

Fear conditioning in women with anorexia nervosa and healthy controls: a preliminary study

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Author Note

Acknowledgements: V. Cardi, E. Lambert, J. Treasure, C. Kan & G. Breen acknowledge funding from the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. V. Cardi is also supported by a grant from MIUR (Dipartimenti di Eccellenza DM 11/05/2017 n. 262) to the Department of General Psychology. K. Purves acknowledges funding from the Alexander von Humboldt Foundation T. McGregor is supported by the UK Medical Research Council (MR/N013700/1). T. C. Eley & G. Breen are part funded by a program grant from the UK Medical Research Council (MR/M021475/1).

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Abstract

Anorexia nervosa is characterized by anxiety-driven behaviors, such as food avoidance and distressing persistent thoughts about weight gain and body image. The present study used a classical fear conditioning procedure to test the processes of fear acquisition and generalization, extinction and renewal in patients with anorexia nervosa and healthy controls. An app-based fear conditioning procedure was administered remotely to 64 patients and 60 healthy controls, over two sessions. A human female scream served as the unconditioned stimulus (US) and two neutral shapes were used as either the paired conditioned stimulus (danger cue; CS+) or the unpaired conditioned stimulus (safe cue; CS-). Patients with anorexia nervosa reported greater threat expectancy in response to the danger cue during the extinction and renewal phases and overall higher levels of negative affect throughout the task, compared to controls. Future research is warranted to replicate these findings and highlight the role that anxiety plays in explaining fear conditioning responses in patients with anorexia nervosa.

Keywords: Anorexia Nervosa, Anxiety, Fear conditioning, Learning, Mobile application

General scientific summary: This preliminary study investigated fear conditioning in anorexia nervosa. Patients with anorexia nervosa had greater threat expectancy in response to stimuli previously associated with a loud scream, even when the stimuli were no longer presented with the scream. If replicated, these results might contribute to explaining patients' difficulties to extinguish fear in relation to eating and its consequences.

Introduction

Anorexia nervosa is a psychiatric disorder primarily characterized by food avoidance and dangerously low body weight. It can develop into a severe form of illness, with studies indicating that only 40-62% of individuals recover over a period of 20 years (Eddy et al., 2017). Over the years, there has been a growing interest in the investigation of fear and anxiety in anorexia nervosa. This is motivated by evidence of the high comorbidity between anxiety disorders and anorexia nervosa, the presence of anxious temperament traits before illness onset and after weight restoration, shared genetic factors and the predictive role of anxiety on eating disorder treatment outcomes (e.g. Hildebrandt et al., 2012; Kaye et al., 2004; Murray et al., 2018; Pallister & Waller, 2008). Furthermore, fear and anxiety around food and the consequences of eating are the core symptoms of anorexia nervosa (Steinglass et al., 2013; Treasure, Cardi, Kan, 2012).

Theoretical models have proposed that anomalies in learning contribute to illness onset and maintenance, such that an evolutionary-driven and potentially rewarding behavior like eating can become an aversive experience (e.g. Strober et al., 2004; Kaye et al., 2004; Hildebrandt et al., 2012; Pallister & Waller, 2008; Treasure, Cardi, Kan, 2011). This proposal has not been tested experimentally, however there is some preliminary evidence, based on patients' narratives, that extreme eating behaviors might develop from abnormal learning experiences (Cardi et al., 2019). These include basic associations between food and traumatic experiences (classical conditioning), vicarious learning of food-related avoidance and aversion (attitudes and behaviors learnt through observing close others), or learning that eating is followed by aversive consequences

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(e.g. negative judgment from others, shame and criticism; operant conditioning) (Cardi et al., 2019). Despite these findings (Cardi et al., 2019), and very sound theoretical predictions (e.g. Strober et al., 2004; Kaye et al., 2004; Hildebrandt et al., 2012; Pallister & Waller, 2008; Treasure, Cardi, Kan, 2011), there is a critical lack of experimental investigations testing whether abnormal learning mechanisms are implicated in the psychopathology of anorexia nervosa, the same way in which these are implicated in anxiety disorders.

Fear conditioning is a well-established behavioral procedure with excellent translational value, which has enabled advances in the understanding of the processes of fear acquisition, extinction, generalisation and renewal (Lonsdorf et al., 2017). The examination of the process of fear conditioning typically consists of different experimental phases: fear acquisition (by pairing a conditioned stimulus, or CS+, which was neutral prior to conditioning, with an aversive event, or aversive unconditioned stimulus US), fear generalization (by testing whether fear transfers to stimuli similar to the CS+), fear extinction (by presenting the CS+ in absence of the aversive event), and fear renewal (by testing whether fear returns after presentation of the CS+ in the absence of the aversive event). In anxiety disorders, this procedure has been used to model experimentally the development, generalization, treatment and relapse of fear and demonstrated that patients with an anxiety disorder have increased fear responses to safety cues (i.e. cues never associated with the aversive US, or CS-) during acquisition and stronger responses to conditioned stimuli during extinction compared to healthy controls (small effect size; Duits et al., 2015; Lissek et al., 2005). There is also

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evidence that enhanced extinction learning is associated with better treatment outcomes among patients with specific phobia (Forcadell et al., 2017).

The aim of this preliminary study was to measure fear conditioning (including fear acquisition, extinction, generalization and renewal) in patients with anorexia nervosa compared to healthy controls. A smartphone application, the Fear Learning and Anxiety Response (FLARe) app, was used to deliver the procedure remotely, using a loud scream as the aversive US. In line with previous studies of fear conditioning, two outcomes were self-reported by participants using visual analogue scales: threat expectancy and affective ratings (Lonsdorf et al., 2017). The FLARe app has been validated against laboratory delivery of fear conditioning in non-clinical community samples and the two procedures produced a similar pattern of findings (Purves, et al., 2019). Based on the literature on fear conditioning in anxiety disorders, and the comorbidity between anxiety disorders and anorexia nervosa, it was hypothesized that patients with anorexia nervosa would demonstrate (1) greater threat expectancy for the CS- during acquisition and (2) greater threat expectancy for the CS+ during extinction. It was also hypothesised that greater threat expectancy for the CS+ during extinction would be associated with greater anxiety and worse eating disorder symptoms in patients with anorexia nervosa. Considering the explorative nature of this study, we also report threat expectancy ratings for fear generalization and renewal, differential threat expectancy scores for the CS+ and negative affective ratings across the phases.

Methods

Participants

Patients with anorexia nervosa were recruited from the Bethlem Royal Hospital in South London and from recruitment websites such as the website of the eating disorder charity BEAT (www.beateatingdisorders.org.uk) and Call for Participants (www.callforparticipants.com). This group included individuals aged 18 or over, with a diagnosis of anorexia nervosa based on the definitions provided by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (American Psychiatric Association, 2013). Healthy controls with no self-reported history of mental health conditions, and who were not currently taking psychiatric medication were recruited through the KCL internal recruitment circular email sent to staff and students. All participants were required to have a smartphone with internet access and were excluded if they could not read English. During screening, participants answered questions related to their age, weight, date of diagnosis, length and type of current treatment (including psychiatric medication, if any), and psychiatric history.

See Figure 1 for a flowchart of study participation and the supplementary text section of the supplementary materials for the sample size power calculation.

Ethics

This study received regulatory approvals from King's College London and South London and Maudsley joint research and development office and by the London Bridge NHS Research Ethics Committee (ref: 18/LO/0121).

Procedure

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Participants completed self-report questionnaires before the fear conditioning procedure. A smartphone app designed for remote administration of a fear conditioning procedure was used, which has been previously tested in a large sample of individuals recruited from the community (Purves et al., 2019). The conditioning task was administered over two sessions and consisted of four phases: fear acquisition (day one), generalisation (day one), extinction (day one) and renewal (day two), with a maximum of seven days between the two sessions. See Supplementary Figure 1 for an overview of the task phases.

During fear acquisition, participants viewed 12 presentations each of a large and small circle on a background image of an outdoor scene (Context A). A loud human female scream served as the unconditional stimulus and was paired with 75% of the presentations of one out of two circles serving as the CS (nine total presentations). The circle paired with the scream was counterbalanced between participants and became the CS+ while the circle never paired became the CS-. During generalization, the CS+ and CS- and another four circles (generalization stimuli: GS1, GS2, GS3, GS4) were presented two times against Context A. Generalization stimuli graduated in size between the CS+ and CS-. The smallest generalization stimulus was 15% larger than the smallest CS, with each subsequent circle being 15% larger again. During generalization, one presentation of the CS+ was paired with the aversive stimulus (50% of the presentations). Fear extinction consisted of eighteen presentations each of the CS+ and CS- on a background image of an indoor living room scene (Context B) with no US. Fear renewal involved four presentations each of the CS+ and CS- on Context A with no US.

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A minimum break of ten minutes occurred between the generalisation and extinction phases, during which time participants were invited to listen to a neutral podcast. The podcast comprised a female voice dictating neutral factual statements such as “The Hope Diamond was shipped from South America to London through the regular mail service” and “The Orient Express travels between Paris and Istanbul”. The reason for the break was to ensure there was sufficient time to allow for consolidation of acquisition learning before commencing extinction.

Measures

Questionnaires: participants provided information related to age, ethnicity, height, weight, relationship status, employment status, treatment status, living status, years of education, use of psychiatric medication, family psychiatric history and current co-morbidities. To assess current eating disorder psychopathology (restraint, eating concern, shape and weight concern) and fear of food, they completed the Eating Disorder Examination Questionnaire (EDE-Q; Fairburn & Beglin, 2008) and the Fear of Food Measure (FOFM; Levinson & Byrne, 2015). To assess trait anxiety and anxiety-related cognitions, they completed the 20-item trait anxiety subscale of the State-Trait Anxiety Inventory (STAI-T; Spielberger, 1983).

Expectancy ratings: for every trial, during each stimulus presentation, participants recorded how much they expected it to be followed by a scream (Likert scale 1: certain no scream, 5: uncertain, 9: certain scream). The scale was available for the last six out of total eight seconds of stimulus presentation. Participants’ first response was recorded.

Differential threat expectancy ratings: Differential learning during acquisition, extinction and renewal was calculated by subtracting the threat expectancy ratings for the CS- from threat expectancy ratings for the CS+.

Affective ratings: participants rated how each stimulus made them feel before the experiment began (baseline), after acquisition (post-acquisition), after extinction (post-extinction), and after renewal (post-renewal) on three Likert scales. These were “unpleasant” (1: happy/satisfied/content/pleasant to 9: unhappy/annoyed/discontent/unpleasant, “anxious” (1: calm/sleepy/dull/unaroused to 9: anxious/aroused/excited/jittery) and “fearful” (1: unafraid/safe/unconcerned to 9: fearful/afraid).

Data processing and analyses

For the questionnaire data, demographic and clinical characteristics were compared between groups using chi-squared tests or t-tests for independent samples, with Bonferroni corrections applied to correct for multiple comparisons. Independent samples t-tests and Bonferroni corrections were also used to compare threat expectancy and affective ratings between samples. The analyses were conducted in SPSS version 22 (SPSS, Inc., USA, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). For the fear conditioning procedure, mean expectancy ratings and affective ratings were calculated for all stimuli across each phase, and differential threat expectancy ratings were calculated for the Acquisition, Extinction and Renewal phases. Data were analyzed using Linear and Generalized Mixed-Effects models (lmer function, lme4 library, Pinheiro 2000) in R v.3.2.2. Four models were calculated; one for threat expectancy ratings towards the CS+, one for threat expectancy ratings towards

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the CS-, one for negative affective ratings towards the CS+ and one for negative affective ratings towards the CS-. These models included Group (healthy controls vs. patients with anorexia nervosa) and Phase (acquisition, extinction and renewal) and their interactions, and also Age and Ethnicity as fixed factors. The subject's IDs were included as random factor. In all analyses, the significant model with the lowest Akaike information criterium value was retained. If significant, interactions were investigated with post-hoc tests using Dunnett's adjustments. The Tukey's test was used for post-hoc corrections when exploring differences between levels of the same factor.

Relationships between threat expectancy ratings and eating disorder psychopathology, body mass index, fear of food and trait anxiety, were assessed using Pearson product-moment correlation coefficients (r). Effect sizes were established using Cohen's d and interpreted as small (0.2), medium (0.5) and large (0.8) (Cohen, 1988).

Results

Clinical and demographic characteristics

Table 1 describes the demographic and clinical characteristics of the groups. As expected, patients with anorexia nervosa had significantly lower body mass index (BMI) and reported significantly higher eating disorder psychopathology, fear of food, and trait anxiety than the control group. Among patients with anorexia nervosa, 57.8% had a current diagnosis of typical anorexia nervosa and the remaining had a diagnosis of atypical anorexia nervosa; 14.28% reported current purging behavior, 19.05% reported current bingeing behaviors, 14.28% reported current use of laxatives, and 3.17% reported current use of diuretics (water tablets). Furthermore, 18.75% reported that they

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currently suffered from anxiety disorders and 31.25% reported that they were taking psychiatric medication at the time of testing.

Fear conditioning outcomes

Threat expectancy towards the CS+: A significant interaction was found between Group and Phase, $X^2(2)=28.20$, $p<0.0001$. Post-hoc analyses revealed that patients with anorexia nervosa reported significantly higher threat expectancy towards the CS+ during Extinction, $Z=-4.41$, $p<0.0001$ and Renewal $Z=-3.17$, $p=0.01$ (Figure 2A). Age and Ethnicity were not significant, $X^2(1)=3.80$, $p=0.051$; $X^2(6)=7.03$, $p=0.31$, respectively. There were no significant between-group differences in threat expectancy ratings for any of the stimuli used during Generalisation, GS1: $t(112)=-1.737$, $p=0.085$; GS2: $t(112)=0.367$, $p=0.715$; GS3: $t(112)=-1.118$, $p=0.266$; GS4: $t(112)=-1.560$, $p=0.122$.

Threat expectancy towards the CS-: A significant interaction was found between Group and Phase, $X^2(2)=8.09$, $p<0.01$ (Figure 2B). However, when calculating post-hoc tests, the between-group comparisons for each phase were not significant ($p>0.54$). Age and ethnicity were not significant either, $X^2(1)=1.42$, $p=0.23$; $X^2(6)=3.31$, $p=0.76$, respectively.

Both patients with anorexia nervosa and healthy controls reported significantly greater threat expectancy towards the CS+ (healthy controls= 6.99, SD=1.46; patients with anorexia nervosa= 7.14, SD=1.45) compared to the CS- (healthy controls= 2.70, SD= 1.36; patients with anorexia nervosa= 3.17, SD= 1.76) during the acquisition phase

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(healthy controls $t(55)=12.37$, $p<.0001$; patients with anorexia nervosa $t(63)=11.88$, $p<.0001$).

Differential threat expectancy: Patients with anorexia nervosa reported higher differential threat expectancy during the extinction phase, suggesting increased discrimination between the CS+ and CS- compared to the healthy controls, $t(116)=-1.973$, $p=0.05$. There were no significant group differences in differential threat expectancy during the acquisition phase, $t(116)=0.609$, $p=0.544$, and the renewal phase, $t(111)=-1.577$, $p=0.118$. Means, standard deviations, standard errors and effect sizes for threat expectancy ratings for the CS+ and CS- are reported in Supplementary Table 1.

Affective ratings towards the CS+: A significant main effect was found for the factors Group, $X^2(1)=12.78$, $p<0.001$ and Phase, $X^2(3)=205.85$, $p<0.0001$. Patients with anorexia nervosa reported overall higher negative affective ratings compared to controls (Patients Mean=6.11, SE=0.12; Controls Mean=5.49, SE=0.11). Negative affect ratings were highest for Acquisition (Mean=7.16, SE=0.13), followed by Renewal (Mean =5.80, SE=0.14), Extinction (Mean = 5.34, SE=0.16) and Baseline (Mean=4.99, SE=0.08). The differences between Baseline and Acquisition ($Z=-13.35$, $p<0.001$), Baseline and Renewal ($Z=4.91$, $p<0.001$), Acquisition and Extinction ($Z=-11.20$, $p<0.001$), Acquisition and Renewal ($Z=-8.27$, $p<0.001$) and Extinction and Renewal ($Z=2.79$, $p=0.02$) were significant. The difference between Baseline and Extinction was not significant ($Z=2.14$, $p<0.13$). The interaction between Group and Phase ($X^2(3)=7.47$, $p=0.058$) and the factors Age ($X^2(1)=0.72$, $p=0.39$) and Ethnicity

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($X^2(6)=2.93$, $p=0.81$), were not significant. Supplementary Figure 2 includes box plots for affective ratings for the CS+ displayed by Group (S2A) and Phase (S2B).

During Generalization, patients with anorexia nervosa reported significantly higher affective ratings for the GS1: $t(113)=-0.234$, $p=0.015$, and for the GS4: $t(97)=-2.234$, $p=0.028$. There were no significant between-group differences in affective ratings for the GS2: $t(113)=-1.717$, $p=0.089$, and for the GS3: $t(113)=-1.247$, $p=0.215$.

Affective ratings towards the CS-: A significant main effect of Group, $X^2(1)=5.42$, $p=0.019$ and Phase, $X^2(3)=33.75$, $p<0.0001$, were found. Patients with anorexia nervosa reported overall higher negative affective ratings for the CS- (patients with anorexia nervosa Mean=4.65, SE=0.16, Healthy Controls Mean =4.12, SE=0.15). Negative affective ratings were highest during Baseline (Mean=4.88, SE=0.08), followed by Acquisition (Mean=4.49, SE=0.17), Extinction (Mean=4.13, SE=0.17) and Renewal (Mean=4.10, SE=0.16). The differences between Baseline and Extinction ($Z=-4.88$, $p<0.001$) and Baseline and Renewal ($Z=-5.01$, $p<0.001$) were significant; whereas those between Baseline and Acquisition ($Z=2.53$, $p=0.055$), Acquisition and Extinction ($Z=-2.35$, $p=0.08$), Acquisition and Renewal ($Z=-2.52$, $p=0.055$) and Extinction and Renewal ($Z=-0.22$, $p=0.99$) were not significant. The interaction between Group and Phase ($X^2(3)=0.29$, $p=0.96$), Age ($X^2=0.06$, $p=0.79$) and Ethnicity ($X^2(6)=7.75$, $p=0.25$) were not significant.

Correlation analysis for threat expectancy ratings

Results of the Pearson product-moment correlation coefficients are presented in Supplementary Table 2. Expectancy ratings for the CS+ during extinction and the mean

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differential expectancy rating during extinction were not significantly correlated to eating disorder psychopathology, body mass index, fear of food or trait anxiety among patients with anorexia nervosa ($p>0.05$).

Discussion

This study used a fear conditioning procedure delivered through a mobile application in patients with anorexia nervosa and healthy controls. Based on the fear conditioning literature in anxiety disorders, and the mechanistic overlap between these disorders and anorexia nervosa, it was hypothesized that patients with anorexia nervosa would display greater threat expectancy for the CS- during acquisition and greater threat expectancy for the CS+ during extinction. It was also hypothesized that greater threat expectancy for the CS+ during extinction would be associated with greater anxiety and worse eating disorder symptoms in patients with anorexia nervosa. The data supported the first hypothesis in part, in that patients had greater threat expectancy towards the CS+ during extinction, but no greater threat expectancy for the CS- during acquisition. The second hypothesis was not supported, in that threat expectancy ratings for the CS+ were not correlated to eating disorder psychopathology, body mass index, fear of food, or anxiety in patients with anorexia nervosa. This might be due to the use of a generally aversive, but disorder non-specific unconditioned stimulus (Lissek et al., 2008), or due to lack of statistical power or to the absence of an effect.

Patients with anorexia nervosa displayed greater threat expectancy during renewal. One possible suggestion is that there is greater availability of the learned

threat association (CS-US pairing) in memory, compared to the inhibitory association (CS-no US) (Carpenter et al., 2019).

Patients also reported higher negative affective ratings in response to the CS+ and the CS- across phases. This might be due to the overall greater anxiety experienced compared to healthy peers. Overall, it is important to note the preliminary nature of the study, and the importance to replicate these initial findings with larger samples and using procedures validated with patients with other psychiatric disorders.

Strengths and limitations

The strengths of the study are that this is one of the first investigations of fear conditioning in anorexia nervosa and that the sample size is adequate for a pilot study. Our results are similar but not identical to results found in anxiety disorders, and therefore are hypotheses generating with regards to potential similarities and differences in fear acquisition between patients with anorexia nervosa and patients with anxiety disorders.

Limitations include the exclusive use of self-report measures of threat expectancy and the lack of a laboratory setting for data collection. The first limitation is mitigated by evidence that verbal and physiological measures of fear tend to converge (Craske, Hermans & Vansteenwegen, 2006; Constantinou et al., 2020). Furthermore, verbal measures of threat expectancy in human fear conditioning research have face validity, diagnostic validity, predictive validity and construct validity (Boddez, Baeyens et al., 2013). Also, the reliability of physiological measures, such as heart rate and skin conductance has been largely questioned in anorexia nervosa, due to the typical

biological profile of the illness (e.g. bradycardia, low blood pressure, poor circulation in feet and hands; Mazurak et al., 2010; Bellodi et al., 2013). The lack of laboratory-based assessments is partly mitigated by the validation of the fear conditioning app against the laboratory-based version of the same procedure in a large non-clinical sample (Purves et al., 2019). However, we note that the validation did not occur with a group of individuals with a psychiatric disorder. Further limitations are that patients with anorexia nervosa differed in age and ethnicity from healthy peers and that anxiety disorders among people with anorexia nervosa were self-reported with reference to the present and were not assessed using a structured clinical interview. The use of self-report questionnaires might contribute to the underestimation of comorbidities. Also, patients were not asked what type of psychiatric medication they were taking at the time of testing. Further studies are warranted to test fear conditioning among patients with comorbid anxiety disorders compared to patients with no lifetime anxiety disorders, and to assess the role of different psychiatric medications on patients' responses to fear conditioning.

Conclusions

Patients with anorexia nervosa reported greater threat expectancy during the Extinction and Renewal phases of a fear conditioning procedure. They also reported higher negative affective ratings compared to healthy controls. If replicated and expanded, these findings might be of translational value to improve the efficacy of exposure treatments for anorexia nervosa.

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Tables

444 **Table 1** Demographic and clinical characteristics

445 Demographic and clinical characteristics of the participants recruited

Characteristic	Patients with anorexia nervosa (n=64)	Healthy Controls (n=60)	95% Confidence Interval		Difference between groups			Effect size
	Mean (SD)	Mean (SD)	Lower	Upper	t	df	p	Cohen's d
Age (years)	28.41 (7.44)	24.69 (6.58)	-6.28	-1.16	-2.88	117	0.005*	0.53
Body Mass Index (kg/m ²)	17.40 (3.08)	21.24 (2.95)	2.69	4.99	6.60	106	<0.001* **	1.27
Eating Disorder Examination Questionnaire (total score)	3.32 (1.52)	0.86 (0.60)	-2.89	-2.04	-11.48	117	<0.001* **	2.13
Fear of Food Measure (total score)	107.00 (38.18)	40.00 (15.31)	-80.05	-53.88	-10.16	95	<0.001* **	2.30
STAI-Trait Anxiety (total score)	60.10 (11.56)	40.09 (9.44)	-23.86	-16.17	-10.31	117	<0.001* **	1.90
	N (% of group)	N (% of group)	X ² (p-value)					
Ethnicity								
Caucasian	53 (82.81%)	39 (65.00%)	18.28 (p=0.003)*					
Black	1 (1.56%)	5 (8.33%)						
Asian	2 (3.13%)	8 (13.33%)						
Mixed Race	0 (0.00%)	2 (3.33%)						
Hispanic/Latino	0 (0.00%)	3 (5.00%)						
Other	8 (12.50%)	3 (5.00%)						
Relationship status								

FEAR CONDITIONING IN ANOREXIA NERVOSA

Single	30 (46.88%)	25 (41.67%)	1.75 (p=0.625)
Married	5 (7.81%)	4 (6.67%)	
Separated/Divorced	3 (4.69%)	1 (1.67%)	
In a couple, not married	23 (35.94%)	27 (45.00%)	
Other	3 (4.69%)	3 (5.00%)	
Educational level			
GCSE	5 (7.81%)	2 (3.33%)	2.15 (p=0.709)
AS level	13 (20.31%)	16 (26.67%)	
A level	13 (20.31%)	16 (26.67%)	
Undergraduate degree	28 (43.75%)	23 (38.33%)	
Postgraduate degree	1 (1.56%)	1 (1.67%)	
Other	4 (6.25%)	2 (3.33%)	

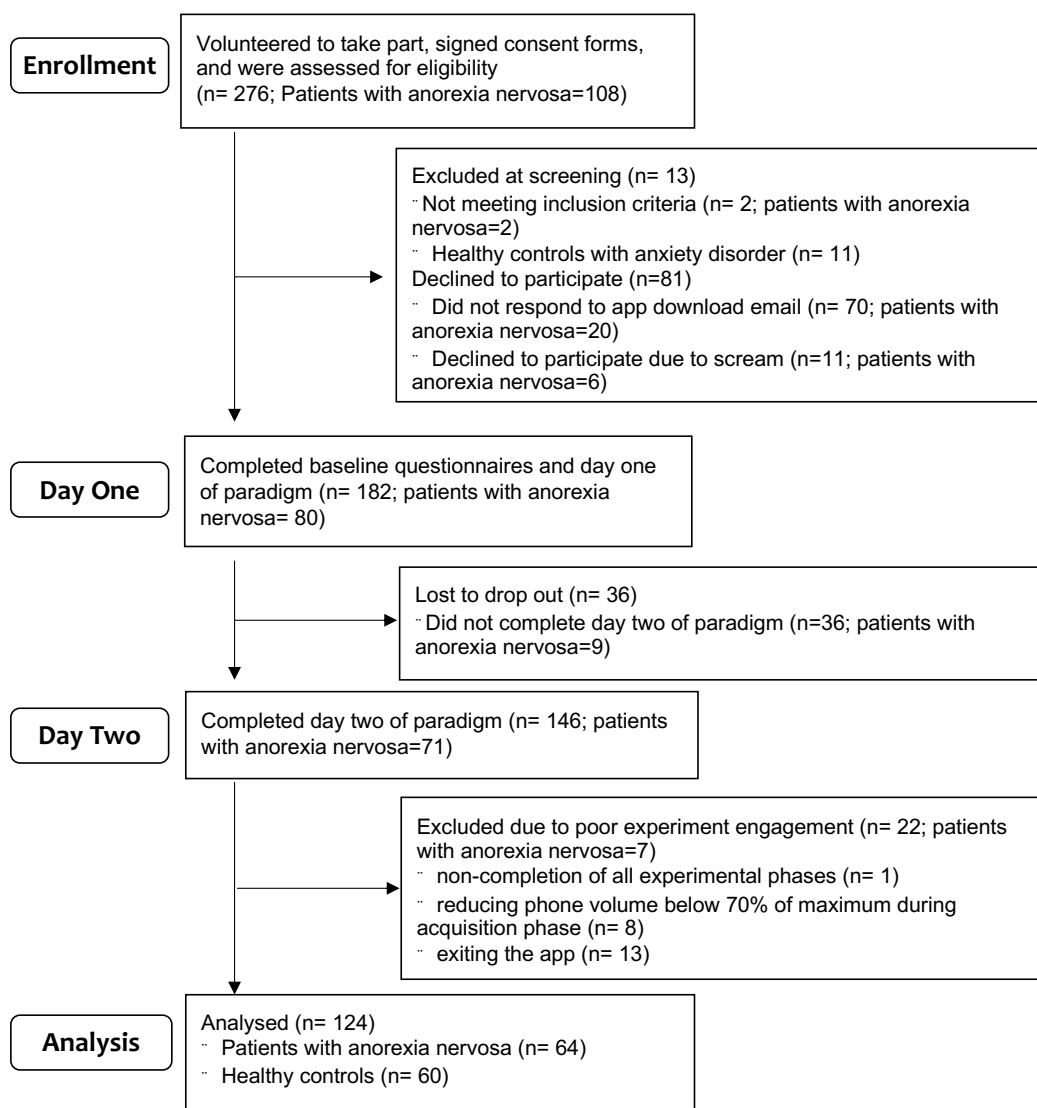
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447 *Note.* SD=standard deviation; N= number of participants; p<0.05; ***p<0.001.

Figures

Figure 1. CONSORT Flow Diagram

This figure describes the processes of enrolment, completion of testing and analyses.



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Figure 2. Threat expectancy ratings for the CS+ and the CS-

Box plots for mean threat expectancy ratings for the CS+ (A) and the CS- (B) displayed by group (Healthy Controls and patients with anorexia nervosa), across phases (“acq”=acquisition, “ext”=extinction, “renew”=renewal)

