Slow to Warm Up: The Role of Habituation in Social Fear

By

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To my husband, Stacy, for his unending support, and my daughter, Sophie, for whom I do everything

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	
α	alpha, threshold significance level of statistical test
AAL	Automated Anatomical Labeling
ANOVA	analysis of variance
ART	Artifact Detection
b	beta value
b'	beta prime value
BDI-II	Beck Depression Inventory II
BOLD	blood oxygen-level dependent
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
EPI	echo planar imaging
FFA	fusiform face area
fMRI	functional magnetic resonance imaging
FOV	field of view
FWHM	full width half maximum
GLM	generalized linear model
gPPI	generalized psychophysiological interaction toolbox
HRF	hemodynamic response function
m	minute
mm	millimeter
MNI	Montreal Neurological Institute
mOFC	medial orbitofrontal cortex
MR	magnetic resonance
MRI	magnetic resonance imaging
n	number of samples
NITRC	Neuroimaging Informatics Tools and Resources Clearinghouse
p	*p*-value
r	correlation value
RCBS	Revised Cheek and Buss Shyness scale
ROIs	regions of interest
s	second
SCID	Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, 4th edition
SD	standard deviation
SENSE	Sensitivity Encoding
SHY-SR	Social Anxiety Spectrum Self-Report
SPM8	Statistical Parametric Mapping, version 8

STAI	State-Trait Anxiety Inventory
T1	T1-weighted magnetic resonance image
TE	echo time
TR	repetition time
V1	primary visual cortex
vmPFC	ventromedial prefrontal cortex
WFU	Wake Forest University

CHAPTER I

Introduction

1.1. The social fearfulness spectrum

Social anxiety disorder—characterized by the fear and avoidance of social interactions—is one of the most common psychiatric illnesses, affecting up to 13% of the population each year (Stein *et al*, 1994; Kessler *et al*, 2005b, 1994). People with social anxiety disorder experience intense distress and discomfort in social situations, especially those that carry the potential for social evaluation or scrutiny from others. The distress and discomfort of social situations is often overwhelming, leading to avoidance of social interaction and disability. Disability associated with social anxiety disorder can range from mild to severe and is often the result of avoidance of important social situations, such as school or work. Although individuals with social anxiety disorder often have fears of public speaking, less than 5% of individuals meet criteria for the diagnosis based exclusively on public speaking fears (Burstein *et al*, 2011; Stein *et al*, 1996; Kessler *et al*, 1998). Instead, the vast majority of individuals with social anxiety disorder experience significant fears in *most* social situations. Social anxiety disorder has a typical onset in adolescence (Kessler *et al*, 2005a, 2010, 1998; Wittchen and Fehm, 2003) and is highly persistent throughout the entire life course (Kessler *et al*, 2010), resulting in reduced educational attainment (Schneier *et al*, 1994; Liebowitz *et al*, 1985), low occupational and financial status (Schneier *et al*, 1994; Wittchen and Fehm, 2003; Patel *et al*, 2002), and reduced quality of life (Saarni *et al*, 2007; Patel *et al*, 2002; Wittchen and Fehm, 2003; Ruscio *et al*, 2008). Social anxiety disorder also has high

comorbidity with other psychiatric illness (Wittchen *et al*, 1999; Wittchen and Fehm, 2003; Beesdo *et al*, 2009, 2007; Buckner *et al*, 2008), particularly depression and substance abuse, making it an important target for early intervention.

However, an expanded view beyond the strict clinical boundaries of social anxiety disorder may be warranted. Social anxiety disorder likely represents the most extreme end of a general dimension of social fearfulness **(Figure 1)** that incorporates traits like shyness (moderate distress in some social situations) and social inhibition (avoidance of social novelty) (Stein *et al*, 1994; Schneier *et al*, 2002; Davidson *et al*, 1994; Stein *et al*, 2004; Furmark, 2002). Although not without controversy (Heiser *et al*, 2009), the view of a social fearfulness spectrum is supported by evolutionary theories

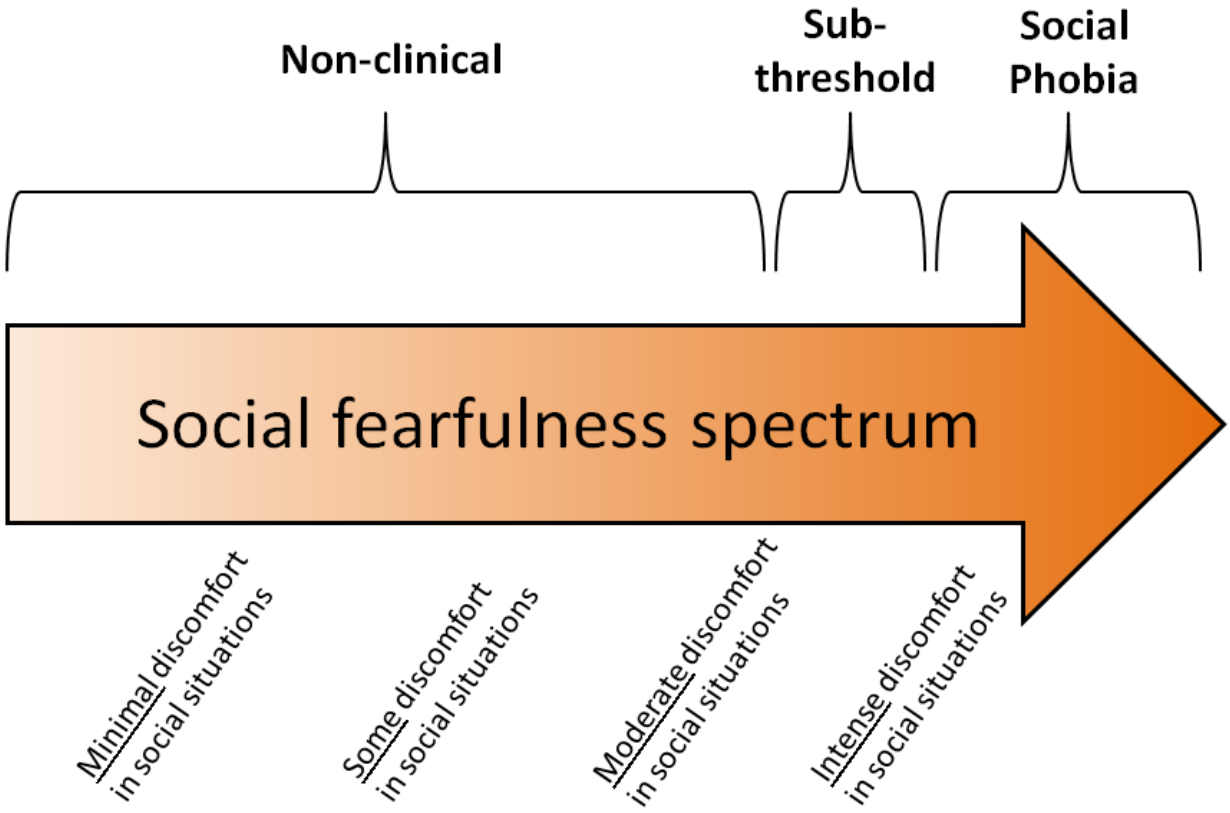


Figure 1. The social fearfulness spectrum. A spectrum encompassing non-clinical, sub-threshold, and clinical manifestations of social fear.

(Hermans and van Honk, 2006), developmental theories (Pérez-Edgar and Fox, 2005), and clinical theories (Hofmann *et al*, 2004) of social anxiety and is in line with observation that these various phenomena share many common neural, behavioral, and cognitive correlates (Pérez-Edgar and Fox, 2005). Additionally, when diagnostic thresholds are extended to include people with sub-threshold social anxiety disorder, it's estimated that up to 18% of the population is affected by significant levels of impairment as a result of social fears (Stein *et al*, 1994; Schneier *et al*, 2002; Davidson *et al*, 1994; Stein *et al*, 2004; Furmark, 2002). Notably, people with sub-threshold social anxiety experience similar functional impairment, including reduced educational attainment, occupational status, and quality of life, as those with a diagnosis (Davidson *et al*, 1994), indicating that a broader understanding of the dimension of social fearfulness might have a significant impact on public health.

The consequences of extreme social fear—on job, career, grades, relationships and self-esteem—are significant (Schneier *et al*, 1994; Wittchen and Fehm, 2003; Patel *et al*, 2002; Liebowitz *et al*, 1985), yet social fear is an easily-minimized phenomenon; for example, the majority of people have had to cope with uncontrollable stage-fright when confronted with a large audience for the first time. Although social anxiety disorder often extends far beyond public speaking fears, it's reasonable to assume that the physiological response to public speaking experienced by an average person overlaps to some extent with the response experienced by people with social anxiety disorder. Therefore, unlike psychotic illness, for instance, the symptoms of social anxiety disorder are at least imaginable for most. For most, the experience of social fear is common and adaptive; in fact, some social fear may improve performance (Eysenck and Calvo,

1992). However, this differs markedly from the experience of a person with extreme social fear, in which anxiety is chronically debilitating and maladaptive. People with extreme social fears have severe autonomic, cognitive, and somatic reactions to even the suggestion of a social situation where evaluation may occur (Cuthbert *et al*, 2003), and the fear is often so great that the person will avoid social situations at all costs, even to the severe detriment of personal goals and relationships. In contrast to adaptive social fear, extreme social fear is associated with distinctly decreased cognitive performance ability (Eysenck and Calvo, 1992), suggesting that the experience of extreme social fear is qualitatively distinct from adaptive social fear.

Social fearfulness encompasses an ecologically-valid spectrum of fear states, including both adaptive and maladaptive expressions. As with most fears, maladaptive social fears develop from an adaptive fear state (Rosen and Schulkin, 1998). Fears have long been recognized as circumscribed to a limited group of categories, including natural situations (e.g., water, heights), predators (e.g., spiders, snakes), and threatening conspecifics (American Psychiatric Association, 2013). A spectrum of fear exists within each of these categories, including both a highly-adaptive range of fear and a pathological extreme. For example, an average person will feel a rush of fear when they nearly step on a snake in their path—a real, present, nearby snake is a potentially serious threat, and in this case, fear is a crucial emotion that protects us from harm. Fear motivates adaptive responses, such as focused attention and sympathetic system activation (“fight or flight”), that increases our chances of navigating a threatening situation successfully (Marks and Nesse, 1994). From an evolutionary perspective, social fear, or fear of threatening conspecifics, is an evolutionarily-

conserved response akin to fear of snakes, spiders, heights, deep water, and all manner of natural threats (Ohman, 1986; Hermans and van Honk, 2006). For social animals, such as humans, the ability to perceive and respond appropriately to an angry encounter has far-reaching consequences. Social groups have historically provided protection, support, and the potential to find a mate. Even in modern society, ostracism from a social circle is a serious outcome which may negatively impact career advancement, friendships, and overall wellbeing. This advantage is illustrated in the "Hawk-Dove" game (Smith, 1982), where bold Hawks and fearful Doves compete for resources. Hawks have the opportunity to gain large rewards (e.g., friends, career advancement) in safe environments but are also more likely to incur costs in threatening environments (e.g., getting into fights, catching communicable diseases). In contrast, Doves are likely to take fewer risks, protecting themselves from negative consequences but also gaining fewer advantages when the environment is safe (Korte *et al*, 2005). In the Hawk-Dove game, the ability to accurately detect threat is critical in determining an appropriate response—over-detection of threat by Doves in a safe environment leads to over-protection from risk and inability to gain necessary resources. However, the experience of fear in the absence of a real and present threat is maladaptive, often leading to distress, avoidance, and disability.

Social fears become maladaptive when they are expressed in situations that present little risk of harm. People with social anxiety disorder chronically detect threat in social situations which pose little threat, such as talking to acquaintances at a party or giving a talk in front of classmates. Detection and assessment of threat is necessary in order to employ an effective survival strategy (e.g., fight or flight). However, over-

detection of threat in social situations may result in chronic avoidance of social encounters, resulting in an inability to gain resources (friends, education) and leading to disability. Therefore, a critical question is whether the ability to accurately evaluate the safety of a social environment contributes to maladaptive social fears. As detection of threat is a critical function in everyday life, subserved by numerous brain regions, it's reasonable to assume that over-detection of threat may stem from neural differences in these regions.

The social fearfulness spectrum represents a pragmatic approach to studying neural detection of threat—the availability of specific dimensional biomarkers, such as markers of response to threat, are essential for the early identification of risk and the assessment of treatment response (Kessler, 2002). However, clinically useful dimensional biomarkers are currently unavailable. Prior studies of social anxiety disorder have overwhelmingly used case/control designs, which are comprised of heterogeneous patient groups and reflect multiple symptoms. While research using case/control designs has made important contributions to broadly defining which brain regions are involved in social anxiety disorder (Freitas-Ferrari *et al*, 2010), the heterogeneity of patient groups may limit the discovery of specific underlying neurobiological mechanisms contributing to social fearfulness.

1.2. Evidence for disrupted threat processing in social fear

Because social anxiety disorder represents an extreme end of the social fearfulness spectrum, studies of patients with social anxiety disorder are ideal in gaining initial insight into which brain regions contribute to social fears. People with social

anxiety disorder show a consistent pattern of altered brain activation in response to potential threats (Lang *et al*, 2000; Walker and Davis, 1997; Gray, 1983; Blanchard *et al*, 2011; Mobbs *et al*, 2009; Adolphs *et al*, 1999; Gray and McNaughton, 2003), suggesting that disrupted threat processing in the brain may contribute to social anxiety symptoms. These regions, shown in **Figure 2**, include the amygdala, hippocampus, medial prefrontal regulatory regions (medial orbitofrontal cortex, ventromedial prefrontal cortex), and visual and face processing regions (primary visual cortex, extrastriate cortex, fusiform face area). Together, these brain regions form an interconnected

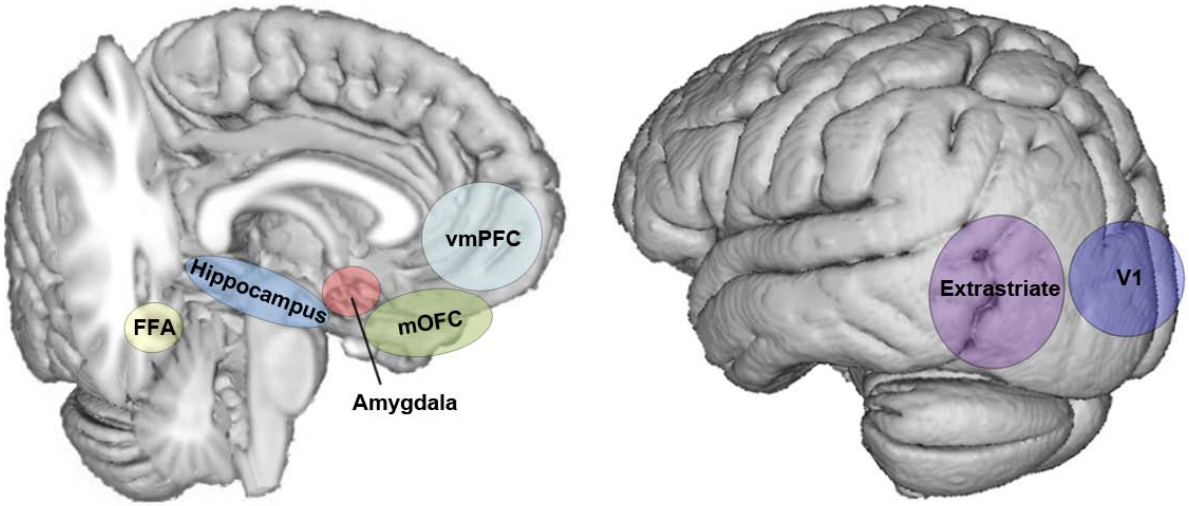


Figure 2. Key brain regions involved in social fearfulness. Fusiform face area (FFA); medial orbitofrontal cortex (mOFC); ventromedial prefrontal cortex (vmPFC); primary visual cortex (V1).

network responsible for visual social threat processing (Stefanacci *et al*, 1996; Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004; Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005; Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006; Wager *et al*, 2009a).

Elevated activity in limbic regions, including the amygdala and hippocampus, has been the most common neural feature associated with social anxiety symptoms. The amygdala, a small almond-shaped structure located in the medial forebrain, is central to the evaluation of social threat. The amygdala is critically important in the detection of environmental threat (Öhman, 2005) and in the expression of fear and anxiety (Lang *et al*, 2000; Davis, 1997), and there is strong evidence for amygdala involvement in social fear, with convergent findings across multiple modalities, species, and threat tasks (for reviews see Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012). Lesions of the amygdala produce a striking lack of fear to environmental and social threat in monkeys (Amaral, 2003; Klüver and Bucy, 1939), and bilateral amygdala damage in humans is associated with difficulty recognizing fearful expressions (Adolphs *et al*, 1999). Functional neuroimaging studies have found elevated amygdala activity in people with social anxiety disorder in response to various types of social threat, such as viewing of threatening faces (Phan *et al*, 2006; Blair *et al*, 2008; Stein *et al*, 2002) or anticipation of public speaking (Tillfors *et al*, 2002; Lorberbaum *et al*, 2004). Significantly, amygdala activity in response to social threat is reduced following successful social anxiety treatment (Furmark *et al*, 2002, 2005). Together, these findings converge to support a critical role for the amygdala in the detection of social threat, and indicate that hyperactivity of the amygdala in response to negative or threatening social stimuli may at least partially underlie social anxiety symptoms.

There is also evidence for altered hippocampal activity in people with social anxiety (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and

Schmidt, 2012). The hippocampus has been associated with the overgeneralization of anxiety (Cannistraro and Rauch, 2003; Gray and McNaughton, 2003)—potentially a critical component of extreme social fearfulness. In people with social anxiety disorder, social threat is associated with elevated activity in the hippocampus and parahippocampal gyrus compared to controls (Stein *et al*, 2002; Straube *et al*, 2004; Tillfors *et al*, 2002); additionally, people with social anxiety disorder show attenuated hippocampal activity to social threat following successful social anxiety treatment (Furmark *et al*, 2005). Although the hippocampus has been less well-studied than the amygdala, these findings suggest that the hippocampus may be important in the development and expression of social anxiety.

The medial prefrontal cortex is involved in the regulation of emotions, such as social fear, and under-engagement of the prefrontal cortex to potential threats may be important in the development of inappropriate fears. Two regions of the medial prefrontal cortex in particular, the medial orbitofrontal cortex (mOFC) and the ventromedial prefrontal cortex (vmPFC), have been implicated in social anxiety. The mOFC, which forms the ventral surface of the medial prefrontal cortex, is involved in tracking the affective value of stimuli and in guiding advantageous choices (Stalnaker *et al*, 2015), potentially guiding the valuation of social experiences. Across a variety of social threat studies, activity in the mOFC is elevated in patients with social anxiety disorder compared to controls (Miskovic and Schmidt, 2012; Freitas-Ferrari *et al*, 2010) suggesting a broad role in social threat evaluation. The vmPFC is a complex region, located dorsal to the mOFC along the medial wall of the prefrontal cortex (Price, 1999), encompassing several functional regions central to social and affective function (Quirk

and Beer, 2006; Zald *et al*, 2002; Milad and Rauch, 2007; Milad *et al*, 2006) and is an important regulator of mood and anxiety symptoms (Myers-Schulz and Koenigs, 2012; Price, 1999). Higher activity in the vmPFC is associated with fewer social anxiety symptoms (Lungwitz *et al*, 2014; Riga *et al*, 2014), and the vmPFC is consistently found to be underactive in people with social anxiety disorder (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012) relative to controls. Following therapeutic treatment for social anxiety symptoms, activity in the vmPFC is increased (Evans *et al*, 2009) suggesting a regulatory role over anxiety symptoms. Together, evidence suggests that the mOFC and vmPFC provide an abstract knowledge of the social world (Krueger *et al*, 2009) critically important for social cognition and social function.

Although limbic and medial prefrontal regions have been more extensively investigated in relation to social threat evaluations, detection of visual social threat may begin early in the visual processing stream—people with social anxiety disorder show structural and functional differences in face processing and early visual processing regions (Freitas-Ferrari *et al*, 2010; Miskovic and Schmidt, 2012). The fusiform face area (FFA), located in the fusiform cortex, is specialized for face processing (Kanwisher *et al*, 1997; Loffler *et al*, 2005), and activity in the FFA has been shown to be elevated in social anxiety patients during a task involving harsh or threatening faces (Frick *et al*, 2013a; Goldin *et al*, 2009; Straube *et al*, 2004, 2005). There are also preliminary indications that people with social anxiety disorder show differences in structure and function within primary visual processing areas; people with social anxiety disorder have greater visual cortex volume (Frick *et al*, 2014) and cortical thickness than controls

(Frick *et al*, 2013b), and show altered visual cortex activity during viewing of faces (McTeague *et al*, 2011). Together, these preliminary findings suggest that visual indicators of potential social threat, such as faces, may be processed differently in people with social anxiety—with evidence of over-detection of threat present even early in the visual processing stream in people with social anxiety.

1.3. Faces are salient social cues

The basic ability to detect and process facial information is critical for gauging appropriate social response, which is the foundation of successful social interactions. Many studies investigating social fear have used face stimuli to elicit a social threat response in the brain. In people with social anxiety disorder, a static picture of a face elicits activity in similar regions as other social stressors (e.g., public speaking) (Freitas-Ferrari *et al*, 2010), indicating that faces are potent signals of potential threat. Faces are one of the most important social cues that we perceive—even a short glimpse of a face conveys a wealth of information about an individual critical for social functioning, including identity, mood, and intent. The importance of face processing is evidenced by three distinct features: 1) humans are born with an innate ability to process and recognize faces (Pascalis and Slater, 2003) and already show processing patterns during infancy similar to adults (Farzin *et al*, 2012); 2) face recognition is highly specific and dissociable from both general intelligence and from other types of recognition memory, like object recognition (Wilmer *et al*, 2010; Zhu *et al*, 2010); and 3) face processing relies on a dedicated neural substrate—the FFA (McKone *et al*, 2007; Tsao and Livingstone, 2008; Tsao *et al*, 2006; Wilmer *et al*, 2010; Kanwisher *et al*, 1997).

Although fearful, negative, or threatening faces are often utilized to elicit neural responses in threat evaluation circuitry, faces don't need to be inherently threatening to elicit similar threat evaluation responses. People with social anxiety disorder show an elevated amygdala response to neutral faces compared to controls (Birbaumer *et al*, 1998; Cooney *et al*, 2006), although to a lesser extent than threatening faces. Neutral expressions are more emotionally ambiguous than other facial expressions (Massaro and Egan, 1996), and there is preliminary evidence that people with social anxiety disorder tend to view neutral expressions as slightly threatening (Winton *et al*, 1995), perhaps due to their ambiguity. Therefore, elevated amygdala activity to neutral faces further supports the hypothesis of an over-perception of social threat.

1.4. Novel faces are cues of potential social threat

Response to novel faces is an important dimension of the affective brain (Weierich *et al*, 2010) and are associated with increased feelings of arousal (Weierich *et al*, 2010) and increased state anxiety (Ousdal *et al*, 2014). As with neutral faces, novel faces are emotionally ambiguous—a novel face can be threatening, rewarding, or inconsequential. Because a novel face must be evaluated quickly to determine the appropriate response, novel faces are highly salient in the brain; novel faces provoke an automatic orienting response (Sokolov, 1963) and rapid reallocation of sensory processing resources that sharpen arousal, perception, motivation, and memory (Schomaker and Meeter, 2015) in order to determine the appropriate behavioral response. The ability of novelty to effectively harness vast neural resources has likely been selected for through evolution; rapid detection and processing of novelty for

potential threat is crucial for survival, as failure to respond quickly to a threat may result in serious harm. In support of this link, infants as young as 6 months old show heightened orienting response and neural activity to novel faces compared to novel objects (Snyder and Keil, 2008).

Novel faces elicit strong activity in threat detection regions—the amygdala and hippocampus both have a well-defined role in face and novelty detection, containing neurons that respond preferentially to faces (Fried *et al*, 1997; Wilson and Rolls, 1993) and neurons which respond only to the first presentation of a stimulus (Fried *et al*, 1997; Wilson and Rolls, 1993; Rutishauser *et al*, 2006). In the medial prefrontal cortex, both the vmPFC and mOFC have been shown to be engaged by novel faces relative to familiar faces (Weierich *et al*, 2010), suggesting that these medial prefrontal regions may also play a role in processing novel social information. Even in early visual processing regions, such as V1 and extrastriate cortex, novel faces elicit differential activation compared to familiar faces (Weierich *et al*, 2010). Activity in the FFA is also modulated by face familiarity (Gobbini and Haxby, 2006)—the FFA is involved in the representation of invariant features of faces and, therefore, is thought to play a key role the recognition of novel face identities.

Importantly, brain regions involved in the detection of novel faces—the amygdala, hippocampus, vmPFC, mOFC, FFA, V1, and extrastriate—also show altered function in social anxiety disorder, suggesting that novel face processing and over-detection of threat are linked. One of the most important aspects of the novelty response is the ability to habituate to a novel stimulus that is not threatening or rewarding. As neutral novel stimuli are repeatedly encountered they become familiar

and safe, and critical neural resources are freed up to detect and process new stimuli. The ability to quickly distinguish a familiar from novel face is likely key in effectively navigating a social environment, and may underlie differences in detection of social threat.

1.5. Habituation as a mechanism for social fearfulness

Habituation, the decrease in response to a repeated stimulus, is one of the simplest forms of learning and memory (Thompson and Spencer, 1966; Thompson, 2009). Although habituation has been well-characterized behaviorally, the neural mechanisms underlying habituation remain largely unknown (Ramaswami, 2014; Wilson and Linster, 2008). It is widely-accepted that neural habituation, often referred to as repetition suppression, is key in filtering novel from familiar sensory experience (Ramaswami, 2014) and focusing attention on important stimuli; therefore, neural habituation is a prerequisite of all other forms of learning. Single-unit recording studies have shown that neural habituation usually occurs rapidly, with the greatest decrease in response observed between the first and second stimulus repetition

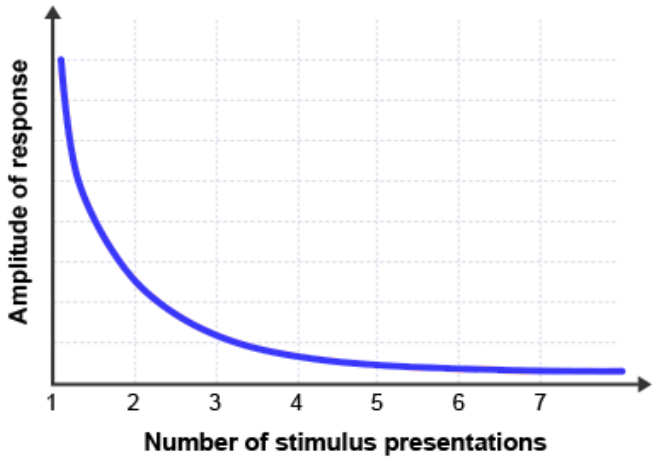


Figure 3. A hypothetical habituation curve in response to repeated stimuli. In single neuron recordings (Wilson and Rolls, 1993) and within regions measured by fMRI (Ishai *et al*, 2004), habituation follows a typical pattern, occurring rapidly between the first and second stimulus presentation, with the most habituation occurring by the third to fifth presentation.

(Figure 3) (Fried *et al*, 1997; Wilson and Rolls, 1993), providing a critical neuronal code for familiarity (Fried *et al*, 1997; Wilson and Rolls, 1993; Gonsalves *et al*, 2005; Dubois *et al*, 1999; Wright *et al*, 2001). In contrast, failure to rapidly habituate to repeated stimuli has been associated with feelings of uncertainty and unfamiliarity (Fried *et al*, 1997; Wilson and Rolls, 1993; Gonsalves *et al*, 2005; Wright *et al*, 2001; Dubois *et al*, 1999). Although habituation is a basic process, individual differences in habituation appear as early as infancy (Bushnell, 1982; Snyder and Keil, 2008) and these differences have been proposed to fundamentally underlie individual differences in mood and anxiety (Davidson, 2002; Schuyler *et al*, 2012). Prolonged neural response within novelty processing regions likely contributes to delayed feelings of safety and familiarity in novel social situations, resulting in feelings of fear and anxiety (Stout *et al*, 2013). Previous work in our lab demonstrated a link between habituation to novelty and social fears; while people with low shyness habituated rapidly to repeated faces, people with high levels of shyness failed to habituate over repeated presentations **(Figure 4)** (Blackford *et al*, 2013). Additional evidence comes from studies of autism, a disorder marked by social difficulties. In people with autism, slow habituation of the amygdala to novel faces has been associated with more severe social impairment (Kleinhans *et al*, 2009). Preliminary findings in social anxiety disorder are less clear. In an early study investigating habituation in people with social anxiety disorder, patients showed an altered pattern of amygdala habituation to novel emotional faces, although group differences in the rate of habituation were not found (Campbell *et al*, 2007). In a recent study, amygdala habituation was found in social anxiety disorder patients, but not controls (Sladky *et al*, 2012). However, in both studies, participants were required to

perform a task while viewing faces; in contrast, habituation studies in shyness have used passive viewing of faces. Task demands may significantly alter amygdala activity (Lieberman *et al*, 2011; Costafreda *et al*, 2008; Zald, 2003).

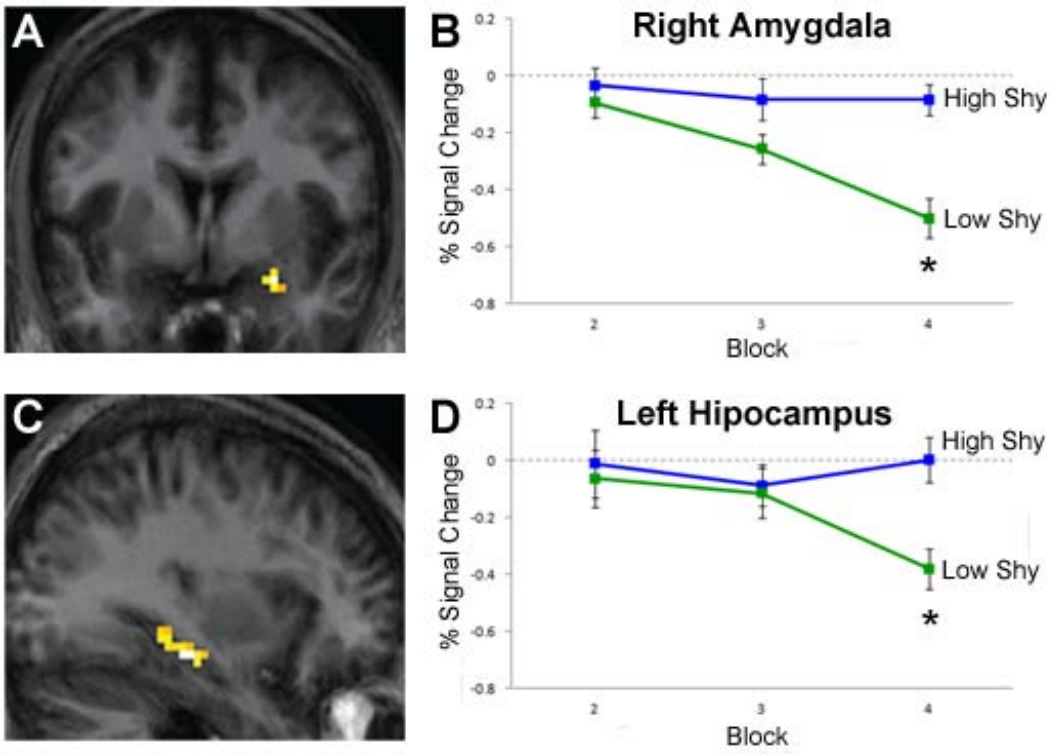


Figure 4. Amygdala and hippocampus fail to habituate in highly shy individuals. (A) The two shyness groups significantly differed in their rate of amygdalar habituation to faces (k=15 voxels). (B) In the low shy group, amygdala fMRI activity was significantly diminished in Block 4 relative to its initial response in Block 1 (Block 4 - Block 1; *p<.05). However, in the highly shy group, amygdala fMRI activity failed to habituate from its initial response in Block 1. fMRI signal for Blocks 2-4 are normalized to Block 1. (C) The two shyness groups also significantly differed in their rate of hippocampal habituation to faces (k=57 voxels). (D) In the low shy group, hippocampal fMRI activity was significantly diminished in Block 4 relative to its initial response in Block 1 (Block 4 - Block 1; *p<.05). However, hippocampal fMRI activity was sustained over time in the highly shy group. fMRI signal for Blocks 2-4 are normalized to Block 1.

1.6. A social fearfulness network

Together, each of the brain regions showing disrupted activity in people with social anxiety disorder—including the amygdala, hippocampus, vmPFC, OFC, FFA, and visual cortex (including V1 and extrastriate cortex—form a structurally and functionally interconnected network of bottom-up and top-down processing of visual social threat **(Figure 5)** (Stefanacci *et al*, 1996; Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004; Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005; Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006; Wager *et al*, 2009a). Activity across this network is likely important in

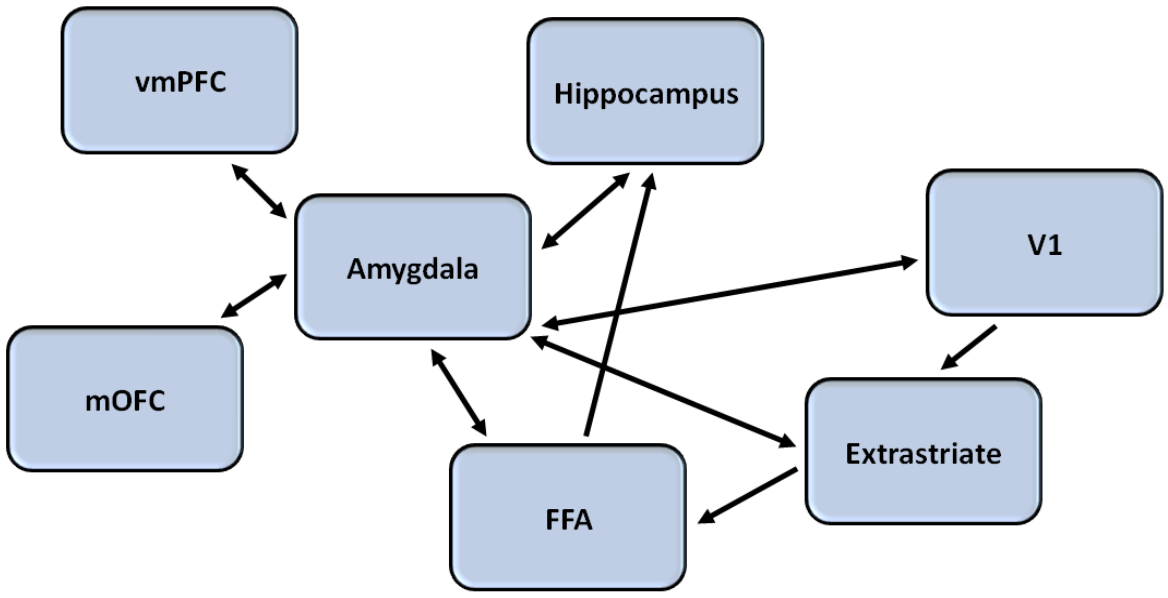


Figure 5. The social fearfulness network. Brain regions implicated in social fearfulness comprise a highly-interconnected network responsible for processing visual social threats.

the experience of social fear. For example, a recent study has shown enhanced connectivity between the two nodes of this network, the amygdala and FFA, in social

anxiety patients (Frick *et al*, 2013a). FFA–amygdala connectivity is critical in the detection of novel faces and the processing of threat. The FFA receives low-level visual information about color, contrast and motion from primary visual processing regions, such as primary visual cortex (V1) and extrastriate cortex, and in turn, forwards information about face identity and expression to the both the amygdala and hippocampus through direct projects (Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005). Through reciprocal connections, the amygdala and hippocampus send highly processed information back to FFA and primary visual processing regions (Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005). Given its central role in face and threat processing, connectivity between the FFA and amygdala may be central to the experience of fear in response to novel faces.

Interactions between the amygdala and hippocampus have also been shown to significantly influence social behavior (Felix-Ortiz and Tye, 2014). The amygdala and hippocampus are densely structurally interconnected (Stefanacci *et al*, 1996) and have important influence over each other in the process of forming and retrieving emotional memories (Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004). Neural processing of the surrounding environment, including evaluation of potential threats, appears to involve a complex interaction between the amygdala and the hippocampus, with the amygdala influencing memory-related plasticity in the hippocampus (Akirav and Richter-Levin, 1999), and the hippocampus providing contextual information to the amygdala that may help modulate amygdala activity during social threat (Iidaka *et al*, 2001; Guyer *et al*, 2008). Amygdala-hippocampal connectivity is likely important in

inhibition of behavior in social situations (Gray, 1983; Gray and McNaughton, 2003) and may be critical in dysfunction and disability associated with extreme social fears.

Connectivity with the medial prefrontal cortex has also been shown to be disrupted in social anxiety disorder; in particular, the mOFC, a region with dense structural connections with the amygdala, shows disrupted functional connectivity with the amygdala in people with social anxiety disorder (Sladky *et al*, 2013). Another region of the medial prefrontal cortex, the vmPFC also has direct structural interconnections with the amygdala (Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006) and lower activity in the vmPFC during a social threat task is associated with sympathetic system activation, a marker of fear and arousal (Wager *et al*, 2009b). This is in line with the vmPFC's hypothesized role in regulation of amygdala activity (Motzkin *et al*, 2014; Hartley and Phelps, 2010; Quirk and Beer, 2006). Connections between the amygdala and medial prefrontal cortex are important in the regulation of activity and may fail to provide external control over the amygdala in social fearfulness. Together, activity across this visual social threat processing network contributes to social anxiety disorder, and there is preliminary evidence that it also varies dimensionally with social fear. However, a comprehensive assessment of activity across this network in social fearfulness is lacking.

1.7. Summary

Initial response and habituation to novelty are two fundamental processes by which we learn about the environment around us, and are key in detecting and filtering salient sensory information. As an individual learns that a stimulus in the environment is

neither threatening nor rewarding, the stimulus becomes safe and familiar, resulting in habituation at both the behavioral and neural levels. However, failure to habituate to non-threatening stimuli in the environment may trigger feelings of uncertainty or unfamiliarity, resulting in fear and anxiety. Evidence in shyness supports the hypothesis that habituation may be related to increased social fearfulness. For example, shy people are typically slow to acclimate to new people and objects, consistent with slower habituation. Additionally, our lab has recently shown that shy individuals fail to show habituation to novel faces in the amygdala and hippocampus, two brain regions associated with fear and evaluation of threat. However, no studies to date have been conducted exploring initial response and habituation as separable processes. Because individual differences in both initial amplitude of response and habituation to social stimuli may provide an important neurobiological marker for risk for psychiatric illness, such as social anxiety disorder, we propose that exploration of these two fundamental processes in individuals ranging from low to high in social fearfulness is critical.

1.8. Specific aims

The goal of this dissertation is to test the hypothesis that neural differences in initial amplitude and habituation to novelty mediate individual differences in social fearfulness. For social animals, initially heightened neural processing of a novel social stimulus is a highly adaptive response that facilitates quick behavioral reaction (Mobbs *et al*, 2009; Blanchard *et al*, 2011) in order to protect one's self from danger. However, social stimuli that are non-threatening are associated with a rapid return to baseline in healthy people (Breiter *et al*, 1996). Our hypothesis is that failure to rapidly habituate to

non-threatening social information may contribute to increased social fearfulness.

To characterize neural habituation in social fearfulness, we study a core set of brain regions that play a critical role in the processing and expression of anxiety and fear (Lang *et al*, 2000; Davis, 1997; Gray and McNaughton, 2003; Gray, 1983; Blanchard *et al*, 2011; Mobbs *et al*, 2009; Adolphs *et al*, 1999), including the amygdala, hippocampus, mOFC , vmPFC, FFA, V1, and extrastriate cortex. Current evidence points to an overall difference in activity in these regions in people with social anxiety disorder (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012); however, the temporal course of the brain's response in these regions is unclear.

The specific aims of this dissertation are to:

1. Characterize neural habituation to repeated faces in a group of adults representative of the full spectrum of social fearfulness (Chapter 2);

2. Examine habituation of neural connectivity to repeated faces across the social fearfulness spectrum (Chapter 3);

3. Examine whether habituation differences are specific to faces by testing whether an association exists between social fearfulness and habituation to repeated objects (Chapters 4 and 5);

4. Examine whether associations between social fearfulness and face processing are specific to social fearfulness by testing for unique effects of social fear, trait anxiety, and depression (Chapter 5).

CHAPTER II

Associations between social fearfulness and neural response to novel and repeated faces

2.1. Introduction

The evaluation of visual social threat is dependent on a key network of brain regions—including the amygdala, hippocampus, mOFC, vmPFC, FFA, V1, and extrastriate cortex **(Figure 2)** (Freitas-Ferrari *et al*, 2010; Miskovic and Schmidt, 2012; Lungwitz *et al*, 2014; Riga *et al*, 2014; Straube *et al*, 2004; McTeague *et al*, 2011)—with each of these brain regions showing dysfunction in people with social anxiety disorder. However, the mechanism underlying disrupted function in these brain regions remains unknown. Many neuroimaging studies have sought to understand social anxiety by studying the magnitude of response to social stimuli, with magnitude representing the average signal across the entire experiment (sometimes 30 minutes or more). One common takeaway from this type of experiment is that social anxiety is a disorder of functional magnitude, with many threat processing regions showing an elevated magnitude of response to social threat, and regulatory regions showing dampened magnitudes. However, the averaging of signal magnitude across an experiment obscures two fundamental elements of the brain's reaction to a stimulus—initial amplitude of response to the social stimulus, and habituation to the stimulus over time.

Initial amplitude and habituation of neural response serve largely differing functions in the brain, and dysfunction in one or both elements implicates distinguishable functional processes underlying social fear. For social animals, initially

heightened neural processing of a potential social threat is a highly adaptive response that facilitates quick behavioral reaction (Blanchard *et al*, 2011; Mobbs *et al*, 2009) in order to protect one's self from danger. Novel stimuli elicit a strong orienting response (Sokolov, 1963) and sharpened attention, perception and memory (Schomaker and Meeter, 2015). Elevated initial amplitudes may reflect a maladaptive over-engagement of these neural resources, potentially leading to an inability to disengage from a stimulus. In contrast, neural habituation is a fundamental learning mechanism, and deficits in this process may reflect a deficit in social learning. As an individual learns that a stimulus is neither threatening nor rewarding, the stimulus becomes safe and familiar, resulting in habituation at the behavioral and neural level. Social stimuli that are non-threatening are associated with a rapid return to baseline in healthy individuals (Breiter *et al*, 1996; Pedreira *et al*, 2010; Rey *et al*, 2014; Blackford *et al*, 2010; Schwartz *et al*, 2003b; Fischer *et al*, 2003; Wright *et al*, 2001). However, failure of habituation is a maladaptive response that may be associated with higher levels of social fear. A recent finding from our lab demonstrated a link between social function and habituation failure; highly shy individuals failed to show habituation in the amygdala and hippocampus, two key brain regions associated with fear and evaluation of threat (Blackford *et al*, 2013, 2011). This finding is consistent with findings of slower behavioral habituation in shyness; shy individuals are typically slow to acclimate to new people and objects (Kagan *et al*, 1987; Garcia-Coll *et al*, 1984). Because initial amplitude and habituation subserve separate processes in the brain, distinguishing the relative contribution of each to social fearfulness may help guide appropriate intervention and treatment strategies.

To determine whether initial amplitude, habituation of response to faces, or both contribute to the experience of social fearfulness, we used functional magnetic resonance imaging (fMRI) to measure brain activity to novel and repeated faces. Participants were selected to represent the social fearfulness spectrum. While case/control studies of social anxiety disorder have contributed to a broad understanding of which brain regions are involved in social fear, dimensional studies are essential in identifying neurobiological mechanisms. The National Institute of Mental Health's Research Domain Criteria project (RDoC) has proposed the use of dimensional approaches, which focus on understanding the neurobiology of specific traits that span the range from normal to pathological, is the critical next step in enabling neuroscience research to inform diagnosis and treatment of psychiatric disorders. An ideal candidate for a dimensional approach is social fearfulness, a trait that ranges from minimal to extreme and encompasses both sub-syndromal and clinical manifestations of social anxiety (Stein *et al*, 1994; Schneier *et al*, 2002; Davidson *et al*, 1994; Stein *et al*, 2004; Furmark, 2002). We propose that understanding the neurobiological correlates of social fearfulness is an essential step in defining a dimensional biological marker for social anxiety. We designed a "repeated faces" task to study response to faces, wherein participants viewed a set of novel face identities repeated up to 7 times throughout the experiment. Because habituation is ubiquitous in the brain, we restricted our analysis to a set of regions previously identified as showing dysfunction in social anxiety **(Figure 2)**.

2.2. Methods

2.2.1. *Participants*

Characteristics. Twenty-nine young adults (13 female) were included in this analysis. Participants were selected based on social fearfulness scores, with an oversampling at the low and high ends of the social fearfulness continuum (see Recruitment and selection, below). Participants were on average 22 years old (SD = 2), Caucasian (72%) and right-handed (79%). There were no associations between social fearfulness and age, sex, race, or handedness **(Table 1)**.

Recruitment and selection. Participants between the ages of 18-25 were recruited from the Vanderbilt University community and surrounding Nashville area using advertisements and recruitment databases. Because observations across the continuum of social fearfulness are necessary for the reliable and precise estimate of the relationship between social fear and neural activity, we used a specialized recruitment strategy—general advertisements seeking individuals to participate in a study “to learn how differences in personality may relate to brain functions” were used to recruit a normally-distributed range of shyness from the population; additionally, targeted advertisements seeking “especially shy” or “especially outgoing” individuals were used to provide an oversampling at the extreme ends of the continuum. Shyness in many ways parallels the physiological, cognitive and behavioral correlates of social anxiety and social fearfulness (Heiser *et al*, 2003).

Prior to enrollment, individuals responding to study advertisements completed an online screening (Revised Cheek and Buss Shyness Scale (RCBS); Hopko *et al*,

2005). We selected the RCBS as a screener because it is a short (13 question), well-validated measure of shyness (Hopko *et al*, 2005). To ensure recruitment of the full

Table 1. Participant characteristics.

	Mean	S.D.	Min	Max	Skewness	Kurtosis
Age (years)	22	2	18	25	-.22	-1.07
Social fearfulness (SHY-SR)	56	34	3	120	.51	-.84
Shyness screen (RCBS)	33	11	15	56	.53	-.67
Trait anxiety (STAI-Trait)	35	12	20	64	.58	-.69
Depression (BDI-II)	7	6	0	27	1.32	2.16
	Count					
Gender (M / F)	(16 / 13)					
Race (C / AA / A)	(21 / 2 / 6)					
Handedness (R / L / Amb)	(23 / 4 / 2)					

Note: Male (M); Female (F); Caucasian (C); African-American (AA); Asian (A); Right (R); Left (L); Ambidextrous (Amb)

shyness distribution we used a stratified recruitment strategy, recruiting approximately equal numbers of participants into each of four levels: not at all shy ≤ 15th percentile; a little shy = 16th – 49th percentile; moderately shy = 50th – 84th percentile; very shy ≥ 85th percentile. Potential scores on the RCBS range from 13 to 65, with higher scores indicating higher levels of shyness. In our sample, participants' screening scores ranged from 15 (not at all shy) to 56 (very shy) and were normally distributed **(Table 1)**. Social fearfulness scores (see below) were well-correlated with shyness screening scores (r = .67, p < .001).

Social fearfulness measurement. We assessed social fearfulness following enrollment in the study using the Social Anxiety Spectrum Self-Report (SHY-SR) (Dell'Osso *et al*,

2014, 2002). The SHY-SR is a 168-item questionnaire specifically developed to assess the dimension of social anxiety and fearfulness, including clinical and sub-clinical symptoms, as well as atypical presentations and isolated symptoms. The SHY-SR ranges from 0-164, with higher scores indicating higher social fearfulness. In our sample, participants' scores spanned the continuum of social fearfulness, ranging from 3 (low social fear) to 120 (high social fear), and were normally distributed across the spectrum **(Table 1)**.

Other measures. High negative affect is a characteristic associated with social fearfulness (Schmidt *et al*, 1997), and is considered a general risk factor for emotional disorders, including social anxiety disorder (Grupe and Nitschke, 2013). We included two commonly-used measures sensitive to negative affect (Watson and Clark, 1984), the State-Trait Anxiety Inventory (STAI) (Spielberger, C, Gorsuch, R, Luschene, R, Vagg, P, Jacobs, 1983) and the Beck Depression Inventory (BDI-II) (Beck, A, Steer, R, Ball, R, Ranieri, 1996). The STAI is comprised of 20 questions assessing general (trait) anxiety and 20 questions assessing current (state) anxiety on a 4-point Likert scale; only trait anxiety scores were included in this study. STAI-trait scores range from 20-80, with higher scores indicating higher trait anxiety and negative affect. Participants' scores ranged from 20 (low trait anxiety) to 64 (high trait anxiety). The BDI-II is a 21-item measure of current depression symptoms. Individual items are scored on a Likert scale ranging from 0-3, with total BDI-II scores ranging from 0-63. Participants' scores ranged from 0 (minimal current depression) to 27 (moderate current depression).

Exclusion criteria. Participants were excluded for past or current psychiatric illness based on the Structured Clinical Interview for the DSM-IV (SCID) (Spitzer *et al*, 1992) with the exception of untreated social anxiety disorder in socially fearful participants. Social anxiety is common in socially fearful individuals; therefore, exclusion of socially fearful individuals with a diagnosis of social anxiety may result in a sample of exceptionally resilient individuals and reduce the generalizability of the study findings. Two participants with high social fear scores (both ≥ 68) had significant social anxiety symptoms based on the clinical interview. Participants were also excluded for current use of psychoactive medications (prev. 6 months) as these may affect brain function. Other exclusion criteria included: significant medical or neurological illness; pregnancy; developmental disability or intellectual deficit; head injury resulting in loss of consciousness; or any conditions that preclude MR scanning (e.g. metal implants). Thirty-two individuals were consented for the study; however, 3 individuals were excluded from the analysis for beginning psychoactive medication treatment between the first and second study visit (n = 1), at the request of the participant (n = 1), and for errors in data collection (n = 1) (see fMRI procedures below), resulting in a final sample of 29 participants.

The Vanderbilt Institutional Review Board approved the study and we obtained written informed consent after providing participants with a complete description of the study.

2.2.2. *Experimental paradigm*

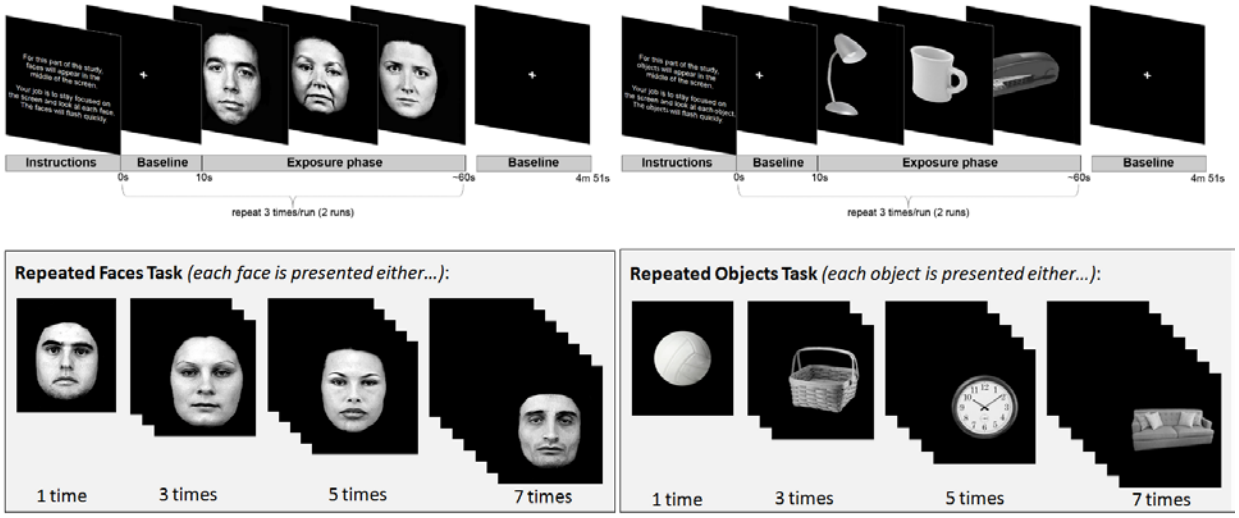


Figure 6. Repeated faces and repeated objects task design. There were a total of 32 neutral face stimuli and 32 neutral object stimuli presented in each task. Each stimulus was presented either 1 time, 3 times, 5 times, or 7 times. Stimuli were presented in pseudo-random order for 1 s followed by a black screen for 2 – 4 s.

Repeated faces task. We designed a "repeated faces" task to investigate neural habituation to social stimuli **(Figure 6)**. The repeated faces task was comprised of 8 face presentation blocks presented across 2 fMRI runs, with each run lasting approximately 4 m, 50 s. Each functional run began with a 10 s fixation crosshair followed by 4 blocks of faces. Blocks consisted of 16 face presentations followed by a 10 s fixation crosshair. Each face presentation lasted a total 1 s followed by a black screen shown for 2 - 4 s. At the beginning of the repeated faces task, participants were told 'In this study faces will appear in the middle of the screen. Your job is to stay focused on the screen and look at each face. The faces will flash quickly'. Participants were shown a series of 32 face identities, with each face identity shown a total of 1 time, 3 times, 5 times, or 7 times, for a total of 128 face presentations. Faces were shown in

pseudorandom order using a jittered, event-related design to maximize fMRI signal measurement efficiency (Friston *et al*, 1999). Stimulus jitter was randomly distributed across presentations. The repeated faces task was presented using E-Prime software (Version 2.0, Psychology Software Tools, Pittsburgh, PA, USA).

Face stimuli. We used faces with a neutral expression, which are ideally-suited to study individual differences in social fearfulness—while strong stimuli (e.g., fear faces) maximize response across individuals, potentially creating a ceiling effect that obscures individual differences, weaker stimuli (e.g., neutral faces) facilitate the detection of individual differences (Lissek et al, 2006). Face stimuli were derived from two standard sets of human face images with neutral-valenced expressions (Gur *et al*, 2001; Lundqvist, D, Flykt, A, Ohman *et al*, 1998). All face stimuli were edited to ensure uniform size, midtone, contrast, level equalization, eye position, and vertical nose bridge position. Extraneous features such as hair and shirt collars were removed from face stimuli. Selection of neutral face stimuli was pseudorandom, counterbalanced for gender and stimulus set.

2.2.3. *MRI data*

Acquisition. Structural and functional MRI data were collected using a 3 Tesla Philips scanner equipped with a 32-channel head coil (Philips Healthcare, Inc., Best, The Netherlands). High-resolution T1-weighted structural images were collected (256 mm FOV, 189 slices, 1 mm slice thickness, 0 mm gap). Functional echo planar images (EPI) were acquired using a sequence optimized to reduce signal loss in the ventral forebrain,

including amygdala and OFC: 2 s TR; 28 ms TE; 90° flip angle; 1.8 SENSE factor; 240 mm FOV; 3 x 3 mm in-plane resolution using an 80 x 80 matrix; and higher order shimming to limit susceptibility artifacts. Each volume contained 38 3.2 mm (0 gap) axial oblique slices (tilted 15° anterior higher than posterior relative to the intercommissural plane), which provided whole-brain coverage.

Preprocessing. MRI data were analyzed using statistical parametric mapping (SPM8; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom) and MATLAB (Version 7.10 64-bit, The MathWorks, Inc., Natick, MA, USA). fMRI data were preprocessed for slice time correction, realigned to the mean slice to correct for motion, spatially normalized into standard stereotactic space (MNI T1 template) using both linear (12-parameter affine) and nonlinear transformations. Data were smoothed with a 6 mm FWHM Gaussian kernel to account for individual differences in brain anatomy. Functional EPI images were visually inspected for artifacts and signal dropout. Volumes with excessive motion (> 3 mm) or signal artifacts (signal > 1.8% of mean) were removed from the analysis using Artifact Detection software (ART; Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC)). Volume artifacts were not correlated with participants' social fearfulness scores or the repeated faces task.

2.2.4. Regions of interest (ROIs)

We selected seven regions previously shown to play a role in social anxiety: the amygdala (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic

and Schmidt, 2012); hippocampus (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012); ventromedial prefrontal cortex (vmPFC; Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012); the medial orbitofrontal cortex (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012); the fusiform face area (see FFA localizer details below; Freitas-Ferrari *et al*, 2010; Miskovic and Schmidt, 2012); the primary visual cortex (Demenescu *et al*, 2013; Frick *et al*, 2014); and the extrastriate visual cortex (Freitas-Ferrari *et al*, 2010; Straube *et al*, 2007; Demenescu *et al*, 2013; Frick *et al*, 2014). Each of these regions has been shown to play a role in expression of fear (Lang *et al*, 2000; Davis, 1997; Gray and McNaughton, 2003; Gray, 1983; Blanchard *et al*, 2011; Mobbs *et al*, 2009; Adolphs *et al*, 1999), and together these regions form a structurally and functionally interconnected network for processing visual social threat (Stefanacci *et al*, 1996; Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004; Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005; Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006; Wager *et al*, 2009a).

Anatomical atlas regions. The amygdala, hippocampus, primary visual cortex (calcarine fissure), and extrastriate cortex (lingual gyrus, inferior and middle occipital cortex) ROIs were defined using the AAL standard masks (Automated anatomical labeling; Tzourio-Mazoyer *et al*, 2002) implemented in Wake Forest University Pick Atlas (WFU Pick Atlas; Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003). The vmPFC and mOFC ROIs were defined according to population masks of human

architectonic areas based on comparative cytoarchitecture in humans and non-human primates (Mackey and Petrides, 2010): the vmPFC was defined as areas 14m, 25, 24 and 32; the mOFC was defined as areas 14r, 14rr, 14c, and 11m.

FFA ROI. FFA ROIs were functionally defined for each participant (see FFA localizer task below). Individual ROIs were maintained for analysis, as previous studies have shown that this approach is stronger, relative to a group overlap ROI, in selectively analyzing face processing signal (Saxe *et al*, 2006). A contrast of faces > scenes was created for each participant and activations within the fusiform gyrus were examined (AAL; WFU pickatlas). A minimum cluster size of 8 voxels (p = .005) provided a cluster-corrected α = .05. Statistical thresholds were adjusted for each participant to constrain activations to a maximum cluster size of 37 voxels (999 mm^3) in the right fusiform and 19 voxels (513 mm^3) in the left fusiform—maximum cluster sizes were based on a review of published studies reporting FFA volumes (Berman *et al*, 2010). Significant clusters were found for the majority of participants in both the right and left hemispheres. Six participants had activation in either the left (n = 3) or the right (n = 3) hemisphere only. Two participants did not have significant FFA activity in either hemisphere. There were no associations between FFA cluster size and social fearfulness, or between detection of FFA clusters and social fearfulness.

FFA localizer task. Because the precise location of the FFA cannot be anatomically defined and differs across individuals, it is necessary to identify the FFA using a functional task. We used a standard FFA localizer task (Wong and Gauthier, 2010) to

functionally define our FFA ROI in each subject. The FFA localizer task consisted of 2 fMRI runs lasting 3 m, 56 s each. Each run began with a 10 s fixation block followed by 9 blocks (16 s each) of face, scene, or scrambled images separated by fixation periods (5 – 16 s), ending with a 10 s fixation block. Face, place and scrambled images were presented for 750 ms, followed by a 250 ms blank screen. Blocks were presented in a pseudorandom order and participants performed a 1-back task (1 – 3 repeated images / block) to promote attention to the images.

2.2.5. *Data analysis*

fMRI data modeling. The first-level (participant) temporal model was estimated using a general linear model (GLM; Friston *et al*, 1995). The design matrices included 4 task regressors, one for each face exposure category (1, 3, 5, 7), convolved to the SPM default hemodynamic response function (HRF). Motion parameters were also included as additional covariates of no interest. Data were high-pass filtered (128 s) to attenuate low frequency signal (linear scanner drift).

Habituation. Habituation is dependent on initial amplitude of response; that is, there is more opportunity for signal to attenuate over time if signal is initially high, while habituation over repeated faces will be minimal (floor effect) if initial signal to faces is low. However, response to novel faces and habituation to repeated faces may be influenced by different mechanisms in the brain. To disentangle habituation from initial amplitude differences, we calculated a normalized habituation slope (*b'*) independent of initial amplitude differences for each participant (Montagu, 1963; Plichta *et al*, 2014).

We first extracted percent signal change from each ROI using MarsBar (Brett *et al*, 2002). Percent signal change in the left and right hemispheres were highly correlated across ROIs; to increase statistical power and minimize type I error, data from left and right ROIs were averaged. However, for completeness, we also include a secondary analysis to specifically test for laterality effects.

Neural habituation slopes were modeled for each participant using the regression

$$Y = bX + a$$

where the mean ROI response (Y) is predicted by the log-transformed face presentation number (X). Face presentations 1, 2, 3, 4, 5, 6, and 7 were natural log-transformed to 0, .69, 1.1, 1.39, 1.61, 1.79, and 1.95. The natural log transform linearizes the habituation curve, which is steepest during early face repetitions, enabling linear regression analysis. We then calculated *b'* for each participant as

$$b' = b - c(a - \bar{a})$$

where *b* is the participant's regression slope, *c* is the mean regression parameter estimate (time) of the sample, and *a* is the initial amplitude estimate (intercept). SAS software (Version 9.3, SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses.

Social fearfulness analysis. Correlations tested for associations between social fearfulness and initial amplitude (intercept) of response to novel faces, and between social fearfulness and normalized habituation slope (*b'*) to repeated faces (habituation from 1st to 7th face presentation). Correlation results were considered significant at $\alpha \leq .05$. R^2 values were computed as a measure of effect size. To visually display the

pattern of results, data were split into three groups (tertiles of social fearfulness scores) and means and standard errors are presented as bar graphs.

Previous results from our lab indicate that shy individuals show a non-linear pattern of behavioral habituation across a similar repeated faces task, with the strongest differences in behavior occurring at the 3rd and 5th face presentation, and with responses across all participants reaching an asymptote by the 7th face presentation (Avery *et al*, 2015). Therefore, we conducted planned secondary analyses to examine associations between social fearfulness and habituation to faces within three discrete repetition windows: 1st to 3rd presentation; 3rd to 5th presentation; 5th to 7th presentation. Secondary habituation analyses were considered significant at $\alpha \leq .0167$ (.05 / 3), Bonferroni-corrected for multiple comparisons.

Arousal/valence. Although face stimuli were derived from neutral-valenced standard sets, differences in how each participant perceived the neutral images could contribute to differences in initial neural response and rate of habituation. Therefore, following all MRI scanning procedures, participants were re-shown each face stimulus on a computer outside the MRI scanner and asked to make arousal and valence ratings. Arousal and valence are dimensional measures used to characterize affective experience. The dimension of arousal ranges from calm to exciting. The dimension of valence ranges from negative to positive. For each face, arousal rating was made first followed by valence rating. Arousal and valence ratings were made on a scale of 1 (“very excited” or “very unpleasant”) to 7 (“very calm” or “very pleasant”). A rating of 4 was indicated as “not excited or calm” or “not pleasant or unpleasant”. Correlations

tested for associations between arousal/valence rating and social fearfulness. Correlations were considered significant at $\alpha \leq .05$.

Laterality. Although neural measures were similar across left and right hemispheres, there is evidence for significant laterality in the brain (Gotts *et al*, 2013; Knecht, 2000; Roth and Hellige, 1998). Therefore, we conducted a secondary analysis to test for initial amplitude and habituation differences across hemispheres. Neural habituation slopes were modeled for each participant by hemisphere and initial amplitude and *b'* slopes were calculated. We first performed *t*-tests between hemispheres to directly test for laterality effects in initial amplitude and habituation. To further explore possible differences in laterality, we conducted the main analysis separately for left and right hemispheres. Using α's consistent with the main analysis; initial amplitude and habituation ($1^{st} – 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary habituation results ($1^{st} – 3^{rd}$; $3^{rd} – 5^{th}$; $5^{th} – 7^{th}$) were considered significant at $\alpha \leq .0167$, Bonferroni-corrected for multiple comparisons.

2.3. Results

2.3.1. *Arousal/valence ratings*

Differences in how participants perceived faces could influence initial reactivity and habituation rates; to ensure that faces were viewed as neutral by all participants, we asked participants to make arousal and valence ratings for each face. Participants rated faces on a scale from 1 to 7 with a rating of 4 indicating a neutral arousal/valence. Participants rated faces as neutral on both arousal and valence (arousal, mean = 4.14,

SD = .60, range = 3.50 – 6.81; valence, mean = 3.87, SD = .32, range = 3.07 – 4.80). There were no associations between social fearfulness and arousal or valence ratings (all *p*'s > .43).

2.3.2. *Initial amplitude*

Social fearfulness was correlated with heightened initial amplitude to novel faces in two regions, the hippocampus and the vmPFC (hippocampus, $r = .49$, $p = .008$; vmPFC, $r = .48$, $p = .008$; **Table 2; Figure 7**). There were no correlations between social fearfulness and smaller initial amplitudes. To illustrate associations, data were split into social fearfulness groups (tertiles). Overall, the low social fear group showed minimal response to novel faces, with small initial amplitudes in the hippocampus and, on average, no response in the vmPFC. In contrast, the high social fear group had a strong initial response to novel faces in both regions **(Figure 8)**. Values for initial response to faces are presented in **Table 3**.

2.3.3. *Habituation*

1^{st} – 7^{th} face presentation. Social fearfulness was correlated with habituation differences in the hippocampus ($r = .46$, $p = .01$; **Table 2; Figure 7**). Visualizing low and high social fear groups, a consistent pattern was illustrated: the low social fear group showed negative *b'* slopes in the hippocampus, indicating habituation across repeated face presentations; in contrast, the high social fear group had *b'* slopes near or above zero, demonstrating a failure to habituate to faces **(Figure 8)**. To visualize neural response to

repeated faces over time, we extracted percent signal change values from each region; values are presented in **Table 3**.

1st – 3rd face presentation. We conducted planned secondary analyses to explore habituation differences during early (1st – 3rd), middle (3rd – 5th), and late (5th – 7th) repetition windows. During early face presentations, social fearfulness was correlated with habituation differences in the extrastriate cortex (r = .43, p = .01; **Table 2; Figure 7**). Visualizing social fearfulness by groups, the low social fear group demonstrated negative *b'* slopes in the extrastriate cortex, indicating rapid habituation to faces during early presentations. In contrast, the high social fear group had *b'* slopes near or above zero, indicating a sustained or increasing response across early face presentations **(Figure 8)**.

3rd – 5th face presentation. During the middle face presentation window (3rd – 5th), social fearfulness was correlated with habituation differences across multiple brain regions—including V1, extrastriate cortex, amygdala, hippocampus, mOFC and vmPFC (V1, r = .52, p = .004; extrastriate, r = .57, p = .001; amygdala, r = .45, p = .01; hippocampus, r = .56, p = .002; mOFC, r = .44, p = .02; vmPFC, r = .51, p = .005; **Table 2; Figure 7**). Visualizing habituation by group, a consistent within-group pattern is illustrated across regions—on average, low social fear had consistently negative *b'* slopes, indicating habituation in low social fear participants, while the high social fear group showed *b'* slopes near or above zero, indicating a sustained or increasing response to repeated faces through the middle presentation window **(Figure 8)**.

Table 2. Correlations between social fearfulness and neural response to faces.

	Initial amplitude (intercept)			**Habituation (*b'*)**											
				$1^{st} - 7^{th}$			**$1^{st} - 3^{rd}$**			**$3^{rd} - 5^{th}$**			**$5^{th} - 7^{th}$**		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.06	.004	.75	-.03	.001	.88	.35	.12	.06	**.52**	**.32**	**.004**	-.07	.005	.72
Extrastriate	.27	.07	.27	.14	.02	.48	**.43**	**.18**	**.01**	**.57**	**.32**	**.001**	.02	.0004	.93
FFA	.11	.01	.59	.25	.06	.22	.39	.15	.04	.33	.11	.09	.18	.03	.37
Amygdala	.36	.13	.06	.31	.10	.11	.34	.12	.07	**.45**	**.20**	**.01**	-.15	.02	.44
Hippocampus	**.49**	**.24**	**.008**	**.46**	**.21**	**.01**	.39	.15	.04	**.56**	**.31**	**.002**	-.21	.04	.28
mOFC	.20	.04	.31	-.05	.03	.79	.35	.12	.06	**.44**	**.19**	**.016**	-.01	.0001	.96
vmPFC	**.48**	**.23**	**.008**	.36	.13	.06	.28	.08	.14	**.51**	**.26**	**.005**	.06	.004	.75

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary analysis results were considered significant at $\alpha \leq .0167$.

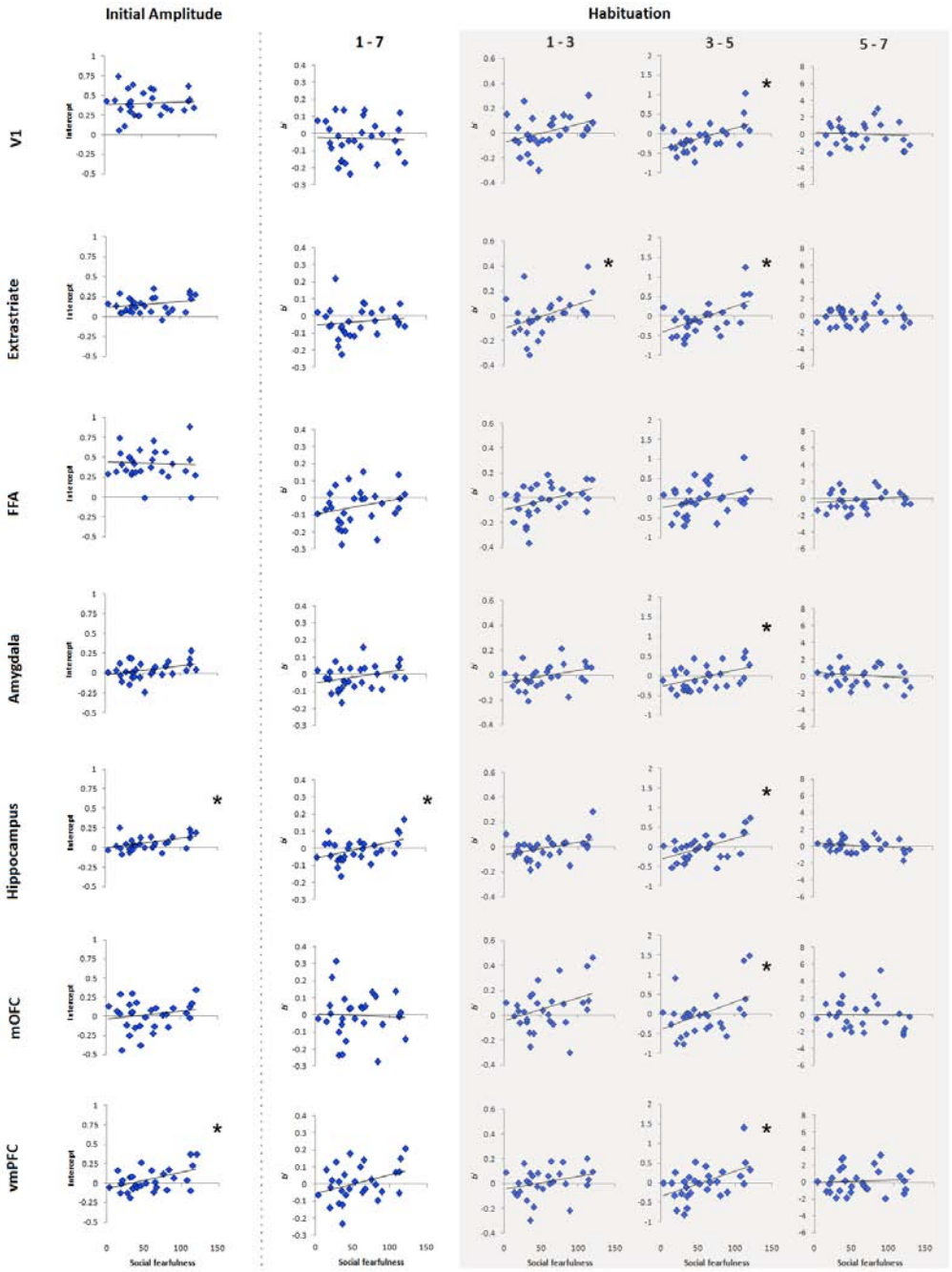


Figure 7. Correlations between social fearfulness and neural response to faces. Scatterplots show the relationship between social fearfulness and neural response to novel and repeated faces. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel faces. Slope (b') values below zero indicate habituation to repeated faces; b' slope values at or above zero indicate sustained or increasing signal to repeated faces, respectively. Social fearfulness was correlated with heightened initial amplitudes to faces in the hippocampus and vmPFC, and with sustained response to repeated faces in the hippocampus (1st - 7th). To further explore habituation differences, we examined signal change over three repetition windows in a secondary habituation analysis (shaded in grey). Secondary habituation contrasts revealed a correlation between social fearfulness and sustained signal in the extrastriate cortex across early (1st - 3rd) face presentations, and in V1, extrastriate cortex, amygdala, hippocampus, mOFC, and vmPFC across middle (3rd - 5th) face presentations. There were no social fearfulness differences in habituation across late (5th - 7th) face presentations.

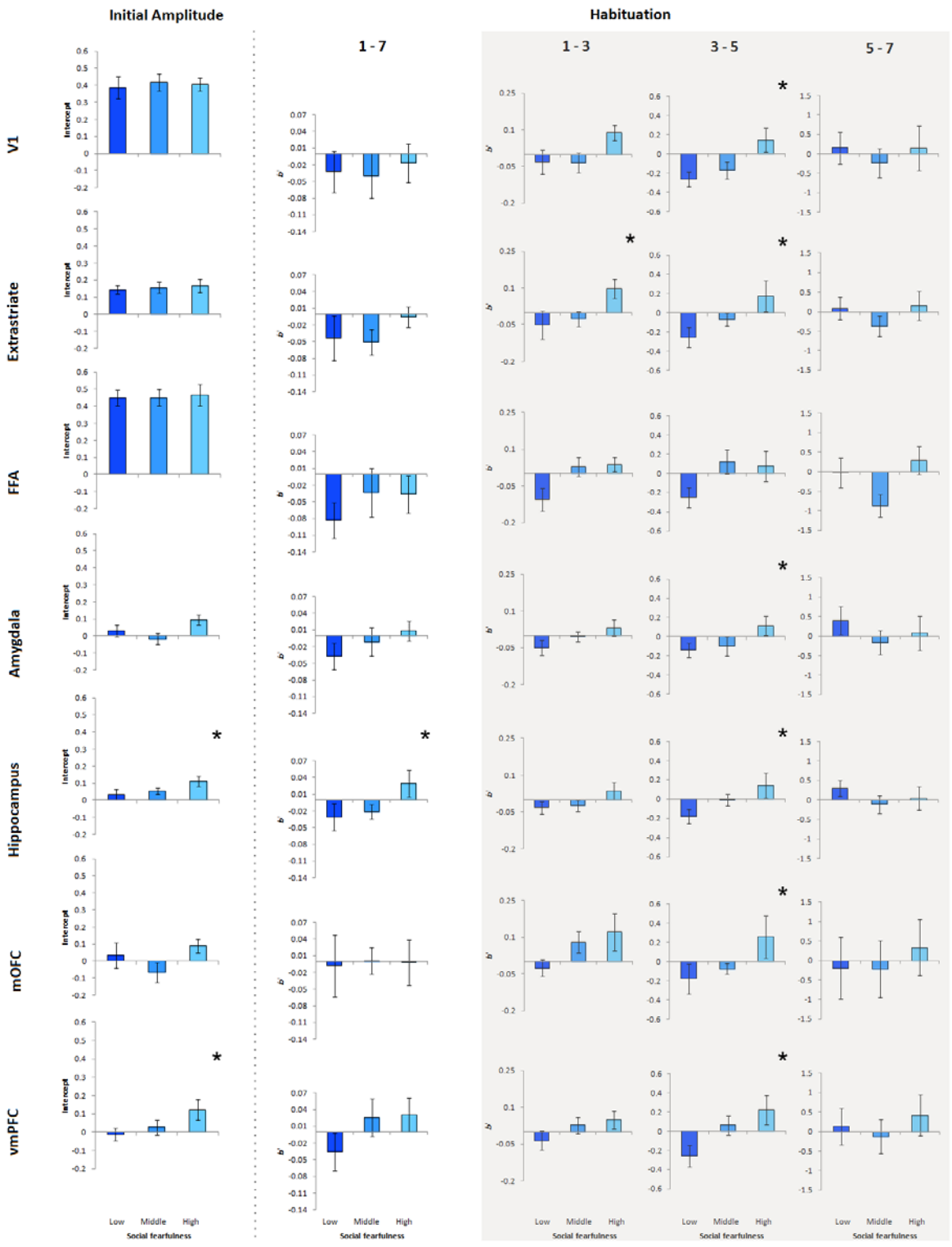


Figure 8. Neural response to faces by social fearfulness tertile. Bar graphs show initial amplitude (intercept) and habituation (b') slope values by social fearfulness tertile for novel and repeated faces. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel faces. Slope (b') values below zero indicate habituation to repeated faces; b' slope values at or above zero indicate sustained or increasing signal to repeated faces, respectively.

Table 3. Percent signal change by face presentation number.

Function	Region	Social fearfulness group	Face presentation 1st mean (sd)	2nd mean (sd)	3rd mean (sd)	4th mean (sd)	5th mean (sd)	6th mean (sd)	7th mean (sd)
Visual processing	V1	All	0.38 (.17)	0.40 (.16)	0.39 (.17)	0.38 (.25)	0.34 (.21)	0.33 (.22)	0.35 (.28)
		High	0.34 (.16)	0.39 (.15)	0.47 (.15)	0.51 (.29)	0.32 (.21)	0.33 (.26)	0.31 (.27)
		Middle	0.42 (.17)	0.42 (.14)	0.35 (.19)	0.32 (.16)	0.31 (.25)	0.33 (.22)	0.36 (.24)
		Low	0.39 (.19)	0.40 (.19)	0.34 (.14)	0.30 (.23)	0.38 (.17)	0.32 (.20)	0.38 (.35)
	Extrastriate	All	0.13 (.12)	0.16 (.13)	0.13 (.16)	0.13 (.17)	0.11 (.14)	0.05 (.17)	0.10 (.24)
		High	0.11 (.14)	0.18 (.11)	0.22 (.16)	0.23 (.16)	0.14 (.12)	0.10 (.14)	0.10 (.21)
		Middle	0.14 (.12)	0.15 (.13)	0.10 (.13)	0.08 (.13)	0.05 (.16)	0.03 (.16)	0.09 (.17)
		Low	0.14 (.11)	0.14 (.15)	0.07 (.16)	0.08 (.19)	0.13 (.14)	0.02 (.22)	0.10 (.33)
Face processing	FFA	All	0.43 (.17)	0.44 (.17)	0.40 (.19)	0.41 (.22)	0.39 (.16)	0.33 (.19)	0.33 (.29)
		High	0.41 (.21)	0.45 (.17)	0.46 (.18)	0.49 (.19)	0.43 (.16)	0.34 (.17)	0.30 (.27)
		Middle	0.42 (.16)	0.47 (.19)	0.45 (.19)	0.40 (.26)	0.36 (.17)	0.34 (.24)	0.44 (.25)
		Low	0.45 (.14)	0.40 (.17)	0.31 (.17)	0.35 (.20)	0.36 (.15)	0.32 (.20)	0.27 (.33)
Novelty / Threat detection	Amygdala	All	0.09 (.13)	0.14 (.14)	0.09 (.17)	0.09 (.25)	0.06 (.13)	0.08 (.18)	0.04 (.24)
		High	0.17 (.16)	0.22 (.13)	0.17 (.23)	0.21 (.24)	0.07 (.11)	0.15 (.12)	0.04 (.27)
		Middle	0.06 (.09)	0.09 (.14)	0.05 (.14)	0.03 (.26)	0.02 (.11)	-0.01 (.20)	0.03 (.14)
		Low	0.05 (.12)	0.11 (.14)	0.04 (.11)	0.02 (.21)	0.08 (.17)	0.09 (.18)	0.05 (.31)
	Hippocampus	All	0.06 (.09)	0.10 (.10)	0.04 (.12)	0.02 (.13)	0.04 (.08)	0.08 (.09)	0.06 (.15)
		High	0.08 (.10)	0.17 (.08)	0.09 (.15)	0.10 (.11)	0.04 (.10)	0.13 (.10)	0.08 (.16)
		Middle	0.06 (.07)	0.06 (.07)	0.04 (.07)	0.00 (.12)	0.02 (.05)	0.04 (.07)	0.07 (.08)
		Low	0.04 (.11)	0.07 (.11)	0.00 (.10)	-0.03 (.12)	0.05 (.09)	0.07 (.09)	0.04 (.19)
Outcome prediction	mOFC	All	-0.01 (.20)	0.06 (.19)	0.04 (.21)	-0.01 (.29)	0.05 (.30)	-0.07 (.43)	0.05 (.59)
		High	0.00 (.16)	0.10 (.19)	0.12 (.29)	0.08 (.36)	-0.01 (.31)	-0.02 (.51)	-0.09 (.50)
		Middle	-0.11 (.21)	0.08 (.19)	0.01 (.10)	-0.05 (.23)	0.04 (.17)	0.03 (.34)	0.08 (.34)
		Low	0.06 (.22)	0.01 (.20)	0.00 (.18)	-0.05 (.28)	0.11 (.40)	-0.20 (.43)	0.15 (.84)
Regulatory control	vmPFC	All	0.04 (.12)	0.10 (.17)	0.04 (.15)	0.01 (.21)	0.05 (.15)	0.04 (.20)	0.10 (.30)
		High	0.07 (.15)	0.17 (.15)	0.10 (.17)	0.10 (.17)	0.03 (.18)	0.06 (.20)	0.02 (.30)
		Middle	0.05 (.11)	0.07 (.17)	0.08 (.12)	0.00 (.18)	0.07 (.12)	0.11 (.23)	0.19 (.22)
		Low	0.00 (.10)	0.05 (.17)	-0.05 (.12)	-0.07 (.25)	0.05 (.14)	-0.03 (.17)	0.10 (.37)

Note: standard deviation (sd)

5th – 7th face presentation. Social fearfulness was not correlated with habituation differences in any region during the late presentation window (all *p*'s > .28; **Table 2; Figure 7**). Overall, *b'* slopes across all groups were near zero during the late presentation window, indicating a sustained pattern of response once faces had been seen many times **(Figure 8)**.

2.3.4. *Laterality*

We tested for laterality of initial amplitude and habituation of neural response in a secondary analysis. We found a single difference in response to novel faces in V1 **(Table 4)** indicating that magnitude of signal across participants was larger in left than right hemisphere; however, social fearfulness correlations in the left and right hemispheres were consistent with the bilateral findings **(Table 2; Table 5)** supporting the combination of hemispheres to examine effects of social fearfulness.

2.4. Discussion

The goal of this study was to investigate the neural basis of social fearfulness, a dimensional characteristic that fundamentally influences social behavior. Using fMRI, we examined two key aspects of neural response to faces—initial response to novel faces, a neural response corresponding to attentional orienting, and habituation to repeated faces, a neural mechanism involved in learning that stimuli are familiar and safe. The main findings from this study are that social fearfulness is associated with 1) higher initial amplitude of neural response to novel faces and 2) failure to habituate to

repeated faces. Social fearfulness was dimensionally associated with sustained signaling across visual social threat processing regions—including the amygdala, hippocampus, mOFC, vmPFC, V1, and extrastriate cortex. Two regions—the hippocampus and vmPFC—also showed heightened initial amplitude of response to

Table 4. T-tests for laterality of neural responses to faces.

	Initial amplitude (left – right)		**Habituation (left – right)**							
			1st – 7th		**1st – 3rd**		**3rd – 5th**		**5th – 7th**	
Region	*t*	*p*-value	*t*	*p*-value	*t*	*p*-value	*t*	*p*-value	*t*	*p*-value
V1	**2.14**	**.04**	.68	.50	.63	.53	-.30	.77	.30	.77
Extrastriate	-1.64	.11	.48	.63	.28	.78	-.03	.97	.23	.82
FFA	.23	.82	.25	.81	-.59	.56	.32	.75	.58	.57
Amygdala	.76	.45	-.81	.42	-.04	.97	-.62	.54	-.18	.86
Hippocampus	.99	.33	-.53	.60	-.36	.72	-.02	.99	-.22	.83
mOFC	.08	.94	.17	.86	.05	.93	.52	.60	-.18	.85
vmPFC	-.08	.94	.14	.89	.19	.85	-.05	.96	-.03	.98

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} – 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

novel faces, suggesting that heightened orienting response is also involved in maladaptive social fear **(Figure 9)**.

Table 5. Correlations between social fearfulness and neural response to faces, by hemisphere.

		Initial amplitude			Habituation											
					$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region		*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	Left	.07	.005	.74	-.08	.006	.69	.30	.09	.12	**.51**	**.26**	**.005**	-.16	.03	.40
	Right	.05	.003	.79	.03	.001	.89	.39	.15	.03	**.50**	**.25**	**.006**	.05	.003	.80
Extrastriate	Left	.21	.04	.28	.11	.01	.56	.37	.14	.05	**.55**	**.30**	**.002**	-.04	.002	.82
	Right	.31	.10	.11	.16	.03	.42	.48	.23	.009	**.58**	**.34**	**.001**	.07	.005	.72
FFA	Left	.03	.001	.90	.14	.02	.51	.39	.15	.06	.30	.09	.29	.10	.01	.64
	Right	.26	.07	.22	.32	.10	.13	.32	.10	.12	.35	.12	.10	.25	.06	.24
Amygdala	Left	**.55**	**.30**	**.002**	**.37**	**.14**	**.05**	.21	.04	.29	**.53**	**.28**	**.003**	-.03	.001	.89
	Right	**.67**	**.45**	**.001**	.29	.08	.13	.27	.07	.15	**.62**	**.38**	**.0004**	-.06	.004	.77
Hippocampus	Left	**.46**	**.21**	**.01**	**.48**	**.23**	**.008**	.41	.17	.03	**.57**	**.33**	**.001**	-.22	.05	.25
	Right	**.49**	**.24**	**.007**	.36	.13	.06	.36	.13	.05	**.52**	**.27**	**.004**	-.19	.04	.34
mOFC	Left	-.16	.03	.41	-.09	.008	.65	.34	.12	.07	.44	.19	.018	.01	.00	.97
	Right	.22	.05	.99	.001	.00	.44	.34	.12	.07	.43	.19	.019	-.03	.001	.89
vmPFC	Left	**.49**	**.24**	**.008**	.34	.12	.07	.30	.09	.12	**.51**	**.26**	**.005**	.07	.005	.73
	Right	**.47**	**.22**	**.01**	**.37**	**.14**	**.05**	.27	.07	.15	**.50**	**.25**	**.006**	.06	.004	.76

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Elevated initial amplitude to novel faces in the hippocampus and vmPFC may underlie heightened novelty/threat detection and attentional orienting responses in socially fearful people. The hippocampus contains neurons that respond selectively to novel stimuli, indicating a critical role in detection of novelty (Fried *et al*, 1997; Wilson and Rolls, 1993; Rutishauser *et al*, 2006; Blackford *et al*, 2010). The hippocampus is hypothesized to encode the "cold hard" facts of a novel or threatening stimulus (Squire and Zola-Morgan, 1991; Eichenbaum, 2001), including the identity of a novel face (Haxby *et al*, 1996) and the context in which it was encountered (LeDoux, 2003). Elevated initial response to novelty in the hippocampus likely reflects heightened orienting to threat stimuli, which has been implicated in automatic orienting responses to novelty; lesions of the hippocampus result in a marked deficit in orienting to novel stimuli (Hendrickson *et al*, 1969). The vmPFC has direct structural interconnections with the hippocampus (Riga *et al*, 2014) and plays a well-documented role in automatic regulation of emotional responses and fear learning through these connections (Motzkin *et al*, 2014; Hartley and Phelps, 2010; Quirk and Beer, 2006; Ray and Zald, 2012).

Social fearfulness was associated with habituation differences across visual threat processing regions. Social fearfulness was associated with an overall failure of hippocampal habituation across the experiment (1^{st} – 7^{th} face presentation). Additional analyses revealed temporally-dependent differences in habituation associated with social fearfulness, with the majority of habituation differences found in the middle presentation window; social fearfulness was associated with sustained signal from the

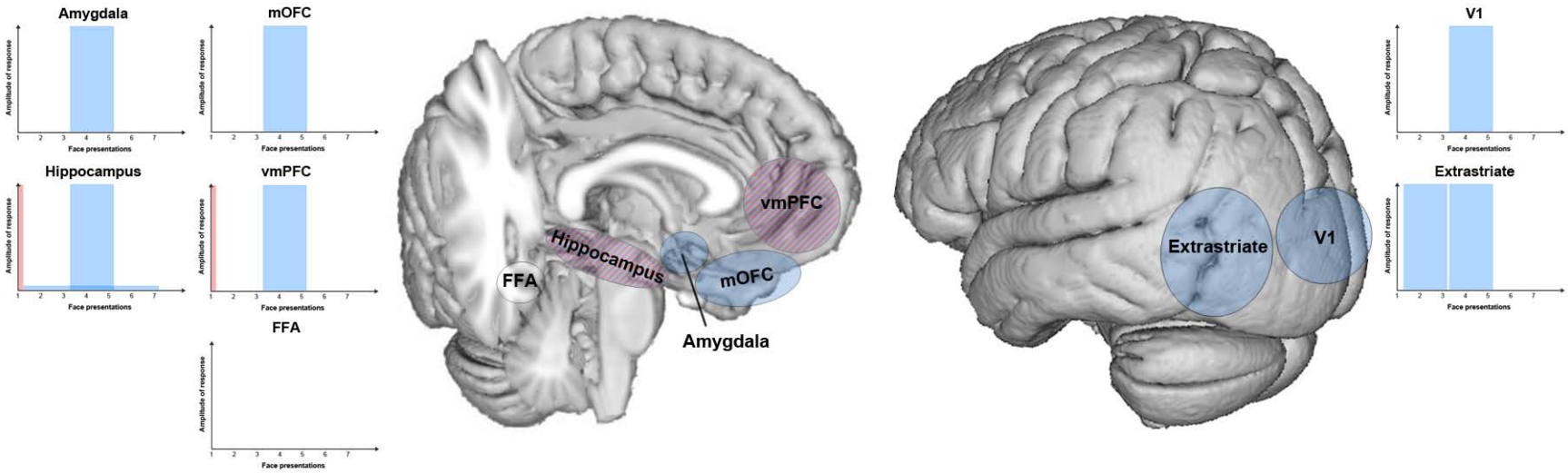


Figure 9. Sumary of social fearfulness differences in response to faces. Social fearfulness was associated with differences in response to novel and repeated faces across visual threat processing regions. The pattern of differences associated with social fearfulness is illustrated on the brain in red and blue. Social fearfulness was correlated with higher initial amplitudes to novel faces in the hippocampus and vmPFC, with both regions also showing sustained signaling over repeated face presentations in socially fearful participants (red/blue stripes). Social fearfulness was also correlated with sustained response in the amygdala, mOFC, V1, and extrastriate cortex (blue). The temporal pattern of initial amplitude and habituation differences for each region is illustrated in graphs surrounding the brain. The hippocampus and vmPFC showed initial amplitude differences (red line). The hippocampus also showed an overall habituation difference across the experiment (blue line, 1st - 7th). Most regions (except FFA) showed habituation differences in the middle presentation window (blue box, 3rd - 5th). The extrastriate cortex also showed habituation differences in the early presentation window (blue box, 1st - 3rd). There were no differences in habituation by social fearfulness during late face presentations (5th - 7th).

3rd to 5th face presentation across most regions, including the amygdala, hippocampus, mOFC, vmPFC, V1, and extrastriate cortex. In contrast, in the earliest time window (1st to 3rd face presentation), only the extrastriate cortex showed a significant difference in rate of habituation by social fearfulness. There were no correlations between social fear and habituation rate differences during the late presentation window (5th to 7th face presentation), indicating that rate of change remained stable across all participants during later face presentations. Although we found no evidence of habituation in people with high social fearfulness—across regions, *b'* slope values in the high social fear group remained near or above zero across all analysis windows —it is possible that people with high social fearfulness would show habituation to a baseline response with further exposures to face stimuli. Future studies should consider increasing the number of face stimulus presentations to determine whether a delay vs. a deficit in habituation exists.

We found strong habituation differences by social fearfulness between the 3rd and 5th face presentation. This finding is in line with previous work in our lab showing behavioral habituation differences by shyness. Using a similar repeated faces task, we measured recognition memory for faces seen 1, 3, 5, or 7 times. We found that shyness was correlated with slower increases in recognition memory with increased exposure, with high shyness participants showing less recognition for faces seen 3, 5, and 7 times than low shyness participants, with the strongest behavioral differences associated with the 5th face presentation (Avery *et al*, 2015). Habituation often occurs rapidly, with the majority of decrease in response occurring across initial stimulus presentation. Indeed, during early face presentations (1st – 3rd) all regions showed moderate, although non-

significant, correlations (all *r*'s > .28) with social fearfulness, suggesting that some habituation differences exist after initial face presentations. However, habituation occurs most rapidly when stimuli are presented sequentially; intervening stimuli and/or time can result in slower habituation or recovery of response (Rankin *et al*, 2009). In this task, face identities were distributed pseudo-randomly across the experiment window (~10 minutes), which may have dampened the rate of habituation in lower social fear participants during early face presentations.

Habituation is one of the most basic forms of non-associative learning and represents a fundamental mechanism for learning about the world. Acting as a sensory filter, neural habituation allows familiar or predictable information to be ignored in favor of devoting neural resources to salient or novel stimuli. Failure to habituate to repeated faces likely reflects a deficit in the ability to learn that a social environment is predictable, familiar and safe. People with high levels of social anxiety fail to show habituation of negative expectations or self-reported nervousness to repeated social threat situations (Eckman and Shean, 1997), and show a corresponding failure of autonomic arousal (heart rate, sweat activity) habituation (Eckman and Shean, 1997), suggesting sustained feelings of environmental threat in people with higher social anxiety.

In healthy adults, neural habituation to repeated faces has been demonstrated across regions involved in salience detection and processing, including the amygdala (Breiter *et al*, 1996; Wright *et al*, 2001; Schwartz *et al*, 2003b; Fischer *et al*, 2003; Plichta *et al*, 2014), hippocampus (Wright *et al*, 2001; Fischer *et al*, 2003), medial prefrontal cortex (Wendt *et al*, 2012), fusiform gyrus (Ishai *et al*, 2004), and occipital

lobe (Ishai *et al*, 2004; Müller *et al*, 2013; Ousdal *et al*, 2014). The amygdala plays a central role in triggering autonomic arousal in response to threat (LeDoux *et al*, 1988), and failure of amygdala habituation has been associated with increased state-based anxiety in healthy adults (Ousdal *et al*, 2014). Similarly, the hippocampus also has a well-established role in habituation to novel environments; lesions of the hippocampus in rats result in deficits in habituation as well as increased anxiety behaviors (Leussis and Bolivar, 2006). Sustained activity in both the mOFC and vmPFC in socially fearful individuals is consistent with the role of the medial prefrontal cortex in regulating emotional responses to threat. The mOFC plays a role in guiding and maintaining emotional responses through extensive connections with the amygdala (Milad and Rauch, 2007), and lesions of the OFC have been shown to reduce anxiety behaviors in monkeys (Fox *et al*, 2010; Kalin *et al*, 2007). The vmPFC critically regulates the amygdala during processing of aversive images (Quirk and Beer, 2006; Shin and Liberzon, 2010; Riga *et al*, 2014; Phelps *et al*, 2004), and elevated activity in the vmPFC is associated with both self-reported anxiety and elevated heart rate during anticipation in healthy adults (Simpson *et al*, 2001). Similarly, in rodents, lesions of the vmPFC result in decreased stress-response (Myers-Schulz and Koenigs, 2012). Both the mOFC and vmPFC play a critical role in extinction learning of fear (Milad *et al*, 2006; Riga *et al*, 2014); although not actively involved in the initial acquisition of extinction learning, which appears to be dependent on the amygdala, the mOFC and vmPFC appear to be involved in the long-term consolidation of fear extinction (Santini *et al*, 2004; Do-Monte *et al*, 2015). Intriguingly, this suggests that failure of habituation in both

the mOFC and vmPFC may underlie deficits in long-term extinction of social fears, likely through disrupted functional interactions with the amygdala.

We found sustained signaling in visual processing regions in socially fearful participants, including V1 and extrastriate cortex. Neuronal signals for salience are evident even in early sensory processing regions, and elevated processing of novel visual stimuli is likely directed through top-down regulation by the amygdala (Kastner and Ungerleider, 2000; Ousdal *et al*, 2014). This regulation likely serves to reallocate visual processing to the most salient features of a stimulus. The amygdala has extensive connections with the visual cortex and likely subserves this enhancement in visual activity. The amygdala also has extensive connections with FFA. However, examinations of FFA activity in social anxiety disorder patients have yielded equivocal results, with some studies finding elevated activity and others finding either no differences or lower activity in patients compared to controls (Freitas-Ferrari *et al*, 2010; Miskovic and Schmidt, 2012). Our results did not show differences in FFA activity by social fearfulness, suggesting that emotion-related enhancements in visual processing may occur earlier in the visual processing stream. Alternately, neutral faces may not enhance activity in the FFA in the same way as more emotional faces (e.g., fear), which are more consistently associated with FFA enhancements (Vuilleumier and Driver, 2007).

We did not find associations between hemispheric lateralization and social fearfulness in any brain region, a finding largely consistent with existing literature regarding hemispheric asymmetries. Early visual areas (V1 and V2) appear to perform identical functions for the left and right halves of the visual field. However, further along

the posterior-anterior visual processing pathway, connections crossing the midline steadily diminish the distinction between separate visual fields, suggesting that processing in higher-order visual areas may become less visual field dependent and more content-dependent and lateralized(e.g., FFA); in line with this hypothesis, early neuroimaging studies suggested that face processing in FFA was lateralized to the right hemisphere (Kanwisher *et al*, 1997; McCarthy *et al*, 1997). Therefore, we were interested in testing for lateralization of social fearfulness associations. However, we did not detect lateralization of FFA activity, a finding in line with recent evidence indicating that both the left and right FFA perform complementary face processing functions that would not be differentially affected by our repeated faces task (Meng *et al*, 2012). Early studies also proposed right lateralization of the amygdala in detection of faces and habituation to emotional stimuli (Phelps *et al*, 2001; Wright *et al*, 2003; Gläscher and Adolphs, 2003); however, studies from our lab (Blackford *et al*, 2013, 2011) and others (Plichta *et al*, 2014; Guyer *et al*, 2008) have found evidence for strong bilaterality in amygdala function. Similarly, laterality findings in the prefrontal cortex and hippocampus have been equivocal, suggesting that subtle differences in task design or analysis techniques may play a role in laterality findings (Ochsner and Gross, 2008; Wager *et al*, 2003).

A frequently observed characteristic of habituation is that weak stimuli produce rapid habituation, while intense stimuli may yield no discernible habituation even after many exposures (Rankin *et al*, 2009). All participants viewed a standardized set of neutral face stimuli during this experiment, which may be considered a “weak” stimulus; however, people with social anxiety disorder tend to rate neutral faces as slightly more

threatening (Winton *et al*, 1995), a finding supported by elevated amygdala activity in patients relative to controls when viewing neutral faces (Birbaumer *et al*, 1998; Cooney *et al*, 2006). In this study, high social fear participants did not rate faces as more intense or arousing than low social fear participants. However, as arousal and valence ratings were collected following repeated face exposures, habituation of emotional reaction to faces may have occurred by the time ratings were made. Therefore, we cannot exclude the possibility that neural habituation differences were the result of neutral faces being perceived as more arousing or intense by high social fear participants. Future studies should consider measuring subjective arousal to face stimuli in real-time, such as through skin conductance response.

2.5. Conclusions

In conclusion, this is the first study to investigate two separate fundamental elements of neural response—initial amplitude and habituation—in relation to trait differences in social fear. Our findings show that both initial amplitude to novel faces and habituation to repeated faces are associated with higher levels of social fear, suggesting that social fears may be maintained by both an overactive orienting response to novel social stimuli and a failure to filter familiar social stimuli, leading to elevated detection of novelty and feelings of anxiety. Together, these findings paint a picture of the temporal characteristics of neural response in social fearfulness—in people with low social fearfulness, novelty evokes an orienting response and threat processing that habituates over time, while in people with high social fearfulness, novelty evokes a strong orienting response and sustained threat processing over an

extended period. Exposure therapy is one of the most successful therapies in the reduction of social fears, and both orienting response and habituation are improved by successful exposure therapy (Matthews *et al*, 2015; Leutgeb *et al*, 2009), suggesting that the critical mechanism underlying successful exposure therapy may be a dampened response and rapid habituation of neural activity to social stimuli. Future studies should directly investigate the effects of exposure therapy on neural responses to novel and repeated social stimuli. Elevated, sustained neural signal may also contribute to risk for social anxiety disorder—future studies should examine amplitude and habituation responses in high and low risk children to determine whether these fundamental differences in response to novelty are present during development.

CHAPTER III

Associations between social fearfulness and functional connectivity during novel and repeated faces

3.1. Introduction

Complex psychiatric disorders are increasingly thought of as disorders of neural connectivity (Vuilleumier and Driver, 2007; Greicius, 2008; van den Heuvel and Hulshoff Pol, 2010). As such, disrupted functional connectivity has been consistently associated with increased anxiety and anxiety disorders (Etkin and Wager, 2007; Etkin, 2010), including social anxiety disorder (Freitas-Ferrari *et al*, 2010; Etkin and Wager, 2007; Goldin *et al*, 2009; Miskovic and Schmidt, 2012). The amygdala is key in fear processing and expression of social anxiety (Freitas-Ferrari *et al*, 2010; Furmark, 2009; Mathew and Ho, 2006; Miskovic and Schmidt, 2012) and is a central hub in the social threat processing network (Freitas-Ferrari *et al*, 2010; Stefanacci *et al*, 1996; Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004; Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005; Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006; Wager *et al*, 2009a); through its vast connections, the amygdala plays an important role in top-down modulation of attentional processes involved in salience detection (Freitas-Ferrari *et al*, 2010; Miskovic and Schmidt, 2012). Disruptions in amygdala circuits may contribute to social anxiety disorder—coupling between the amygdala and vmPFC is disrupted in anxiety disorders (Rauch *et al*, 2006; Milad *et al*, 2006) and recent functional connectivity studies have shown greater connectivity between the amygdala and visual cortices, and

lower connectivity between the amygdala and medial prefrontal cortex in social anxiety disorder patients relative to controls (Miskovic and Schmidt, 2012). Communication between the amygdala and regulatory regions has been hypothesized to play a key role in social anxiety; impaired communication between amygdala and the mOFC and vmPFC likely contributes to the elevated amygdala activity seen in people with social anxiety, resulting in increased amygdala responsiveness to salient stimuli and sustained threat processing (Akirav and Maroun, 2007). Directional connectivity studies have supported this hypothesis, demonstrating dampened regulatory influence from the medial prefrontal cortex over the amygdala in patients with social anxiety disorder relative to controls, along with a corresponding heightened influence of the amygdala on visual cortices (Liao *et al*, 2010; Sladky *et al*, 2013).

Neuronal habituation in threat processing regions signals safety and familiarity (Fried *et al*, 1997; Wilson and Rolls, 1993; Gonsalves *et al*, 2005; Wright *et al*, 2001; Dubois *et al*, 1999) and the ability to habituate is critical in the regulation of emotion. However, little is known about habituation of functional connectivity to anxiety-provoking stimuli. Findings of dampened functional connectivity between medial prefrontal regulatory areas and the amygdala in social anxiety patients suggest a failure of this circuit across repeated exposures to salient stimuli (Liao *et al*, 2010). However, a recent study directly examining habituation of functional connectivity found that amygdala-medial prefrontal regulatory circuits habituate normally in social anxiety disorder patients (Sladky *et al*, 2012); although, note that in this study the control group did not show the expected pattern of amygdala-regulatory circuit habituation, suggesting that task demands may have altered amygdala signaling. Recent findings have also

indicated heightened connectivity between the amygdala and visual cortex plays a role in anxiety (Ousdal *et al*, 2014; Liao *et al*, 2010); communication between the amygdala and visual cortex may be important for attention reallocation and boosting sensory processing of novel, salient stimuli (Vuilleumier, 2005; Padmala and Pessoa, 2008; Weierich *et al*, 2010). However, the temporal dynamics of this functional connection also remain unexplored. Therefore, the question of whether temporally-altered signaling between the amygdala and threat processing regions contributes to social anxiety remains unresolved.

In this study we examined the temporal pattern of amygdala functional connectivity across social fearfulness. Amygdala connectivity was explored across the visual threat processing network, including the hippocampus, mOFC, vmPFC, FFA, V1, and extrastriate cortex **(Figure 5)**. To examine differences related to detection of novel stimuli, we first tested for social fearfulness differences in initial amplitude of amygdala connectivity to novel faces. Next, to explore the temporal pattern of connectivity, we tested for social fearfulness differences in habituation of functional connectivity to repeated faces. Based on previous findings, we hypothesized that socially fearful people would show stronger amygdala connectivity during novel face presentations, and would also show a slower habituation of amygdala connectivity over repeated face presentations.

3.2. Methods

3.2.1. *Participants*

Characteristics. All participants (n = 29) were included in the functional connectivity analysis. See Chapter 2 for recruitment and screening procedures. Participant characteristics are detailed in **Table 1**.

3.2.2. *Functional connectivity*

Experimental design. To determine whether social fearfulness is associated with altered habituation of functional connectivity during repeated face presentations, we used a generalized psychophysiological interaction analysis (gPPI; McLaren *et al*, 2012)—gPPI assesses how brain regions interact in a task-dependent manner and can reveal important insights into brain-behavior relationships. gPPI analyses were conducted between the amygdala (seed region) and functionally- and structurally-connected brain regions involved in social fear (see Chapter 2 for ROI details). For the amygdala seed region, we used an amygdala mask from the AAL atlas (Automated anatomical labeling; Tzourio-Mazoyer *et al*, 2002) implemented in the Wake Forest University Pick Atlas (WFU Pick Atlas; Maldjian *et al*, 2003, 2004). The target ROIs for the gPPI analysis were: the hippocampus, V1, and extrastriate cortex, which were defined using the AAL standard masks; the FFA, defined using a functional localizer task (see Chapter 2 for FFA localizer details); and the vmPFC and mOFC, defined according to population masks of human architectonic areas based on comparative cytoarchitecture in humans and non-human primates (Mackey and Petrides, 2010).

3.2.3. *Data analysis*

gPPI. Using the gPPI toolbox, average fMRI time series data were extracted from the amygdala seed region during the repeated faces task (Figure 4; also, see Chapter 2 for a detailed description of the repeated faces task paradigm). gPPI analysis uses three regressors: the physiological regressor, the psychological regressor, and the interaction regressor. The physiological regressor was the fMRI time series, and the psychological regressor was the habituation contrast of 1^{st} – 7^{th} face presentation. Planned secondary analyses also examined habituation of functional connectivity in three discrete time windows: 1^{st} – 3^{rd} face presentation; 3^{rd} – 5^{th} face presentation; and 5^{th} – 7^{th} face presentation. The interaction regressor modeled the change in amygdala connectivity between the conditions in the contrast (i.e., change from 1^{st} to 3^{rd} face). The regressors were used to model the fMRI time series in each participant, producing an estimate of connectivity between the amygdala and each ROI for each contrast.

Habituation of functional connectivity. We calculated a normalized habituation of connectivity slope (*b'*) for each participant independent of initial connectivity differences (Montagu, 1963; Plichta *et al*, 2014). Normalized habituation of connectivity was calculated as described in Chapter 2. Because percent signal change in the left and right hemispheres were highly correlated, signal was averaged across hemispheres and *b'* connectivity slopes were calculated bilaterally. SAS software (Version 9.3, SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses.

Social fearfulness analysis. Correlations tested for associations between social fearfulness and initial amygdala connectivity to novel faces, and between social fearfulness and normalized habituation of amygdala connectivity slopes (*b')* to repeated faces across participants (habituation from 1^{st} to 7^{th} face presentation). Results were considered significant at $\alpha \leq .05$. R^2 values were computed as a measure of effect size. Means and standard errors of social fearfulness groups are presented as bar graphs.

To explore potential non-linear differences in habituation of connectivity, we conducted a secondary analysis examining correlations between habituation of connectivity within constrained windows ($1^{st} – 3^{rd}$; $3^{rd} – 5^{th}$; $5^{th} – 7^{th}$) and social fearfulness ($\alpha \leq .0167$, Bonferroni-corrected for multiple comparisons).

Exploratory functional connectivity analysis. We conducted a *post hoc* analysis to explore functional connectivity across the visual threat processing network. Percent signal change for novel and repeated faces was extracted from each ROI and correlations tested for relationships in initial amplitude and habituation across regions; to reduce type I error, we limited the analysis to the habituation contrast ($3^{rd} – 5^{th}$), as this contrast yielded the strongest habituation findings (Chapter 2, **Table 2**). To determine the effect of social fearfulness on correlations between regions, correlations were performed with and without controlling for social fearfulness and were compared by converting *r* values to *z* scores to test for differences ($\alpha \leq .05$).

3.3. Results

3.3.1. *Initial connectivity*

Social fearfulness was not associated with differences in amygdala connectivity during novel face presentations **(Table 6, Figure 10)**. Initial connectivity beta values are presented in **Table 7**.

3.3.2. *Habituation of functional connectivity*

1st – 7th face presentation. To test for social fearfulness differences in habituation of connectivity, we contrasted amygdala connectivity during the 1st face presentation with connectivity during the 7th face presentation. Social fearfulness was not associated with differences in habituation of connectivity between the amygdala and other regions (all *p*'s > .09; **Table 6; Figure 10**). Across regions, *b'* slope values were near or below zero for most participants, indicating an overall pattern of sustained or habituating connectivity across regions by the last face presentation **(Figure 11)**. To visualize response to repeated objects over time, we extracted percent signal change values by region; values are presented in **Table 7**.

1st – 3rd face presentation. We next conducted planned secondary analyses to test for differences in habituation of connectivity across early (1st – 3rd), middle (3rd – 5th), and late 5th – 7th) repetition windows. Social fearfulness was not associated with differences in habituation of amygdala connectivity in the early face presentation window (all *p*'s > .08; **Table 6; Figure 10**). Across most participants, *b'* slope values were near or below

Table 6. Correlations between social fearfulness and amygdala connectivity during face viewing.

	Initial amplitude			Habituation											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.09	.008	.64	.02	.00	.93	.27	.07	.16	**.45**	**.20**	**.01**	-.09	.008	.64
Extrastriate	.16	.03	.40	.26	.07	.17	.33	.11	.08	**.49**	**.24**	**.007**	-.05	.003	.80
FFA	.08	.006	.70	.17	.03	.43	.21	.04	.31	.25	.06	.24	.36	.13	.09
Hippocampus	-.02	.00	.93	.18	.03	.35	.16	.03	.40	.36	.13	.05	-.21	.04	.27
mOFC	.15	.02	.43	-.06	.004	.78	.21	.04	.27	.31	.10	.10	.02	.00	.93
vmPFC	.30	.09	.12	.32	.10	.09	.26	.07	.18	.40	.16	.03	.05	.003	.78

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation (1^{st} - 7^{th}) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

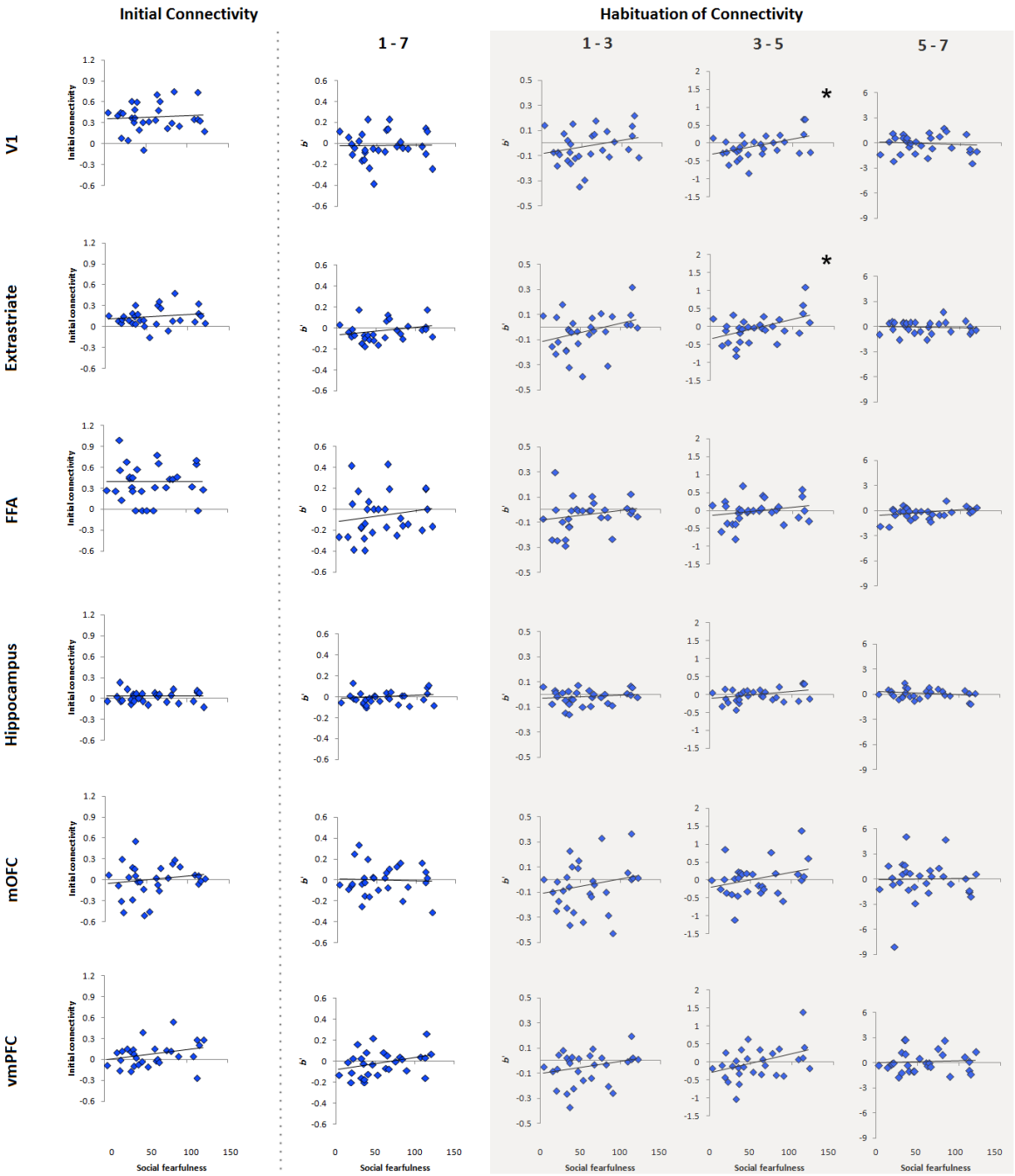


Figure 10. Correlations between social fearfulness and amygdala functional connectivity during face presentations. Scatterplots show relationships between social fearfulness and amygdala functional connectivity during novel and repeated faces. Asterisks (*) denote significant correlations. Intercept values above zero indicate initial amygdala connectivity (greater than baseline) to novel faces. Slope (b') values below zero indicate habituation of amygdala connectivity to repeated faces; b' slope values at or above zero indicate sustained or increasing amygdala connectivity to repeated faces, respectively. There were no social fearfulness differences in initial amygdala connectivity or overall change in amygdala connectivity (1st - 7th) across the experiment. Secondary habituation contrasts (shaded in gray) revealed a correlation between social fearfulness and sustained amygdala connectivity in V1 and extrastriate cortex across middle (3rd - 5th) face presentations.

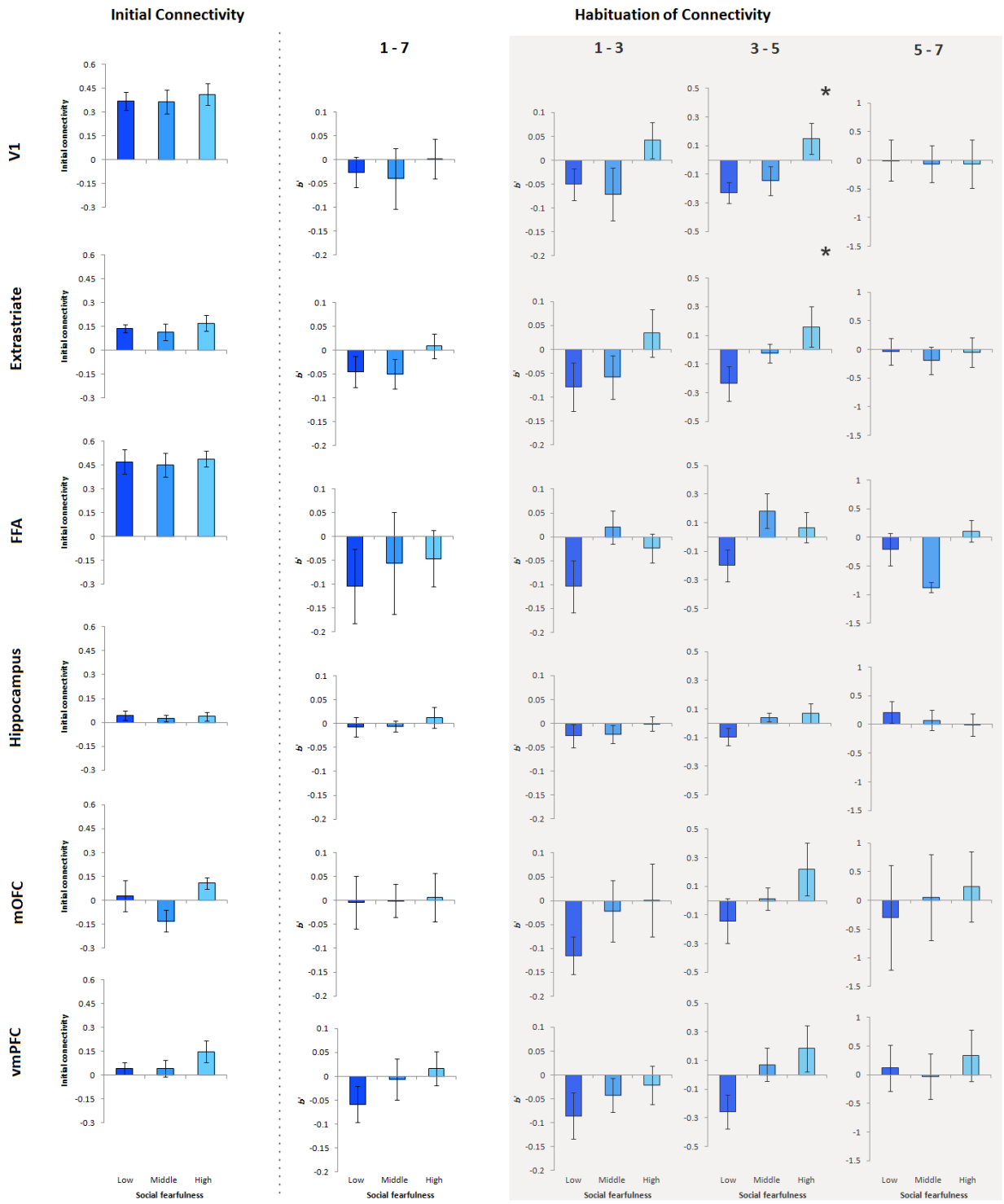


Figure 11. Functional connectivity to faces by social fearfulness tertile. Bar graphs show initial amplitude of amygdala connectivity (intercept) and habituation (b') slope values of functional connectivity by social fearfulness tertile over novel and repeated face presentations. Asterisks (*) denote significant correlations. Intercept values above zero indicate initial amygdala connectivity (greater than baseline) to novel faces. Slope (b') values below zero indicate habituation of amygdala connectivity to repeated faces; b' slope values at or above zero indicate sustained or increasing amygdala connectivity to repeated faces, respectively.

Table 7. Amygdala functional connectivity beta values by face presentation number.

Function	Region	Social fearfulness group	Face presentation 1st mean (sd)	2nd mean (sd)	3rd mean (sd)	4th mean (sd)	5th mean (sd)	6th mean (sd)	7th mean (sd)
Visual processing	V1	All	0.42 (.22)	0.37 (.37)	0.45 (.19)	0.41 (.30)	0.41 (.22)	0.39 (.26)	0.40 (.32)
		High	0.40 (.21)	0.42 (.28)	0.55 (.18)	0.51 (.41)	0.36 (.17)	0.38 (.27)	0.37 (.31)
		Middle	0.43 (.28)	0.40 (.22)	0.40 (.19)	0.36 (.21)	0.40 (.30)	0.41 (.28)	0.38 (.30)
		Low	0.44 (.19)	0.31 (.55)	0.40 (.17)	0.37 (.22)	0.46 (.18)	0.39 (.27)	0.45 (.36)
	Extrastriate	All	0.10 (.13)	0.08 (.28)	0.12 (.13)	0.16 (.29)	0.10 (.12)	0.03 (.18)	0.08 (.21)
		High	0.09 (.14)	0.16 (.15)	0.19 (.11)	0.32 (.43)	0.11 (.08)	0.06 (.12)	0.10 (.18)
		Middle	0.09 (.17)	0.06 (.16)	0.08 (.11)	0.06 (.15)	0.06 (.17)	0.02 (.17)	0.04 (.19)
		Low	0.13 (.06)	0.01 (.42)	0.08 (.16)	0.08 (.13)	0.12 (.09)	0.02 (.24)	0.08 (.27)
Face processing	FFA	All	0.44 (.19)	0.51 (.46)	0.39 (.17)	0.43 (.19)	0.40 (.13)	0.30 (.27)	0.36 (.31)
		High	0.43 (.23)	0.42 (.27)	0.44 (.17)	0.46 (.21)	0.44 (.14)	0.28 (.24)	0.38 (.27)
		Middle	0.40 (.24)	0.53 (.34)	0.42 (.17)	0.44 (.28)	0.34 (.15)	0.29 (.46)	0.41 (.38)
		Low	0.48 (.14)	0.57 (.65)	0.34 (.17)	0.41 (.10)	0.38 (.12)	0.32 (.20)	0.33 (.33)
Novelty / Threat detection	Hippocampus	All	0.03 (.07)	0.08 (.12)	0.02 (.07)	0.01 (.13)	0.03 (.06)	0.06 (.10)	0.05 (.11)
		High	0.03 (.09)	0.11 (.11)	0.04 (.07)	0.09 (.16)	0.01 (.07)	0.09 (.09)	0.05 (.11)
		Middle	0.03 (.04)	0.02 (.08)	0.03 (.05)	-0.01 (.11)	0.03 (.03)	0.03 (.10)	0.06 (.13)
		Low	0.04 (.07)	0.10 (.14)	-0.01 (.08)	-0.04 (.09)	0.05 (.08)	0.04 (.10)	0.03 (.11)
Outcome prediction	mOFC	All	0.01 (.27)	-0.10 (.49)	0.03 (.21)	0.02 (.47)	0.05 (.30)	-0.10 (.47)	0.04 (.61)
		High	0.00 (.33)	0.06 (.27)	0.07 (.27)	0.17 (.69)	-0.05 (.29)	-0.04 (.46)	-0.12 (.50)
		Middle	-0.11 (.23)	-0.03 (.45)	0.01 (.10)	-0.06 (.28)	0.07 (.16)	0.07 (.37)	0.07 (.44)
		Low	0.12 (.19)	-0.31 (.65)	0.00 (.24)	-0.05 (.31)	0.13 (.41)	-0.31 (.52)	0.18 (.82)
Regulatory control	vmPFC	All	0.05 (.17)	0.06 (.24)	0.03 (.15)	0.10 (.45)	0.03 (.13)	0.04 (.24)	0.09 (.27)
		High	0.03 (.21)	0.12 (.15)	0.07 (.15)	0.35 (.66)	0.00 (.15)	0.04 (.25)	0.02 (.29)
		Middle	0.07 (.18)	0.01 (.23)	0.09 (.11)	0.03 (.19)	0.07 (.11)	0.12 (.25)	0.14 (.20)
		Low	0.05 (.10)	0.04 (.33)	-0.05 (.14)	-0.08 (.22)	0.04 (.15)	-0.05 (.21)	0.10 (.31)

Note: standard deviation (sd)

zero, suggesting a pattern of sustained or habituating connectivity across regions **(Figure 11)**.

*3rd – 5th face pre*sentation. Social fearfulness was associated with differences in habituation of connectivity between the amygdala and two brain regions—V1 and extrastriate cortex—in the middle repetition window (V1, r = .45, p = .01; extrastriate, r = .49, p = .007; **Table 6; Figure 10**). For both regions, the low social fear group demonstrated negative *b'* connectivity slopes, indicating a decrease in amygdala connectivity with repeated face presentations, while the high social fear group had *b'* connectivity slopes near or above zero, indicating sustained or increasing amygdala connectivity across repeated face presentations **(Figure 11)**.

5th – 7th face presentation. Social fearfulness was not associated with differences in habituation of amygdala connectivity in the late face presentation window (all p's > .09; **Table 6; Figure 10**). Across regions, *b'* slope values were near zero for most participants, indicating sustained amygdala connectivity during later face presentations **(Figure 11)**.

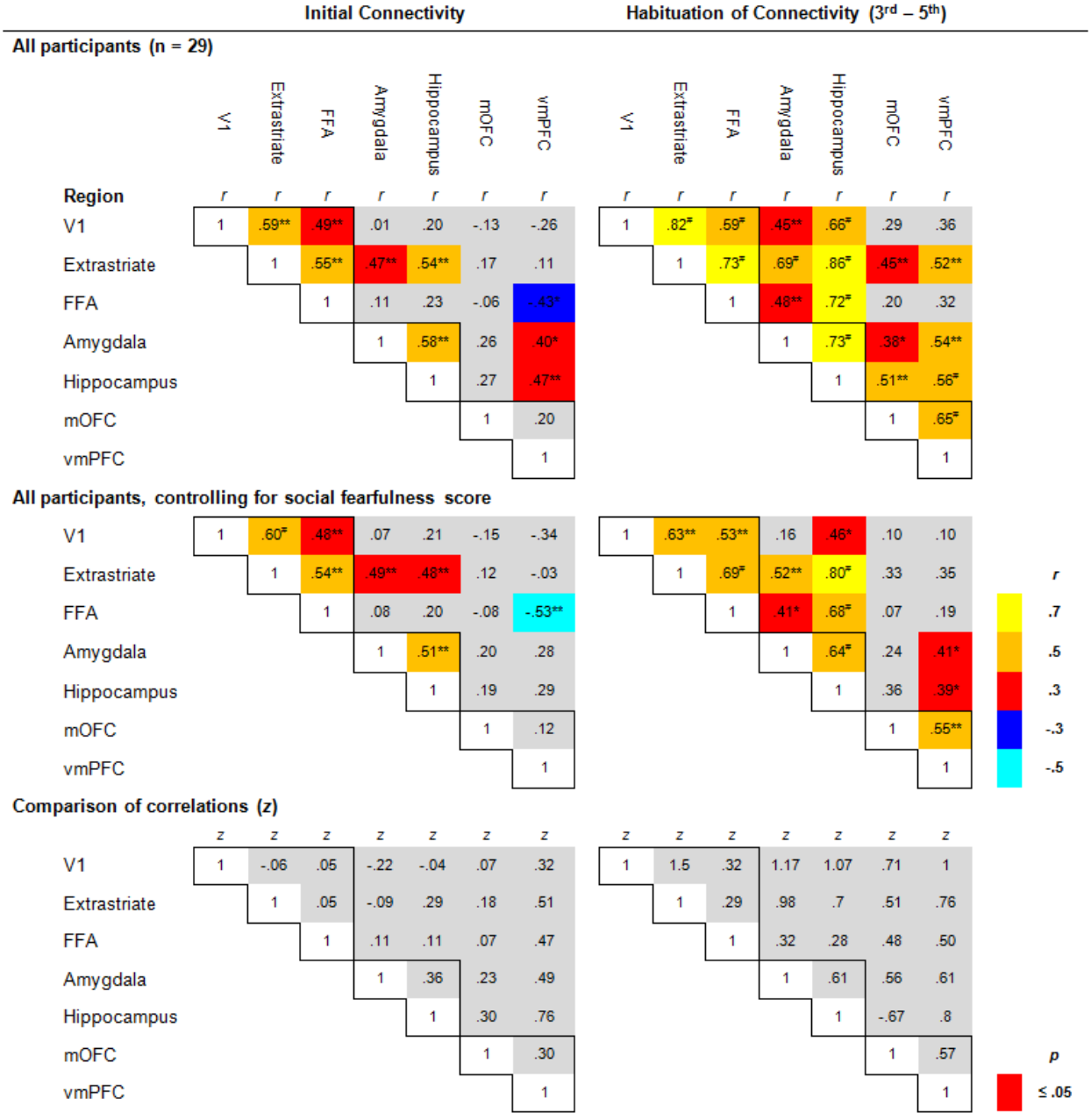
3.3.3. *Exploratory functional connectivity across regions*

Initial connectivity to novel faces. The visual threat processing network is densely structurally and functionally interconnected, with many direct connections between regions. To explore associations between social fearfulness and the rest of the network, we performed a secondary functional connectivity analysis. We first tested for

correlations in initial response to novel faces across the sample. Overall, areas performing similar functions showed strong correlations with each other; visual and face processing regions showed a similar initial amplitude of response (V1, extrastriate cortex, FFA), as did novelty and threat processing regions (amygdala, hippocampus). In contrast, medial prefrontal regions (mOFC, vmPFC) showed a weak correlation in initial amplitude of response **(Table 8)**. We found no associations between functional connectivity and social fearfulness (all *p*'s > .05, **Table 8**), suggesting that overall functional connectivity between regions is not significantly influenced by trait social fear.

3rd – 5th face presentation. Because rate of habituation in the middle presentation window (3rd – 5th presentation) showed the strongest correlation with social fearfulness, we explored connectivity for this middle repetition window across regions. Across participants, habituation between the 3rd and 5th face presentation was positively correlated across regions, with regions performing similar functions showing the strongest correlations with each other. Additionally, visual and face processing regions also showed strong positive correlations with novelty and threat detection regions **(Table 8)**. There were no associations between functional connectivity and social fearfulness (all *p*'s > .05, **Table 8**), suggesting that overall functional connectivity between regions is not significantly influenced by trait social fear.

Table 8. Exploratory functional connectivity across regions.



Note: $^{*}p \leq .05$; $^{**}p \leq .01$; $^{\#}p \leq .001$.

3.4. Discussion

The goal of this study was to investigate temporal changes in amygdala functional connectivity during processing of novel and repeated social stimuli and the relationship with social fearfulness. Using gPPI functional connectivity analysis, we examined both initial connectivity to novel faces and habituation of connectivity to repeated faces. Our findings indicate that socially fearful people have sustained amygdala-visual cortex connectivity across repeated face presentations—social fearfulness was dimensionally associated with sustained amygdala connectivity with two primary visual processing regions, V1 and extrastriate cortex **(Figure 12)**.

Associations between social fearfulness and sustained amygdala-visual cortex connectivity were found in the middle face presentation window (3^{rd} – 5^{th}), which is in line with our previous findings showing strong sustained signal in the same regions—amygdala, V1 and extrastriate cortex—in the middle face presentation window (Chapter 2; **Table 2**). The amygdala may be key in driving sustained visual cortex activity in socially fearful individuals—a previous study in social anxiety disorder patients demonstrated a driving role of the amygdala over visual processing regions (Liao *et al*, 2010). Our current results provide further evidence that amygdala-visual cortex connectivity may be an important neural substrate of social fearfulness. However, as recent findings have suggested that the amygdala is sufficient, but not necessary, to enhance emotion-related visual cortex activity (Edmiston *et al*, 2013), future studies should investigate the role of a broader set of brain regions (Pessoa and Adolphs, 2010)in emotion-related enhancement of visual cortex activity in social fear.

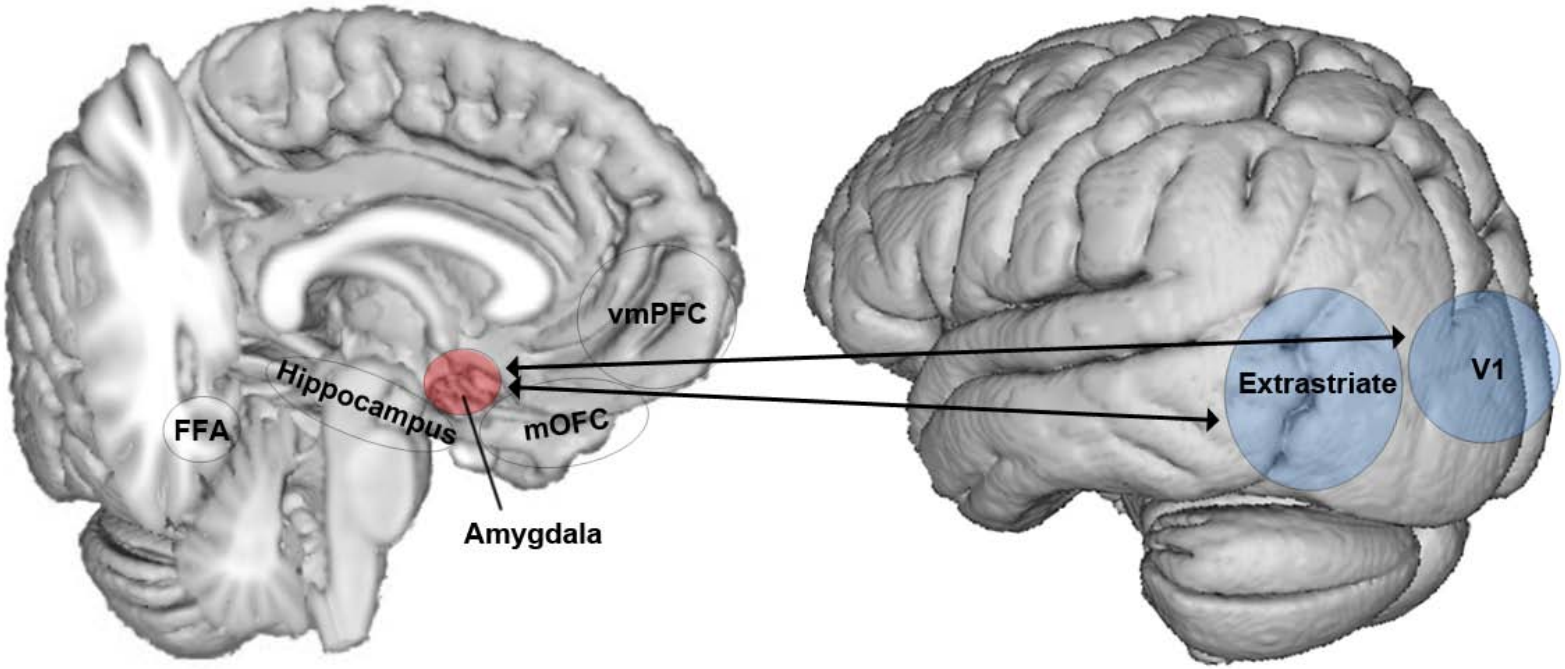


Figure 12. Summary of social fearfulness differences in amygdala functional connectivity during face viewing. Social fearfulness was associated with differences in amygdala connectivity across repeated face presentations in visual and prefrontal cortex. Amygdala connectivity findings are illustrated on the brain, with arrows showing significant amygdala connections with brain regions (shaded in blue). Social fearfulness was correlated with sustained amygdala connectivity with both V1 and extrastriate cortex. Both visual cortex regions showed sustained amygdala connectivity in the middle presentation window (3rd - 5th). There were no connectivity differences by social fearfulness in early or late face presentation windows, nor were there differences in initial connectivity to novel faces by social fearfulness.

Previous studies have shown elevated visual cortex activity in response to novel (vs. familiar) stimuli (Schwartz *et al*, 2003a, 2003b; Ousdal *et al*, 2014; Weierich *et al*, 2010). Elevated visual cortex activity may serve to enhance visual processing of salient stimuli (Padmala and Pessoa, 2008; Vuilleumier, 2005). Activity in primary visual cortex has been associated with increased memory for affective visual stimuli (Padmala and Pessoa, 2008). Neural habituation in visual cortex may mechanistically underlie increased memory; recent work has identified a link between synaptic plasticity in V1, resulting in neural habituation of signaling, and long-term behavioral memory in mice (Cooke *et al*, 2015). Importantly, increased visual processing, and resulting increased visual attention and memory, are thought to be driven by amygdala circuits (Vuilleumier and Driver, 2007). Our findings of sustained amygdala-visual cortex connectivity in social fearfulness are in line with a previous finding of heightened connectivity in social anxiety (Liao *et al*, 2010). Taken together, these findings suggest an enhancement in visual attention to novel social stimuli in people with social anxiety subserved by increased functional connectivity between amygdala and visual cortex.

We did not find significant associations between social fearfulness and amgydala-medial prefrontal connectivity, although there was a trend for sustained amygdala-vmPFC connectivity in social fearfulness. This finding is in contrast to the inverse coupling between vmPFC and amygdala that has been demonstrated in anxiety disorders (Rauch *et al*, 2006; Milad *et al*, 2006) including social anxiety (Liao *et al*, 2010). However, sustained amygdala-vmPFC connectivity is in line with more recent studies showing that the vmPFC can also have an excitatory influence over amygdala activity. The vmPFC exerts regulatory control over the amygdala during processing of

aversive stimuli through heterogeneous connections that serve to both inhibit and enhance amygdala outputs (Myers-Schulz and Koenigs, 2012; Shin and Liberzon, 2010; Riga *et al*, 2014; Phelps *et al*, 2004). Consistent with an excitatory influence, increased activity in the vmPFC has been associated with increased glucocorticoid response to stress in healthy adults (Jahn *et al*, 2010). Therefore, sustained functional interaction between the vmPFC and amygdala may enhance anxiety by potentiating amygdala output (Myers-Schulz and Koenigs, 2012). Our trend-level association consistent with an excitatory amygdala-vmPFC circuit and suggests that increased amygdala-vmPFC connectivity may be important in social fearfulness, although future studies should replicate this preliminary finding.

While the amygdala is a central driver of communication across the visual threat processing network, extensive structural and functional connections exist between regions, with many important connections bypassing the amygdala (Stefanacci *et al*, 1996; Akirav and Richter-Levin, 1999; Iidaka *et al*, 2001; Phelps, 2004; Amaral *et al*, 2003; Mohedano-Moriano *et al*, 2007; Muñoz and Insausti, 2005; Gabbott *et al*, 2005; Roberts *et al*, 2007; Ghashghaei and Barbas, 2002; Quirk and Beer, 2006; Wager *et al*, 2009a). To further explore connectivity differences within the visual threat processing network, we conducted an exploratory connectivity analysis across all regions. Our findings indicate widespread connectivity across the visual threat processing network **(Table 6)**. The strongest correlations were within processing modalities (e.g., visual and face processing regions, novelty/threat detection regions), and between novelty/threat detection regions (amygdala, hippocampus) and visual and face processing regions (V1, extrastriate cortex, FFA), indicating that increased

interactions across these modalities may be important in social fear. In contrast, interactions between medial prefrontal regulatory areas (mOFC, vmPFC) and other nodes of the visual threat processing network showed mostly small, non-significant correlations. However, correlations were similar when controlling for social fearfulness, suggesting that, across the brain, social fearfulness did not account for a significant amount of variance in connectivity values. Overall, this finding is in line with recent research showing that functional connectivity within subcortical and primary sensory (unimodal) regions is largely independent of participant characteristics, showing relatively low individual variability, while functional connectivity with higher-level multimodal regions shows greater individual variability in connectivity and are more strongly related to cognitive differences (Mueller *et al*, 2013).

3.5. Conclusions

In conclusion, here we show that sustained signaling between the amygdala and the visual cortex is associated with individual variability in social fearfulness. Connections between the amygdala and visual cortex are key in the rapid detection of visual threat and in focusing attentional processing on salient visual stimuli. These findings support the notion that social anxiety is subserved by sustained amygdala-visual cortex activity, and suggest that enhanced attention to salient visual stimuli may be important in social fearfulness.

CHAPTER IV

Specificity of neural response to faces in social fearfulness

4.1. Introduction

In the previous chapters we showed that social fearfulness is related to altered neural response to faces. An important question is whether these findings represent an overall deficit in the processing of novelty, or rather are specific to the processing of social stimuli. Heightened processing of social threat is considered a core feature of social anxiety, and studies have overwhelmingly used social stimuli (e.g., faces) and social situations (e.g., public speaking) to study brain regions related to social threat detection and processing (Freitas-Ferrari *et al*, 2010). Social processing differences show some specificity for social anxiety symptoms (Schofield *et al*, 2009), and heightened response to social novelty is hypothesized to be more closely linked to risk for social anxiety disorder than response to non-social novelty (Dyson *et al*, 2011).

Evolutionary theories, however, suggest that detection and processing of social and non-social novelty are major functions of the brain, and are fundamentally biologically linked (Chang *et al*, 2013). In humans, the tendency to respond to both social and non-social novelty with wariness and avoidance—inhibited temperament—is often assessed together as the construct of behavioral inhibition (Clauss *et al*, 2015). Behavioral inhibition has been shown to be heritable (Clauss *et al*, 2015; Robinson *et al*, 1992), providing evidence for a biological link in trait response to social and non-social novelty. A recent study investigating the ‘shy-bold’ continuum in baboons found that

boldness in exploring novel non-social and social stimuli is heritable (Johnson *et al*, 2015). Boldness in exploring social and non-social stimuli is also predictive of later social behavior in peer groups (Johnson *et al*, 2015), suggesting an association between novelty response and social function.

However, little is known about the specificity of social processing differences in the brain. The goal of this study was to determine whether social fearfulness is uniquely related to disrupted processing of social stimuli. We used a “repeated objects” task to examine non-social neural responses. Participants viewed a set of novel objects repeated up to 7 times while neural responses were measured using fMRI. Initial amplitude of response to novel objects and habituation of response to repeated objects was measured across brain regions previously identified as showing dysfunction in social fearfulness, including the amygdala, hippocampus, mOFC, vmPFC, FFA, V1 and extrastriate cortex **(Figure 2)**.

4.2. Methods

4.2.1. *Participants*

Characteristics. All participants (n = 29) were included in the object habituation analysis. See Chapter 2 for recruitment and screening procedures. Participant characteristics are detailed in **Table 1**.

4.2.2. *Experimental paradigm*

Repeated objects task. We used a “repeated objects” task to investigate neural habituation to non-social stimuli **(Figure 6)**. The repeated objects task used an identical

design as the repeated face task (see Chapter 2 for a detailed description). Briefly, participants were shown a series of 32 neutral objects, with each object shown a total of 1 time, 3 times, 5 times, or 7 times, for a total of 128 object presentations. Objects were shown in pseudorandom order using a jittered, event-related design to maximize fMRI signal measurement efficiency (Friston *et al*, 1999). The repeated objects task was presented using E-Prime software (Version 2.0, Psychology Software Tools, Pittsburgh, PA, USA).

Object stimuli. Images of common, neutral objects (e.g. an umbrella, a vase, a lamp) were obtained from internet photo databases. All stimuli were edited to ensure uniform size, midtone, contrast, and level equalization. Selection of neutral object stimuli for the familiar or novel groups was random.

4.2.3. *MRI data*

Acquisition and preprocessing. Structural and functional MRI data were collected using a 3 Tesla Philips scanner (Philips Healthcare, Inc., Best, The Netherlands) as described in Chapter 2. MRI data were analyzed using statistical parametric mapping (SPM8; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom) and MATLAB (Version 7.10 64-bit, The MathWorks, Inc., Natick, MA, USA). fMRI data were preprocessed for slice time correction, realigned to the mean slice to correct for motion, spatially normalized into standard stereotactic space (MNI T1 template), and smoothed using 6 mm FWHM Gaussian kernel. Functional and structural data were visually inspected for artifacts. Volumes with excessive motion (> 3 mm) or

signal artifacts (signal > 1.8% of mean) were removed from the analysis using Artifact Detection software (ART; Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC)). Volume artifacts were not correlated with participants' social fearfulness or with the repeated object task.

4.2.4. *Regions of interest (ROIs)*

Anatomical and functional ROIs. Habituation analyses were conducted within seven ROIs (amygdala, hippocampus, mOFC, vmPFC, FFA, V1, and extrastriate cortex). Functional connectivity analyses were conducted between the amygdala and each of the ROIs. For a full description of ROI selection, see Chapter 2.

4.2.5. *Data analysis*

fMRI data modeling. The first-level (participant) temporal model was estimated using a general linear model (GLM; Friston *et al*, 1995). The design matrices included 4 task regressors, one for each object exposure category (1, 3, 5, 7), convolved to the SPM default hemodynamic response function (HRF). Motion parameters were also included as additional covariates of no interest. Data were high-pass filtered (128 s) to attenuate low frequency signal (linear scanner drift).

Habituation. Habituation slopes normalized for initial amplitude of response (*b'*) were calculated for each participant (Montagu, 1963; Plichta *et al*, 2014). Normalized habituation slopes were calculated as described in Chapter 2. Percent signal change in the left and right hemispheres were highly correlated across ROIs; therefore, to

increase statistical power and minimize type I error, data from left and right ROIs were averaged. SAS software (Version 9.3, SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses.

Functional connectivity. To determine whether social fearfulness is associated with altered habituation of functional connectivity during repeated object presentations, we used a generalized psychophysiological interaction analysis (gPPI; McLaren *et al*, 2012). gPPI analyses were conducted as described in Chapter 3. Briefly, gPPI analyses were conducted between the amygdala (seed region) and each ROI.

Habituation of functional connectivity. Habituation of connectivity slopes normalized for initial connectivity (*b'*) were calculated for each participant. Normalized habituation of connectivity was calculated as previously described (see Habituation, above). Because percent signal change in the left and right hemispheres were highly correlated, signal was averaged across hemispheres and *b'* connectivity slopes were calculated bilaterally.

Social fearfulness analysis. Statistical analyses were conducted as described in Chapters 2 and 3. To examine differences in response to novel and repeated objects, we used correlations to test for associations between social fearfulness and initial amplitude (intercept) of response to novel objects and between social fearfulness and normalized habituation slope (*b'*) to repeated objects (habituation from 1st to 7th object presentation). To examine differences in amygdala functional connectivity in response

to novel and repeated objects, we conducted correlations between social fearfulness and initial amygdala connectivity (intercept) to novel objects, and between social fearfulness and normalized habituation of amygdala connectivity slopes (*b')* to repeated objects across participants (habituation from 1st to 7th face presentation). Results were considered significant at $\alpha \leq .05$. R^2 values were computed as a measure of effect size. To visualize patterns of response, data were also split into three groups (tertiles of social fearfulness scores) and means and standard errors are presented as bar graphs.

As habituation differences may not occur linearly but rather at varying rates between the 1st and 7th presentation, planned secondary analyses were conducted to examine associations between social fearfulness and habituation to objects within three discrete repetition windows: 1st to 3rd presentation; 3rd to 5th presentation; 5th to 7th presentation. Secondary analyses were considered significant at $\alpha \leq .0167$, Bonferroni-corrected for multiple comparisons.

Specificity to faces. Correlations between response to faces and objects were performed to explore overall associations across participants. Results were considered significant at $\alpha \leq .05$. To partial the unique effects of faces, we performed correlations between social fearfulness and neural responses to faces while controlling for responses to objects. To test whether responses to objects significantly explained the variance in neural responses to faces, we directly compared social fearfulness correlations with and without correction for objects by converting *r* values to z scores and computing *p*-values. Consistent with the main analysis, partial correlations with initial amplitude and overall habituation (1st – 7th) were considered significant at $\alpha \leq .05$;

secondary results (habituation contrasts $1^{st} – 3^{rd}$, $3^{rd} – 5^{th}$, $5^{th} – 7^{th}$) were considered significant at $\alpha \leq .0167$, Bonferroni-corrected for multiple comparisons. R^2 values were computed as a measure of effect size.

4.3. Results

4.3.1. *Response to objects*

Initial amplitude. Here we report associations between social fearfulness and neural response to objects. Social fearfulness was correlated with a dampened initial response to novel objects in the mOFC (r = -.39, p = .04; **Table 9; Figure 13**). There were no correlations between social fearfulness and heightened initial response to novel objects. In the mOFC, the low social fear group had the expected response to novelty, showing an initial response greater than baseline to novel objects. However, the high social fear group had an initial response below baseline to novel objects **(Figure 14)**, suggesting that initial response to novel objects may be suppressed in this group. To visualize neural responses to novel objects, we extracted percent signal change values from each region; values are reported in **Table 10**.

Table 9. Correlations between social fearfulness and neural response to objects.

	Initial amplitude (intercept)			Habituation (*b'*)											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	-.27	.07	.16	.07	.005	.72	.06	.004	.76	-.23	.05	.24	-.15	.02	.43
Extrastriate	-.24	.06	.21	-.05	.003	.81	.004	.00	.98	-.29	.08	.13	-.08	.006	.66
FFA	-.32	.10	.10	-.23	.05	.26	.01	.00	.98	-.07	.005	.71	.01	.00	.95
Amygdala	-.30	.09	.12	-.16	.04	.41	.13	.02	.50	-.06	.004	.74	-.03	.001	.87
Hippocampus	.02	.00	.93	.12	.01	.55	.14	.02	.46	.08	.006	.68	-.06	.004	.76
mOFC	**-.39**	**.15**	**.04**	-.29	.08	.12	-.24	.06	.22	**-.53**	**.28**	**.003**	-.002	.00	.99
vmPFC	-.34	.12	.07	-.18	.03	.36	-.28	.08	.14	**-.50**	**.25**	**.006**	.06	.004	.78

Note: significant correlations in bold; shaded area indicate secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

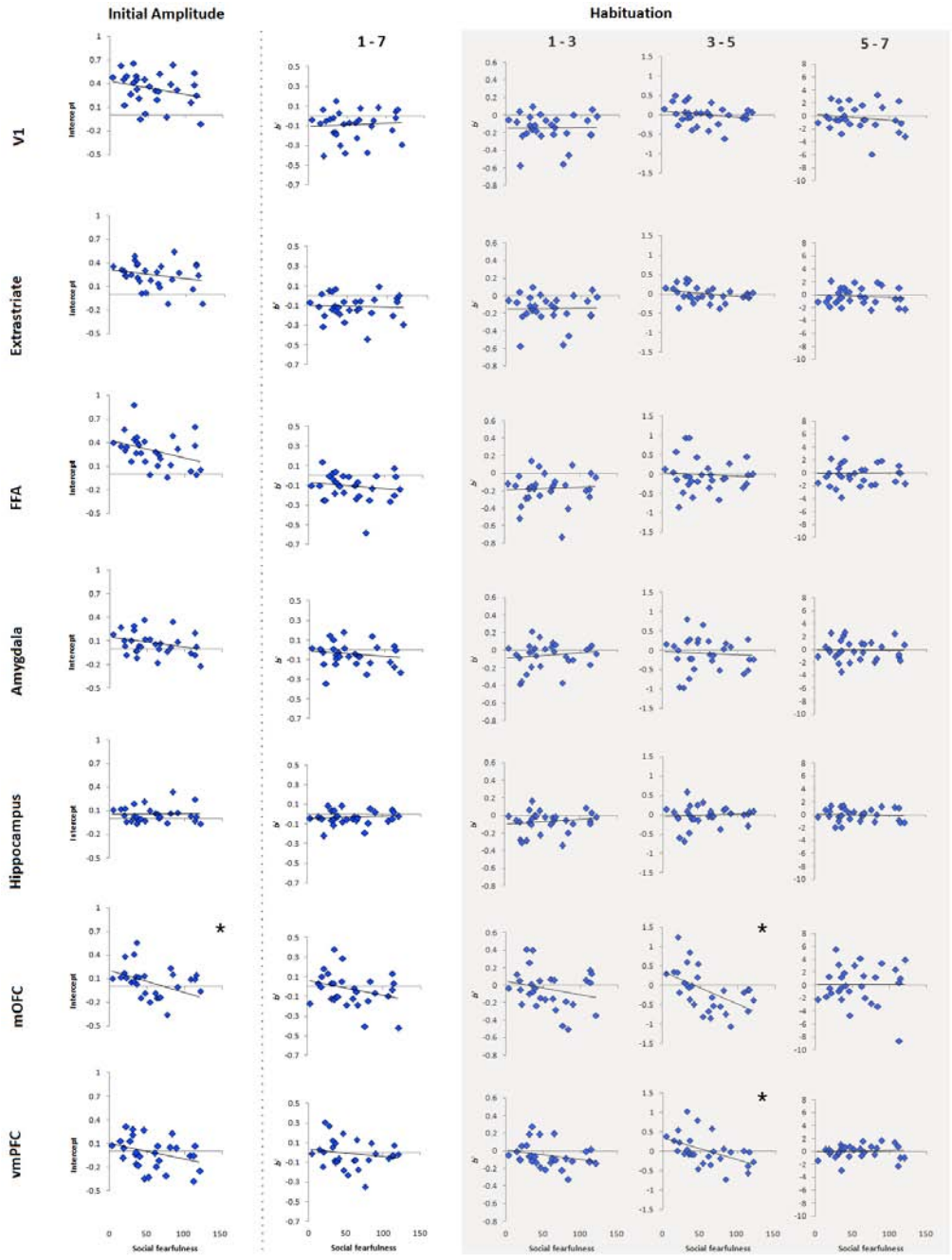


Figure 13. Correlations between social fearfulness and neural response to objects. Scatterplots show the relationship between social fearfulness and neural response to novel and repeated objects. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel objects. Slope (b') values below zero indicate habituation to repeated objects; b' slope values at or above zero indicate sustained or increasing signal to repeated objects, respectively. Social fearfulness was correlated with dampened initial amplitude of response in the mOFC to novel objects. Secondary habituation contrasts (shaded in gray) revealed a correlation between social fearfulness and greater habituation in the mOFC and vmPFC across middle (3rd - 5th) object presentations. There were no social fearfulness differences in habituation across early (1st - 3rd) or late (5th - 7th) object presentations, nor were there overall differences in habituation (1st - 7th) by social fearfulness.

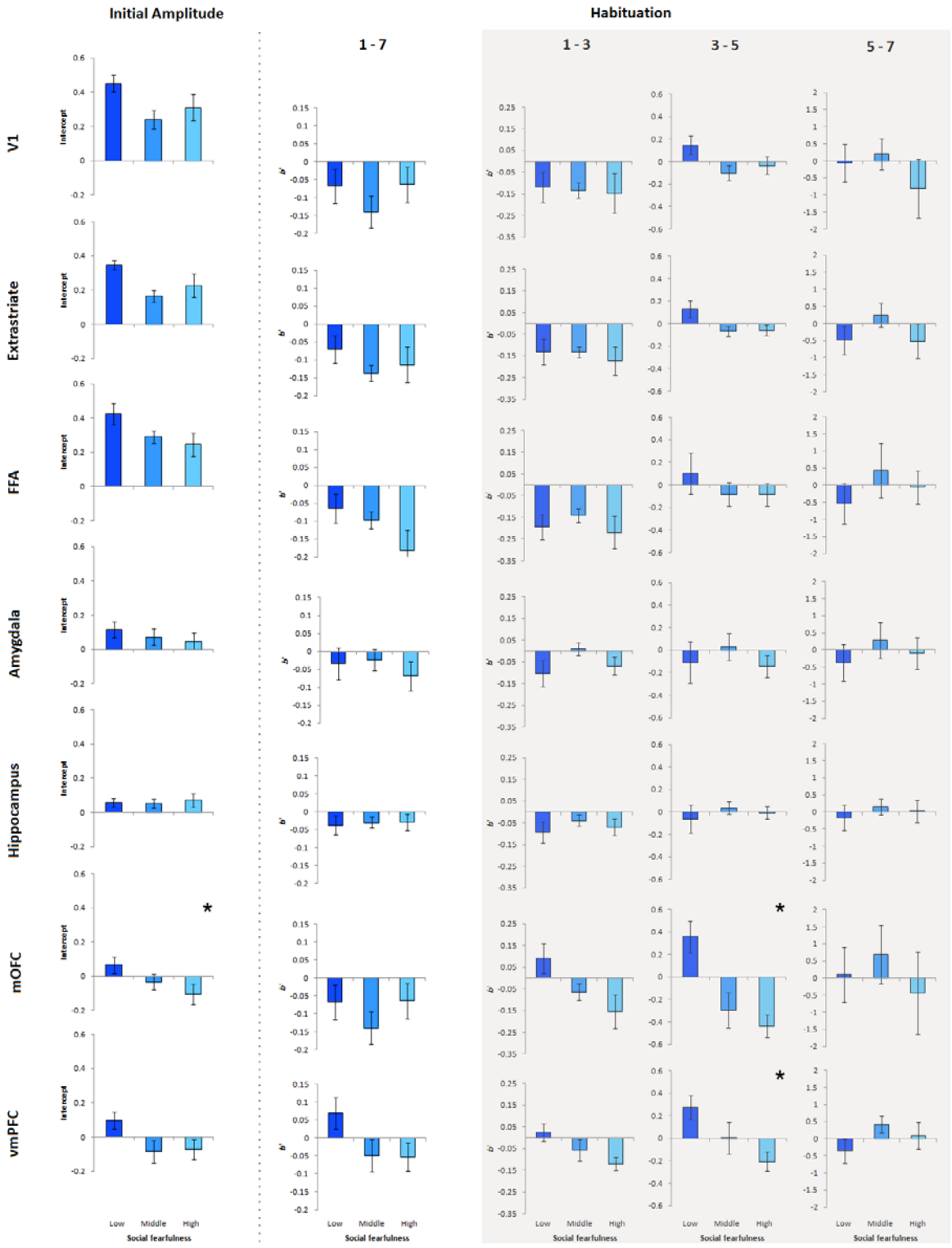


Figure 14. Neural response to objects by social fearfulness tertile. Bar graphs show initial amplitude (intercept) and habituation (b') slope values by social fearfulness tertile for novel and repeated objects. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel objects. Slope (b') values below zero indicate habituation to repeated objects; b' slope values at or above zero indicate sustained or increasing signal to repeated objects, respectively.

Table 10. Percent signal change by object presentation number.

Function	Region	Social fearfulness group	Object presentation 1st mean (sd)	2nd mean (sd)	3rd mean (sd)	4th mean (sd)	5th mean (sd)	6th mean (sd)	7th mean (sd)
Visual processing	V1	All	0.36 (.23)	0.26 (.31)	0.21 (.19)	0.18 (.27)	0.22 (.29)	0.23 (.24)	0.14 (.35)
		High	0.36 (.32)	0.25 (.36)	0.20 (.19)	0.20 (.30)	0.27 (.37)	0.22 (.26)	0.30 (.23)
		Middle	0.22 (.13)	0.22 (.21)	0.12 (.16)	0.18 (.25)	0.18 (.21)	0.21 (.24)	0.01 (.28)
		Low	0.47 (.09)	0.31 (.35)	0.30 (.20)	0.16 (.27)	0.20 (.29)	0.28 (.23)	0.09 (.45)
	Extrastriate	All	0.26 (.19)	0.17 (.25)	0.10 (.14)	0.08 (.21)	0.11 (.22)	0.10 (.17)	0.01 (.25)
		High	0.26 (.26)	0.15 (.33)	0.07 (.14)	0.07 (.26)	0.12 (.26)	0.06 (.22)	0.07 (.19)
		Middle	0.16 (.09)	0.12 (.12)	0.06 (.10)	0.11 (.11)	0.12 (.13)	0.10 (.07)	-0.06 (.13)
		Low	0.37 (.10)	0.22 (.26)	0.17 (.16)	0.07 (.24)	0.09 (.25)	0.14 (.19)	0.03 (.37)
Face processing	FFA	All	0.37 (.19)	0.23 (.25)	0.16 (.19)	0.09 (.26)	0.16 (.18)	0.18 (.25)	0.13 (.32)
		High	0.31 (.21)	0.10 (.31)	0.11 (.22)	0.04 (.31)	0.12 (.23)	0.08 (.31)	0.06 (.23)
		Middle	0.30 (.11)	0.26 (.18)	0.16 (.10)	0.17 (.14)	0.23 (.13)	0.24 (.23)	0.13 (.30)
		Low	0.47 (.18)	0.34 (.19)	0.20 (.22)	0.06 (.29)	0.14 (.18)	0.21 (.20)	0.19 (.41)
Novelty / Threat detection	Amygdala	All	0.09 (.20)	0.04 (.22)	0.03 (.15)	0.02 (.25)	-0.02 (.15)	0.08 (.22)	-0.05 (.32)
		High	0.05 (.21)	0.02 (.25)	0.00 (.10)	-0.01 (.31)	-0.03 (.08)	0.03 (.26)	-0.06 (.28)
		Middle	0.04 (.17)	0.07 (.22)	0.08 (.09)	0.12 (.17)	0.02 (.15)	0.10 (.20)	-0.06 (.17)
		Low	0.17 (.20)	0.04 (.22)	0.00 (.22)	-0.04 (.23)	-0.04 (.21)	0.11 (.22)	-0.02 (.47)
	Hippocampus	All	0.08 (.12)	0.03 (.15)	0.00 (.10)	0.00 (.20)	0.00 (.09)	0.04 (.17)	-0.01 (.22)
		High	0.09 (.16)	0.02 (.20)	0.01 (.06)	0.02 (.26)	0.01 (.09)	0.01 (.20)	0.00 (.18)
		Middle	0.04 (.06)	0.04 (.13)	0.01 (.07)	0.06 (.10)	-0.01 (.09)	0.06 (.11)	-0.04 (.09)
		Low	0.10 (.12)	0.02 (.13)	-0.01 (.15)	-0.07 (.19)	-0.01 (.10)	0.07 (.19)	0.01 (.33)
Outcome prediction	mOFC	All	0.04 (.23)	0.03 (.35)	-0.01 (.25)	0.02 (.47)	-0.07 (.32)	0.10 (.42)	-0.04 (.61)
		High	-0.01 (.24)	-0.07 (.37)	-0.16 (.18)	-0.15 (.45)	-0.14 (.33)	-0.15 (.35)	-0.05 (.90)
		Middle	-0.05 (.14)	-0.04 (.18)	-0.09 (.12)	0.11 (.54)	-0.05 (.15)	0.13 (.39)	-0.13 (.38)
		Low	0.19 (.23)	0.21 (.39)	0.23 (.23)	0.11 (.41)	-0.02 (.43)	0.33 (.42)	0.04 (.45)
Regulatory control	vmPFC	All	0.00 (.23)	-0.02 (.18)	-0.06 (.17)	-0.07 (.19)	-0.04 (.17)	0.01 (.27)	-0.03 (.34)
		High	-0.04 (.22)	-0.06 (.18)	-0.15 (.09)	-0.11 (.25)	-0.05 (.15)	-0.02 (.33)	-0.06 (.34)
		Middle	-0.10 (.17)	-0.06 (.21)	-0.08 (.18)	-0.04 (.18)	-0.07 (.08)	0.05 (.25)	-0.14 (.14)
		Low	0.13 (.25)	0.04 (.16)	0.06 (.16)	-0.04 (.12)	-0.01 (.24)	0.00 (.23)	0.09 (.45)

Note: standard deviation (sd)

1st – 7th object presentation. Social fearfulness was not correlated with habituation differences between the 1st and 7th object presentation (all *p*'s > .12; **Table 9; Figure 13)**. Across social fearfulness groups *b'* slopes were mostly negative, indicating an overall pattern of habituation across participants between the 1st and 7th object presentation **(Figure 14)**. To visualize neural responses to objects over time, we extracted percent signal change values from each region; values are reported in **Table 10**.

1st – 3rd object presentation. We conducted secondary analyses to test for differences in habituation across early, middle, and late repetition windows. Social fearfulness was not correlated with habituation differences in any region during the early presentation window (all *p*'s > .14; **Table 9; Figure 13**). Across social fearfulness groups *b'* slopes were mostly negative, indicating rapid habituation during early object repetitions **(Figure 14)**.

3rd – 5th object presentation. Social fearfulness was correlated with habituation differences in the mOFC and vmPFC during the middle repetition window (mOFC, $r = -.53$, $p = .003$; vmPFC, $r = -.50$, $p = .006$; **Table 9; Figure 13**). The low social fear group had positive *b'* slope values in both the mOFC and vmPFC, indicating a sustained or increasing response to repeated objects. In the context of rapid habituation during early object repetitions, this suggests that responses were maintained near baseline during the middle repetition window. However, the high social fear group had negative *b'* slope

values in both regions, indicating continued habituation to repeated objects during the middle presentation window **(Figure 14)**.

5th – 7th object presentation. Social fearfulness was not correlated with habituation differences in any region during the late repetition window (all *p*'s > .43; **Table 9; Figure 13**). Across social fearfulness groups, *b'* slopes were near zero indicating an overall sustained response to objects during later repetitions **(Figure 14)**.

4.3.2. *Functional connectivity to objects*

Initial connectivity. Social fearfulness was not correlated with differences in initial connectivity during novel object presentations (all *p*'s > .10; **Table 11, Figure 15**). Beta values for initial amygdala connectivity are reported in **Table 12**.

1st – 7th object presentation. Social fearfulness was not correlated with differences in habituation of amygdala connectivity between the 1st and 7th object presentation (all *p*'s > .12; **Table 11, Figure 15**). Across regions, *b'* slope values were near or below zero for most participants, indicating that participants showed an overall pattern of sustained or habituating connectivity between the 1st and 7th object presentation **(Figure 16)**. To visualize patterns of amygdala connectivity over time, we extracted functional connectivity beta values for each region **(Table 12)**.

Table 11. Correlations between social fearfulness and amygdala connectivity during object viewing.

	Initial amplitude			Habituation											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	-.18	.03	.36	.03	.001	.88	-.15	.02	.45	-.22	.05	.26	-.18	.03	.36
Extrastriate	-.08	.006	.69	.007	.00	.97	-.37	.14	.05	-.29	.08	.13	-.09	.008	.66
FFA	-.35	.12	.10	-.33	.11	.12	-.36	.13	.09	-.18	.03	.41	.12	.01	.59
Hippocampus	.17	.03	.39	.19	.04	.33	-.06	.004	.75	-.01	.00	.94	-.07	.005	.70
mOFC	-.10	.01	.62	-.26	.07	.17	-.32	.10	.10	**-.48**	**.23**	**.008**	.03	.001	.88
vmPFC	-.15	.02	.43	-.09	.008	.65	-.36	.13	.05	**-.51**	**.26**	**.004**	.08	.006	.67

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

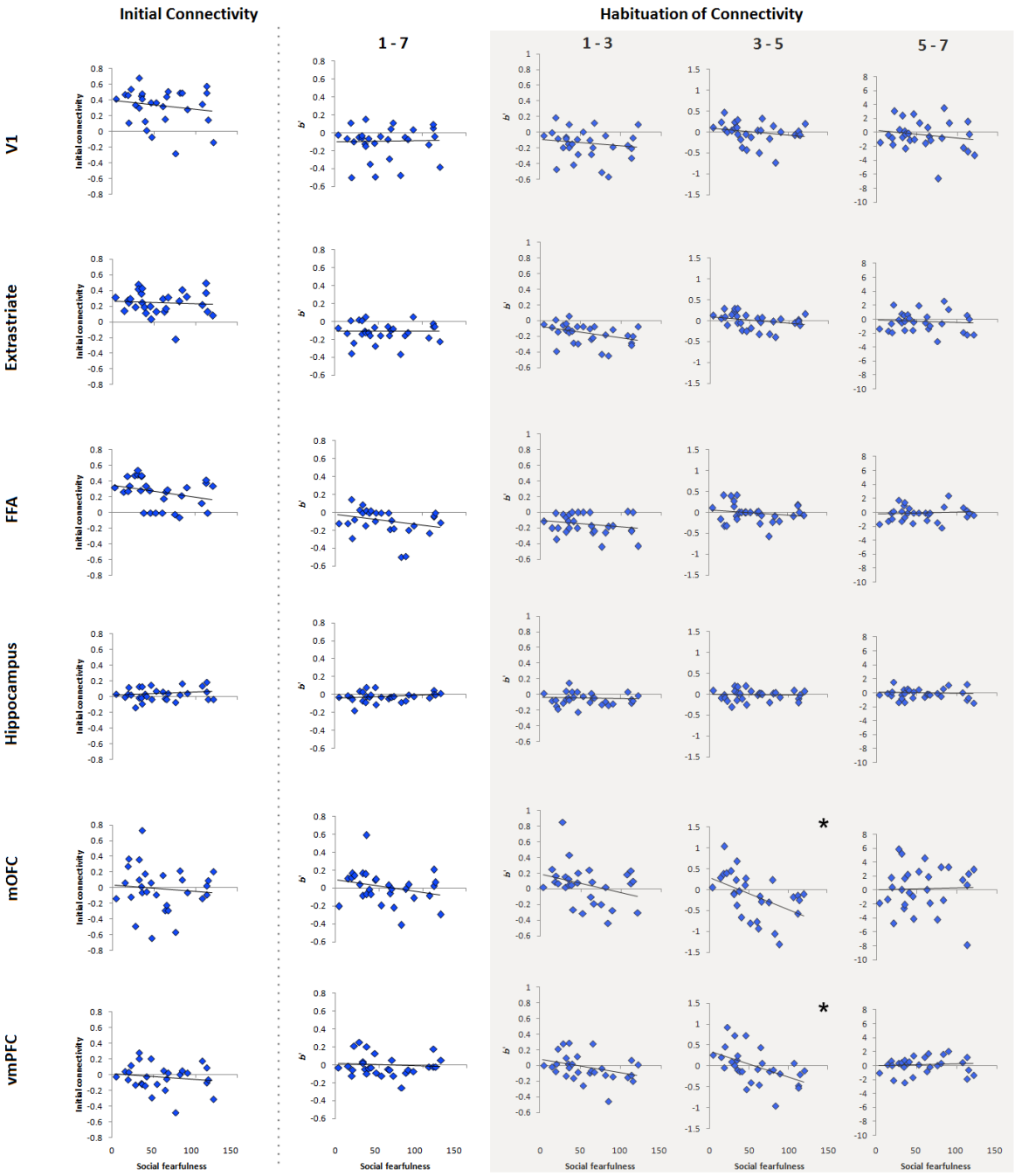


Figure 15. Correlations between social fearfulness and amygdala functional connectivity during object presentations. Scatterplots show relationships between social fearfulness and amygdala functional connectivity during novel and repeated objects. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel objects. Slope (b') values below zero indicate habituation to repeated objects; b' slope values at or above zero indicate sustained or increasing signal to repeated objects, respectively. Secondary habituation contrasts (shaded in gray) revealed correlations between social fearfulness and greater habituation of amygdala connectivity with the mOFC and vmPFC across middle (3rd - 5th) object presentations. There were no social fearfulness differences in initial amygdala connectivity to novel objects, habituation of amygdala connectivity in early (1st - 3rd) or late (5th - 7th) object presentations, or in overall habituation to objects (1st - 7th).

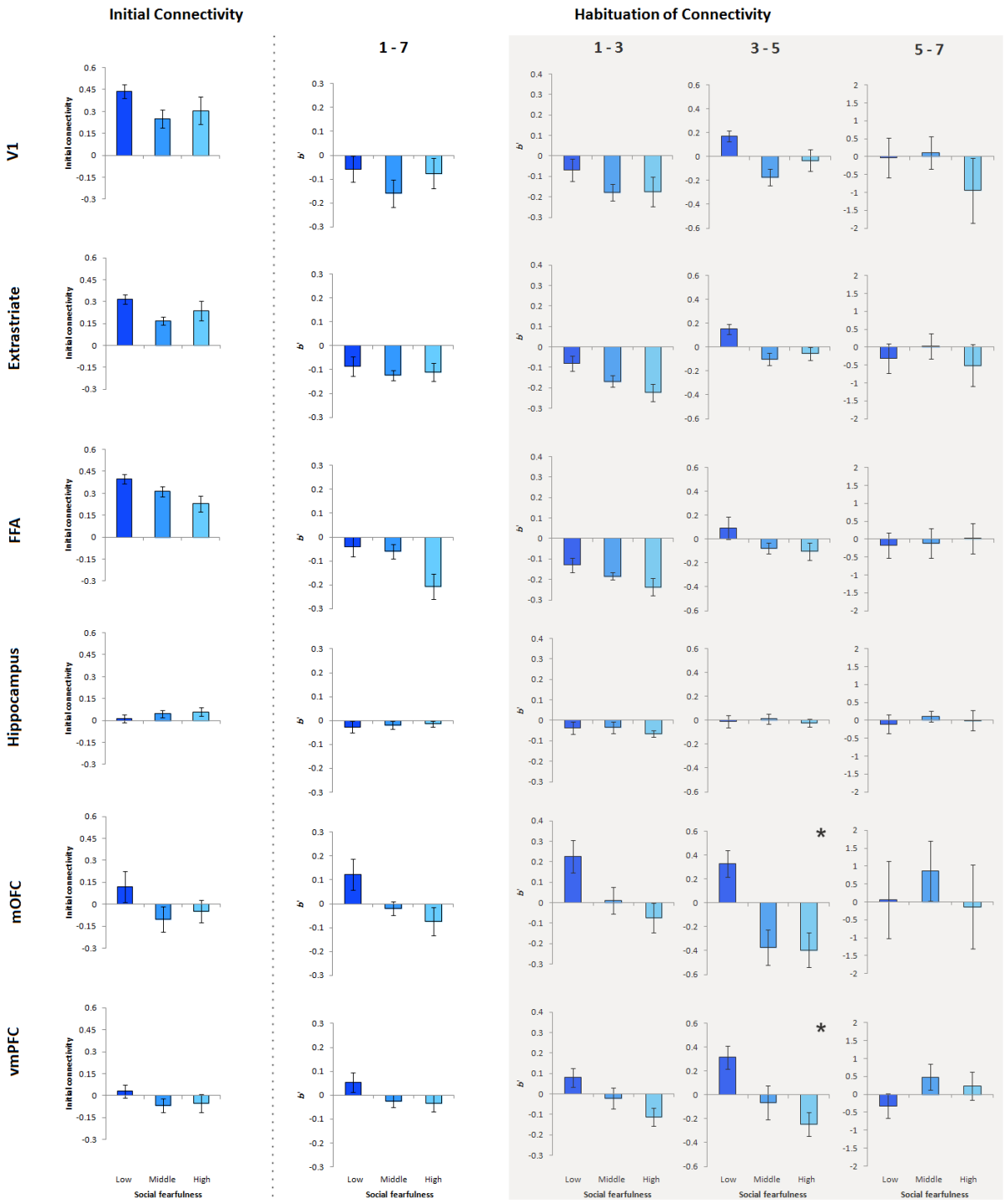


Figure 16. Functional connectivity to objects by social fearfulness tertile. Bar graphs show initial amplitude of amygdala connectivity (intercept) and habituation (b') slope values of functional connectivity by social fearfulness tertile over novel and repeated object presentations. Asterisks (*) denote significant correlations. Intercept values above zero indicate an initial response (greater than baseline) to novel objects. Slope (b') values below zero indicate habituation to repeated objects; b' slope values at or above zero indicate sustained or increasing signal to repeated objects, respectively.

Table 12. Amygdala functional connectivity beta values by object presentation number.

Function	Region	Social fearfulness group	Object presentation 1st mean (sd)	2nd mean (sd)	3rd mean (sd)	4th mean (sd)	5th mean (sd)	6th mean (sd)	7th mean (sd)
Visual processing	V1	All	0.38 (.25)	0.25 (.32)	0.32 (.15)	0.35 (.35)	0.28 (.18)	0.28 (.19)	0.26 (.26)
		High	0.39 (.31)	0.25 (.21)	0.39 (.14)	0.41 (.26)	0.30 (.19)	0.33 (.26)	0.25 (.29)
		Middle	0.36 (.22)	0.16 (.44)	0.27 (.18)	0.44 (.51)	0.23 (.21)	0.27 (.18)	0.22 (.26)
		Low	0.37 (.23)	0.33 (.29)	0.30 (.11)	0.22 (.24)	0.32 (.14)	0.25 (.11)	0.31 (.25)
	Extrastriate	All	0.20 (.24)	0.06 (.33)	0.14 (.16)	0.16 (.23)	0.11 (.14)	0.09 (.19)	0.08 (.25)
		High	0.22 (.35)	0.07 (.21)	0.22 (.14)	0.19 (.22)	0.17 (.12)	0.14 (.19)	0.13 (.25)
		Middle	0.18 (.15)	-0.03 (.56)	0.12 (.13)	0.22 (.24)	0.05 (.16)	0.10 (.15)	0.02 (.21)
		Low	0.19 (.19)	0.15 (.10)	0.06 (.17)	0.06 (.22)	0.11 (.12)	0.03 (.23)	0.07 (.29)
Face processing	FFA	All	0.45 (.25)	0.37 (.30)	0.39 (.20)	0.40 (.27)	0.36 (.19)	0.32 (.23)	0.24 (.27)
		High	0.49 (.26)	0.39 (.21)	0.45 (.18)	0.43 (.23)	0.41 (.17)	0.31 (.29)	0.21 (.31)
		Middle	0.43 (.31)	0.42 (.16)	0.47 (.20)	0.47 (.28)	0.39 (.23)	0.41 (.21)	0.34 (.31)
		Low	0.44 (.18)	0.29 (.46)	0.26 (.18)	0.30 (.27)	0.28 (.16)	0.24 (.17)	0.17 (.09)
Novelty / Threat detection	Hippocampus	All	0.05 (.09)	0.03 (.19)	0.01 (.07)	0.00 (.14)	0.01 (.07)	0.07 (.10)	0.05 (.13)
		High	0.04 (.08)	0.01 (.18)	0.02 (.07)	0.04 (.15)	0.02 (.08)	0.09 (.11)	0.07 (.13)
		Middle	0.06 (.08)	-0.01 (.24)	0.04 (.04)	0.04 (.12)	0.01 (.08)	0.07 (.08)	0.05 (.10)
		Low	0.03 (.11)	0.09 (.14)	-0.02 (.09)	-0.07 (.12)	0.01 (.09)	0.05 (.12)	0.04 (.15)
Outcome prediction	mOFC	All	0.10 (.37)	-0.08 (.42)	0.02 (.23)	0.03 (.54)	0.04 (.31)	0.00 (.40)	0.05 (.68)
		High	0.22 (.38)	-0.06 (.23)	0.09 (.28)	-0.03 (.47)	0.00 (.30)	0.05 (.55)	-0.06 (.51)
		Middle	-0.09 (.21)	-0.27 (.60)	0.01 (.15)	0.24 (.79)	0.03 (.17)	0.03 (.28)	0.03 (.37)
		Low	0.16 (.44)	0.07 (.35)	-0.03 (.23)	-0.10 (.28)	0.08 (.42)	-0.07 (.36)	0.17 (1.01)
Regulatory control	vmPFC	All	0.14 (.25)	0.08 (.19)	0.03 (.14)	-0.01 (.35)	0.03 (.13)	0.06 (.21)	0.08 (.29)
		High	0.22 (.35)	0.11 (.17)	0.07 (.14)	0.02 (.26)	0.01 (.15)	0.07 (.19)	0.00 (.31)
		Middle	0.13 (.16)	-0.01 (.15)	0.07 (.12)	0.00 (.55)	0.06 (.11)	0.14 (.23)	0.18 (.19)
		Low	0.05 (.17)	0.14 (.22)	-0.04 (.13)	-0.05 (.21)	0.03 (.15)	-0.03 (.20)	0.06 (.34)

Note: standard deviation (sd)

1st – 3rd object presentation. Social fearfulness was not correlated with habituation of amygdala connectivity from the 1st to the 3rd object presentation (all p's > .05; **Table 11; Figure 15**). Across most participants, *b'* slopes were below zero indicating habituation of connectivity to objects during early repetitions **(Figure 16)**.

3rd – 5th object presentation. In the middle repetition window, social fearfulness was correlated with differences in habituation of connectivity between the amygdala and two regions, the mOFC and vmPFC (mOFC, r = -.48, p = .008; vmPFC, r = -.51; p = .004; **Table 11; Figure 15**). There was a similar pattern across both regions—the low social fear group had positive *b'* connectivity slopes in the mOFC and vmPFC, indicating sustained or increasing connectivity with the amygdala during repeated object presentations. In the context of minimal initial response to novel objects **(Table 10)** and habituation of connectivity during early object presentations, this suggests that the low social fear group maintained connectivity near baseline during repeated object presentations. In contrast, the high social fear group had negative *b'* connectivity slopes in the mOFC and vmPFC, suggesting continued habituation of amygdala connectivity below baseline during the middle object presentation window **(Figure 16)**.

5th – 7th object presentation. There were no social fearfulness differences in habituation of connectivity in the late repetition window (all p's > .36; **Table 11; Figure 15)**. Across participants, *b'* connectivity values were near zero indicating sustained connectivity with the amygdala across regions during later object repetitions **(Figure 16)**.

4.3.3. *Specificity of effects of social stimuli*

Initial amplitude. To determine whether social fearfulness effects were unique to faces, we performed correlations with faces controlling for effects of objects. Social fearfulness showed similar correlations with initial amplitudes to faces when controlling for objects compared to not controlling for objects **(Table 2; Table 13)**, with social fearfulness associated with higher initial amplitudes in the hippocampus and vmPFC in both analyses, indicating that objects accounted for little variance in neural response to novel faces. To further test whether initial responses to faces were unique, we directly compared social fearfulness correlation values when controlling for objects compared to not controlling for objects; direct comparison of correlation values revealed no significant differences **(Table 14)**.

Habituation. Similarly, correlations between social fearfulness and habituation to faces were similar when controlling for objects compared to not controlling for objects **(Table 2; Table 13)**. Social fearfulness was associated with dampened rate of habituation across most regions, with predominant effects in the middle (3^{rd} – 5^{th}) face presentation window in both analyses, indicating that objects accounted for little variance in habituation to faces. Direct comparison of correlation values revealed no significant differences **(Table 14)**.

Table 13. Correlations between social fearfulness and neural response to faces, controlling for objects.

	Initial amplitude (intercept)			Habituation (*b'*)											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.20	.04	.31	-.06	.004	.75	.35	.12	.07	**.57**	**.33**	**.002**	-.08	.006	.70
Extrastriate	.30	.09	.13	.14	.02	.49	.44	.19	.02	**.55**	**.30**	**.003**	.007	.00	.97
FFA	.26	.07	.21	.27	.07	.18	.39	.15	.05	.35	.12	.08	.19	.04	.36
Amygdala	**.38**	**.14**	**.04**	.34	.12	.08	.36	.13	.06	**.45**	**.20**	**.016**	-.15	.02	.45
Hippocampus	**.49**	**.24**	**.009**	**.45**	**.20**	**.016**	.39	.15	.04	**.56**	**.31**	**.002**	-.20	.04	.31
mOFC	.28	.08	.15	-.06	.004	.78	.33	.11	.09	**.53**	**.28**	**.004**	-.01	.00	.96
vmPFC	**.42**	**.18**	**.03**	.37	.14	.06	.24	.06	.23	.36	.13	.06	.07	.005	.74

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 14. Specificity of neural response to faces: comparison of response to faces with and without correction for response to objects.

	Initial amplitude (intercept)		Habituation (*b'*)							
			$1^{st} - 7^{th}$		$1^{st} - 3^{rd}$		$3^{rd} - 5^{th}$		$5^{th} - 7^{th}$	
Region	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value
V1	-.51	.61	.11	.91	0	1	-.26	.80	.04	.97
Extrastriate	-.12	.91	0	1	-.04	.97	.11	.91	.05	.96
FFA	-.56	.58	-.08	.94	0	1	-.08	.94	-.04	.97
Amygdala	-.08	.94	-.12	.91	-.08	.94	0	1	0	1
Hippocampus	0	1	.05	.96	0	1	0	1	-.04	.97
mOFC	-.31	.76	.61	.54	.08	.94	-.43	.67	0	1
vmPFC	.27	.79	-.04	.97	.15	.88	.67	.50	-.04	.97

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Initial connectivity. We found similar correlations between social fearfulness and initial amygdala connectivity to novel faces when controlling for objects compared to not controlling for objects **(Table 6; Table 15)**, indicating that objects accounted for little variance in connectivity to novel faces. Direct comparison of correlation values showed no significant differences **(Table 16)**.

Habituation of connectivity. Similarly, habituation of amygdala connectivity to faces showed a similar associations with social fearfulness when controlling for objects compared to not controlling for objects **(Table 6; Table 15)**, with social fearfulness associated with higher amygdala connectivity with V1 and extrastriate cortex in both analyses, indicating little influence of objects on neural activity to faces. Direct comparison of correlation values showed no significant differences **(Table 16)**.

4.4. Discussion

The goal of this study was to determine whether social fearfulness was uniquely related to differences in social (vs. non-social) neural processing. We found that neural responses to faces were associated with social fearfulness even when controlling for neural responses to objects. Our findings indicate that rather than a difference in novelty processing *per se*, which would suggest differences in general neuronal function in socially fearful people, social fearfulness is associated with specific differences in face processing. These findings are consistent with studies demonstrating that social inhibition has a stronger relationship with social anxiety disorder symptomatology than non-social inhibition

Table 15. Correlations between social fearfulness and amygdala connectivity to faces, controlling for objects.

	Initial amplitude (intercept)			Habituation (*b'*)											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.22	.05	.26	.003	.00	.99	.27	.07	.16	**.45**	**.20**	**.016**	-.08	.006	.70
Extrastriate	.22	.05	.25	.26	.07	.18	.26	.07	.19	**.45**	**.20**	**.016**	-.05	.003	.79
FFA	.21	.04	.34	.27	.07	.21	.26	.07	.23	.26	.07	.23	.34	.12	.11
Hippocampus	-.03	.001	.90	.17	.03	.38	.15	.02	.44	.37	.14	.05	-.22	.05	.26
mOFC	.22	.05	.25	-.05	.003	.79	.28	.08	.14	.42	.18	.03	.01	.00	.96
vmPFC	.28	.08	.16	.34	.12	.07	.35	.12	.07	.21	.04	.30	.06	.004	.78

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 16. Specificity of amygdala connectivity to faces: comparison of amygdala connectivity to faces with and without correction for response to objects.

	Initial amplitude (intercept)		**Habituation (*b'*)**							
			1^{st} – 7^{th}		**1^{st} – 3^{rd}**		**3^{rd} – 5^{th}**		**5^{th} – 7^{th}**	
Region	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value
V1	-.48	.63	.06	.95	0	1	0	1	-.04	.97
Extrastriate	-.22	.83	0	1	.28	.78	.19	.85	0	1
FFA	-.48	.63	-.38	.70	-.19	.85	-.04	.97	.08	.94
Hippocampus	.04	.97	.04	.97	.04	.97	.13	.90	.99	.32
mOFC	-.26	.80	-.04	.97	-.27	.79	-.46	.65	.04	.97
vmPFC	.08	.94	-.08	.94	-.36	.72	.76	.45	-.04	.97

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation (1^{st} – 7^{th}) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

(Van Ameringen *et al*, 1998; Schofield *et al*, 2009; Dyson *et al*, 2011), and with the finding that deficits in social processing are specifically associated with social anxiety symptoms (Schofield *et al*, 2009).

Evolutionary theories suggest that brain regions critical for survival in lower species—that is, brain regions that detect novelty—have been elaborated and repurposed to incorporate social processing in species more dependent on social groups, such as primates (Chang *et al*, 2013). A striking example is mormyrid fish, whose electrosensory system, originally purposed for orienting and detection of motion, now subserves social function absent in ancestral states (Katz, 2006; Chang *et al*, 2013). Another example of this repurposing exists in the evolution of oxytocin signaling in the brain—while oxytocin serves an ancestral role in decreasing anxiety and approach behavior, it is has evolved to support parenting, maternal bonding, and mating in primates (Chang *et al*, 2013). Because social function is critical for human health, welfare and survival, it is intuitive that brain regions specialized for the rapid detection of non-social novelty would also develop the ability to respond to social novelty and evaluation of social threat. However, our findings provide support for functional specialization within these brain regions, with differences in response to social information coexisting alongside normal, adaptive responses to non-social information.

In examining responses to novel objects, we found that social fearfulness was associated with alterations in medial prefrontal cortex responses to objects. Socially fearful participants showed a dampened initial response to novel objects in the mOFC and greater habituation to objects in the mOFC and vmPFC. Exploring functional connectivity, we found greater habituation of amygdala connectivity with the mOFC and

vmPFC in socially fearful participants during repeated object presentations. Together, these findings may indicate a disruption in amygdala-medial prefrontal connectivity in socially fearful people during processing of non-social stimuli. These findings suggest that assessing medial prefrontal-amygdala circuit function to both social and non-social stimuli may be important for future studies.

4.5. Conclusions

In conclusion, we show that differences in processing of social stimuli are unique in social fearfulness—we found no evidence of a generalized deficit in novelty processing across stimulus types. As heightened response to social novelty has been more closely linked to risk for social anxiety disorder (relative to response to non-social novelty) (Dyson *et al*, 2011), these findings suggest that investigation of the neural basis of social fearfulness may inform risk for development of social anxiety disorder. These findings may have implications for evaluating response to treatment in patients with social anxiety disorder; given this preliminary evidence that overall response to non-social novelty is not disrupted in people with high levels of social fear, non-social novelty may serve as a valuable baseline against which to gauge change in neural responses to social stimuli following treatment in social anxiety disorder.

CHAPTER V

Specificity of effects to social fearfulness

5.1. Introduction

A major challenge in psychiatric neuroimaging is showing specificity of associations between neural responses and traits of interest. We have shown that neural response to social stimuli varies along the dimension of social fearfulness. A component of social fearfulness is high negative affect, a stable trait in which people tend to view themselves negatively and experience a broad range of negative emotions including nervousness, fear, anxiety, and guilt (Watson and Clark, 1984). Traits such as social fearfulness, anxiety, and depression share a common component of high negative affect, and therefore show consistently strong correlations with each other (Schmidt *et al*, 1997; Watson *et al*, 1988).

A critical question is whether social fears show a unique neural signature in the brain, or whether components of the social fearfulness response to faces are related to the separate but overlapping characteristic of high negative affect. In general, negative affect is associated with higher incidence of psychopathology, although there's little evidence that negative affect increases risk for a specific type of diagnosis (Watson and Clark, 1984). However, for accurate identification and effective early treatment of at-risk individuals, a specific neural signature of risk for social anxiety is critical. Here, we tested for specificity of associations between neural response to faces and social fearfulness. To determine whether the association between social fearfulness and neural response to faces could be explained by negative affect, we tested for unique

effects of trait anxiety and depression—two characteristics that strongly overlap with negative affectivity—on initial amplitude and habituation.

5.2. Methods

5.2.1. *Participants*

Characteristics. All participants (n = 29) were included in the specificity analysis. See Chapter 2 for recruitment and screening procedures. Trait anxiety was measured using the State-Trait Anxiety Inventory (STAI) and depression was measured using the Beck Depression Inventory (BDI-II) (see Chapter 2 for details). Participant characteristics are detailed in **Table 1**.

5.2.2. *Data analysis*

Regions of interest (ROIs). Habituation analyses were conducted within seven ROIs (amygdala, hippocampus, mOFC, vmPFC, FFA, V1, and extrastriate cortex). For a full description of ROI selection, see Chapter 2.

Specificity to social fearfulness. Correlations between social fearfulness, anxiety, and depression were performed to explore overall associations across participants. To partial the unique effects of social fearfulness, we performed correlations between social fearfulness and neural response to faces while controlling for 1) trait anxiety (STAI-trait) or 2) depression (BDI-II). Consistent with the main analysis, partial correlations with initial amplitude and overall habituation ($1^{st} – 7^{th}$) were considered significant at $\alpha \leq .05$; secondary results (habituation contrasts $1^{st} – 3^{rd}$, $3^{rd} – 5^{th}$, $5^{th} – 7^{th}$)

were considered significant at α ≤ .0167, Bonferroni-corrected for multiple comparisons. R^2 values were computed as a measure of effect size. To test whether trait anxiety or depression significantly explained associations between social fearfulness and neural responses to faces, we directly compared social fearfulness correlations with and without correction for 1) anxiety or 2) depression by converting *r* values to z scores and computing *p*-values (primary analyses were set at α ≤ .05; secondary comparisons were determined significant at α ≤ .0167, Bonferroni corrected for multiple comparisons).

5.3. Results

5.3.1. *Specificity of associations with social fearfulness*

We first determined correlations between measures; as expected, social fearfulness scores were highly correlated with both trait anxiety and depression scores across participants, indicating shared variance across measures (*r*'s > .66, *p*'s < .001; **Table 17**).

Trait anxiety. Trait anxiety and social fearfulness were associated with unique patterns of activity in response to novel faces. Trait anxiety and social fearfulness had opposing effects on neural response to novelty in the extrastriate cortex and FFA—trait anxiety was correlated with lower initial amplitudes in both regions (extrastriate, *r* = -.43, *p* = .03; FFA, *r* = -.41, *p* = .04; **Table 18**) while social fearfulness was correlated with higher initial amplitudes (extrastriate, *r* = .47, *p* = .02; FFA, *r* = .39, *p* = .05; **Table 19**). Trait anxiety was also uniquely correlated with higher initial amplitudes in the vmPFC (*r* = .45, *p* = .02; **Table 18**) while social fearfulness was uniquely correlated with sustained signal

Table 17. Correlations between social fearfulness, trait anxiety and depression scores across participants.

	Social fearfulness		Trait anxiety		Depression	
Measure	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Social fearfulness	1	--	**.78**	**< .001**	**.66**	**< .001**
Trait anxiety			1	--	**.71**	**< .001**
Depression					1	--

Note: significant correlations in bold; results were considered significant at α ≤ .0167.

in the extrastriate cortex (*r* = .48, *p* = .01; **Table 19**). Direct comparisons revealed that the only brain region that showed a significant difference after controlling for trait anxiety was the vmPFC (initial amplitude, *p* = .05; **Table 20**). The correlation between social fearfulness and initial amplitude in the vmPFC was no longer significant after controlling for trait anxiety scores.

Depression. Similarly, depression and social fearfulness accounted for unique patterns of activity in response to faces. Depression was uniquely correlated with lower initial amplitudes in the FFA (*r* = -.44, *p* = .03; **Table 21**) and dampened rates of habituation in the hippocampus (habituation (1^{st} – 3^{rd}), *r* = .46, *p* = .01) **(Table 21)**. In contrast, social fearfulness was uniquely associated with elevated initial amplitudes in the amygdala and extrastriate cortex (amygdala, *r* = .44, *p* = .02; extrastriate, *r* = .42, *p* = .03; **Table 22**). The direct comparison demonstrated that depression scores did not significantly account for any effects of social fearfulness **(Table 23)**.

Table 18. Correlations between trait anxiety and neural response to faces, controlling for social fearfulness.

	Initial amplitude (intercept)			**Habituation (*b'*)**											
				$1^{st} - 7^{th}$			**$1^{st} - 3^{rd}$**			**$3^{rd} - 5^{th}$**			**$5^{th} - 7^{th}$**		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	-.24	.06	.24	-.12	.01	.57	-.08	.006	.69	-.24	.06	.24	.22	.05	.28
Extrastriate	**-.43**	**.19**	**.03**	-.05	.03	.81	.06	.004	.77	-.21	.04	.30	.26	.07	.19
FFA	**-.41**	**.17**	**.04**	-.19	.04	.35	-.04	.002	.83	-.27	.07	.18	.25	.06	.22
Amygdala	-.05	.03	.83	-.04	.002	.86	.25	.06	.22	.13	.02	.52	.31	.10	.12
Hippocampus	.12	.01	.57	.02	.00	.94	-.05	.03	.81	-.15	.02	.46	.37	.14	.07
mOFC	-.24	.06	.24	.31	.10	.13	.41	.17	.04	.16	.03	.43	-.04	.002	.83
vmPFC	**.45**	**.20**	**.02**	.26	.07	.21	.28	.08	.17	.43	.19	.03	.23	.05	.25

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 19. Correlations between social fearfulness and neural response faces, controlling for trait anxiety.

	Initial amplitude (intercept)			Habituation (*b'*)											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.22	.05	.29	.007	.00	.97	.23	.05	.27	.45	.20	.02	-.16	.03	.45
Extrastriate	**.47**	**.22**	**.02**	.08	.006	.70	.18	.03	.38	**.48**	**.23**	**.01**	-.18	.03	.37
FFA	**.39**	**.15**	**.05**	.30	.09	.13	.29	.08	.14	.41	.17	.04	-.07	.005	.72
Amygdala	.23	.05	.27	.18	.03	.37	.02	.00	.93	.16	.03	.44	-.32	.10	.11
Hippocampus	.22	.05	.29	.26	.07	.19	.27	.07	.19	.44	.19	.03	-.38	.14	.06
mOFC	.28	.08	.17	-.28	.08	.17	-.08	.006	.71	.18	.03	.39	.06	.004	.78
vmPFC	-.03	.001	.89	.005	.00	.98	-.02	.00	.93	.02	.00	.94	-.11	.01	.60

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 20. Specificity of effects of social fearfulness: comparison of social fearfulness effects with and without correction for trait anxiety.

	Initial amplitude (intercept)		**Habituation (*b'*)**							
			$1^{st} - 7^{th}$		**$1^{st} - 3^{rd}$**		**$3^{rd} - 5^{th}$**		**$5^{th} - 7^{th}$**	
Region	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value
V1	-.59	.56	-.13	.90	.47	.64	.33	.74	.33	.74
Extrastriate	-.84	.40	.22	.83	1	.32	.45	.65	.73	.47
FFA	-1.09	.28	-.20	.84	.41	.68	-.33	.74	.91	.36
Amygdala	.51	.61	.5	.62	1.2	.23	1.17	.24	.62	.52
Hippocampus	1.13	.26	.83	.41	.49	.62	.58	.56	.67	.50
mOFC	-.31	.76	-.47	.64	1.61	.11	1.05	.29	-.25	.80
vmPFC	**1.99**	**.05**	1.34	.18	1.11	.27	1.96	.05	.18	.86

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 21. Correlations between depression and neural response to faces, controlling for social fearfulness.

	Initial amplitude (intercept)			Habituation (*b'*)											
				$1^{st} - 7^{th}$			$1^{st} - 3^{rd}$			$3^{rd} - 5^{th}$			$5^{th} - 7^{th}$		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	-.31	.10	.11	-.03	.001	.90	.22	.05	.26	-.08	.006	.70	.06	.004	.75
Extrastriate	-.34	.12	.08	.17	.03	.40	.42	.18	.03	.06	.004	.75	.03	.001	.90
FFA	**-.44**	**.19**	**.03**	-.01	.00	.96	.17	.03	.42	-.27	.07	.18	.19	.04	.35
Amygdala	-.28	.08	.15	-.13	.02	.52	.28	.08	.15	.11	.01	.58	.12	.01	.56
Hippocampus	.06	.004	.76	.28	.08	.16	**.46**	**.21**	**.01**	.11	.01	.58	.23	.05	.25
mOFC	.17	.03	.38	.20	.04	.32	.43	.19	.02	.41	.17	.03	.10	.01	.60
vmPFC	.27	.07	.17	.28	.08	.14	.17	.03	.40	.04	.002	.86	.19	.04	.34

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation ($1^{st} - 7^{th}$) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 22. Correlations between social fearfulness and neural response to faces, controlling for depression.

	Initial amplitude (intercept)			Habituation (*b'*)											
				1 - 7			1 - 3			3 - 5			5 - 7		
Region	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value	*r*	r^2	*p*-value
V1	.26	.07	.19	-.006	.00	.98	.14	.02	.48	.46	.21	.02	-.09	.008	.63
Extrastriate	**.42**	**.18**	**.03**	-.008	.00	.97	.09	.008	.66	.43	.19	.02	-.003	.00	.99
FFA	.38	.14	.06	.20	.04	.34	.21	.04	.30	.42	.18	.03	.01	.00	.94
Amygdala	**.44**	**.19**	**.02**	.31	.10	.11	.09	.008	.64	.30	.09	.12	-.19	.04	.34
Hippocampus	.35	.12	.07	.21	.04	.29	.01	.00	.95	.40	.16	.04	-.30	.09	.12
mOFC	.04	.002	.86	-.17	.03	.39	.002	.00	.99	.11	.01	.60	-.08	.006	.70
vmPFC	.24	.06	.22	.10	.01	.61	.11	.01	.57	.38	.14	.04	-.08	.006	.69

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation (1^{st} – 7^{th}) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

Table 23. Specificity of effects of social fearfulness: comparison of social fearfulness effects with and without correction for depression.

	Initial amplitude (intercept)		Habituation (*b'*)							
			1 - 7		1 - 3		3 - 5		5 - 7	
Region	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value	z	*p*-value
V1	-.74	.46	-.09	.93	.81	.42	.28	.78	.07	.94
Extrastriate	-.62	.54	.54	.56	1.33	.18	.68	.50	.08	.94
FFA	-1.04	.30	.19	.85	.72	.47	-.38	.70	.62	.54
Amygdala	-.34	.73	0	1	.95	.34	.63	.53	.15	.88
Hippocampus	.62	.54	1.02	.31	1.45	.15	.75	.45	.35	.73
mOFC	.59	.56	.44	.66	1.31	.19	1.3	.19	.25	.80
vmPFC	1	.32	1	.32	.64	.52	.59	.56	.51	.61

Note: significant correlations in bold; shaded area indicates secondary tests; initial amplitude and overall habituation (1^{st} – 7^{th}) results were considered significant at $\alpha \leq .05$; secondary results were considered significant at $\alpha \leq .0167$.

5.4. Discussion

The goal of this analysis was to determine the specificity of the effect of social fearfulness on initial amplitude and habituation of neural responses to faces. Overall, we found evidence for specificity of social fearfulness in neural response to faces, with trait anxiety accounting for only one unique effect. Trait anxiety significantly accounted for the relationship between social fearfulness and elevated initial amplitude to novel faces in the vmPFC. Because trait anxiety is associated with generally increased risk for developing psychopathology, including anxiety disorders and depression (Hankin and Abela, 2005), this suggests that elevated activity in the vmPFC may account for more general risk for illness in socially fearful participants while activity across the rest of the circuit provides a specific dimensional marker of risk for social anxiety. Depression did not account for any unique effects of neural activity. Together, these findings indicate that social fearfulness is associated with a signature neural response, particularly in novelty detection and visual processing regions, that is specific to social fear and not accounted for the more general trait of negative affect.

Of interest, we found a dissociation in visual cortex responses by trait anxiety and social fearfulness. In the extrastriate cortex and FFA, social fearfulness was uniquely associated with higher initial amplitudes to novel faces, while trait anxiety was uniquely associated with lower amplitudes during novel face presentations. Studies have shown enhanced orienting response and “hyperscanning” of faces, including direct fixations on the eyes, in people with high trait anxiety (Bradley *et al*, 2000; Mogg *et al*, 2000), while shyness has been associated with fewer eye movements around a face and avoidance of the eyes (Wang *et al*, 2012). This suggests differences in viewing of a

face as a possible mechanism for differences in neural response to novel faces. An additional possibility is that people with trait anxiety employ stronger regulation of visual processing regions during face viewing—trait anxiety was uniquely related to elevated initial amplitude in the vmPFC, a region with extensive connections to the visual cortex (Sesack *et al*, 1989; Chiba *et al*, 2001). Functional interactions between the vmPFC and visual cortex have been associated with expectation-based top-down regulation of visual search (Pantazatos *et al*, 2012). Although trait anxiety and social fearfulness are partially overlapping traits, with a shared component of negative affect, these findings suggest that important differences between the two traits may exist in neural function, and in orienting to and evaluation of faces. Future studies assessing dissociable responses to novel faces may uncover important mechanisms differentiating these overlapping traits.

A limitation of the study is the high degree of overlap between trait anxiety and social fearfulness. Participants with high social fearfulness were also highly likely to have high trait anxiety, with approximately 60% shared variance in the two traits. Therefore, it's possible that results are driven by unique participants showing divergence in trait anxiety and social fearfulness scores. Future studies are necessary to replicate these findings in larger sample.

5.5. Conclusions

Our results support a specific neural signature for social fearfulness. Trait anxiety was also associated with dampened visual cortex and face processing responses to novel faces, providing preliminary evidence for a neural dissociation between the

overlapping traits of anxiety and social fearfulness. These findings may have important implications for guiding future studies in at-risk populations. Negative affect is associated with a broad, non-specific risk in psychopathology while social fearfulness is associated with a relatively specific elevated risk for social anxiety disorder; therefore, our findings provide initial evidence for neural mechanisms contributing to both broad and specific risk for development of mental illness.

CHAPTER VI

Discussion and future directions

6.1. Discussion

Social anxiety disorder is highly prevalent and chronic illness affecting more than 1 in 10 Americans each year. Due to its early onset during adolescence, social anxiety disorder has cascading consequences throughout development, resulting in decreased educational attainment, lower occupational status, and decreased quality of life. Social anxiety disorder is also a significant risk factor for other major psychiatric illness, such as depression and substance abuse. Early identification and effective treatment of social anxiety disorder would have a substantial impact on public health. The availability of specific dimensional biological markers is essential for the early identification of risk and the assessment of treatment response; however, clinically useful dimensional biological markers are currently unavailable.

In this study, we identified a dimensional relationship between neural response to novelty and trait social fearfulness. We describe two neural mechanisms—response to novel faces and habituation to repeated faces—in socially fearful people that fundamentally influence individual variability in response to social stimuli. Neural response to novelty is critical in attentional orienting responses; however, equally important is the ability to habituate to novel stimuli that are safe. Here we show that socially fearful people have both an elevated response to novel faces and fail to habituate to repeated faces in multiple brain regions involved in processing of novelty and threat; specifically, we found elevated response to novel faces in the hippocampus

and vmPFC, and a significant difference in habituation rate in the amygdala, hippocampus, mOFC, vmPFC, V1, and extrastriate cortex. Critically, we show that altered brain activity did not reflect a general impairment in novelty processing; social fearfulness was not associated with an elevated or sustained response to novel objects, indicating a unique deficit in social novelty processing.

The neural mechanisms of habituation. Habituation is a fundamental mechanism by which we learn about the world around us. Habituation of sensory stimuli is critical in filtering information that is safe and familiar while allowing neural resources to new, potentially threatening stimuli. While the neural mechanisms of habituation remain partially unknown (Ramaswami, 2014), inhibitory signals between brain regions are key in focusing attentional processing and may play a role in habituation. In particular, top-down feedback from the amygdala to primary sensory systems has been shown to play a key role in focusing sensory processing on behaviorally-relevant stimuli (Vuilleumier and Driver, 2007). We found sustained functional connectivity between the amygdala and early visual processing areas in social fearfulness—including V1 and extrastriate cortex—suggesting that amygdala-visual circuits may be specifically enhanced in social fear. Together, these findings comprise a neural signature of social fearfulness, including differences in novelty detection, habituation, and connectivity across a visual threat processing network.

A model of failed medial prefrontal regulation in social fear. Elevated initial response in the vmPFC was uniquely explained by trait anxiety, suggesting that activity in vmPFC

may be a general risk factor for psychopathology rather than social anxiety *per se*. Additionally, we found that overall amplitude remained higher in socially fearful participants throughout face viewing, despite a similar rate of habituation across participants. Although vmPFC activity may not be a specific marker of risk for social anxiety, examination of differences in this region may help elucidate the neural factors that increase the risk for developing comorbid illness in people with social anxiety. In particular, depression is common in people with social anxiety disorder (Beesdo *et al*, 2007). Depression is also consistently linked with altered vmPFC activity (Myers-Schulz and Koenigs, 2012). Consistent with findings in depression, our combined results suggest a model of weak, chronically-engaged vmPFC regulation of amygdala activity (Myers-Schulz and Koenigs, 2012). For example, during neutral face viewing, low social fear participants had little response in the amygdala or vmPFC; in contrast, high social fear participants had an elevated amygdala response to neutral faces. We suggest that during viewing of neutral faces, low social fear participants required little vmPFC regulation of the amygdala, while high social fear participants required an elevated vmPFC response in an attempt to regulate elevated amygdala activity. However, as vmPFC-amygdala activity remained high throughout the task in socially fearful participants. Based on these findings, we propose that vmPFC regulation fails during viewing of neutrally-valenced faces.

The strength of a stimulus is critical in eliciting neural responses. The use of strong stimuli (e.g., fear faces) may create uniformity in response, while weak stimuli (e.g., neutral faces) better elicit individual differences (Lissek *et al*, 2006). Numerous studies have demonstrated inverse functional connectivity between the medial

prefrontal cortex and amygdala in anxiety patients (Goldin *et al*, 2009; Kim *et al*, 2011). However, each of these studies used negatively-valenced faces to examine neural response; no studies to date have investigated medial prefrontal-amygdala connectivity using neutrally-valenced faces, as we did here. Based on our findings, we propose that medial prefrontal cortex regulation fails during viewing of both negatively- and neutrally-valenced faces, with the only difference between the two conditions being a stronger amygdala response to negative stimuli that far exceeds medial prefrontal response, leading to findings of inverse coupling. In further support of this model, we found a dissociation in prefrontal-amygdala regulation within our own participants dependent on stimulus strength (neutral faces vs. neutral objects)—while we found a trend for sustained vmPFC-amygdala activity to faces (a stronger stimulus), vmPFC-amygdala activity showed a stronger rate of habituation in response to objects (a weaker stimulus) in high social fear participants. As social animals, social stimuli are an inherently salient and relatively strong stimuli (Lissek *et al*, 2006); in contrast, non-social stimuli are a relatively weak stimulus and have little salient value. In accordance, the medial prefrontal cortex and amygdala both respond strongly to social stimuli, with the strongest response in socially fearful people, while amygdala activity is similar across all participants to non-social stimuli. Overall, these findings suggest a weak, chronically-engaged vmPFC-amygdala regulatory circuit may contribute to trait anxiety in social fearfulness.

Evidence for hippocampal involvement. We found strong evidence of hippocampal differences in social fearfulness. In our habituation analysis, the hippocampus was the

only region where we detected both elevated response to novel faces and an overall sustained response to repeated faces across the entire course of the experiment in socially fearful participants. Although we did not specifically investigate habituation of connectivity of the hippocampus in this study, correlations between the hippocampus and other nodes of the social threat network were among the strongest detected in our exploratory connectivity analysis. We also found evidence that habituation differences were not distinctly related to negative affect, an emotional trait largely attributed to amygdala function (Gray and McNaughton, 2003), providing further indication that brain regions other than the amygdala play an important role in social fearfulness. These findings are in line with Gray's theory of septo-hippocampal inhibition, which states that the hippocampus plays a central role in production of anxiety. In this theory, the drive to approach novelty competes with the drive to avoid potential threats, with the hippocampus playing a critical role in behavioral inhibition in order to avoid potential threat (Gray and McNaughton, 2003; Gray, 1983). Our findings broadly support a central role for the hippocampus in social fearfulness, providing support for this theory. We suggest that further investigation of the hippocampus in social fearfulness is strongly warranted, particularly in its role in habituation to social novelty.

From adaptive to maladaptive neural response. Our findings suggest that social fearfulness is driven by hyperactivity of threat detection circuits. However, little is known about the progression from adaptive, healthy social fear to maladaptive, extreme variants. Preliminary evidence suggests that two mechanisms play a role: 1) a reduced threshold for activation in threat detection regions; and 2) hyperexcitability of brain

regions developed through a sensitization process (Rosen and Schulkin, 1998). During an adaptive fear response, activity in threat detection regions increases then subsides as the threat is reduced or eliminated. However, in people sensitive to social threat, chronic activation of threat detection circuits may lead to hyperexcitability, wherein circuits are more sensitive to threat and more readily activated in the future. Subsequent social fear responses would be more easily triggered by less-threatening stimuli, leading to a pattern of chronic social fear response to everyday situations. It's possible that a biological predisposition for slow habituation may underlie sensitization processes in the brain. Sensitization to threat has been shown to be driven by release of glucocorticoids and corticotropin-releasing hormone, a response critically controlled by activity in the amygdala and hippocampus. Prolonged activation of the amygdala and hippocampus, through slow habituation, may lead to increased release of glucorticoids and trigger long-term changes in how the brain processes threatening information. This suggests that identification of biological determinates of habituation in the brain may aid in identifying those individuals most at risk for developing psychopathology.

However, the molecular mechanisms underlying neural habituation remain unclear, although molecular processes known to support learning and memory are hypothesized to play a role. For example, cyclic guanacine monophosphate (cGMP), a second messenger molecule underlying long-term cellular changes, is likely important in both short and long-term cellular habituation (Soibam *et al*, 2013). cGMP is crucially involved in synaptic plasticity (Kleppisch and Feil, 2009) and plays a significant role in learning and memory formation (Bernabeu *et al*, 1996). Inhibiting the enzymes that break down cGMPs has been shown to increase cognitive function and improve

recognition memory in aged rats (Baratti and Boccia, 1999; Prickaerts *et al*, 2004), as well as ameliorate cognitive deficits in Huntington's chorea and Alzheimer's disease by increasing cGMP in the hippocampus (Puzzo *et al*, 2009; Cuadrado-Tejedor *et al*, 2011; Saavedra *et al*, 2013). Recent findings in humans have also described a link between memory, the amygdala, and a gene encoding cGMP degradation enzymes (Knowles *et al*, 2015). As habituation is a fundamental memory process, it's likely that differences in cGMP regulation of cellular plasticity play a role in neural habituation processes. Disruptions in four key neurotransmitter systems—the serotonergic, dopaminergic, GABAergic, and endocannabinoid systems—have also been shown to regulate neural habituation in rodents (Leussis and Bolivar, 2006; Salomons *et al*, 2013; Patel and Hillard, 2008; Gunduz-Cinar *et al*, 2012), with most systems implicated in human behavioral habituation as well (Wiggins *et al*, 2013; Bunzeck *et al*, 2013; Conzelmann *et al*, 2012; Hariri *et al*, 2009). Follow up studies in humans should directly test for genetic and memory differences that may link to disruptions in molecular habituation processes.

Our results may also have implications for targeted therapies aimed at regulating amygdala response. Treatments regulating norepinephrine signaling may be particularly useful in strengthening vmPFC-amygdala regulation in socially fearful people. Because social situations are ubiquitous, social fearfulness likely results in greater daily stress and chronic stress exposure. Stress is a potent catalyst for change in the brain and has profound effects on both the medial prefrontal cortex and the amygdala, resulting in strengthening of amygdala function, reduced firing of medial prefrontal neurons, and strengthened norepinephrine signaling in both regions (Arnsten *et al*, 2015). High levels of norepinephrine released during stress exposure impairs medial prefrontal cortex

function via actions at alpha-1 receptors. Treatments that block norepinephrine signaling in the brain (e.g., alpha-1 receptor antagonists) have been shown to strengthen medial prefrontal regulatory function and weaken amygdala response (Arnsten, 2009) and are effective in reducing symptoms in post-traumatic stress disorder (Raskind *et al*, 2003). A possible mechanism for norepinephrine-mediated regulation of anxiety symptoms may be strengthening of habituation of neural activity in the medial prefrontal cortex and amygdala; future studies should determine whether treatments targeting norepinephrine signaling regulate habituation of neural response. Gray's theory of septo-hippocampal inhibition in anxiety further emphasizes a potential role for noradrenergic signaling in the neural basis of social fear. Norepinephrine signaling is critical in regulation of hippocampal function, and both hippocampal lesions (Gray and McNaughton, 2003) and lesions of the dorsal ascending noradrenergic pathway (McNaughton and Mason, 1980) cause strong anxiolytic effects.

At the circuit level, the dynamics of habituation also remain obscure (Ramaswami, 2014). Neural habituation likely involves many processes in the brain, including molecular signaling at the synapse, activation of local inhibitory circuits, and activation of top-down regulatory processes, with current evidence suggesting that each of these processes plays a partial role. From a circuit perspective, habituation may occur either between regions that comprise a circuit or within local inhibitory circuits. Although influences within and across regions are difficult to discern, models that predict different habituation outcomes based on circuit interactions may be useful in designing studies. From one perspective habituation is a top-down regulatory response that reflects integration of multiple forms of sensory information within higher-level

regions. In this view, habituation of sensory systems (e.g., visual) is simply a mirror of regulatory feedback (e.g., amygdala). Key to this view of habituation, habituation to one stimulus promotes generalization to stimuli within the same modality (Rankin *et al*, 2009). For example, in a centralized system, habituation to one type of visual threat (individual face) in the amygdala would generalize to similar types of visual threat (all faces), because the habituation is being regulated by a single central region. Our findings, from a centralized circuit viewpoint, would suggest that failure of habituation of amygdala activity is the basis for failed habituation in the visual cortex. However, we did not detect associations between social fearfulness and overall habituation to faces as a category (regardless of being novel or familiar), suggesting that differences in centralized habituation are not key in social fear.

In an opposing viewpoint, habituation processes occur within sensory modalities themselves and are transmitted upstream to integration regions such as the amygdala to guide novelty and threat detection processes. From this viewpoint, habituation to one stimulus does not easily generalize to other stimuli in the same modality; in other words, habituation is specific to a single stimulus, such as a specific person's face. Our results provide preliminary support for this de-centralized view of habituation in social fear—across randomized face presentations where more novel and more familiar face presentations were mixed, participants generally showed higher activity to faces that were more novel and lower neural activity to faces that had been seen many times. This suggests that differences in neural habituation (or failure of habituation) in social fearfulness are driven by local inhibitory circuits within individual regions.

Specificity to social fear. Finally, we found that the majority of neural differences related to social fearfulness were specific to this trait. A specific neural signature of risk for social anxiety is critical for accurate identification and effective early treatment of at-risk individuals. Here, we find that although social fearfulness, trait anxiety, and depression share core characteristics, social fearfulness uniquely explained neural differences in response to social stimuli.

Limitations. There are several limitations that should be taken into account when evaluating the findings from these studies. Because our primary goal was to examine response to faces, participants always viewed face blocks prior to object blocks in order to maximize attention to faces. Therefore, it's possible that neural responses to objects are an effect of time/fatigue. A direct investigation of objects in future studies may be beneficial in further untangling individual differences in processing of "strong" vs. "weak" stimuli (Lissek *et al*, 2006). Online arousal/anxiety ratings were not collected during stimulus presentations as cognitive tasks, such as conscious ratings of anxiety levels, have been shown to alter neural response to stimuli (Pérez-Edgar *et al*, 2007). However, differences in arousal/anxiety during the task could influence differences in neural response (Choi *et al*, 2012). Future studies should consider collecting an online measure of arousal during face presentations, such as skin conductance response.

Conclusions. In sum, we found that level of social fearfulness predicted response to novelty and habituation across a core network of brain regions involved in social information processing. Individuals who were high in social fearfulness displayed both

an elevated and sustained response to faces across a network of regions involved in visual threat processing, and a sustained pattern of functional connectivity between the amygdala and visual cortices. In individuals who were low in social fearfulness, response to faces was characterized by low initial amplitudes and habituation of neural activity. This dimensional neural signature is specific to social stimuli and is independent of trait anxiety and depression, indicating a specific association with social fear.

6.2. Clinical implications

The ability to feel familiar and safe in social situations is important and may have far reaching consequences. The presence of a familiar social partner greatly enhances the ability to overcome specific fears and subsequent anxiety (Lungwitz *et al*, 2014). Socially-familiar peers may serve as a robust safety signal in a variety of situations. Lacking this safety signal, socially fearful people may show greater inhibition in fear-provoking non-social situations (e.g., riding a rollercoaster, watching a scary movie), with cascading consequences for both social and non-social development. Recent research has also identified a link between slow habituation of autonomic arousal to stressful events and greater body mass index (Feda *et al*, 2015), suggesting that habituation differences contribute broadly to physical health risk.

Characterizing a specific neural mechanism underlying the dimension of social fearfulness has the potential to: 1) Guide individualized treatment selection. Psychiatric treatment selection often involves trial and error, which can delay provision of effective treatment. Using knowledge about an individual patient to guide treatment choice has innumerable benefits and may provide insight into which individuals would benefit most

from particular therapies; 2) Identify individuals at risk for social anxiety disorder. A specific biological marker of social fearfulness will provide a more precise predictive tool for risk assessment; 3) Guide development of scientifically-based treatments. Characterization of neural habituation in social fearfulness can provide the first step toward the development of novel therapeutics. Using animal models of habituation, new drugs and therapies could be developed; 4) Address the need for dimensional neurobiological measures of psychopathology across diagnostic categories. Social fearfulness is a trait that cuts across multiple diagnostic categories, including autism (van Steensel *et al*, 2011) and schizophrenia (Pallanti *et al*, 2004), leading to significant increased disability, and preliminary studies have suggested that neural habituation may play a role in each of these illnesses (Kleinhans *et al*, 2009; Holt *et al*, 2005). Therefore, neural habituation may provide a useful neurobiological marker across multiple disorders. This approach is consistent with the National Institute of Mental Health's RDoC initiative calling for "the development, for research purposes, of new ways of classifying psychopathology based on dimensions of observable behavior and neurobiological measures".

6.3. Future directions

Social anxiety disorder is a highly prevalent and costly illness, and early identification and effective treatment of social anxiety would have a substantial impact on public health. The availability of specific dimensional biological markers are essential for the early identification of risk and the assessment of treatment response (Kessler, 2002). Our findings provide preliminary evidence that two fundamental elements of

neural response to social stimuli—initial amplitude and habituation—vary dimensionally with social fearfulness, a spectrum including both clinical and sub-threshold social anxiety. We hope that these results serve as a springboard for future studies of the temporal dynamics of neural response and their impact on social functioning. We suggest the following future research directions.

1. Examine novelty response and habituation across development. The ability to predict who is at risk for developing social anxiety disorder is an important goal and has the potential to significantly impact early treatment and prevention. Most cases of social anxiety disorder occur during early adolescence, at a time when the 'social brain' is undergoing major structural and functional development (Blakemore, 2008). This early onset has cascading implications for social development, school performance, and career choice. Detection of brain changes during this period that contribute to risk for social anxiety disorder could have a profound impact on development of new, preventative therapies. Investigation of fundamental aspects of response to social novelty—both initial amplitude of response and habituation to repeated stimuli—in adolescents may shed new light on this developmental process and provide a valuable mechanisms for early treatment.

2. Determine the relationship with functional impairment. How habituation to novelty contributes to the development of functional impairment—the hallmark of social anxiety disorder—remains unknown. While the diagnosis of social anxiety

disorder is associated with high levels of social fear, and will therefore fall at the upper end of the social fear continuum, social anxiety disorder is not synonymous with social fearfulness; it requires an additional consideration of impairment, or the extent of dysfunction and distress in a person's life resulting from the social anxiety (Rapee and Spence, 2004). Although functional impairment is related to social fear severity, it is also a distinct factor. Studies within social anxiety disorder patients determining associations between neural response to novel and repeated social stimuli and extent of impairment are necessary to distinguish the contribution of these neural mechanisms to this critical factor.

3. Identify effective treatments. Psychiatric treatment selection often involves trial and error, which can delay effective treatment. Using knowledge about an individual patient to guide treatment choice has innumerable benefits. For example, although there are several effective treatments for social anxiety disorder, these treatments only work for approximately 50% of social anxiety disorder patients (van Vliet *et al*, 1994; Stein *et al*, 1998; Heimberg *et al*, 1998). Exposure therapy, one of the most effect treatments for social anxiety disorder, is fundamentally a learning process, and we would predict that habituation is one of the target mechanism of this type of therapy (Protopopescu *et al*, 2005). Indeed, preliminary evidence has linked faster rate of habituation following exposure therapy with lowered behavioral avoidance of phobic stimuli (Matthews *et al*, 2015). The ability to characterize an individual's neural habituation response may

provide insight into which individuals would benefit most from exposure therapy. Characterization of neural habituation in people with high social fearfulness can also provide the first step toward the development of novel therapeutics. Using rodent models of behavioral habituation (Salomons *et al*, 2013; Leussis and Bolivar, 2006; Patel and Hillard, 2008), studies have suggested that behavioral habituation may serve as a new behavioral test for anxiolytic response to therapy.

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