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Affective Modulation of the Startle Reflex Is an Ineffective Methodology to Examine Depression-Linked Interpretative Biases

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Cognitive theory predicts that depression is associated with a bias to interpret ambiguous information in a mood-congruent fashion. This negative interpretative bias may serve as a maintenance factor for the continuation of a depressed mood state. The majority of studies investigating such interpretative biases suffer from a variety of methodological problems. This research has utilized an objective physiological measure involving the affective modulation of the human eye blink reflex in 25 depressed and 25 control subjects by depressive, depressive-ambiguous, and distorted stimuli. Almost half of the depressed subjects suffered from a comorbid anxiety disorder. In contrast to previous research utilizing the same methodology, depressed participants did not react differently to non-depressed participants in terms of their blink reflex response to the various stimuli types. This outcome is ascribed to the exclusion of anxiety-related stimuli in the current study. Depression-related stimuli failed to augment blink amplitudes in both subject groups. Therefore, affective modulation of the startle reflex is an ineffective methodology for the detection of depression-linked interpretative biases, as there is no difference to how individuals react to depressive and neutral stimuli. In this study, patients diagnosed with social anxiety disorder reacted to difficult-to-identify stimuli with augmented blink amplitudes, interpreted as an anxiety response.

Keywords: Depression, Social Anxiety Disorder, Ambiguous, Interpretative Bias, Startle Reflex

Introduction

The cognitive distortion known as interpretative bias—a propensity to interpret ambiguous or innocuous events and situations in a negative fashion—has long been associated with depressive illnesses (Beck, 1967, 1976). It is possible that such a phenomenon may act as a cognitive vulnerability to a depressive disorder, and also as a maintenance mechanism for the continuation of an existing depressed mood state. Previous papers investigating the existence of negative interpretative biases for ambiguous information in depression have relied primarily upon subjective experimental methodologies such as self-report (e.g., Watkins & Rush, 1983; Nunn, Mathews, & Trower, 1997) or evaluative feedback (e.g., Gotlib, 1983; Vestre & Caulfield, 1986) experiments. Unfortunately, this body of research is plagued by mixed results and methodological limitations such as experimenter demand effects, response selection biases, and depression-linked autobiographical memory influences (Clark & Teasdale, 1985; Williams & Broadbent, 1986; MacLeod & Mathews, 1991).

The difficulties and criticisms associated with previous research into depression-linked negative interpretative biases prompted the development of a novel objective methodology that was seemingly immune to such concerns. Lawson, MacLeod, and Hammond (2002) utilised the phenomenon of human startle reflex affective modulation in their investigation of interpretative biases in depression. The affective modulation of the human startle reflex was discovered by Vrana, Spence, and Lang (1988) and since confirmed by a multitude of studies over a diverse range of stimulus modalities and experimental conditions. The startle reflex is a protective mechanism neurophysiologically linked to the defensive motivational system's response to an aversive environmental cue. Therefore, the reflex is facilitated when an organism is exposed to an aversive or negative stimulus and inhibited when the appetitive motivational system is activated by a hedonic or positive stimulus (Lang, Bradley, & Cuthbert, 1992).

The affective modulation of the startle reflex allows for a psychophysiological test of the existence of a negative interpretative bias in depression that appears to be free from the major limitations of the various subjective methodologies. By presenting ambiguous stimuli to depressed individuals, it can be inferred from their startle reflex response to a loud burst of noise as to what particular interpretation they imposed upon the stimuli. Accordingly, Lawson et al. (2002) developed a set of negative, neutral, and ambiguous stimuli in order to test for an interpretative bias in depressed individuals.

The stimuli of Lawson et al.'s (2002) study consisted of recorded spoken words of either a negative affective valence (e.g., *alone*, *fright*, *lonely*), neutral affective valence (e.g., *dreamy*, *prime*, *wheat*), or an acoustically distorted hybrid word (e.g., *cancer* + *dancer*, *scream* + *stream*) that served as the ambiguous category of stimuli. Two groups of participants were recruited based on their scores on the Beck Depression Inventory (Beck, Steer, & Brown, 1996)—depressed and non-depressed university students. All of the participants were presented with one of the negative, neutral, or ambiguous words on each trial of the experiment and instructed to imagine a situation involving themselves and that particular word. An acoustic startle probe was generated during this imagery period on the majority of the trials and the blink reflex of participants was recorded as the measure of startle.

The results of Lawson et al.'s (2002) experiment revealed that both groups exhibited increased blink amplitudes after presentation of the negative words in comparison to the neutral words, which is consistent with previous studies of blink reflex affective modulation. However, the two groups differed in their reaction to the ambiguous stimuli. Non-depressed participants reacted to the ambiguous words in a neutral manner whereas depressed participants reacted to them as if they were negative. This finding suggests that the depressed group considered the ambiguous stimuli unpleasant or negative, and therefore, gives support to the hypothesis made by Beck's (1967, 1976) cognitive theory that depression is associated with the tendency to impose a negative interpretation upon ambiguous information.

Although it appears that Lawson et al.'s (2002) study had demonstrated that a negative interpretative bias for ambiguous information exists in depressed individuals, three major limitations can be identified with the research. Firstly, the participants consisted of a homogeneous student population and the depressed group did not experience clinical levels of depressive symptomatology. Secondly, a large number of the negative words used in the study accessed emotional themes of anxiety rather than depression. Anxiety and fear related words such as *rape*, *attack*, and *scream*, for example, may have induced greater levels of emotional arousal than depression related words such as *alone*, *gloom*, and *grief*, for example. The amplitude of the human startle reflex is not only subject to the affective valence of a stimulus, but also to its emotional arousal (Sabatinelli, Bradley, & Lang, 2001), and previous research indicates that sad stimuli fails to evoke augmented startle responses (Bradley, Codispoti, Cuthbert, & Lang, 2001). Finally, the ambiguous stimuli differed from the non-ambiguous stimuli on a property other than ambiguity of meaning; they were also more difficult to understand. Participants with elevated levels of anxiety may have worried that their inability to correctly determine the ambiguous words would have embarrassing or unpleasant consequences, thus increasing the negative affect and emotional arousal of the stimuli, and subsequent blink amplitudes.

Several modifications to Lawson et al.'s (2002) methodology were introduced in the present study to determine if their results were representative of clinical depressive disorders, and dependent on participant anxiety rather than depression. Firstly, a clinically depressed sample was recruited for participation. Secondly, only negative words of a primarily depressive theme were retained in the auditory stimulus set. Finally, a fourth category of stimuli was created in order to control for possible anxiety effects stemming from indecision over the ambiguous words. This category was similar to the ambiguous category, but was composed of acoustically distorted neutral words. If depressed individuals found both the distorted and ambiguous categories of stimuli aversive, then it could be assumed that it was the uncertainty over stimulus identity that they found unpleasant, rather than resorting to the notion of a depression-linked interpretative bias for the ambiguous stimuli.

Methods

Participants

Twenty-five clinically depressed psychiatric outpatients and 25 healthy control subjects matched with the patient group for age and gender participated in the study. All of the subjects reported English to be their primary language. Participants in the clinically depressed group were outpatients recruited from the Centre for Clinical Interventions in Perth, Western Australia, and all were about to commence cognitive-behavioural therapy for a depressive episode. Psychologists at the clinic selected those patients they deemed appropriate for the study based on diagnosis, current symptomatology, and capability to give informed consent. Exclusion criteria for the study included the presence of brain damage, neurological disorder, psychosis, mania, or substance abuse disorder. Seventeen (68%) of the patients were diagnosed with Major Depressive Disorder, six (24%) were diagnosed with Bipolar Disorder (currently in a depressive phase lasting at least two weeks), and two patients (8%) were diagnosed with a Major Depressive Episode superimposed on Dysthymic Disorder.

Twelve (48%) of the depressed outpatients suffered from a comorbid anxiety disorder (social anxiety disorder = 5, generalised anxiety disorder = 3, agoraphobia with panic = 2, agoraphobia without panic = 1, and panic disorder = 1). Fourteen (56%) of the patients were currently prescribed antidepressant medication (selective serotonin re-uptake inhibitors). Comorbid anxiety was considered a factor for covariate analysis.

Healthy control subjects were recruited from the general community through the use of advertisements and information sheets. Mean ages of the patient and control group were 42.2 (range 24 - 69) and 37.7 years (range 19 - 59) respectively. Mean years of education was 13.8 (range 9 - 22) and 15.8 (range 10 - 24) respectively. Both groups consisted of fifteen (60%) females.

Target Stimuli

The auditory stimulus sets used in this experiment were generously provided by Lawson et al. (2002) and were therefore identical to those employed in their own study. Eighteen healthy volunteers rated words from the negative category on two five-point Likert measures, the first assessing the degree to which each individual word accessed emotional themes of anxiety and/or depression, and the second assessing the emotional arousal level of the word. Twenty of the negative words that were deemed to be the most closely related to sad and depressed affect were selected for use in the experiment. Negative words such as *attack*, *battle*, *cancer*, *explode*, *fear*, *fright*, *rape*, and *threat*, for example, were discarded, leaving only words related primarily to depressive themes for use in the experiment. The remaining twenty depression-linked negative words included *alone*, *gloom*, *grief*, *guilt*, *inferior*, *lonely*, *loser*, and *sorrow*, for example. In contrast to the anxiety related words (mean = 3.28; $t_{(38)} = 11.3$, $p < 0.05$), the depressive words (mean = 1.23; $t_{(38)} = 1.7$, $p > 0.05$) were not rated significantly higher than the neutral words (mean = 0.73) in terms of emotional arousal.

Ten of the depressive words were left intact and the remaining half were used in their ambiguous form as created by Lawson et al. (2002), for example, *lonely* + *lovely* = *lo*ely*. Ten intact neutral words were selected at random from Lawson et al.'s (2002) neutral category set to act as the control stimuli. The distorted neutral category of words was developed by mixing the neutral words with their reversals. For example, the word foot was fused together with its reversal *teef* to produce a distorted neutral word that was quite difficult to hear and understand correctly.

In summary, the auditory stimuli for this study consisted of 40 single spoken words divided into four separate categories: depression-linked negative words (e.g., *alone*, *grief*), depression/neutral ambiguous words (e.g., *gloom*/*bloom*, *guilt*/*built*), neutral words (e.g., *dancer*, *river*), and distorted neutral words (e.g., *caring*/*gnirac*, *silent*/*tnelis*).

Experimental Hardware

An Acer TravelMate 212 TXV notebook was used to present the visual and auditory stimuli involved in the experiment, and to record subject eye blink responses. These responses, defined as increases in electromyographic (EMG) activity from the orbicularis oculi muscle of the left eye, were essentially electric signals produced by the motor structures that elicit muscle contractions in that region. The signals were recorded using two tin-cup surface electrodes 6-mm in diameter and 2-mm in depth (Electro-cap International, model E-21, USA). The electrodes were filled with conductive paste (Ten 20 conductive EEG paste) in order to provide some consistency to the hydration and conductivity of the skin beneath the electrodes. One electrode was placed approximately 1-cm below the pupil while the other was positioned 1-cm apart from the first, at the lateral canthus. A third electrode, the ground electrode, was a silver/silver-chloride disposable adhesive patch placed on the participant's left hand.

The EMG signal detected by the electrodes was conveyed to a high performance AC preamplifier (Grass Instruments, model CP511, West Warwick, USA) where it was amplified and broadly filtered using a low pass cut-off of 1-kHz and a high pass cut-off of 30-Hz, with an additional 50-Hz notch filter. The filtered signal was relayed to a connector box (National Instruments, San Diego, USA), and then transmitted to a 12-bit analog-to-digital converter (DAQ card, model 6062E, National Instruments, San Diego, USA) where it was digitised and sampled at a rate of 1-kHz. The system recorded the signal for a time period of 500-ms for each individual trial (100-ms prior to onset of the blink-eliciting stimulus and 400-ms after its occurrence). The recorded EMG activity was then stored on the notebook's hard drive for subsequent offline scoring purposes.

The auditory stimuli and the blink-eliciting stimuli were both presented by the notebook. The former consisted of the recorded spoken words and the latter consisted of a 30-ms burst of white noise with a near-instantaneous rise time created by a LabVIEW program (National Instruments, San Diego, USA). A white noise generator (Goldline, USA) provided background white noise, and this input was combined with that of the notebook by a 2-channel stereo preamplifier performance mixer (Stanton Magnetics, model ESM-11, China). This ensured the background white noise was presented at a level of 60-dB (A), the auditory stimuli were presented at 62-dB (A), and the blink-eliciting stimuli were presented at 97-dB (A). All of these sounds were conveyed to participants through a pair of headphones (Optimus Pro, model XB-100, Intertran, Australia), which also attenuated ambient noises from the environment. A sound level meter (Bruel & Kjaer, model 2237A, Australia) was used to calibrate the levels of these auditory stimuli prior to each session of testing.

Experimental Software

A LabVIEW version 6.1 (National Instruments, San Diego, USA) program developed by two of the authors controlled the presentation and timing of all the visual and auditory stimuli, with the exception of the background white noise, which was on continuously during the startle testing procedure. In addition to experimental control, the program also collected the physiological data from participants and stored it in raw form on the notebook's hard drive for later analysis. The LabVIEW program rectified the data and digitally filtered the rectified data using a finite impulse response band-pass filter, with a low pass filter set at 250-Hz and a high pass filter set at 78-Hz. Finally, a second-order low-pass Chebyshev filter smoothed the data.

A second LabVIEW program was devised for the purposes of scoring the signal. The first function completed by the program was to convert the filtered, rectified, and smoothed EMG signal from digital to metric units (μV). The second function of the program was to score the data. The scoring program detected the onset amplitude threshold of each blink (defined as the mean plus three standard deviations of value of the baseline amplitude of the 100-ms of activity occurring before the onset of the startle stimulus), the peak amplitude of each blink (the maximum point of the signal exceeding onset amplitude threshold), and the end of response of each blink (defined as the first time that the signal fell under the onset amplitude threshold since the beginning of the blink).

The program applied three criteria to the scoring of blink traces to ensure that only those blinks included in the analysis could be confidently linked to the effect of the blink-eliciting stimuli and not to randomly occurring endogenous blinks. Firstly, blink responses were excluded if the onset amplitude threshold did not transpire within 17-ms to 150-ms of the blink-eliciting stimulus. Blinks occurring outside of this window were unlikely to be time-locked to the stimulus. Secondly, blink responses were discarded if a blink occurred in the 100-ms prior to the blink-eliciting stimulus, because such blinks could produce paired-pulse inhibition. Finally, if the program was unable to detect any blink at all (i.e., the onset amplitude threshold was not reached and pre-startle baseline activity was normal), then the trace was rejected from the study. Approximately 10% of blink traces were excluded from the study on the basis of these three criteria.

Procedure

Prior to the arrival of the participant, the equipment was calibrated for internal voltage readings and for the appropriate sound levels. Calibration was conducted in the same fashion for every individual testing session. After the participants had completed the information and consent procedure, they were seated before the computer and had electrodes attached to the orbicularis oculi muscle under their left eye and to the area of skin on top of their left hand. The subjects next received verbal instructions as to what the task entailed and what was required of them. They were then given a set of headphones to wear for the purposes of auditory stimuli presentation. Participants were informed that the screen would display three colored lights, which would guide them through each trial of the experiment. The illumination of the green light signaled the beginning of a trial, and it remained lit for the duration of the entire trial. After a two-second interval, an auditory target stimulus (a neutral, negative, ambiguous, or distorted word) was played. An amber light was then illuminated 100-ms after the presentation of the auditory stimulus, indicating that the participant should begin to imagine a situation involving themselves that was evoked by that particular word. To encourage compliance, participants were told that the experimenter would question them as to what imagery they created for specific words at the conclusion of the experiment.

Eight-seconds after the amber light illumination, both of the lights were terminated and a red light was illuminated to signal the end of that particular trial. Participants were asked to stop imagining and to relax when the red light was turned on. An intertrial interval of thirteen-seconds elapsed before the commencement of the next trial, which was signaled by the illumination of the green light. Blink-eliciting stimuli were presented on 71.4% of the trials, always occurring 5.5-seconds into the imagery period. This was to ensure that the startle stimulus was relatively unpredictable. The startle stimulus was also played randomly on approximately 16.7% of the intertrial intervals, always occurring 5-seconds into the intertrial interval. The purpose of this intertrial interval startle was also to make the blink-eliciting stimulus less predictable to participants. The subjects were then informed that the first three trials were practice trials, and that after successful completion of these trials, they would embark on the actual experiment, which consisted of the 56 trials.

Results

Three control subjects and one patient were excluded from the results for failure to blink in response to the blink-eliciting stimuli. The criterion for exclusion was if a participant blinked on less than 50% of the trials in any one of the four auditory stimulus categories. The study therefore analysed the data from 22 controls and 24 patients. The recorded blink amplitude EMG data was analysed in a mixed-design General Linear Model of analysis of variance (ANOVA). Pair-wise comparisons were conducted using the Sidak adjustment for multiple comparisons. Unless stated otherwise, sphericity of the data was violated as determined by Mauchly's test and the Greenhouse-Geisser adjustment was used in the analysis of results.

Figure 1 shows that the overall size of the blink amplitudes of depressed participants (n = 24) was not significantly different from that of the control subjects (n = 22; $F_{(1,44)} = 1.52$, $p > 0.05$, $\eta_2 = 0.03$, power = 0.23). There was no significant difference in the amplitude of blink responses to the various stimuli types, with all participants displaying approximately equivalent responses to each stimulus category ($F_{(2.4,107.2)} = 0.17$, $p > 0.05$, $\eta_2 = 0.00$, power = 0.08, $\varepsilon = 0.81$). There was also no significant interaction involving participant mood and stimuli type, indicating that there were no fundamental differences in how depressed and control subjects reacted to each of the stimulus types ($F_{(2.4,107.2)}$ = 0.28, $p > 0.05$, $\eta_2 = 0.01$, power = 0.10, $\varepsilon = 0.81$).

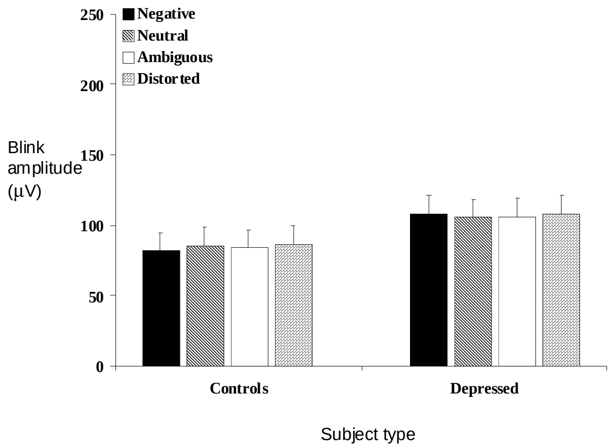

Figure 1.
Blink amplitude data with standard error bars for each stimulus type as a function of participant mood.

Discussion

The results of this study, did not replicate Lawson et al.'s (2002) finding that depressed individuals exhibit an interpretative bias for the negative plausible alternative of the ambiguous words. The depressed patients did not exhibit increased blink reflex amplitudes in response to the ambiguous stimuli. In addition, neither the control group nor the depressed group responded to the negative-depressive category of stimuli with augmented blink amplitudes with anxiety-related negative words excluded from the study. Thus, the larger startle responses displayed by both the control subjects and the depressed subjects in Lawson et al.'s (2002) study may be attributed to the presence of anxiety-related negative words such as *rape* and *attack* in that experiment. The failure of the depression-related negative words to evoke larger blink amplitudes in response to a startling stimulus supports previous affective modulation research (Bradley et al., 2001; Kreibig, Wilhelm, Roth, & Gross, 2011) This may be due to the effects of the low arousal level of the depressive words or to the inherent difference between the emotions of anxiety and depression with regard to startle reflex response.

Bradley et al. (2001) discovered that in contrast to anxiety-provoking visual images (e.g., animal attack and mutilation), pictures of a depressive nature (e.g., loss and pollution) failed to evoke a heightened blink response in their sample of healthy university students. In addition to the probable low arousal effect, Bradley et al. (2001) hypothesized that negative stimuli can vary in its theoretical "relevance" to life and immediate survival. Anxiety-related negative stimuli are perceived to be most threatening to an individual's existence, and thus activate the defensive motivational system, while depression-related negative stimuli are considered to be of far less immediate consequence and result in minimal activation of the system. The result of the present study replicates that of Bradley et al. (2001) with regard to the effects of depressive stimuli.

Covariate analysis controlling for comorbid anxiety in the depressed group revealed no significant effects. However, an interesting result was discovered in the five patients with a comorbid diagnosis of social anxiety disorder. They exhibited

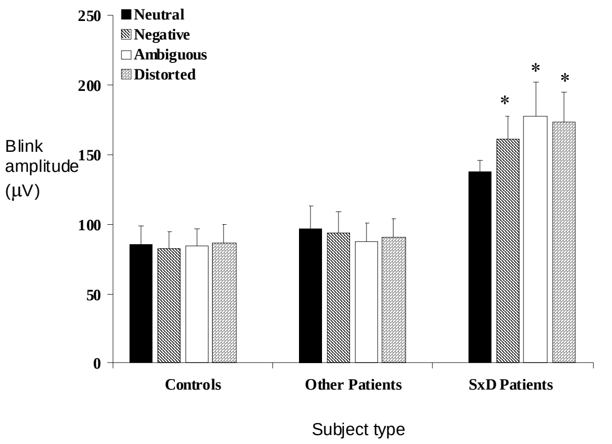

Figure 2.
Mean blink amplitude with standard error bars for each stimulus type as a function of participant mood and social anxiety disorder diagnosis (*asterisks denote a significant difference* [$p < 0.05$] *from the neutral category within each group*).

significantly larger overall blink amplitudes compared to the control group and the other depressed patients ($F_{(2,43)} = 3.67$, $p < 0.05$, $\eta_2 = 0.15$, power = 0.64). A significant main effect was discovered for stimulus type ($F_{(2.6,111.8)} = 3.10$, $p < 0.05$, $\eta_2 = 0.07$, power = 0.67, $\varepsilon = 0.87$), and there was also a significant interaction involving participant type and stimuli category, indicating that a diagnosis of social anxiety disorder had an influence upon the amplitude of blink responses to the various categories of stimuli ($F_{(5.2,111.8)} = 4.35$, $p < 0.01$, $\eta_2 = 0.17$, power = 0.96, $\varepsilon = 0.87$). Pairwise comparisons revealed that in contrast to the control group and the other depressed patients, socially anxious patients exhibited significantly larger blink amplitudes to the negative, ambiguous, and distorted stimuli in comparison to the neutral stimuli (Figure 2).

It can be inferred from this increased amplitude of the startle response that the socially anxious patients were in a highly anxious state throughout the experiment. This could be attributed to the experimental context itself, which involved aspects of social interaction, observation, and perceived evaluation. The DSM-IV describes social anxiety disorder as a "marked and persistent fear of one or more social or performance situations in which the person is exposed to unfamiliar people or possible scrutiny by others" (APA, 1994). Therefore, it is likely that the experimental situation evoked significant anxiety in socially phobic individuals due to fact that it involved observation and scrutiny by an unfamiliar person (the experimenter).

It is possible that patients with social anxiety disorder may have felt that they were 'failing' the experiment by not correctly identifying the various words, and this would result in them being evaluated negatively by the experimenter and appearing foolish in the eyes of others. Such a perception would render the ambiguous and distorted stimuli aversive and threatening in this patient group, leading to further worry and anxiety, and a subsequently augmented startle response. The results from the social anxiety subgroup primarily indicate that the procedure has sufficient power to detect a real effect. However, due to the small sample size of the social anxiety disorder subgroup, replication with a larger number of patients with social anxiety disorder is required.

The current finding that neither words with depressing content nor ambiguous stimuli that can be plausibly interpreted as either a neutral or depressive word are sufficiently aversive to affect the startle reflex has a major implication for the utility of Lawson et al.'s (2002) procedure. The use of depressive stimuli for the affective modulation of the eye blink reflex indicates that, while considered unpleasant in ratings of affective valence, depressive stimuli do not increase blink amplitudes relative to neutral stimuli in healthy controls and depressed patients without a diagnosis of social anxiety disorder. Therefore, Lawson et al.'s (2002) methodology is not capable of detecting the presence of a depression-linked interpretative bias, as there seems to be little difference in how individuals react to depressive and neutral stimuli (Bradley et al., 2001; Kreibig et al., 2011). Based on this observation, it would be impossible to determine whether an individual interpreted an ambiguous stimulus in a depressive fashion from their startle response to that stimulus.

The results of Lawson et al.'s (2002) experiment may therefore be attributed to the presence of highly arousing anxiety-related words. Their anxious subjects may have exhibited an interpretative bias for threat-related words in the ambiguous stimuli, or alternatively, simply found the ambiguous stimuli aversive for some other reason. An important implication from the results of the current study is that participant social anxiety levels may act as a major confounding factor in experimental research designs investigating depressive illnesses or symptoms, particularly those employing physiological assessments such as the startle response.

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