions by emitting pulses that relate to physical time. Panel (a) shows that a number of seconds a corresponds with a number of pulses x emitted by the internal clock. Panel (b) shows that when the internal clock is sped up (e.g., under conditions of arousal), the x pulses are reached before the a seconds have passed. Such would result in a premature onset of preparatory fear responding. The a seconds now correspond with y pulses. Panel (c) shows that subsequently judging the duration of a seconds would result in a retrospective overestimation if participants base their judgment on the reference memory of y pulses: The y pulses correspond with a longer actual time interval when the internal clock is no longer sped up by arousal.

Figure 2. The temporal locus of the fear response is shifted away from CS onset, with its maximum intensity just before onset of the US (fictitious data).

Figure 3. Subjective fear plotted against time interval for the short CS+ (50 sec) in the non-counting and counting condition, and for the long CS+ (90 sec) in the non-counting and counting condition. Subjective fear was rescaled to a range from 0 to 1.

Figure 4. Fitted function and observed mean for the short CS+ (50 sec) in the non-counting (Panel A) and counting (Panel B) condition. Subjective fear was rescaled to a range from 0 to 1.

Figure 5. Fitted function and observed mean for the long CS+ (90 sec) in the non-counting (Panel A) and counting (Panel B) conditions. Subjective fear was rescaled to a range from 0 to 1.

Figure 6. Estimation of CS durations (in ms) plotted against reinforcement of CS as a function of CS duration. The top panel shows the results for the counting condition, the bottom panel shows the results for the non-counting condition.