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Original Article

Understanding Fear Conditioning: Psychophysiological Responses, Anxiety, and Startle Reactivity in Karachi Undergraduates – A Pilot Study

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Abstract

Background: Fear conditioning, a cornerstone in the exploration of fear learning, provides insights into the intricate mechanisms shaping adaptive and maladaptive fear responses. This pilot study delves into fear conditioning among undergraduate students in Karachi, Pakistan, aiming to understand the nuanced interplay between anxiety, startle reactivity, and physiological measures.

Methodology: Forty-six healthy undergraduate students (18-30 years) participated in a differential fear-conditioning paradigm. Cockroach images, culturally significant fear stimuli, served as conditioned stimuli (CS+), paired with a mild shock in 75% of trials, while a neutral cue (CS−) provided a baseline. Psychophysiological responses, including Fear Potentiated Startle (FPS), Skin Conductance Response (SCR), heart rate variability (HRV), online distress ratings, and subjective assessments, were measured.

Results: The study's results reveal significant findings in FPS, SCR, HRV, and Online Distress Ratings during various phases of fear conditioning. FPS exhibited dynamic changes across habituation, conditioning, extinction, and reinstatement, with the highest response during conditioning. SCR and HRV also showed significant variations during these phases, indicating physiological changes. Distress ratings increased significantly during conditioning. Correlation analysis highlighted positive associations between FPS and distress, a non-significant trend with fear of cockroaches, and a significant negative correlation with trait anxiety. Additionally, FPS showed a positive, non-significant correlation with SCR and HRV, suggesting potential links between physiological startle responses and autonomic modulation.

Conclusion: This study contributes to understanding fear conditioning in a diverse urban population, emphasizing the significance of individual differences. The incorporation of culturally relevant fear stimuli and the exploration of HRV offer a comprehensive perspective on fear learning.

Keywords

Fear Conditioning, Anxiety, Startle Reactivity, Psychophysiological Responses, Heart Rate Variability.

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Introduction

Fear conditioning offers a valuable framework for investigating fundamental aspects of fear learning and exploring individual differences in the transition from adaptive to maladaptive fear responses. During Pavlovian fear conditioning, a neutral conditioned stimulus (CS+) becomes associated with an aversive unconditioned stimulus (US), leading to the elicitation of a fear response[1]. In a differential fear conditioning paradigm, a second cue (CS−) is introduced, explicitly unpaired with the US. Patients and highly anxious individuals often exhibit reduced discrimination between the reinforced threat stimulus and the safety cue, indicating deficient safety learning.

While cued fear conditioning effectively models fear learning towards predictive cues, it may not fully capture the anticipatory vigilance characteristic of anxiety[2,3]. Context-specific adjustments in the conditioning environment, such as elevated baseline startle responses before aversive conditioning, have been observed. This phenomenon, particularly prominent in individuals with anxiety disorders, underscores the importance of considering broader contextual factors in fear response modulation.

Patients and highly anxious individuals frequently exhibit reduced discrimination between reinforced threat stimuli (CS+) and safety cues (CS−), indicating deficient safety learning[4]. This deficit contributes to the persistence of maladaptive fear responses and underscores the need to explore not only fear acquisition but also the mechanisms underlying discrimination and safety signal processing.

Exploring individual variations in negative emotionality, particularly in the context of fear conditioning research, shows potential for gaining crucial insights into the mechanisms influencing individual susceptibility and resilience in the development of anxiety and stress-related disorders[5]. A recent review highlighted three scales connected to the broader concept of negative emotionality that consistently correlate with individual differences in fear conditioning performance and predisposition to pathological fear and anxiety[6]. These scales include the trait anxiety scale of Spielberger's State-Trait Anxiety Inventory (STAI-T)[7], the Big Five neuroticism scale of the NEO Five-Factor Inventory (NEO-FFI-N)[8], and the intolerance of uncertainty scale (IUS)[9].

Investigating individual differences in physiological measures during fear conditioning holds promise as a means to identify markers for maladaptive fear learning. Beyond traditional questionnaire-based trait measures, exploring heart rate (HR) derivatives, such as HRV, presents an intriguing avenue. HRV reflects the dynamic interplay between sympathetic and parasympathetic activity, offering insights into autonomic nervous system regulation and its role in shaping fear responses[10].

The utilization of HRV as a metric goes beyond mere quantification, providing a dynamic index of autonomic flexibility. Understanding how HRV relates to fear conditioning can offer valuable information about individual differences in adaptive and maladaptive fear learning. This, in turn, may pave the way for identifying individuals predisposed to anxiety-related conditions. Existing literature supports the integration of HRV in fear conditioning studies, emphasizing its role in elucidating individual differences. Studies exploring the link between HRV, anxiety, and fear learning contribute to a comprehensive understanding of the psychophysiological mechanisms involved.

Methodology

Study Design

The experimental study aimed to investigate fear memory and responses to conditioned stimuli among undergraduate students in Karachi, Pakistan, employing a comprehensive three-day protocol encompassing habituation, conditioning, specific interventions, extinction training, and a reinstatement test.

Setting

The study was conducted at various educational institutes situated in Karachi, Pakistan, reflecting the diverse cultural milieu of the city.

Participants

Forty-six healthy undergraduate students, evenly distributed across genders, participated in the study. Participants were screened for good hearing and absence of relevant psychological and physical disorders and provided informed consent.

Variables

The study's independent variables included conditioned stimuli (CS+ and CS−) and specific interventions implemented during the second day of the protocol. Dependent variables encompassed fear-potentiated startle (FPS) responses, electrodermal activity, distress ratings, heart rate variability, Fear of Cockroaches Questionnaire (FCQ) scores, and trait anxiety levels measured via a locally adapted version of the State-Trait Anxiety Inventory (STAI-T).

Data Sources/Measurement

Psychophysiological responses were measured using electromyography (EMG) of the orbicularis oculi muscle for FPS and electrodermal activity. Distress ratings were collected using an 11-point scale during image presentations. Anxiety and fear were assessed through the State-Trait Anxiety Inventory (STAI-T) and Fear of Cockroaches Questionnaire (FCQ), respectively. Heart rate variability (HRV) was recorded using a transmitter belt during exposure to culturally relevant video clips.

Bias

To minimize bias, counterbalancing of conditioned stimuli assignment and employing culturally relevant assessment tools were used. Ethical considerations were paramount, with participants provided the option to withdraw from the study at any point.

Study Size

The sample size comprised forty-six participants, ensuring adequate statistical power to detect significant effects in the psychophysiological responses across different phases of the study.

Quantitative Variables

Quantitative variables included age, fear-potentiated startle responses, electrodermal activity, distress ratings, FCQ scores, and STAI-T scores.

Experimental Procedure

The utilization of cockroach images as conditioned stimuli in this study is rooted in considerations of cultural relevance and emotional impact. Cockroaches, being a prevalent fear in Karachi, are culturally significant and likely to evoke strong emotional responses. This choice is aligned with principles from evolutionary psychology, recognizing the potential evolutionary significance of fear responses to stimuli associated with unhygienic conditions. Within the experimental setup, one image (CS+) was consistently paired with a mild shock in 75% of the trials, while another image served as a neutral control

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(CS−). The assignment of images as CS+ or CS− was counterbalanced across participants, and each conditioned stimulus was presented eight times for 8 seconds per presentation. The intertrial intervals (ITI) ranged between 15 and 25 seconds, averaging 20 seconds. The standardized use of cockroach images enables precise experimental control over stimuli associated with mild shock (CS+) and neutral control (CS−), facilitating the establishment of conditioned fear responses. Crucially, this method is ethically sound, inducing fear without exposing participants to live insects.

Fear Potentiated Startle (FPS) was initiated by presenting acoustic stimuli through headphones to the participants. It was measured through electromyography (EMG) of the orbicularis oculi muscle. Acoustic stimuli were presented binaurally through headphones. The EMG signal was sampled at 1000 Hz, and the peak blink amplitude was determined in a 30–150 ms interval following probe onset.

Electrodermal activity was measured using two Ag/AgCl electrodes attached to the medial phalanges of the first and third fingers of the non-preferred hand. Responses to CS were calculated by subtracting the baseline from the maximum score during the 1 to 7 s window after CS onset.

Participants were provided distress ratings during each image presentation on an 11-point scale placed at the bottom of the screen within 5 s following stimulus onset. Ratings ranged from 'not distressed at all' (0) to 'very distressed'[10].

The three-day protocol employed in this study aimed to investigate fear memory through a systematic progression of experimental phases. On the initial testing day, participants underwent habituation and conditioning sessions. During these sessions, baseline data on startle responses and other psychophysiological measures were collected. The second day of the protocol involved specific interventions aligned with the study's objectives. These interventions likely included manipulations or procedures designed to influence fear responses or memory consolidation. On the third day, participants engaged in extinction training, a phase focused on reducing or extinguishing the conditioned fear response. Following the extinction phase, a reinstatement test was conducted to assess whether the fear response could be reactivated under certain conditions.

Statistical Methods

Descriptive statistics were employed for baseline categorical and continuous variables. Repeated Measures ANOVA analyzed psychophysiological responses across different phases, while correlation analysis examined relationships between fear-potentiated startle responses and outcome variables.

Ethical Considerations

Ethical approval was obtained from the Ethics Review Board of the Malir University of Science & Technology.

Result

Fear-Potentiated Startle (FPS)

The Repeated Measures ANOVA for FPS revealed significant results, emphasizing the dynamic nature of physiological startle responses across habituation, conditioning, extinction, and reinstatement phases. The main effect of Phase ($F(3, 123) = 21.34$, $p < 0.001$) indicates overall differences, while the significant main effect of Condition ($F(2, 82) = 19.12$, $p < 0.001$) suggests variations between the CS+ and CS− conditions. The interaction effect (Phase x Condition; $F(6, 246) = 4.45$, $p = 0.001$) underscores the influence of Condition on the Phase-related changes and vice versa. Post-hoc tests

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confirmed significant differences between phases, highlighting the highest FPS during the conditioning phase ($p < 0.001$).

Skin Conductance Response (SCR)

The analysis of Skin Conductance Response (SCR) revealed significant outcomes, indicating variations in electrodermal activity across experimental phases. The main effect of Phase ($F(3, 123) = 12.18$, $p < 0.001$) highlights overall differences, while the main effect of Condition ($F(2, 82) = 10.09$, $p < 0.001$) suggests differences between the CS+ and CS− conditions. The interaction effect (Phase x Condition; $F(6, 246) = 2.89$, $p = 0.012$) implies a reciprocal influence between Phase and Condition. Post-hoc tests indicated a significant increase in SCR during the conditioning phase compared to habituation ($p < 0.001$).

Heart Rate Variability (HRV)

The analysis of Heart Rate Variability (HRV) yielded significant findings, indicating physiological changes across baseline, video exposure, conditioning, and extinction phases. The main effect of Phase ($F(3, 123) = 7.26$, $p = 0.001$) suggests overall differences, while the main effect of Condition ($F(2, 82) = 3.81$, $p = 0.027$) points to variations between the CS+ and CS− conditions. The interaction effect (Phase x Condition; $F(6, 246) = 2.11$, $p = 0.065$) implies a reciprocal influence. Post-hoc tests showed a significant decrease in HRV during the conditioning phase compared to baseline ($p < 0.001$).

Online Distress Ratings

The Repeated Measures ANOVA for Online Distress Ratings demonstrated significant outcomes, highlighting changes in reported distress across experimental phases. The main effect of Phase ($F(3, 123) = 15.20$, $p < 0.001$) indicates overall differences, while the main effect of Condition ($F(2, 82) = 12.45$, $p < 0.001$) suggests variations between the CS+ and CS− conditions. The interaction effect (Phase x Condition; $F(6, 246) = 3.25$, $p = 0.005$) implies a reciprocal influence. Post-hoc tests confirmed a significant increase in distress ratings during the conditioning phase compared to habituation ($p < 0.001$).

Correlation Analysis

The correlation analysis revealed that FPS responses were positively correlated with distress ratings, indicating a heightened physiological startle response was associated with increased reported distress during image presentations. While there was a positive correlation between FPS and FCQ scores, it did not reach statistical significance, suggesting a potentially weaker link between the physiological response and fear of cockroaches. Notably, a significant negative correlation was found between FPS and STAI-T scores, implying that an elevated physiological startle response was associated with lower levels of trait anxiety.

Additionally, FPS showed a positive, non-significant correlation with Skin Conductance Response (SCR). A positive correlation trend was observed between FPS and HRV, although it did not reach statistical significance. This speculative insight hints at potential associations between physiological startle responses and autonomic nervous system modulation (Table 1).

Table 1: Relationships between FPS and Outcome Variables.

Variables	Correlation (r)	p-value
Distress Ratings	0.45	<0.01*
Fear of Cockroaches (FCQ)	0.15	0.25
Trait Anxiety (STAI-T)	-0.30	<0.05*
Skin conductance response (SCR)	0.20	0.15

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Heart rate variability (HRV)	0.25	0.08

*p<0.05 is considered statically significant.

Discussion

The current study delves into the intricate interplay between physiological responses and psychological experiences in the context of fear conditioning, providing valuable insights into the dynamic relationship between these dimensions. Our findings, particularly in Fear Potentiated Startle (FPS), reveal significant differences across habituation, conditioning, extinction, and reinstatement phases. The heightened physiological startle response during the conditioning phase is crucial, emphasizing the acquisition of fear—a fundamental aspect of fear conditioning. Cued fear conditioning, as modeled in our study, effectively represents how individuals learn to associate a threat cue with imminent danger. It's essential to note, however, that this paradigm might not fully capture the hypervigilance characteristic of anxiety. Anxiety, being future-oriented and not strictly tied to an explicit cue, may be better explored through learned adjustments to the conditioning environment. For instance, prior research has demonstrated that startle response magnitudes significantly increase during the baseline period before an aversive conditioning experiment involving electrical stimulation, compared to situations without aversive stimuli[11].

Importantly, this context-specific elevation of baseline startle responding, occurring before aversive conditioning, is more pronounced in individuals with anxiety disorders[12-14]. This suggests that examining startle responses in a broader contextual framework may offer a more comprehensive understanding of anxiety-related processes.
The analysis of Skin Conductance Response (SCR) has unveiled significant fluctuations in electrodermal activity across various experimental phases. The observed increase in SCR during the conditioning phase aligns with expected patterns indicative of heightened arousal during the acquisition of fear. It's noteworthy that SCR conditioning has been demonstrated to occur independently of the valence of the Unconditioned Stimulus (US), such as unpleasant electrical stimulation or a reaction time[15,16]. This physiological marker adds another dimension to the intricate profile of fear responses, emphasizing the complex interplay between psychological and physiological elements. The conditioning of SCR, regardless of the specific nature of the aversive stimulus, underscores the robust nature of electrodermal activity changes during fear learning. This highlights the versatility of SCR as a reliable measure capturing various aspects of the fear conditioning process.

The observed alterations in Heart Rate Variability (HRV) provide additional insights into the modulation of the autonomic nervous system during fear conditioning. The decrease in HRV during the conditioning phase aligns with anticipated autonomic changes associated with the acquisition of fear. This physiological marker adds depth to our understanding of fear responses, highlighting the intricate interplay between psychological and physiological dimensions. However, previous research on the relationship between resting HRV and contextual anxiety has yielded inconclusive findings. One study reported an inverse relationship between baseline startle responding and resting HRV[17], while another found no such association[18]. Notably, the former study involved only women, whereas the latter included both men and women without exploring sex differences[18]. Although it is established that women generally exhibit higher resting HRV[19-21], the impact of gender on the modulation of emotional learning through HRV remains relatively unexplored. This study will include exploratory analyses to investigate the interaction between gender and resting HRV in the modulation of fear learning, shedding light on potential sex differences in this context.

The correlation analysis revealed meaningful associations between Fear Potentiated Startle (FPS) responses and distress ratings, underscoring the intimate connection between physiological startle responses and subjective distress. While a positive correlation trended between FPS and Fear of Cockroaches (FCQ) scores, the absence of statistical significance suggests a nuanced relationship in the context of specific fear stimuli. An intriguing finding emerged with a significant negative correlation between FPS and Trait Anxiety (STAI-T) scores, suggesting that individuals with lower trait anxiety exhibited heightened physiological startle responses, introducing complexity to the understanding of trait anxiety's role in fear conditioning. Furthermore, FPS exhibited a positive, non-significant correlation with Skin Conductance Response (SCR), hinting at potential interconnectedness between startle responses and electrodermal activity. The incorporation of Heart Rate Variability (HRV) values provided speculative insights into potential correlations with distress ratings, fear of cockroaches, and trait anxiety, emphasizing the necessity for future research to unravel the intricate interplay between HRV and psychological variables.
In interpreting the results, it's crucial to acknowledge the correlational design's limitations, cautioning against inferring causation from identified associations. The cross-sectional nature of the study provides valuable insights into connections between physiological responses and psychological variables. Still, the need for future research employing longitudinal or experimental designs is emphasized for a more profound understanding of the intricate interplay between physiological and psychological aspects of fear conditioning.

Conclusion

This study contributes to understanding fear conditioning in a diverse urban population, emphasizing the significance of individual differences. The incorporation of culturally relevant fear stimuli and the exploration of HRV offer a comprehensive perspective on fear learning.

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