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Is Fear Generalization influenced by the unknown?

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IS FEAR GENERALIZATION INFLUENCED BY THE UNKNOWN?

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ABSTRACT

Social anxiety is characterized by intense fear or discomfort in social situations, such as public speaking, meeting new people, or interacting in groups (National Institute for Health Excellence and Care, 2013). This fear may be out of proportion to the actual threat and may lead to avoidance of these social situations. This is described as fear generalization and mainly consists of transferring a learned fear response associated with one specific stimulus to a new one (Huggins et al., 2021). Excessive fear generalization (fear overgeneralization) has been proved to be involved in the perpetuation and intensification of social anxiety symptoms (Aslanidou et al., 2023; Lemmens et al., 2021). Actually, some studies distinguish fear and anxiety not only by their duration (phasic vs sustained), but also by the nature of the threat (predictable vs unpredictable) (Schmitz & Grillon, 2012; Wieser et al., 2016). Thereby, it is reasonable to think that all these concepts are related. Previous findings have been proving that the more an event is perceived as unpredictable, the more likely it is of inducing anxiety (Schmitz & Grillon, 2012; Zvolensky et al., 2000).

Hence, although it is beyond doubt that fear generalization and unpredictability contribute to the development of social anxiety symptoms, the direct relationship between unpredictability and fear generalization remains unclear. Considering this gap, the purpose of this study is to determine if different levels of unpredictability influence fear generalization and at what extent it affects social anxiety (Lemmens et al., 2021).

In this research, two groups were submitted to a fear conditioning paradigm with a generalization test, with different levels of unpredictability. One of the groups was the Unpredictable Group and the other the Predictable Group, with different instructions given at the beginning of the experiment. Subjective (expectancy, valence, and arousal ratings), psychophysiological (skin conductance response, SCR), and visuocortical (steady-state visual evoked potentials, ssVEPs) indices of fear were recorded. Levels of social anxiety (Liebowitz Social Anxiety Scale, LSAS) and intolerance of uncertainty (Intolerance of Uncertainty Scale, IUS) were quantified for each participant, as well as depression (Beck's Depression Inventory, BDI-II) and anxiety levels (State-Trait Anxiety Inventory, STAI).

The research showed that predictability of cues did not significantly influence participants' subjective responses to the conditioned stimulus (CS+), regardless of the unpredictability created. In addiction, participants found the CS+ more unpleasant, more arousing, and more likely to be followed by the unconditioned stimulus (US) as indicated by the valence, arousal, and US-expectancy ratings respectively. This study also suggests that the unpredictability of cues does not lead to a notable enhancement in the brain's response to the CS+.

In summary, the present study implies that fear generalization is unaffected by unpredictable threats. Although it is unlikely that unpredictability alone may cause fear to overgeneralize, the unpredictability of a threat may not be completely separated from the generalization of fear reactions. This suggests that understanding the underlying mechanisms behind fear reactions could also potentiate the understanding of unpredictable events and the mechanisms related

to it. However, further research is required to address the unanswered questions this study raised. Additionally, higher levels of social anxiety symptoms were found to be related with higher intolerance of uncertainty.

Keywords: social anxiety, fear generalization, unpredictability, steady-state visual evoked potentials, electroencephalography

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INTRODUCTION

Everything that we do has a reason. As humans we make plans, and we expect them to work out to succeed personally and professionally. However, not everything that we plan will have the exact outcome that we predicted. Almost nothing occurs exactly the way we designed it. The world is unpredictable. Work, relationships, business, everything is influenced by so many circumstances that is impossible to predict the result of every decision that we make. Unpredictability is a reality that we need to accept throughout our lives, but each one of us has a different way of dealing with and responding to it. People who suffer from anxiety-related disorders, such as social anxiety disorder, generalized anxiety disorder, and panic disorder, for example, are more likely to experience unpredictability in a much more negative way than healthy individuals (Zvolensky et al., 2000). In this study, we will explore if unpredictability and the excessive generalization of fear are affected by each other and to what extent they act together on potentiating social anxiety symptoms.

1. Social Anxiety Disorder

Social anxiety is when people feel symptoms of anxiety or fear in circumstances where they can be judged or observed, such as speaking in public, meeting new people, or even doing everyday activities, such as eating in front of others (Grupe & Nitschke, 2013). Overall, social anxiety disorder is among the most prevalent of the anxiety disorders in Europe, being the lifetime prevalence

of it in the general population close to 7%. It usually manifests during late childhood, and it is more common in women (NIH, 2002).

Patients with social anxiety disorder have significant functional impairment in initiating and maintaining social relationships and in the educational and work fields, such as work productivity and unemployment (Lecrubier et al., 2000). Because of that, it is relevant to understand what leads to this disorder and the mechanisms related to it, supporting the development of effective treatments that could improve the quality of life of the individuals.

Symptoms of social anxiety can differ from patient to patient. However, they include blushing or sweating, increase in heart rate, feeling "sick to their stomach", having a solid body posture, avoiding places with other people, and feeling self-conscious that people will judge them negatively. Although social anxiety has a degree of heritability, researchers found that stress and environmental factors play an important role in the disorder. Treatments for social anxiety include psychotherapy, medication, or a combination of both (NIH, 2002).

Neuroimage research on social anxiety suggested dysfunction in distributed circuits involving the amygdala, insula, hippocampus, and orbital frontal regions of the brain (Haley & Freeman, 2018) and in serotonin regulation (Stein & Andrews, 2015). These brain areas form interconnected networks with visual processing areas, playing crucial roles in modulating attention, emotion, and cognition, which can influence how individuals perceive and respond to social stimuli, including

visual information. Thus, the literature tells us that symptoms of social anxiety can be associated with visuocortical system engagement (Stegmann et al., 2020).

The visuocortical system refers to the neural pathways and structures involved in processing visual information; from the moment our eyes capture light to the interpretation of that information in the brain. In the retina of the eyes, many cells have different functions in these mechanisms. When light stimulates a photoreceptor cell within the retina, these cells transform light energy into an electrical signal. Then, the electrical signal will be directed to bipolar cells, which will send the signal as information to the brain. At the same time, neighboring horizontal cells are activated. These horizontal cells inhibit the activity of adjacent photoreceptor cells through inhibitory neurotransmitters, such as GABA (gamma-aminobutyric acid). This is referred as lateral inhibition and results in an enhancement of the contrast between regions of light and dark, sharpening the perception of edges and boundaries in the visual scene. Recent studies found that the essential aspects of visual processing rely on lateral inhibitory feedback to photoreceptors by horizontal cells (Barnes et al., 2020; Stegmann et al., 2020). When studying anxiety-related symptoms, it was found that the accentuation of the lateral inhibition pattern increases with the severity of social anxiety (Stegmann et al., 2020).

As it will be discussed in the next chapter, anxiety symptoms are usually related to an excessive generalization of fear, that is, an excessive tendency to recognize ambiguous stimuli as threatening to perceptual similarity to a previously learned threat, leading to the avoidance of the new scenario, even though they

are not the same (Aslanidou et al., 2023; Huggins et al., 2021). This becomes problematic when individuals respond with fear to environmental cues that signal safety (Aslanidou et al., 2023; Lissek et al., 2014). Thereby, fear conditioning protocols are valuable approaches to go deeper in the understanding of this disorder and the underlying mechanisms that occur in the visual cortical engagement (Ahrens et al., 2016; Cushman & Fanselow, 2010; Friedl & Keil, 2021). A fear conditioning protocol consists of the process through which a neutral conditioned stimulus (CS+; e.g. a neutral female face) elicits fear by being followed by an aversive unconditioned stimulus (US; e.g., a loud female scream) (P. Pavlov, 1927). Researchers found that patients with anxiety disorders differ from healthy participants in fear learning processes by the enhanced acquisition, resistance to extinction, and overgeneralization of conditioned fear (Ahrens et al., 2016).After introducing the participant to the CS+, similar stimuli called generalized stimuli (GSs) are presented to create generalization of fear, even though the US is still only related to the CS+. In other words, the participants learn to fear a specific cue. So, when other similar cues are presented, they will probably anticipate fear and be in an enhanced state of alert and discomfort by the possibility of the occurrence of the aversive US (Ahrens et al., 2016; Cushman & Fanselow, 2010).

The present study aims to evoke fear generalization in different groups, one that receives the threat as an expected cue (Predictable Group) and another that hears it randomly (Unpredictable Group). Comparing the generalization created in individuals from the different groups, it is possible to understand the impact of unpredictability in fear overgeneralization, that is the excessive generalization of

fear responses. Complementary to this, questionnaires were used, including a scale that measures social anxiety symptoms (LSAS) (Safren et al., 1999). Then, analyzing generalization responses in participants with higher scores of social anxiety could be an important for this study if both groups were compared and differences between them were found, since they represent the different scenarios of unpredictability created. This aims to investigate the impact of unpredictable events in fear overgeneralization and to what extent this relationship has an effect in social anxiety symptoms.

Using a similar fear conditioning paradigm, a previous study made in the same research group as the present one showed that CS+ was equally unpleasant and arousing to the participants compared to the other GSs, regardless of low expectations of the threat. This can be compared to the challenges experienced by individuals suffering from clinical anxiety in controlling their protective responses, even when they are aware that these responses are exaggerated (Aslanidou et al., 2023) .

2. Fear Generalization and Overgeneralization

Fear generalization is a process that enables individuals to respond appropriately to new or harmful situations. Fear generalization can help an organism survive potential threats as an adaptive process that relies on the presence of perceptual features that are similar to a learned threat cue (Huggins et al., 2021). This can be seen almost every day in daily situations with higher or lower aversive threats. For example, when a person is fast driving a car while it is

raining and suddenly loses control of it causing an accident, the next time they drive in the rain, their attention and caution will be much higher, because they learned that it can be a very dangerous situation. This unconscious learning mechanism that usually everyone develops throughout their lives is crucial to avoid unsafe situations and of course to guarantee their survival. With personal growth and life experiences, every individual learns to be careful in obvious situations like this, but others who experience traumatic events, for example being in a war, will make this association even when it is not necessary. This leads to excessive defensive responses resulting in a maladaptive process called fear overgeneralization. In short, fear overgeneralization refers to the excessive spreading of fear from genuine threat-related stimuli to similar but innocuous cues (Aslanidou et al., 2023; Huggins et al., 2021).

Clinical studies showed that this overexpression of fear can cause high levels of distress and impairment in an individual's daily functioning, contributing also to avoidance of daily activities that provide positive reinforcement. This refers to the process of increasing the likelihood of a desired behavior by adding a stimulus following behavior, for example, if every time that you work out, you receive a flower, you are going to like this activity not only of the activity itself but also because of the reward as the form of a flower. However, when an individual experiences overexpression of fear because of some previous experience, it can cause the person to avoid these activities that would typically make them feel good. For example, if you have a fear of social situations, as in social anxiety disorder, if the gym where you work out is starting to become famous, there are

going to be a lot more people in there and working out starts to become something that makes you uncomfortable and eventually you may start avoiding it because of the distress that it causes you (Huggins et al., 2021).

Researchers discovered that anxiety disorders are associated with disrupted function in brain regions relevant to fear generalization, such as the insula, amygdala, and ventromedial prefrontal cortex (vmPFC). The meta-analysis made by Cooper et al. (2022) discusses this relationship in a well-detailed manner. Neuroscience research showed that the molecular explanation of fear generalization focuses on the hippocampal function in pattern separation of related but distinct experiences. In other words, prolonged stress may lead to an impairment in the hippocampal ability to differentiate patterns, making it more difficult to distinguish between danger and safety, leading to overgeneralization. The paper relies on 20 studies and concludes that heightened fear generalization is a marker of anxiety-related disorders, including social anxiety disorder. Knowing that stress causes difficulty in differentiating stimuli, differential fear conditioning remains the most common protocol to measure fear-based processes in humans (Aslanidou et al., 2023; Cooper et al., 2022; Huggins et al., 2021; Lissek et al., 2014; Stegmann et al., 2020; Zhao et al., 2022). Also, generalization tests are better approximations of the clinical reality of anxiety symptoms, even though the experience of living with pathological anxiety can be much more complex due to the unpredictability of the events in the daily life of the patients (Cooper et al., 2022).

As explained before, fear conditioning is a type of associative learning task in which the subject associates a particular previously neutral stimulus (CS+) with an aversive one (US), such as an electric shock or, as in this study, a loud noise. This association results in a Conditioned Response (CR) to the CS+. When other similar stimuli (GSs) are then presented, individuals generalize their responses, since they learned to fear the CS+ leading to the anticipation of the same threat from these similar stimuli. These protocols aim to give the researcher a quantification of participants' fear responses elicited by each GS. This way, it allows the assessment of how fear generalizes across stimuli that vary gradually from being highly similar to the CS+ to being a lot different from it, even though they are all very similar (Ahrens et al., 2016).

The relationship between fear generalization and anxiety starts in a failure to extinguish the fear-conditioned response. In other words, pathological anxiety results from a failure to inhibit the fear response in the presence of a safety event. The meta-analysis showed that patient-control differences in the acquisition of simple conditioning (with only CS+ and US, as used in the present study) were significantly larger than such differences in the acquisition of discrimination learning (with both excitatory and inhibitory associations). The results showed greater excitatory conditioning to danger cues among patients with anxiety disorders, meaning that they present increased learning and sensitivity to cues associated with danger or fear, making their responses more pronounced when presented with these cues compared to healthy individuals. Thus, anxious participants also presented impaired inhibitory conditioning to safety signals,

implying that individuals with anxiety disorders have difficulty learning to associate safety cues, since they may not effectively learn to suppress fear responses in the presence of these safety signals. This also contributes to their heightened fear responses even in non-threatening situations (Cooper et al., 2022; Lissek et al., 2005).

In this study we used a single-cue fear-conditioning protocol and the CS+ was a neutral female face. There were six additional neutral female faces during the task (GSs) varying in similarity to the CS+, but only CS+ was followed by the female scream (US). The strength of the CS-US association represents the magnitude of the CR (Aslanidou et al., 2023; Cushman & Fanselow, 2010).

Using fear conditioning, researchers found that overgeneralization of conditioned fear propagates anxiety cues in the individual's environment that are capable of evoking and maintaining anxiety (Lissek et al., 2014). Lissek et al. (2014) discovered that overgeneralization of fear may contribute crucially to the psychopathology of generalized anxiety disorder and that individuals who suffer from anxiety need less dangerous information for a stimulus to activate the fear system. They also found that although diverse types of danger signals may generate overgeneralization, once activated, the fundamental processes involved are likely similar across various disorders, including social anxiety, PTSD or GAD (Lissek et al., 2014).This supports another study (Lissek et al., 2010) that showed that overgeneralization contributes to the psychopathology of panic disorder, since it increases the likelihood of encountering panic reminders after an attack, increasing the risk of additional attacks and eventual panic disorder. In addition to

this, researchers gathered various studies to prove that anxiety also involves alterations to attentional processes that make threat detection easier, leading to a higher perception of dangerous situations. Individuals with social anxiety disorder, when presented with ambiguous social scenarios and facial expressions, were biased with their fear responses, usually overgeneralizing them. This represents the important role attention and excessive fear responses have on participants with social anxiety disorder (Grupe & Nitschke, 2013).

Overall, findings suggest that the high correlation between fear overgeneralization and social anxiety might be potentiated by the unpredictability of events, being important to have a better understanding of unpredictability and how individuals deal with ít (Grupe & Nitschke, 2013; Lissek et al., 2010, 2014).

3. Unpredictability

Unpredictability of threat is defined as the objective probability of an aversive event to occur, and it has been considered central in inducing anxiety (Grupe & Nitschke, 2013). Unpredictable threat is shown to be highly relevant for anxiety disorders, which are thought to result from maladaptive responses to uncertainty. During social interactions, people diagnosed with social anxiety disorder anticipate and worry about others' judgment, which can be ambiguous and certainly unpredictable. Even though a lot of them are certain that they will be negatively judged, being expecting it, they cannot accurately predict how that will happen. In social situations, there are often subtle and nuanced cues that influence social judgments, making it even more challenging for individuals with social anxiety to predict how others will perceive them. This ambiguity starts because

social interactions involve complex interpersonal dynamics, diverse social rules, and individual perception differences, suggesting that unpredictable situations are critical to the disorder (Clauss et al., 2020).

Zvolensky et al. (2000) developed the Perceived Predictability Index (PPI) which measures the unpredictability of internal and external anxiety-provoking stimuli. The study shows that people who perceive aversive events as more unpredictable are more likely to experience greater anxiety and fear in response to them, as unpredictability ratings for various stimuli are positively related to greater self-reported fear for those stimuli. When facing an unpredicted event, people develop different cognitive, emotional, and behavioral reactions, since different physiological systems respond differently to varying similarity with a fear stimulus.

In short, cognitive reactions involve mental processes such as thoughts, perceptions, and interpretations, for example how an individual interprets some cue or what the individual believes to be likely to happen, including their expectation of something to happen and even the realization of a dangerous situation when in one. On the other hand, emotional reactions involve subjective feelings or affective responses to stimuli. For example, fear is an emotional response to a perceived threat. In the present study, participants gave the researchers information about how arousing and unpleasant the faces (stimuli) made them feel, registering their emotional reactions. Alternatively, changes in behavior or observable actions are behavioral reactions that can also be observed

in response to stimuli, for example, the use of maladaptive strategies to manage or regulate emotions or stress, as in the case of fear overgeneralization.

In Zvolensky's et al. (2000) paper, three studies were made using different approaches to measure these reactions. Questionnaires were used to assess perceptions of predictability of cues for a large variety of anxiety-related events, including anxiety sensitivity, state and trait anxiety, and suffocation fear. This measured individuals' cognitive and emotional reactions, allowing the understanding of how anxiety or fear can be different from person to person. Direct measures of the central and/or peripheral nervous system can also give insights into different types of participants' reactions. In the cited research, they tested these different reactions by seeing that individuals responded with higher or lower degrees of predictability depending on the stressful life events that they have experienced or even just one that had more impact on them. People low in perceived predictability of internal events responded with greater cognitive and affective distress compared to their equivalents high in predictability for that same dimension (Zvolensky et al., 2000).

In the present study, participants also answered their expectation of a threat related to the stimuli (US-expectancy), giving researchers feedback on their cognitive reactions and questionnaires to access to participants' emotional reactions. Electroencephalography (EEG) was utilized to measure participants' electrophysiological brains activity, which can give insights into cognitive processes involved in the neural processing of a given stimuli, such as attention, perception, and even emotion regulation, that was observed with steady-state

visually evoked potentials (ssVEPs) later in the analysis. Steady-state visually evoked potentials are oscillatory responses in the visual cortex that occur at the same frequency as the modulation rate of the stimulus. Also, by measuring Skin Conductance Response (SCR), researchers in the current investigation quantified the stimulus-locked electrodermal activity, which allowed the evaluation of individuals' emotional arousal evoked by the stimuli.

Previous studies discovered that social anxiety moderates the relationship between the bed nucleus of the stria terminalis (BNST) and other brain regions in response to unpredictability. Studies in models of anxiety implied that lesions to the BNST reduced responses to contextual cues, while lesions to the amygdala reduced responses to explicit or direct threats. The BNST is responsible for responses to unpredictable threats, being highly important to the study of anxiety disorders due to the relationship between unpredictable events and the development of anxiety symptoms. Findings discovered greater activation of the BNST in response to unpredictable cues and that socially anxious individuals may encounter strong BNST-amygdala connectivity. These findings lead researchers to affirm that great sensitivity to contexts of unpredictability may be a trait that contributes to heightened social anxiety and may also serve to maintain anxiety over time (Clauss et al., 2020).

Regarding the relationship between unpredictability and fear, a study conducted in 2023 revealed that individuals with greater fear symptomatology at baseline (at the beginning of the study) were characterized by increased anticipation of the predictable threat. However, greater anticipation of

unpredictable threats at baseline was associated with higher increases in anxiety 1.5 years later (Wilson & MacNamara, 2023). Also, other findings showed that threat uncertainty did not lead to overgeneralization of the threat responses in any measure. Nevertheless, they also found that higher trait intolerance of uncertainty was associated with wider generalization in threat expectancy ratings (Aslanidou et al., 2023). However, the impact of intolerance of uncertainty is still unclear (Aslanidou et al., 2023).

4. Steady-state Visually Evoked Potentials

When individuals experience fear, defensive mechanisms are activated to give them instructions on what to do regarding the threat. These mechanisms can be measured by somato-visceral measures, such as fear-potentiated startle, skin conductance, and cardiovascular responses. In addition to these systems, either autonomic or motor, the human body automatically enhances its sensory processing. In other words, the perception and attention to threat becomes higher. Previous studies showed that heightened attention to threatening cues is pronounced in patients with anxiety disorders and that excessive attention to threatening stimuli has been considered influential in the basis of anxiety disorders. Neurophysiological measures from the visual cortex have been used to quantify the visuocortical responses and investigate more about attention mechanisms and processes that may be related to it. To quantify these responses, we used steady-state visually evoked potentials (ssVEPs) (Stegmann et al., 2020).

SsVEPs are oscillatory responses to luminance-modulated stimuli, such as flickering, in which the electrocortical response recorded from the scalp resonates at the same frequency as the driving stimulus (Kritzman et al., 2022; Norcia & Appelbaum, 2015). This electrophysiological measure of the visuo-cortical processing is modulated by the allocation of attention to visual stimuli in the external environment (Kritzman et al., 2022). These potentials allow the direct quantification of visuocortical responses to a specific stimulus.

This technique appears to be a good approach for monitoring processes related to attention and emotional arousal based on studies that showed enhancement of the ssVEPs amplitudes when associated with emotional (pleasant or unpleasant) pictures, compared to neutral ones. Also, unpleasant pictures can lead to slightly higher brain response amplitudes (Keil et al., 2003). This is in line with the statement that visual selective attention may be allocated to external stimuli according to their respective motivational or affective significance (Keil et al., 2003). Accordingly, there are specialized circuits that process emotionally significant information within the brain's visual neuronal network. These circuits may have developed stronger connections and could be more extensively distributed throughout the brain compared to circuits responsible for processing less emotionally arousing information. This suggested a prioritization or specialization within the brain's visual processing system for processing emotionally significant stimuli (Keil et al., 2003). Thereby, heightened ssVEPs amplitudes indicate increased visuocortical activation and enhanced attention to the driving stimulus (Müller et al., 1998; Stegmann et al., 2020).Previous studies

suggest that the discrimination of threat and similar stimuli by the visual cortex may be a pattern observed in neuroimaging and electrophysiology studies (Friedl & Keil, 2021; McTeague et al., 2015).

A study made by McTeague et al. (2015) showed that visual cortical activation corresponding to the lateral inhibition pattern emerged during acquisition. Then, Stegmann et al. (2020) used ssVEPs and fear conditioning protocols to study social anxiety. They found that during habituation and acquisition, individuals with higher social anxiety showed amplified visuocortical responses to the face stimuli. Also, ssVEPs amplitudes for the CS+ in the study were increased, followed by an immediate reduction for the closest GS (Stegmann et al., 2020). This supports the assumption that there is an enhanced visuocortical engagement to the CS+ and suppression of the stimuli with the highest similarity to the CS+.

Furthermore, several mental illnesses, including social anxiety disorder, have been linked to the inability to adaptively switch attention internal and external directed. So, studies that use ssVEPs to study neural mechanisms of maladaptive attention allocation can be a potential strength for science (Kritzman et al., 2022).

Using ssVEPs, researchers found in previous studies that high social anxiety is characterized by exaggerated brain responses to seeing emotional faces (angry, fearful, and happy) compared to neutral facial expressions, being the negative faces (such as angry stimuli and then fearful stimuli) the ones with higher ssVEPs amplitudes. A significant interaction was seen between high and low socially anxious participants in relative sensitivity to expressions. On the other hand, stimuli that implied rejection, disapproval, or interpersonal challenge were not the

only ones that caused increased neuronal mass activity in the visual cortex. Conversely, some unpleasant (fearful), as well as pleasant (happy) emotions, elicited equivalent occipitocortical facilitation, suggesting a broad visual sensitivity to emotional facial displays in social anxiety (McTeague et al., 2011).

Bearing this in mind, ssVEPs seem to be an interesting approach to studying the nature of fear generalization and its role in social anxiety disorder when the presence of unpredictability.

It is also relevant to notice that by measuring the brain's activity of participants, researchers also discovered that there are some brain regions sensitive to the effects of generalization such as fear excitation (amygdala and insula) and inhibition (ventromedial prefrontal cortex, vmPFC). Also, the hippocampus is proven to have an important role in fear generalization (Huggins et al., 2021).

5. Reasoning and Goals

The primary goals of this research is to examine if an unpredictable cue would lead to the overgeneralization of conditioned responses and how can that be a marker of social anxiety.

For this, we aim to deepen the understanding of social anxiety disorder and its underlying mechanisms. As mentioned before, social anxiety disorder can have a huge impact on individuals' lives. Thus, by delving into the factors contributing to social anxiety, such as fear generalization and unpredictability, researchers aim to identify potential targets for intervention and treatment. The study aims to

investigate the relationship between fear generalization and unpredictability, providing valuable insights into the development and maintenance of social anxiety symptoms. With that, it can be an enabler to identify predictors of social anxiety, by examining variables such as intolerance of uncertainty and physiological responses to fearful stimuli.

In the present study, the authors assess the activity of the central nervous system through electroencephalogram recordings, in which the ssVEPs were quantified. ssVEPs were analyzed after CS+ is presented on the screen during Acquisition and after all 7 stimuli (GS1, GS2, GS3, CS+, GS5, GS6, GS7) are presented during Generalization. This aims to understand if there is any significant difference between their amplitudes after the different stimuli. The activity of the peripheric nervous system was analyzed through skin conductance responses (SCR), and ratings of arousal, valence, and US-expectancy we also collected through self-report measures. Arousal and valence were collected regarding CS+ after Habituation and Acquisition and regarding all 7 stimuli after Generalization. US-expectancy was registered regarding the expectancy of each one of the 7 stimuli being followed by the US (female scream). To create different scenarios of predictability, the participants were randomly divided into two groups: one that receives instructions about the threat becoming an expected cue (Predictable Group) and another that hears it randomly without any instructions about the timing of the scream (Unpredictable Group).

6. Hypothesis

In this study, we want to examine if an unpredictable cue would lead to the overgeneralization of conditioned responses and how can that be a marker of social anxiety. For that, we used a fear generalization protocol with habituation to the CS+ (neutral face), acquisition with the CS+ associated with a loud scream (US), and generalization where similar stimuli to the CS+ were presented (GSs), but without the loud scream.

During generalization, we expect both groups (Predictable and Unpredictable) to find the conditioned stimulus (CS+) more unpleasant, more arousing, and more likely to be followed by the unconditioned stimulus (US) compared to the other stimuli, as indicated by the valence, arousal and US-expectancy self-report ratings, respectively (Aslanidou et al., 2023). Based on previous findings, we expect higher amplitudes in ssVEPs for the CS+ compared to the other generalized stimulus (GSs: GS1, GS2, GS3, GS5, GS6, GS7) (Stegmann et al., 2020).

We also expected participants from the Unpredictable Group to associate the CS+ with a more negative valence compared to those in the Predictable group, since anticipation can emerge from the unpredictability and the lack of control associated with unpredictable situations (Wieser et al., 2016). In the same line of thinking, the Unpredictable Group may experience higher arousal levels in response to the CS+ compared to those in the Predictable Group since the anticipation of an unpleasant outcome in unpredictable events can lead to heightened physiological arousal (Wieser et al., 2016). Also, individuals in the Unpredictable Group are also hypothesized to have higher expectancy of the

scream following the neutral face (CS+) compared to the Predictable one, due to the anticipation created in the unpredictable scenario they experienced (Wieser et al., 2016). We expect higher amplitudes in ssVEPs for the CS+ in the Unpredictable group compared to the Predictable one. Consequently, we predict an enhanced activation of the visuocortical system in the Unpredictable group after the CS+.

We expect participants with higher scores of social anxiety (Liebowitz Social Anxiety Scale) to present higher visuocortical responses (Stegmann et al., 2020; Zvolensky et al., 2000). We expect ssVEPs measures to give us a better understanding of the visuocortical system engagement associated with social anxiety symptoms. Based on previous research, we suppose that the cortical engagement in response to the stimuli associated with the US is highly related to social anxiety (Stegmann et al., 2020). We expect participants with higher scores of intolerance of uncertainty (Intolerance of Uncertainty Scale) to show higher scores on the social anxiety scale compared to participants with lower levels.

METHODS

1. Participants

A total of 56 participants were recruited through advertisements at Erasmus University of Rotterdam in exchange for course credit. Participants were excluded if they had a family history of photic epilepsy and/or suffer from tinnitus. We used G*power 3.1.9.4 to do a power analysis for acquisition to calculate the interaction between the seven stimuli (GS1, GS2, GS3, CS+, GS5, GS6, GS7) and the two groups (Predictable and Unpredictable), with a small to moderate effect

size f = .20, alpha set at .05 and power at 85. We used a mixed ANOVA and reached a total sample size of 30 participants. In this study, it was considered the power analysis of generalization, giving the information that a total sample of 30 participants would be sufficient, however we collected 56 to account for exclusions due to technical problems. All participants had normal or corrected-to-normal vision. Each participant was randomly assigned to one of the groups.

There was no statistical significance between groups (all p-values > .05) regarding sex ratio, age, social anxiety (LSAS), intolerance of uncertainty levels (IUS), depression (BDI-II), and anxiety (STAIS and STAIT). The sex ratio analysis was done with a chi-square analysis and the rest of the variables were analyzed with t-tests.

Means and standard deviations for all questionnaires, sex, and age can be found in Table 1 as well as the scores of que questionnaires: LSAS, IUSD, BDI-II, STAIS, and STAIT.

The experimental procedure of the current study was approved by the Ethical Committee of the Erasmus University Rotterdam in accordance with the declaration of Helsinki.

Table 1

Mean and standard deviations of the participants of each group (Unpredictable and Predictable) regarding sex, age, and the scores of LSAS, IUSD, BDI-II, STAIS, and STAIT

Variable	Unpredictable Group	Predictable Group	p-value		
N	29	27	χ^2 (df)		
Sex	F: 24 M: 4 NB:1	F: 21 M: 6 NB:1	1.531 (2)	.291	
	M (SD)	M (SD)	F(Sig.)		t-value
Age	20.14 (1.71)	20.26 (2.65)	2.79 (0.101)	.838	-0.205
LSAS	49.66 (23.61)	44.37 (24.47)	0.08 (0.778)	.415	0.822
IUS	66.14 (18.48)	58.60 (14.80)	1.64 (0.207)	.108	1.636
BDI-II	10.53 (6.46)	9.39 (5.97)	0.13 (0.909)	.495	0.687
STAIS	24.55 (5.12)	24.33 (4.21)	0.35 (0.555)	.863	0.174
STAIT	27.46 (4.27)	26.78 (3.00)	3.67 (0.061)	.495	0.687

Note. F = female / M = male / NB = nonbinary.

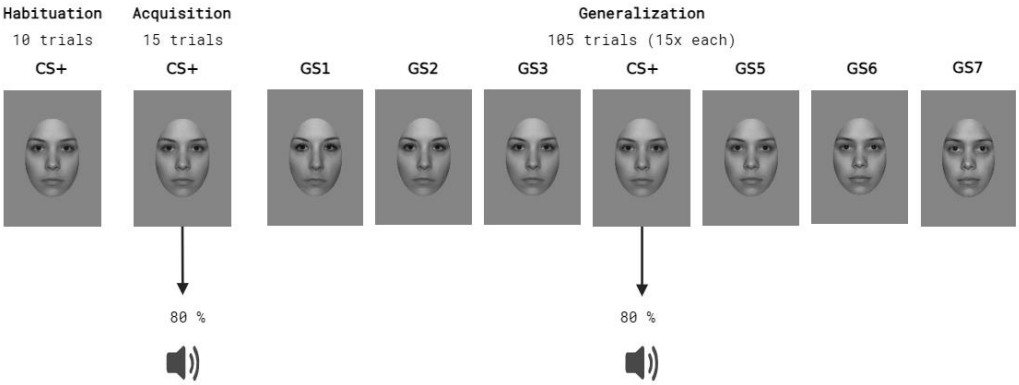
2. Materials

2.1 Stimuli

Using a face-morphing software named Sqirlz Morph, the stimuli were created from two female faces from the NimStim Set of Facial Expressions (03F NE C, 10F NE C) (Tottenham et al., 2009). The images were converted to a gray scale and luminance and brightness were kept constant. Those two faces were morphed in steps of 16.667% to create 5 morphs, being the CS+ the third morphed face. In total, there was GS1 (female face 1), GS2 (first morphed), GS3 (second morphed), GS4 (third morphed, Cs+), GS5 (fourth morphed), GS6 (fifth morphed) and GS7 (female face 2). GS1-GS3 and GS5-GS7 were the generalized stimuli (GSs) while morph 4 was the conditioned stimulus (CS+). Each picture was presented on a gray background (R: 133, G: 133, B:133) flickering at 15 Hz. Participants were seated 120 cm away from the screen. The US was a female scream combined with a white noise of 90 dB which lasted for 1s and was presented through four field speakers. No other stimulus than CS+ received US reinforcement.

Figure 1

Experimental procedure and conditioning protocol



Note. In the habituation phase, no US was presented. In acquisition, the reinforcement rate for the CS+ - US coupling was the same for both groups (80%), but the Unpredictable Group was told that the US was going to be randomly, and the Predictable Group was told that the US was going to follow the CS sometimes. In generalization, the same instructions were given, but not only the CS+ was presented but also 6 GSs.

2.2 Questionnaires

Before the EEG task, participants filled out four surveys to measure underlying psychopathological traits: second edition of Beck's Depression Inventory (BDI-II), State-Trait Anxiety Inventory (STAI), Liebowitz Social Anxiety Scale (LSAS), and Intolerance of Uncertainty Scale (IUS).

Depression levels were assessed with the second edition of Beck's Depression Inventory (BDI-II) (Beck et al., 2015), which contains 21 items evaluated in a four-point Likert scale (0 = "I do not feel sad" and 3 = "I am so sad or unhappy that I can't stand it").

Anxiety was measured with the State-Trait Anxiety Inventory (STAI) (Bieling et al., 1998), which contains 20 items measuring state anxiety (STAI-S) and 20 measuring trait anxiety (STAI-T). The items were also evaluated on a four-point Likert scale (1 = Not at all/ Almost never, 4 = Very much so/Almost always).

Social anxiety levels were measured with Liebowitz Social Anxiety Scale (LSAS) (Safren et al., 1999), which includes 24 items divided into two subscales: 13 items regarding performance anxiety and 11 regarding social situations. Participants rated on a four-point Likert scale of 0-3 (Sullivan & Artino, 2013), reporting the level of fear during the situations (0 = "none fear" and 3 = "severe fear". Then, using the same scale, participants rated the avoidance of the situation (0 = "never avoid" and 3 = "usually avoid").

To measure participants' levels of intolerance of uncertainty we used the Intolerance of Uncertainty Scale (IUS) (Buhr & Dugas, 2002), which consists in 27 items that assesses emotional, cognitive, and behavioral reactions to different situations and consequences of being uncertain or attempts to control the future. Participants rate on a Likert scale of 1-5 (1 = "not at all characteristic of me" and 5 = "entirely characteristic of me").

2.3 Subjective Rating

During the experiment, we collected participants' valence and arousal ratings of all stimuli, using the Self-Assessment Manikin scale (Bradley & Lang, 1994). This ratings were collected after Habituation, Acquisition and Generalization, always after the end of each phase. Valence and arousal were measured on a 1-9 scale,

being 1 “very pleasant” (valence) or “very calm” (arousal) and 9 “very unpleasant” (valence) or “very arousing” (arousal).

Additionally, US-expectancy was measured with the question “How likely is this face to be followed by the scream?” presented along with each face. This was done after Acquisition for the expectancy to hear the scream after CS+ and after Generalization for the expectancy of hearing the scream after each one of the 7 stimuli. In Habituation there was no US, so this variable was not registered. Participants answered on a 0-100 scale by dragging a red bar to the most appropriate point in the scale with their cursor.

The participant’s ability to discriminate the different stimuli was also measured to further explore if the responses in the generalization phase were due to the inability to perceptually differentiate between the different stimuli. That was assessed using a Discrimination task (Greenaway, 2017) with six trials, by comparing each face with the CS+. In each trial, two stimuli (CS+ and one of the other pictures) were presented one by one (no interstimulus interval) for 1s each, in a random order. After each presentation, participants answered whether the two pictures showed the same face by pressing either “y” for yes or “n” for no using the keyboard. All ratings’ questions were presented until a response was given.

3. Study Design

After signing the informed consent and filling out the questionnaires, participants were seated in a separate soundproof EEG room, where the EEG and

SCR electrodes were applied. The seat was positioned 1.5 meters away from a 22-inch iiyama HM204DT-A computer screen with a 120 Hz refresh rate. Participants were instructed to remain as motionless as possible during the experiment and were informed that they would see female neutral faces and sometimes a loud and unpleasant scream. The whole experiment lasted approximately 40 minutes.

The experiment is based on a single-cue fear conditioning protocol with a generalization test divided into three stages: Habituation, Acquisition, and Generalization. In the Habituation phase, the participants need to become familiar with a neutral female face (CS+). This face appeared on the screen 10 times without any unconditional stimulus (US). At the end of this stage, ratings about valence and arousal were also recorded. In the Acquisition phase, CS+ was presented 15 times. This stage is different for each group: in the Predictable Group, a female scream (Unconditioned Stimulus, US) follows CS+ at its offset, with an 80% reinforcement rate. To the participants from this group the following instructions were given: "You will sometimes hear a loud scream only after the cue appears on the screen". On the other hand, in the Unpredictable Group, the US follows the CS+ randomly during acquisition, with the same frequency as in the other group. The participants were instructed with the following notes: "You can hear a scream at any time". After that, participants also filled valence, arousal, and US-expectancy ratings. Finally, the Generalization test assesses participants' responses to stimuli with varying similarity to the original CS+. The goal is to evaluate how well participants can generalize their learned responses to stimuli

that are similar but not identical to the original CS+. All stimuli (GS1, GS2, GS3, CS+, GS5, GS6, GS7) were presented 15 times each. All faces flickered at 15 Hz for 5 s, to evoke ssVEPs and the order of the stimulus is pseudorandomized with the requirement that there are no more than two presentations of the same face in a row. After that, ratings of valence, arousal, and US-expectancy were measured. At the end of the Generalization phase, participants answered to a Discrimination task to register their ability to distinguish CS+ from the other stimuli.

4. Psychophysiological Recording and Analysis

4.1 Electroencephalography, EEG

During all phases, brain activity was recorded with an electroencephalogram (EEG) using a Biosemi Active-Two amplifier system from 64 Ag/AgCl active electrodes attached to an elastic cap according to the 10/20 system. The Biosemi Active-Two system includes two extra electrodes: Common Mode Sense (CMS) and Driven Right Leg (DRL), which act as online reference and ground. Electro-ocular activity was also measured with an electrooculogram (EOG) with four flat type active electrodes: two electrodes placed on the two outer canthi of both eyes to record horizontal eye activity and two on the infraorbital and a supraorbital region of the right eye to register eye vertical movements. The signal is digitized at a 512 Hz sampling rate and 24-bit analog-to-digital conversion and the threshold of impedance is kept below 30 kΩ.

The electrodes were attached from the beginning of the task, however, only ssVEPs of Acquisition and Generalization were analyzed since there was only one stimulus (CS+) flickering for only 10 times during Habituation.

4.2 Skin Conductance Response, SCR

Skin conductance response (SCR) was measured during the experiments using the same Biosemi Active-Two amplifier system with two 8 mm Ag/AgCl electrodes that contained 0.05M NaCl electrolyte medium. The electrodes were attached to the second phalanx of the middle and ring finger of the non-dominant hand after lightly cleaned with water.

Even though this data was collected, it will not be used in this study, however, it will be analyzed in a future project of the research group in which this study is included.

4.3 Processing data

EEG recordings were analyzed offline with the software BrainVision Analyzer 2.3 (BVA). Data was filtered with a low cutoff of 0.1 Hz and a high cutoff of 40 Hz. In BVA, ocular artifacts were detected and corrected with the Gratton-Cole artifact correction procedure for 5 participants (101, 106, 109, 134, 146) and with an ICA correction for the rest of them. Then, data was re-referenced to the average of all electrodes and segmented into time windows of 500 ms pre-stimulus until 5500 ms after stimulus onset The signal was then transformed into the frequency domain using a Fast-Fourier-Transformation for the last 2000 ms of

stimulus presentation to eliminate initial non-stationary ssVEPs components and to highlight ssVEPs power which has shown to be more sensitive to conditioning effects in the second half of CS presentation (Miskovic & Keil, 2013; Moratti et al., 2006).

Based on previous studies (Aslanidou et al., 2023; Stegmann et al., 2020), the ssVEPs signal was averaged across electrodes *Iz, Oz, O1* and *O2*, and both mean activity from these electrodes and individual values of each electrode were used in the statistical analyzes.

4.4 Statistical analyzes

All the statistical analyzes were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

For habituation, the ratings (valence, arousal) were analyzed with an *independent samples t-test* to compare the results between groups (Unpredictable or Predictable Group).

For acquisition, the ratings (valence, arousal, and US-expectancy) were also analyzed with an *independent samples t-test* to compare the results between groups (Unpredictable or Predictable Group). ssVEPs were analyzed considering the average of the four electrodes (Iz, Oz, O1, O2) for each participant. In this scenario, the average of ssVEPs was considered a dependent variable in an *independent samples t-test* analysis to compare the results between groups (Unpredictable or Predictable Group).

For generalization, a *repeated measures ANOVA* was made to analyze valence, arousal, and US-expectancy with Stimuli (GS1, GS2, GS3, CS+, GS5, GS6, GS7) as a within-subjects factor and Group (Unpredictable or Predictable) as between-subjects factor. For variables that showed significance, post-hoc pairwise comparisons with a *paired t-test* were made, which was the casa of the main effect Stimuli for valence, arousal and US-expectancy ratings. ssVEPs were also analyzed considering the average of the four electrodes (Iz, Oz, O1, O2). In this case, we used a *repeated measures ANOVA* with Stimuli (GS1, GS2, GS3, CS+, GS5, GS6, GS7) as within-subjects factor and with Group (Unpredictable or Predictable) as between-subjects factor. To analyze the differences between the amplitudes of the ssVEPs after CS+ compared to the other 6 stimuli, post-hoc pairwise comparisons with a *paired t-test* were made.

It was also important to execute a Discrimination Task to understand the degree of discrimination of the CS+ from all other stimuli (GS1, GS2, GS3, GS5, GS6, GS7). To see if some stimuli where better discriminated from CS+ than other a *repeated measures ANOVA* was made with Stimuli (GS1, GS2, GS3, GS5, GS6, GS7) as within-subjects factor and with Group (Unpredictable or Predictable) as between-subjects factor.

For questionnaires, *Bivariate Pearson correlations* were made. Correlating LSAS and IUS and BDI-II, we expected that the higher the scores of LSAS, the higher the scores of IUS and BDI-II. Correlating IUS with BDI-II, it was expected that individuals with higher scores in of IUS also present higher scores in BDI-II. Then, the LSAS' levels of each participant were also correlated with the respective

ssVEPs. It was expected that the higher the mean of the ssVEPs of the participant, the higher their LSAS' levels.

The threshold for statistical significance was set at $\alpha = .05$ for all analyses. In case of a violation of sphericity, Greenhouse-Geisser corrected results were reported. Post-hoc analyses were corrected for multiple comparisons using the Bonferroni procedure.

RESULTS

1. Main Analysis

1.1 Habituation

1.1.1 Ratings. The *independent sample t-tests* for valence, t(52) = -1.085 , p = .283, and arousal ratings, t(51) = - 1.234, p = .816, returned no significant effects. Means and standard deviations for all variables in Habituation can be found in Table 2.

Table 2

Valence and Arousal during Habituation for both Unpredictable and Predictable Groups regarding the Conditioned Stimuli (CS+)

Stimuli	CS+			
Variable	Unpredictable Group M (SD) N (28)	Predictable Group M (SD) N (27)	t-value	p-value

Valence (1-9)	3.54 (1.35)	3.96 (1.54)	-1.085	.283
Arousal (1-9)	4.71 (1.96)	4.85 (2.19)	-0.234	.816

Note. N = Number of individuals, M = Mean of the variable, SD = Standard Deviation

1.2 Acquisition

1.2.1 Ratings. An *independent sample t-test* for valence ratings returned no significant effects, t(52) = -0,842, p = .403, but the *independent sample t-test* for arousal showed a nearly significant difference between Groups, t(52) = -1.965, p = .055. The effect of arousal is close to the significance threshold (.05 < p < .10), suggesting that the arousal induced by the stimuli after acquisition is borderline and the current study may have a lack of statistical power to find this effect. Means and standard deviations for all variables in Acquisition can be found in Table 3.

An *independent sample t-test* for US-expectancy returned significance, t(52) = -2,578, p = .013, when equal variances are assumed. Looking at the mean values of this variable (Table 3), it is possible to affirm that the Predictable Group (M = 75.8, SD = 15.2) was expecting the scream when seeing the CS+ more often than the Unpredictable Group (M = 62.0, SD = 23.1).

Table 3

Ratings of valence, arousal and US-expectancy and steady-state Visually Evoked Potentials (ssVEPs) recordings during Acquisition for both Unpredictable and Predictable Groups regarding the Conditioned Stimuli (CS+)

Variable	CS+			
	Unpredictable Group M (SD) N (28)	Predictable Group M (SD) N (26)	t-value	p-value
Valence (1-9)	5.75 (1.48)	6.12 (1.70)	-0.842	.403
Arousal (1-9)	6.11 (1.69)	7.00 (1.65)	-1.965	.055
ssVEPs (power FFT)	0.31 (0.55)	0.30 (0.05)	0.154	.878
US-exp (0 -100)	61.96 (23.12)	75.81 (15.20)	-2.578	.013

1.2.2 Psychophysiology. Doing the analysis of the ssVEPs with the average of the four electrodes, the *independent sample test* showed no significant main effect of Group in acquisition for ssVEPs, t(53) = 0.154, p = .878.

1.3 Generalization

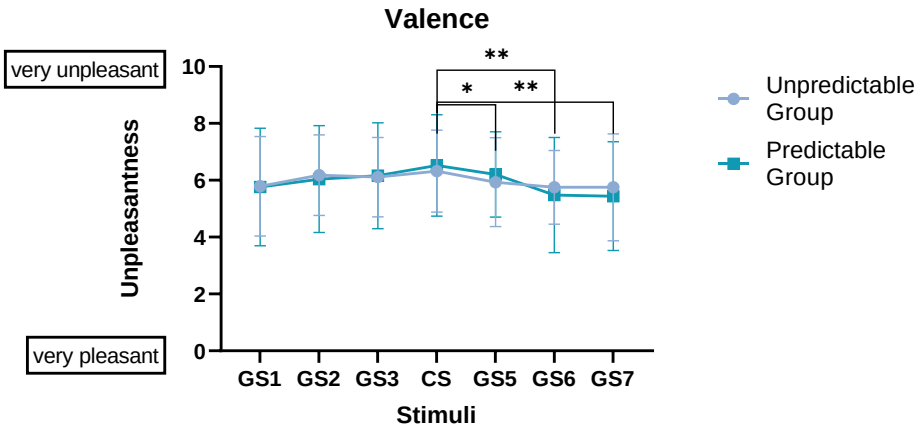
1.3.1 Ratings. A *repeated-measures ANOVA* was performed to evaluate the effect of Group, Stimuli and Stimuli*Group interaction on valence. We found no

significant main effect of Group ($F < 1$, $p = .930$) nor a significant Stimuli*Group interaction ($F < 1$, $p = .757$). The effect of Stimuli on valence was significant at the .05 level, $F(4,189) = 3.413$, $p = .012$, $\eta_p 2 = .063$, $\varepsilon = .616$.

Post-hoc pairwise comparisons with a *paired t-test* adjustment indicated that there was a significant difference between the valence for CS+ and GS5 ($p = .05$), GS6 ($p = .002$) and GS7 ($p = .008$). Ratings for valence were significantly higher for CS+ than for GS5, GS6 and GS7, suggesting that participants found CS+ significantly more unpleasant than the reported stimuli. A near significance ($.05 < p < .10$) was also found between the valence for CS+ and GS1 ($p = .052$). Ratings for valence were slightly higher for CS+ than for GS1, suggesting that participants found GS1 slightly more unpleasant than CS+. Means and standard deviations for valence ratings regarding all stimuli after Generalization can be found in Figure 1.

Figure 1

Means and standard error of the means for all stimuli regarding valence ratings from participants from both Unpredictable and Predictable Group after Generalization phase



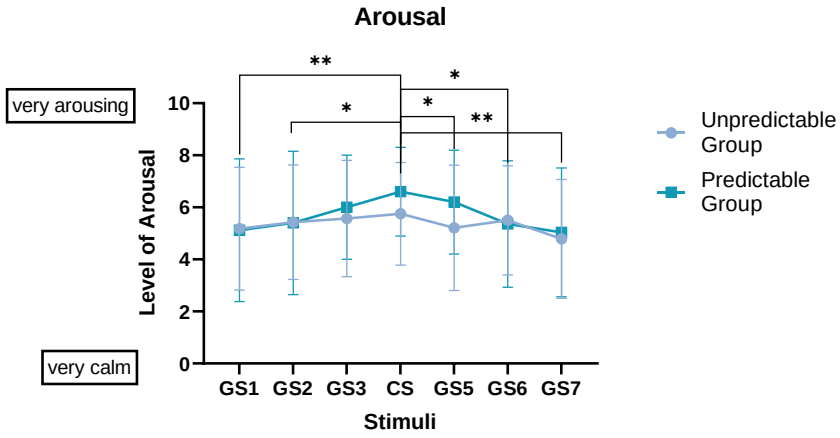
Note. * and ** indicate significant difference from CS+ at the 0.05 and 0.01 level, respectively.

A *repeated-measures ANOVA* was performed to evaluate the effect of Group, Stimuli and Stimuli*Group interaction on arousal. We found no significant main effect of Group ($F < 1$, $p = .518$) nor a significant Stimuli*Group interaction ($F < 1$, $p = .296$). The effect of Stimuli on arousal was significant at the .05 level, $F(4,193) = 3.413$, $p = .003$, $\eta_p 2 = .078$, $\varepsilon = .631$.

Post-hoc pairwise comparisons with a *paired t-test* adjustment indicated that there was a significant difference between the arousal for CS+ and GS1 ($p = .008$), GS2 ($p = .040$), GS5 ($p = .041$), GS6 ($p = .030$) and GS7 ($p = .001$). Ratings for arousal were significantly higher for CS+ than for GS1, GS2, GS5, GS6 and GS7, suggesting that participants found CS+ significantly more arousing than the reported stimuli. Means and standard deviations for arousal ratings regarding all stimuli after Generalization can be found in Figure 2.

Figure 2

Means and standard error of the means for all stimuli regarding arousal ratings from participants from both Unpredictable and Predictable Group after Generalization phase.



Note. * and ** indicate significant difference from CS+ at the 0.05 and 0.01 level, respectively.

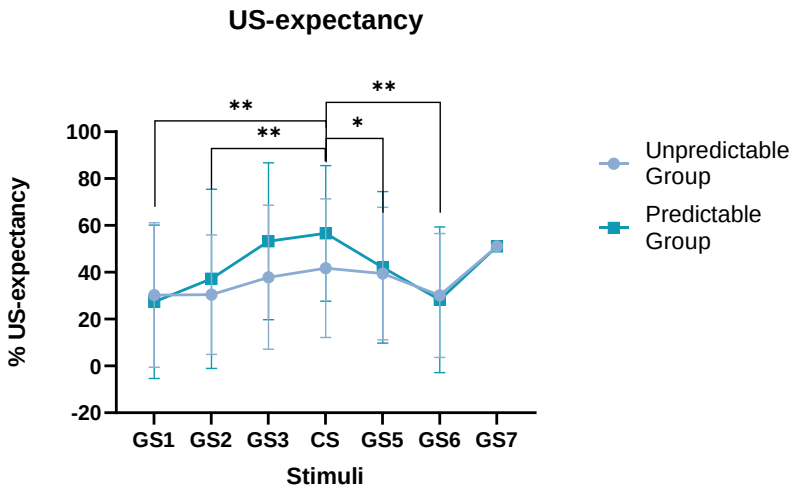
A *repeated-measures ANOVA* was performed to evaluate the effect of Group, Stimuli and Stimuli*Group interaction on US-expectancy. We found no significant main effect of Group ($F < 1$, $p = .386$) nor a significant Stimuli*Group interaction ($F = 1.731$, $p = .135$). The effect of Stimuli on US-expectancy was significant at the .05 level, $F(5,234) = 10.250$, $p < .001$, $\eta_p 2 = .167$, $\varepsilon = .763$.

Post-hoc pairwise comparisons with a *paired t-test* adjustment indicated that there was a significant difference between US-expectancy for CS+ and GS1 ($p < .001$), GS2 ($p = .003$), GS5 ($p = .038$) and GS6 ($p < .001$). Ratings for arousal were significantly higher for CS+ than for GS1, GS2, GS5 and GS6, suggesting that participants found CS+ significantly more likely to be followed by the scream than the reported stimuli. Means and standard deviations for US-expectancy ratings regarding all stimuli comparing to the CS+ and comparing to all of them after Generalization can be found in Figure 3.

Means and standard deviations for valence, arousal, and US-expectancy in Generalization can be found in Table 3 for the total values and in Table 4 for both Unpredictable and Predictable Groups.

Figure 3

Means and standard error of the means for all stimuli regarding US-expectancy ratings from participants from both Unpredictable and Predictable Group after Generalization phase



Note. * and ** indicate significant differences comparing to the CS+ at the 0.05 and 0.01 levels, respectively.

Table 3

Ratings of Valence, Arousal and US-expectancy; and steady-state Visually Evoked Potentials (ssVEPs) recordings during Generalization for all participants regardless of the group regarding the Conditioned Stimuli (CS+) and all other 6 Generalized Stimulus (GS1, GS2, GS3, GS5, GS6, GS7)

	Stimuli

Variable	GS1 *M(SD)*	GS2 *M(SD)*	GS3 *M(SD)*	CS+ *M(SD)*	GS5 *M(SD)*	GS6 *M(SD)*	GS7 *M(SD)*
Valence (1-9)	5.77 (1.89)	6.11 (1.64)	6.13 (1.62)	6.42 (1.60)	6.06 (1.52)	5.62 (1.67)	5.60 (1.92)
Arousal (1-9)	5.15 (2.53)	5.42 (2.45)	5.77 (2.12)	6.15 (1.89)	5.68 (2.26)	5.43 (2.24)	4.91 (2.36)
ssVEPs (Power FFT)	0.21 (0.04)	0.24 (0.05)	0.38 (0.14)	0.35 (0.08)	0.30 (0.06)	0.27 (0.05)	0.25 (0.04)
US-exp (0-100)	28.92 (31.49)	33.62 (32.01)	45.13 (32.70)	48.75 (29.97)	40.70 (30.01)	29.23 (28.47)	*

*Note.** refers to a problem in collecting the data of the US-expectancy for GS7, so it is not going to be countable for the statistics.

Table 4

Ratings of Valence, Arousal and US-expectancy; and steady-state Visually Evoked Potentials (ssVEPs) recordings during Generalization for both Unpredictable and Predictable Groups regarding the Conditioned Stimuli (CS+) and all other 6 Generalized Stimulus (GS1, GS2, GS3, GS5, GS6, GS7)

Variable	Stimuli						
	GS1	GS2	GS3	CS+	GS5	GS6	GS7
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
Group	Unpredictable Group						
Valence (1-9)	5.79 (1.75)	6.18 (1.42)	6.11 (1.40)	6.32 (1.44)	5.93 (1.56)	5.75 (1.30)	5.75 (1.88)
Arousal (1-9)	5.18 (2.36)	5.43 (2.20)	5.57 (2.24)	5.75 (1.97)	5.21 (2.41)	5.50 (1.10)	4.79 (2.28)
US-exp (0-100)	30.29 (30.85)	30.43 (25.52)	37.89 (30.76)	41.71 (29.61)	39.46 (28.29)	30.11 (26.49)	*
ssVEPs (Power FFT)	0.21 (0.05)	0.24 (0.07)	0.49 (0.19)	0.43 (0.12)	0.31 (0.09)	0.26 (0.06)	0.23 (0.05)
Group	Predictable Group						
Valence (1-9)	5.76 (2.07)	6.04 (1.88)	6.16 (1.86)	6.52 (1.78)	6.20 (1.50)	5.48 (2.02)	5.44 (1.98)
Arousal (1-9)	5.12 (2.74)	5.40 (2.75)	6.00 (2.00)	6.60 (1.71)	6.20 (2.00)	5.36 (2.43)	5.04 (2.48)
US-exp (0-100)	27.40 (32.76)	37.20 (38.25)	53.24 (33.51)	56.64 (28.94)	42.08 (32.36)	28.24 (31.07)	*
ssVEPs (Power FFT)	0.21 (0.05)	0.25 (0.07)	0.26 (0.19)	0.28 (0.12)	0.29 (0.09)	0.29 (0.06)	0.27 (0.05)

Note. Asterisk * refers to a problem in collecting the data of the US-expectancy for GS7, so it is not going to be countable for the statistics.

1.3.2 Psychophysiology. A *repeated-measures ANOVA* was performed to evaluate the effect of Group, Stimuli and Stimuli*Group interaction on ssVEPs using the mean of the four electrodes used: Iz, Oz, O1, O2. We found no significant main effect of Group ($F < 1$, $p = .689$), Stimuli ($F = 1.531$, $p = .224$) nor significant Group*Stimuli interaction ($F = 1.122$, $p = .313$). The effect of Stimuli on ssVEPs was not significant at the .05 level, $F(1,73) = 1.443$, $p = .241$, $\eta_p 2 = .028$, $\varepsilon = .229$.

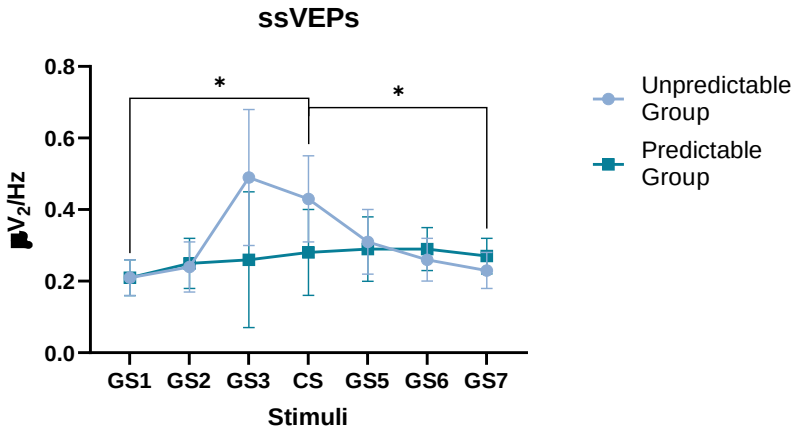
On the other hand, post-hoc pairwise comparisons with a *paired t-test* adjustment indicated that there was a significant difference between ssVEPs for CS+ and GS1 ($p = .018$) and GS7 ($p = .069$). It is therefore relevant to note that this is a small effect, since the ANOVA did not show any significance with the effect of Stimuli.

An *independent samples t-test* was performed to evaluate the effect of Group on ssVEPs only after CS+. We found no significant main effect of Group, $t(53) = 0.891$, $p = .377$.

Means and standard deviations for ssVEPs in Generalization can be found in Table 3 for the total values, and in Table 4 and Figure 5 for both Unpredictable and Predictable Groups.

Figure 5

Means and standard error of the means for all stimuli regarding steady-state visually evoked potentials (ssVEPs) during the Generalization phase.



Note. * indicate significant difference from CS+ at the 0.05 level.

2. Correlation Analysis

To analyse if the average amplitude of the ssVEPs of the four electrodes (Iz, Oz, O1, O2) and the questionnaires are correlated, *Bivariate Pearson correlations* were made.

The results from acquisition indicate a non-significant positive relationship between ssVEPs' amplitudes and BDI-II, STAIS, STAIT and LSAS. In addition, there was a non-significant negative relationship between ssVEPs' amplitudes during acquisition and IUS.

The results from generalization indicate a non-significant positive relationship between ssVEPs' amplitudes and BDI-II, STAIT, LSAS and IUS. In addition, there was a non-significant negative relationship between ssVEPs' amplitudes during generalization and STAIS.

Correlation coefficients and p-values for all questionnaires (BDI-II, STAIT, STAIS, LSAS, IUS) during Acquisition and Generalization are presented in Table 5.

Table 5

Correlation between steady-state Visually Evoked Potentials (ssVEPs) during Acquisition and Generalization and all questionnaires (BDI-II, STAIT, STAIS, LSAS, IUS) for all participants

Comparing to ssVEPs	BDI-II	STAIS	STAIT	LSAS	IUS
Phase	Acquisition Phase				
Correlation coeficiente (df)	0.194 (53)	0.049 (53)	0.133 (52)	0.070 (53)	-0.023 (51)
p-value	.156	.720	.415	.612	.873
Phase	Generalization Phase				
Correlation coeficiente (df)	0.139 (53)	-0.025 (53)	0.186 (52)	0.042 (53)	0.009 (51)
p-value	.312	.857	.178	.758	.948

Note. df = degrees of freedom

3. Discrimination Task

The two groups were additionally compared in the degree of discrimination of the CS+ from all other stimuli. A *repeated-measures ANOVA* was performed to evaluate the effect of Group, Stimuli and Stimuli*Group interaction on participants' ability to discriminate stimuli from CS+. We found no significant main effect of Group ($F < 1$, $p = .718$) nor significant Group*Stimuli interaction, $F(4,193) = 0.413$, $p = .789$. The effect of Stimuli on the Discrimination Task was

significant at the .05 level, $F(4,193) = 20.671$, $p < .001$, partial $\eta_p 2 = 0.288$, $\varepsilon = .758$. This suggests that participants' ability to discriminate the CS+ from other stimuli varied significantly depending on the type of stimulus presented. Means and standard deviations for Discrimination Task answers regarding all stimuli compared to the CS+ can be found in the next paragraph.

UP: M = 0.238, SD = 0.034

P: M = 0.220, SD = 0.036

Overall participants discriminated well the CS+ from the other test stimuli, regardless of the predictability of the cue.

4. Exploratory Analysis

As an exploratory analysis, we correlated all questionnaires with each other with *Bivariate Pearson Correlations*.

The results indicate a significant positive relationship between BDI-II and LSAS, $r(54) = 0.577$, $p < 0.001$; and IUS, $r(52) = 0.499$, $p < 0.001$. In addition, we found a significant negative relationship between BDI-II and STAIS, $r(54) = -0.379$, $p = .004$.

The correlations also indicate a significant negative relationship between LSAS and STAIS, $r(54) = -0.342$, $p = .010$. Thus, there is a significant positive relationship between LSAS and STAIT, $r(53) = 0.284$, $p = .035$; and IUS, $r(51) = 0.333$, $p = .015$. Additionally, a significant positive relationship between LSAS and IUS was also found, $r(52) = 0.620$, $p < 0.001$.

DISCUSSION

This study aimed to answer two related questions: (1) can different scenarios of unpredictable or predictable threat lead to overgeneralization of the conditioned responses? and (2) can a relationship between fear overgeneralization and unpredictability act as a marker in social anxiety disorder? For that, we compared both groups, since they were given different scenarios of unpredictability, and then all participants, regardless of the group.

During habituation, the lack of significance means that participants' perceptions of the emotional valence of the stimuli and levels of arousal induced by them did not change significantly for the first phase.

During acquisition, participants from the Predictable Group were expecting the scream when seeing the CS+ more often than the Unpredictable Group, as indicated by US-expectancy ratings. This is in line with the fact that the Predictable Group was not totally blind regarding the time that the scream appeared, which was the case of the Unpredictable Group, that had no idea when the scream could appear. However, no significant differences were found between them regarding their subjective emotional responses, indicated by valence and arousal ratings. This suggests that the predictability of the aversive stimulus presentation influenced participants' anticipatory responses over the course of the acquisition phase. Because the Unpredictable Group did not had access to explicit cues regarding the timing of the threat, they may have experienced greater anticipation due to the unpredictability of the task.

This is consistent with literature that suggests that unpredictable aversive events may lead to a state of anxiety and anxious apprehension due to the chronic expectation of threat (Wieser et al., 2016). Shankman et al. (2011) observed that unpredictable timing and intensity of aversive stimuli can lead to increased aversive responding and that the anxiety-related symptoms of unpredictability may generalize to situations beyond unpredictable timing (Aslanidou et al., 2023). Lin et al. (2012) showed that expected emotional stimuli can provoke stronger behavioral and brain responses. Conversely, the similar subjective emotional reactions (measured by valence and arousal ratings) suggest that while predictability influenced anticipatory responses, it may not necessarily alter the immediate emotional experience associated with stimuli.

On the other hand, looking at the analysis of arousal ratings comparing Groups, it is possible to say that, even though the differences are not significant, there is a near significance between individuals that received a predictable threat comparing to individuals that were blind regarding the timing of it. Taking this into account, this result suggests that the effect of Group may be relevant for future research with higher statistical power. This can be explained because these individuals received some information about when the scream will happen, making them to anticipate it after the CS+, provoking stress, and discomfort in those individuals in a stronger way compared to the ones in the Unpredictable Group.

Later in the task, during generalization, no significant differences were found between the Unpredictable and Predictable groups regarding valence and arousal evaluations. This suggests that the predictability of cues did not significantly

influence participants' responses to the conditioned stimulus (CS+). Contrary to the hypothesis that individuals who experienced unpredictable threat may find CS+ more unpleasant and arousing than individuals who experienced predicted threats (Wieser et al., 2016), our findings suggest that the emotional impact that CS+ has on participants was consistent across different scenarios of unpredictability, as measured by valence and arousal (Iberico et al., 2008; Wieser & Keil, 2020). On the other hand, there was a statistically significant difference among the stimuli regarding the level of unpleasantness and arousal those stimuli made participants feel. In addition, participants found CS+ more unpleasant than GS5, GS6 and GS7; and more arousing than GS1, GS2, GS5, GS6 and GS7, showing that regardless of the group, individuals could perceive different emotions regarding CS+ and other stimuli. Previous studies showed that the expectation of the threat was found to be higher in unpredictable situations compared to predictable ones, after a generalization paradigm (Iberico et al., 2008). However, that did not happen in the present study, since the effect of Group failed to reach significance when analyzing US-expectancy during Generalization. This suggests that both groups showed similar levels of expectancy regarding when the scream would occur, independent of the unpredictability created. However, there was a statistically significant difference among the stimuli regarding their expectancy of hearing the scream. Individuals particularly found CS+ more expected to be followed by the scream when comparing to GS1, GS2, GS5 and GS6, which is consistent with the fact the GS1 is an original face, and GS2, GS5 and GS6 are the less similar stimuli to CS+

(knowing that GS7 data could not be analyzed, so we are not considering their expectancy to this face). This allowed participants to distinguish them clearly during generalization, preparing them for the US. Overall, the analysis did not show any other statistically significance. This also suggests that there was no evidence to support the idea that the relationship between the type of stimuli presented and the participants' expectancy of hearing the loud scream varied depending on the group, meaning that it did not vary depending on the unpredictable or predictable cue.

So, overall, participants found the CS+ more unpleasant, arousing, and more likely to be followed by the unconditioned stimulus (US) as indicated by the valence, arousal, and US-expectancy ratings, respectively, as we hypothesized based on previous experiments (Ahrens et al., 2016; Aslanidou et al., 2023). As it can be seen in Table 3 as well as in Figures 2-5, the highest mean for valence, arousal, and US-expectancy ratings is in response to CS+ (6.42, 6.15, and 48.75, respectively). Along with the statistics, this suggests that participants could associate the CS+ with a negative outcome, regardless of the presence of an unpredictable or predictable cue. Thus, these results suggest that both groups learned equally well to associate the CS+ with fear and negative experiences, resulting in the absence of different generalization gradients between the two groups in our study due to the successful acquisition of conditioned fear in both groups.

The ssVEPs showed no significant differences between Groups during both Acquisition and Generalization. However, it is known from previous findings that

an unpredictable event leads to an increase in social anxiety levels and that excessive fear generalization is a potential biomarker of social anxiety (Ahrens et al., 2016; Nelson et al., 2015). Hence, it was expected to confirm that the lower the predictability of an event (assessing to the Unpredictable Group), the greater the overgeneralization of the fear-conditioned response (higher ssVEPs' amplitudes). However, the Unpredictable Group did not differ from the Predictable Group regarding generalization of fear, due to the lack of significant differences in the amplitudes of the ssVEPs. This suggests that there was no significant increase in the activation of the visuocortical system in the Unpredictable Group after the presentation of the stimuli, suggesting that the unpredictability of cues does not lead to a significant enhancement in the brain's response to the CS+. These results differs from those from previous investigations that showed higher amplitudes for unpredictable compared to predictable threats (McTeague et al., 2015; Miskovic & Keil, 2013; Stegmann et al., 2020).

Comparing the seven stimuli presented during Generalization, ssVEPs amplitudes were significantly higher for CS+ comparing to GS1 and GS7, meaning that participants' visuocortical engagement was higher for the CS+ when comparing to the two original faces with post hoc comparisons. This suggests that participants' brain responses to CS+ were enhanced and that participants are generalizing learned responses to stimuli that share perceptual features with the original one, but not when looking at clearly different stimuli than CS+ (GS1 and GS7). In other words, since a threat cue was related to the CS+, participants

probably also exhibited fear responses to GSs due to their perceptual similarity to the CS+. Hence, the significance suggests that CS+ elicited increased visuocortical responses reflecting elevated sensory engagement in participants when facing a threat. It is therefore relevant to note that this is a small effect, since the ANOVA did not show any significance when comparing stimuli, regardless of the unpredictability created.

One reason for the inconsistent findings may be the use of simple stimuli that can directly activate cells in the visual cortex that are sensitive to orientation. This happens because the brain's responses to these basic stimuli can be more readily measured and differentiated due to their direct impact on specific visual processing areas (Aslanidou et al., 2023). However, complex stimuli such as faces have multiple characteristics, including both threat-related features and non-threat-related ones. Thus, detecting these differences can be challenging for the brain, making it harder to distinguish between faces since different stimuli may share many similarities between these threat and non-threat-related features (Aslanidou et al., 2023; McTeague et al., 2015). Aslanidou et al. (2023) showed that threat uncertainty does not influence on fear generalization and one of the reasons that was presented to justify the lack of higher ssVEPs for the Uncertainty Group was a possible influence of the viewing distance of the stimuli (Aslanidou et al., 2023).

In studies where significance was observed for the ssVEPs using complex stimuli (Stegmann et al., 2020; Wieser et al., 2014), participants were sitting 100 cm away. Nevertheless, in the present study, as well as in Aslanidou et al. (2023)'s

study, participants were sitting 120 cm away. The greater distance can lead to small cortical representation (Murray et al., 2006) and that, along with the complexity of the stimuli used, can influence the visuocortical engagement and create differences that were too small to be detected (Aslanidou et al., 2023).

People with anxiety also have difficulty in differentiating similar stimuli and responding appropriately in terms of their fear response, meaning that they have difficulties in identifying which situations are genuinely dangerous and which ones are safe (Aslanidou et al., 2023; Duits et al., 2015). This is referred as an impaired discrimination fear learning. This impairment in anxiety individuals leads to the generalization of their fear responses to a broader range of similar stimuli. This process leads to less steep generalization gradients, meaning that instead of experiencing a sharp increase in fear or anxiety limited to that specific trigger, these individuals may experience a more gradual increase in fear across a range of similar stimuli, and not only to the actual dangerous one (Ahrens et al., 2015; Huggins et al., 2021; Lissek et al., 2009; Milad et al., 2007).

In fact, social anxiety individuals are known to display elevated startle potentiation in response to unpredictable events, which enhances the importance of the interaction between unpredictability and social anxiety. However, the interaction between fear and anxiety also plays a role in making stimulus fearful. So, we suppose that unpredictability is a significant factor, but it is not the sole contributor to the overgeneralization of fear responses. These insights emphasize the complexity of the relationship between unpredictability and fear responses. Understanding these dynamics is not only essential for unraveling the relationship

between these concepts, but it's also highly valuable to our comprehension of fear-related processes and potentially informing about interventions for anxiety-related disorders.

The study aims to provide insights into the development and maintenance of social anxiety symptoms such as the underlying mechanisms that the disorder involves. For that, questionnaires were analyzed indicating, as we expected, that individuals more intolerant to uncertainty are more likely to suffer from social anxiety disorder. This confirmed what was studied in other studies where the need for predictability and control in uncertain situations may contribute to heightened social anxiety symptoms as individuals may perceive social interactions as unpredictable and potentially threatening (Aslanidou et al., 2023; McTeague et al., 2011).

Our findings further indicate significant correlations between social anxiety levels and other psychological constructs, including depression, state, and trait anxiety. This enhances the importance of these interrelationships for developing comprehensive assessment protocols and evidence-based interventions tailored to the individual needs of patients experiencing SAD. Then, the present study also observed that individuals with a higher intolerance of uncertainty are also more likely to experience higher trait anxiety and depression. Trait anxiety is characterized by persistent feelings of apprehension and worry, characterized by the tendency to respond fearfully to a wide variety of unspecific stressors, and is considered a risk factor for anxiety disorders (Spielberger, 1972). Thereby, it is reasonable to think that when someone is intolerant to uncertain events, they

experience these feelings of hopelessness and dysphoria, contributing also to symptoms of depression. As an exploratory analysis, we also found that participants with more tendency to develop depression symptoms, presented lower levels in the state-anxiety questionnaire.

It was also expected that participants with the tendency to develop social anxiety symptoms to present higher visuocortical responses during generalization, but there was no significant correlations between these two factors when a correlation between social anxiety scores (LSAS) and ssVEPs activation was made. This means that the association between the visuocortical system and social anxiety still needs more study to produce robust conclusions. Comparing all questionnaires, they all failed to reach a significant correlation with the ssVEPs amplitudes after both acquisition and generalization. The lack of significance emphasizes the possibility that studying humor disturbances like social anxiety assessing to healthy participants might not have been the best option to study this many symptoms and such complex disorder. Besides that, the relationship between visuocortical processing and social anxiety disorder symptoms may be influenced by multiple factors (National Institute for Health Excellence and Care, 2013; Spielberger, 1972), such as attentional processes, emotional regulation strategies, and cognitive biases, and the study may not have accounted for all these factors adequately leading to a non-significant result.

By identifying individuals at risk of developing social anxiety, professionals could inform early intervention strategies and give them better quality of life in the future. Following this line of thinking, by targeting fear generalization,

unpredictability, and other contributing factors, clinicians may be better equipped to tailor interventions to individual needs and improve treatment outcomes. Overall, the study contributes to the broader scientific understanding of anxiety disorders and fear processing, leading to the advance of theoretical models of anxiety and informing future research directions in the field, by elucidating the mechanisms underlying social anxiety disorder.

CONCLUSION

Based on the results of this study, fear generalization is not influenced by the unknown (unpredictability). Different scenarios of unpredictable or predictable threat do not lead to overgeneralization of the conditioned responses.

This study gives insights into participants visuocortical system activation. The presentation of CS+ leads to slightly higher visuocortical activation, comparing to the presentation of original faces (GS1 and GS7). However, regarding the possibility of an interaction between unpredictability and fear generalization, based on our results, we can suppose that unpredictability by itself is not strong enough to create fear overgeneralization, but that unpredictable threat may not be dissociated with the generalization of fear responses in totality.

This research reinforces previous studies (Aslanidou et al., 2023) by proving that the more intolerant to uncertainty a person is, the more likely s/he is to develop social anxiety symptoms.

To potentiate the impact in generalization to find more about this association, we could use an approach that resorted to stimuli that were different in how arousing they were, resulting in not only having the US related to the CS+,

but also more arousing GSs that could generate different emotional responses in the participant (Aslanidou et al., 2023).

1. Future approaches

Advanced analytical techniques such as machine learning could be applied to uncover patterns, associations, or classifications that might not be immediately apparent through traditional analyzes. Future studies could also rely on network analysis to explore the connectivity patterns within the brain during unpredictable threat and their relationship to social anxiety, providing insights into the functional architecture of the brain in the context of fear overgeneralization. The use of real-time neurofeedback based on EEG or SCR measures could also be another interesting approach to improve this research. This, however, could have implications for the development of personalized therapeutic interventions for individuals with social anxiety. It would also be valuable to include a comparison with a clinical sample, such as individuals diagnosed with social anxiety disorder to explore the generalizability of our findings to clinical populations, adding clinical relevance to the research. These future steps lie in their potential to advance our understanding of the neural and psychological mechanisms underlying the overgeneralization of fear responses during unpredicted situations. Collectively, these factors can contribute to the advancement of more efficacious and individualized interventions for individuals contending with social anxiety disorder.

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