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The role of development and anxious disposition in fear regulation

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Abstract

The ability to discriminate and update the threat or safety value of stimuli in the environment has clear health benefits. A common hallmark of many anxiety disorders is pervasive and sustained responding to stimuli that no longer signal threat, suggesting impaired fear regulation. Unfortunately, some populations, such as adolescents and those with anxious dispositions are particularly vulnerable to anxiety disorders. This body of work examines how individual differences in development and anxious disposition impact fear extinction, the key fear regulatory processes studied in this thesis. In a series of fear conditioning experiments adapted for developmental samples, we demonstrated individual differences in development and anxious disposition to predict substantial variability in fear extinction ability, as measured with psychophysiological and neural correlates. In a developmental sample, we found that younger age and age-related structural changes in the ventromedial prefrontal cortex (vmPFC) are important predictors of continued responding in the amygdala to learned threat vs. safety cues during fear extinction. In adult samples, however, we found intolerance of uncertainty to specifically predict elevated responses to both learned threat and safety cues in psychophysiological correlates and the amygdala during fear extinction, over and above other general measures of anxious disposition. More broadly, these findings highlight the potential of developmental and intolerance of uncertainty-based mechanisms to help understand pathological fear in anxiety disorders and inform future treatment targets.

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Declaration:

I confirm that this is predominantly my own work and the use of all material from other sources has been properly and fully acknowledged. In Chapters 2 and 4, students helped me collect the data (Karoline Tokarska, Natalia Kontoudaki, and Helen Warwick).

The research presented in Chapters 2-4 has been reported in articles that are published, under review, or are currently in preparation for submission:

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1. Introduction

1.1 Fear generation and regulation

'Fear' can be described as an evolutionarily conserved defence state that arises in response to imminent threat in the environment (Panksepp, 1998). For example, fear initiates a cascade of behavioural and physiological changes that prepare the organism for response readiness, heightened awareness and rapid evaluation of threat, in order to avoid harm (LeDoux, 1998). Such responses are contextually sensitive, are found across species (Panksepp, 1998), and vary both interindividually and intraindividually (Davidson, 2002; Frijda, 1986). Fear responses are thought to originate from a combination of innate (Panksepp, 1998) and learnt adaptations throughout development (Izard, 2011).

Definition of fear generation

An organism's response to a stimulus evaluated as (or deemed) fearful. Fear generation can be operationalised by measuring responses to potential threat stimuli (subjective feelings, expressive behaviour, and physiology).

Definition of fear regulation

Voluntary or involuntary modulation of response intensity or duration to a stimulus evaluated as fearful. Fear regulation may be operationalised by measuring changes in responses to potential threat stimuli as a function of time or context.

1.1.1 Neurobiology of fear

Classically, fear-generative and regulatory processes have been captured through experimental manipulations that present pain (e.g. shock), predator (e.g. spider, snake, course shapes), conspecific (e.g. faces) and unpredictable stimuli (Gamer & Büchel, 2009; Herry et al., 2007; Larson, Aronoff, Sarinopoulos, & Zhu, 2009; Larson, Aronoff, & Stearns, 2007; Mobbs et al., 2010; Ohman, Flykt, & Esteves, 2001; Öhman, Lundqvist, & Esteves, 2001; Rhudy & Meagher, 2000; Thomas, Drevets, Whalen, et al., 2001; Whalen et al., 2001). Typically, across species, aversive stimuli, compared to neutral stimuli, evoke defensive responses (e.g. freezing, avoidance), indexed by increased vigilance and arousal within behavioural, physiological, and neural systems (Lang & Bradley, 2010; LeDoux, 1998). Neural circuits within the amygdala, anterior cingulate cortex, hypothalamus, and brainstem are primarily involved in fear expression across species (Gross & Canteras, 2012; Oler et al., 2012; Shackman et al., 2011). Initially, the amygdala was thought to be specialised in threat detection (Morris, Öhman, & Dolan, 1999; Whalen et al., 2004), but recent evidence refutes this claim (Williams et al., 2006), suggesting that the amygdala is generally involved in vigilance and broader monitoring of salience (Rosen & Donley, 2006; Sander, Grafman, & Zalla, 2003; Whalen, 2007). For example, a landmark study by Herry et al. (2007) found sustained amygdala activation and anxiogenic behaviour in both humans and animals to unpredictable neutral tones vs. predictable neutral tones. Furthermore, in humans, Herry et al. (2007) found unpredictable neutral tones vs. predictable tones to increase activation in the amygdala to angry faces during a concurrent dot probe task, suggesting unpredictability to modulate attention to aversive

stimuli (Herry et al., 2007). Quelling of the amygdala can be seen over time through habituation (Fisher et al., 2009), or by simply manipulating the proximity and predictability of aversive stimuli (Fisher et al., 2009; Herry et al., 2007; Mobbs et al., 2010; Sarinopoulos et al., 2009).

During exposure to fearful stimuli, parts of the prefrontal cortex that are thought to be responsible for signalling safety, such as the ventromedial prefrontal cortex (vmPFC) have been found to inhibit the amygdala (Fisher et al., 2009; Mobbs et al., 2010). Complementary to these findings, recent intracranial electroencephalography and lesion studies in humans support a causal role of the vmPFC in the regulation of the amygdala (Christen & Grandjean, 2010; Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2015), and other fear generative regions (Motzkin, Philippi, Oler, et al., 2015; Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2014). For example, patients with damage to the vmPFC, compared to healthy controls, have been shown to exhibit heightened right amygdala activity whilst viewing aversive pictures and at rest (Motzkin, Philippi, Wolf, et al., 2015).

A large body of work has also studied fear (and other emotions) generative and regulatory processes through experiments that present cognitive tasks during or after fear-relevant stimuli (Blair et al., 2007; Brown, van Steenbergen, Band, de Rover, & Nieuwenhuis, 2012; Dolcos & McCarthy, 2006; Ihssen, Heim, & Keil, 2007; Kanske, Heissler, Schönfelder, Bongers, & Wessa, 2010; Morriss, Taylor, Roesch, & van Reekum, 2013; Schönfelder, Kanske, Heissler, & Wessa, 2013; Van Dillen, Heslenfeld, & Koole, 2009a; Wangelin, Löw, McTeague, Bradley, & Lang, 2011; Weinberg & Hajcak, 2011). During these experiments, as participants attempt to maintain attention on task

demands, in spite of competing emotional stimuli, increased recruitment of the ventral lateral prefrontal cortex (vlPFC), dorsal lateral prefrontal cortex (dlPFC) and dorsal medial prefrontal cortex (dmPFC), and decreased recruitment of the amygdala has been found (Blair et al., 2007; Dolcos & McCarthy, 2006; Kanske et al., 2010; Van Dillen, Heslenfeld, & Koole, 2009b). Whilst prefrontal activity in these experiments varies by task, similar activation is found in the amygdala across these experiments and in fMRI studies only using fear stimuli (Fisher et al., 2009; Mobbs et al., 2010)

In an attempt to separate fear (and other emotion states) generative and regulatory processes further, researchers have typically used intentional or instructed tasks, where participants are asked to maintain, suppress (or decrease), enhance (or increase) or reframe responses to emotional stimuli such as pictures (Hajcak & Nieuwenhuis, 2006; Jackson, Malmstadt, Larson, & Davidson, 2000; Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner et al., 2004; Urry et al., 2006; van Reekum et al., 2007), films (Goldin, McRae, Ramel, & Gross, 2008) and threat of shock (Delgado, Nearing, LeDoux, & Phelps, 2008; Kalisch, Wiech, Herrmann, & Dolan, 2006). Decrease, down-regulate and reframe instructions have been found to reduce physiological and event related potential correlates to affective picture stimuli (Hajcak, Moser, & Simons, 2006; Jackson et al., 2000; Moser, Hajcak, Bukay, & Simons, 2006; Moser, Krompinger, Dietz, & Simons, 2009; Schönfelder et al., 2013) and learned threat cues (Delgado et al., 2008). In the brain, reduced activity in the amygdala and increased activity in the prefrontal cortex are found to be common neural signatures of successful down-regulation of fear (Delgado et al., 2008) and more general negative affect

(Johnstone et al., 2007; Ochsner et al., 2002; Ochsner et al., 2004; Urry et al., 2006), suggesting shared amygdala-prefrontal cortical mechanisms in instructed and uninstructed fear regulation (Blair et al., 2007; Dolcos & McCarthy, 2006; Fisher et al., 2009; Mobbs et al., 2010; Van Dillen et al., 2009a). However, the exact site of activation in the prefrontal cortex varies depending on the timing and contents of emotion regulation instructions. For example, Urry et al. (2006) & Johnstone et al. (2007) presented auditory instructions during picture stimuli and found significant inverse coupling between the amygdala-vmPFC (but see, van Reekum et al., 2007). Conversely, Ochsner and colleagues (2004) presented written instructions before picture stimuli and found significant increases in dmPFC activation when participants were asked to make the negative pictures self-focused and significant increases in dlPFC and dmPFC when participants were asked to make the negative pictures situational-focused.

In summary, fear generative and regulatory processes have been examined by manipulating fear stimulus proximity, relevance, and context. The research outlined above points to an extensive network of brain regions that respond to fearful situations. Notably, the amygdala and vmPFC have been identified to play critical roles in fear generative and regulatory processes, respectively.

1.1.2 Neurobiology of fear conditioning

Another approach in which to probe uninstructed fear generation and regulation is through fear conditioning paradigms (Quirk, 2011). Fear can be conditioned by repeatedly presenting a neutral stimulus (conditioned stimulus,

CS+) with an aversive stimulus, such as a shock or loud tone (unconditioned stimulus, US). Eventually, the presentation of the CS+ alone can induce a conditioned fearful response (e.g. increases in skin conductance or freezing). After conditioning, repeatedly presenting the CS+ alone results in the diminishment of conditioned responses to the CS+. This process is known as fear extinction. The reduction of the conditioned response over time during extinction is thought to reflect changes in contingency beliefs and harm expectancy (for review see, (Hofmann, 2008)). Conditioned responses can be prolonged during extinction by using a very aversive stimulus such as an electric shock, increasing unpredictability during conditioning (e.g. using a 50% pairing) and by including neutral stimuli (CS-) without the US (e.g a control and safety signal comparison to the CS+) (LeDoux, 1996).

Early lesion work in animals and fMRI studies have implicated the amygdala to be responsible for the storage of cued and contextual conditioning associations (Büchel, Morris, Dolan, & Friston, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; LaBar, LeDoux, Spencer, & Phelps, 1995; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Phelps, Delgado, Nearing, & LeDoux, 2004), while is has been suggested that the hippocampus stores contextual conditioning associations only (Milad et al., 2007; Phillips & LeDoux, 1992). Unfortunately, amygdala subnuclei activation during fear conditioning cannot be captured with the resolution of fMRI. Experimental work with animals, however, has provided the field with substantial gains in elucidating the differential roles of the amygdala subnuclei and their connections to other regions (prelimbic, hippocampus) important in fear conditioning (Pare & Duvarci, 2012): The lateral amygdala (LA) receives input from the auditory cortex and thalamus, and is

thought to be responsible for converging CS-US associations. The medial central nucleus of the amygdala (Cem) projects to the thalamus, hypothalamus, and brainstem, and is therefore crucial in generating fear expression. Initially, it was hypothesised that the LA directly projected to the Ce. However, recent data indicates that LA neurons project directly to the lateral Ce (Cel) and indirectly to the medial Ce (Cem) via the basolateral amygdala (BLA) and intercalated cell masses (ITC) (Maren, 2011; Pape & Pare, 2010).

Fear extinction relies on the coordinated action of the amygdala, hippocampus and vmPFC (or sgACC) (Quirk, 2011), which share extensive connections (McDonald, 1998; Pape & Pare, 2010). In human extinction studies using fMRI, and single cell recording studies in rodents, activity in the amygdala and subsequent fear expression (e.g. electrodermal activity in humans and freezing in rodents) have been shown to gradually decrease to CS+ relative to CS- stimuli across extinction (Büchel et al., 1998; LaBar et al., 1998; Phelps et al., 2004; Repa et al., 2001). A large body of work suggests the vmPFC to be responsible for the inhibition of the amygdala across the course of extinction. For example, infralimbic (vmPFC) lesioned rats display smaller decrements in freezing behaviour to CS+ trials across extinction. Thus, these rats require far more presentations of CS+ trials for successful fear diminishment (Milad, Vidal-Gonzalez, & Quirk, 2004; Morgan & LeDoux, 1995; Morgan, Romanski, & LeDoux, 1993). Furthermore, stimulation of the infralimbic cortex during CS+ onset reduces fear expression from the Ce (Kim, Jo, Kim, Kim, & Choi, 2010; Maroun, Kavushansky, Holmes, Wellman, & Motanis, 2012; Milad & Quirk, 2002; Quirk, Repa, & LeDoux, 1995). Continuing this line of work, more recent animal findings suggest the vmPFC to excite ITC, and the ITC to inhibit the

Cem. For example, impaired extinction is found when: (1) the ITC are lesioned (Likhtik, Popa, Apergis-Schoute, Fidacaro, & Paré, 2008), (2) the ITC are injected with neuropeptide S antagonists that reduce glutamatergic transmission (Jüngling et al., 2008) and vmPFC inputs to the ITC are silenced through optogenetics (Bukalo et al., 2015; Do-Monte, Manzano-Nieves, Quiñones-Laracuente, Ramos-Medina, & Quirk, 2015). Furthermore, in human fMRI studies, increased activity in the vmPFC is found for the CS+ during late extinction learning (Milad et al., 2007) and increased activity in both hippocampus and vmPFC in subsequent extinction recall sessions conducted a few days after initial fear acquisition (Kalisch, Korenfeld, et al., 2006; Phelps et al., 2004). These findings suggest that both the hippocampus and vmPFC are responsible for providing contextual information, and the vmPFC is directly involved in the long term inhibition of the central nucleus of the amygdala and subsequent fear expression (see the fear conditioning and extinction circuits in Fig 1, (Sotres-Bayon & Quirk, 2010)).

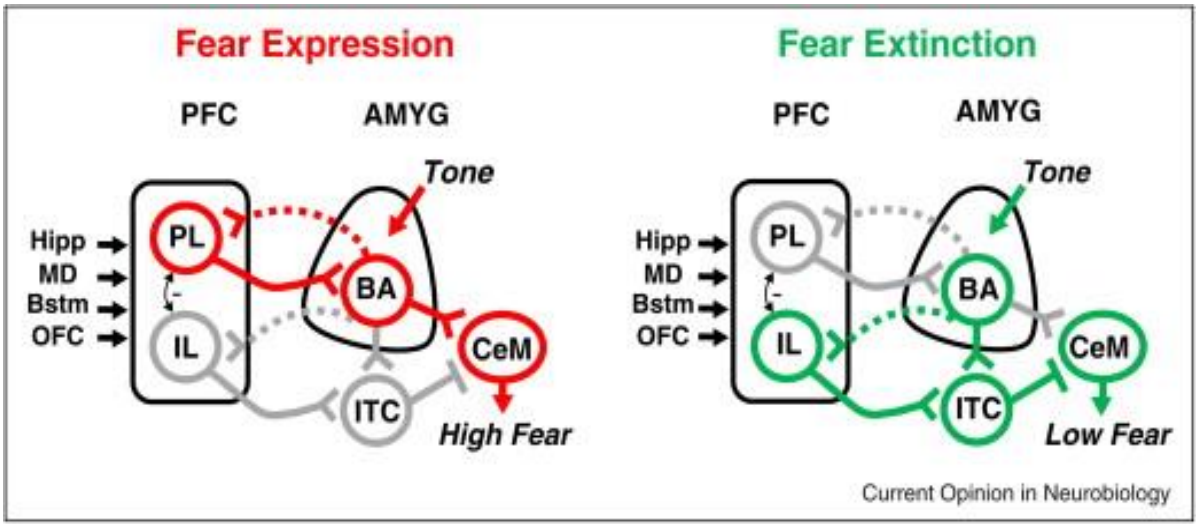

Figure 1. Prefrontal control of fear expression and extinction. During fear expression (left) and extinction (right) of auditory fear conditioning, tone responses from the amygdala (amyg) get integrated by the prelimbic (PL) or infralimbic (IL) prefrontal cortex with converging information from diverse sources such as hippocampus (Hipp), brainstem monoamines (Bstm), mediodorsal thalamus (MD), and orbital prefrontal cortex (OFC) to determine whether or not to produce a fear response. Fear excitation involves PL projections back to basal amygdala (BA), whereas fear inhibition involves IL projections to amygdala-intercalated cells (ITC). In turn, BA excites neurons in the medial division of the central nucleus of the amygdala (CeM) to produce fear responses, while ITCs inhibit these amygdala output neurons thereby inhibiting fear responses. Thus, the same conditioned stimulus (e.g. a tone) signals either high fear (red) or low fear (green) states in the appropriate circumstances. Figure and caption taken from Sotres-Bayon & Quirk, 2010.

1.1.3 Overlapping neurobiology of fear generative and regulatory processes

Overall, the evidence above suggests that the amygdala and vmPFC share overlapping functions during fear habituation, extinction and time-locked reappraisal-based regulation (Delgado et al., 2008; Fisher et al., 2009; Ochsner et al., 2009; Schiller & Delgado, 2010; Urry et al., 2006). More specifically, the amygdala gives rise to fear generative processes, such as expression, through associative learning and monitoring of salience in the environment, whilst the vmPFC is implicated in fear regulatory processes, such as inhibition of the amygdala, through habituation, extinction, and cognitive modification (see Fig 2, (Etkin, Egner, & Kalisch, 2011)). The review above also identified other areas of the prefrontal cortex such as the dmPFC, dlPFC and vlPFC that are involved in down regulating fearful expression in the amygdala, however these areas of the prefrontal cortex appeared to be specific to certain contexts (see Fig 2), such as dual task demands (Blair et al., 2007; Dolcos & McCarthy, 2006; Van Dillen et al., 2009a) and anticipatory reappraisal-based regulation (Ochsner et al., 2002; Ochsner & Gross, 2005; Ochsner et al., 2004).

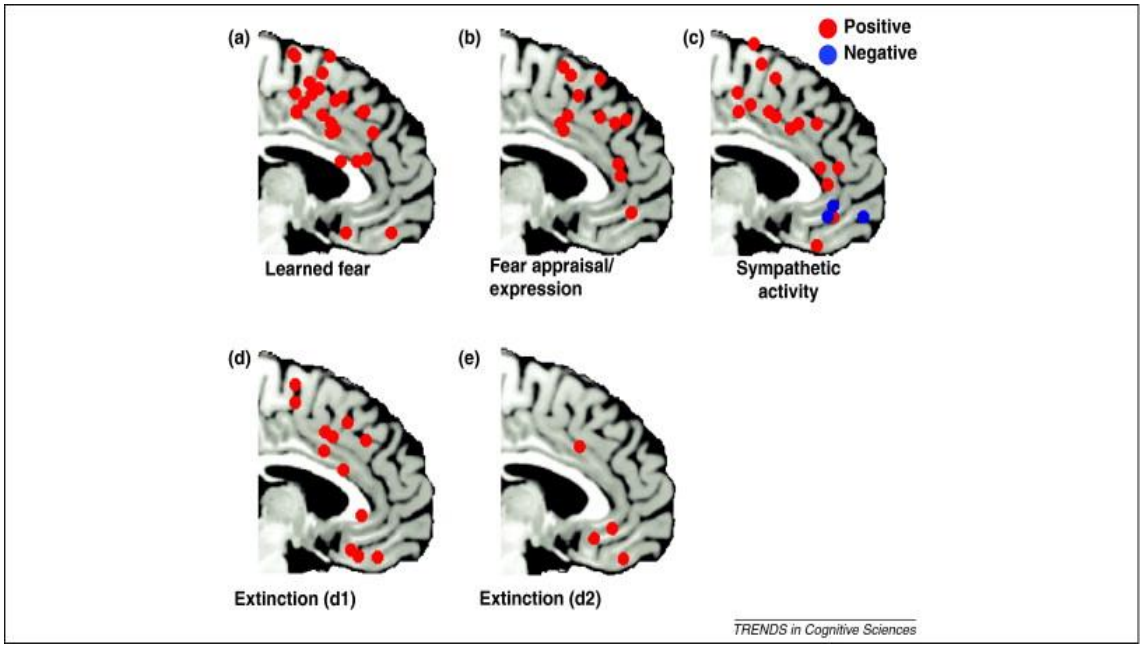

Figure 2. Activation foci associated with fear and its regulation. Predominantly dorsal ACC and mPFC activations are observed during classical (Pavlovian) fear conditioning (a), as well as during instructed fear paradigms, which circumvent fear learning (b). Likewise, sympathetic nervous system activity correlates positively primarily with dorsal ACC and mPFC regions and negatively primarily with ventral ACC and mPFC regions, which supports a role for the dorsal ACC and mPFC in fear expression (c). During within-session extinction, activation is observed in both the dorsal and ventral ACC and mPFC (d), whereas during subsequent delayed recall and expression of the extinction memory, when the imaging data are less confounded by residual expression of fear responses, activation is primarily in the ventral ACC and mPFC (e). Figure and caption taken from Etkin, Egner, & Kalisch, 2011.

1.2 Individual differences in fear generation and regulation

Fear generative and regulatory processes are essential for health and protecting against psychopathology (Davidson, 1998). The extent to which these processes develop across the lifespan for different individuals is of particularly importance, given the prevalence of fear-related behaviours seen in anxiety disorders, which are commonly reported to begin in youth and early adulthood (Kessler et al., 2005). The current section will review the functional and structural neurobiology (e.g. amygdala-vmPFC circuitry) of fear generative and regulatory processes in: (1) anxious disposition and anxiety disorders within adult and paediatric samples (e.g. between 8-18yrs), and (2) adolescence, which is a key developmental window of vulnerability to anxiety disorders. The following review will assess the commonalities in patterns shared across individual differences in anxious disposition and anxiety disorders, and developmental stage separately, with particular focus on the relevance of subclinical anxiety and developmental stage in identifying fear generative and regulatory processes associated with anxiety disorder risk. Differentiating between the different types of anxiety disorder is beyond the current scope of the thesis

1.2.1 Anxious disposition and the anxiety disorders

Fear is described as a phasic state of arousal to imminent threats, whilst anxiety is described as a tonic state of arousal to anticipated future threats (Grupe & Nitschke, 2013; Tovote, Fadok, & Lüthi, 2015). Both states are typically adaptive and share substantial neurobiological overlap (Tovote et al.,

2015). However, chronic fear and anxiety can lead to adverse health effects and anxiety disorder development. Unfortunately, anxiety disorders are common (e.g. lifetime prevalence of 28.8%) and tend to begin during adolescence and early adulthood (Kessler et al., 2005). Anxiety disorders are characterised by exaggerated, inappropriate and persistent fear and anxiety to perceived threat, as well as pervasive avoidance behaviours of fear- and anxiety-inducing situations (Shin & Liberzon, 2009). Crucially, healthy individuals with anxious traits have been found to be at greater risk for anxiety disorder development (Beesdo, Knappe, & Pine, 2009; Maller & Reiss, 1992). Anxious disposition represents individual differences in the frequency, intensity and stability of experiences of fear and anxiety over time (Buhr & Dugas, 2002; Davidson, 2002; Meyer, Miller, Metzger, & Borkovec, 1990; Spielberger, 2010), and is typically measured in adults through self-report scales, such as the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, & Lushene, 1970), Intolerance of Uncertainty (IU) (Buhr & Dugas, 2002), and many others (Meyer et al., 1990; Reiss, Peterson, Gursky, & McNally, 1986).

More recently, researchers have been examining the neurobiological underpinnings of fear and anxiety in subclinical and clinically anxious populations, in order to separate fear and anxiety processes that: (1) are due to anxiety disorder vulnerability and extend to anxiety disorders, and (2) are consequential to an anxiety disorder. In the adult anxiety literature, a large corpus of data suggests anxious disposition to predict hyperactivity to potential threat stimuli in regions associated with negative affect, uncertainty and hyper-vigilance such as the amygdala (Barrett & Armony, 2009; Etkin et al., 2004; Mujica-Parodi et al., 2009; Schienle, Köchel, Ebner, Reishofer, & Schäfer,

2010; Somerville et al., 2013; Stein, Simmons, Feinstein, & Paulus, 2007), and hypoactivity in fear regulatory control regions such as the vmPFC (Mujica-Parodi et al., 2009; Sehlmeyer et al., 2011; Somerville et al., 2013; Xu et al., 2013). Notably, such patterns are found for a variety of tasks, including viewing of fearful faces unconsciously (Etkin et al., 2004) and consciously (Mujica-Parodi et al., 2009), matching facial expressions (Stein et al., 2007), uncertainty manipulations (Schienle et al., 2010; Somerville et al., 2013), decision making (Xu et al., 2013) and cognitive tasks with and without fearful content (Bishop, 2009). In addition, high trait anxiety is associated with increased arousal to learned threat and safety cues during fear learning, extinction and generalisation (Barrett & Armony, 2009; Browning, Behrens, Jocham, O'Reilly, & Bishop, 2015; Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011; Gazendam, Kamphuis, & Kindt, 2013; Haddad, Pritchett, Lissek, & Lau, 2012; Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011; Sehlmeyer et al., 2011). Anxious individuals even display differences to non-anxious individuals within the amygdala-prefrontal circuit at rest. For example, at rest, they show greater relative right prefrontal activation (Blackhart, Minnix, & Kline, 2006; Davidson, 2002), as well as poorer connectivity between amygdala and prefrontal cortical regions (Kim, Gee, Loucks, Davis, & Whalen, 2011). Similar patterns of activation within the amygdala and prefrontal cortex are found for a range of tasks and at rest for those with anxiety disorders (Etkin & Wager, 2007; Rauch, Shin, & Wright, 2003; Sylvester et al., 2012), suggesting that both subclinically anxious individuals and anxiety disorder patients share some basic disturbances in fear generative and regulatory processes (Davidson, 2002).

Furthermore, recent research suggests that subclinically anxious individuals and anxiety disorder patients may share underlying structural markers. For example, weaker structural integrity of the uncinate fasciculus, a white matter tract that communicates information between the amygdala-vmPFC, has been shown in high trait anxious individuals, (Baur, Hänggi, & Jäncke, 2012; Kim & Whalen, 2009; Soliman et al., 2010) and in anxiety disorder patients (Baur et al., 2013; Phan et al., 2009; Tromp et al., 2012). In addition, there is consistent evidence for reduced grey matter volume in the cingulate and medial prefrontal regions for non-clinically anxious individuals (Kühn, Schubert, & Gallinat, 2011; Van Schuerbeek, Baeken, De Raedt, De Mey, & Luypaert, 2011) and anxiety disorder patients (Na et al., 2013; Shang et al., 2014; van Tol et al., 2010). However, the grey matter volume sizes of the amygdala are inconsistent across trait anxious and anxiety disorder patients, with some studies reporting smaller volumes (Alemany et al., 2013; Fisler et al., 2013; Hayano et al., 2009) and others larger volumes (Baur et al., 2012; Cerasa et al., 2013; Redlich et al., 2014; Schienle, Ebner, & Schäfer, 2011), which appear to be lateralised mainly to the right amygdala. Importantly, these structural differences within amygdala-vmPFC circuitry may underlie the functional deficits observed in subclinically anxious individuals and anxiety disorder patients.

Crucially, surmounting evidence suggests shared functional markers in adults and paediatric samples (defined here as late childhood into adolescence, i.e. 8-18yrs) with anxious disposition and anxiety disorders (Beesdo, Knappe, et al., 2009). Children and adolescents with anxiety disorders have been reported to display heightened arousal in general (Bakker, Tijssen, van der Meer,

Koelman, & Boer, 2009; Krämer et al., 2012; Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011). Behavioural and imaging studies have found children and adolescents with anxiety disorders, relative to healthy controls, to show attentional biases to threat-relevant stimuli, indexed by faster behavioural responses and higher amygdala activity during passive viewing of fearful or angry faces (Beesdo, Lau, et al., 2009; Thomas, Drevets, Dahl, et al., 2001), dot probe tasks with fearful and angry faces (McClure et al., 2007; Monk et al., 2006; Monk et al., 2008; Roy et al., 2008), faces following uncertain cues (Williams et al., 2014), and peer evaluation (Guyer et al., 2008), relative to neutral control conditions. In comparison, many studies have reported anxious youth to display heightened arousal during safe cues or contexts, suggesting proneness to threat generalisation. For example in fear conditioning paradigms, heightened arousal to learned safety cues has been shown in high trait anxious children (Haddad, Bilderbeck, James, & Lau, 2015; Jovanovic et al., 2014; Kadosh et al., 2015), children and adolescents with anxiety disorders (Lau et al., 2008b; Liberman, Lipp, Spence, & March, 2006; Waters, Henry, & Neumann, 2009) and in those who later develop an anxiety disorder (Craske et al., 2012). Furthermore, poorer connectivity between the amygdala and prefrontal regions is also observed in anxious youth, compared to non-anxious youth, during attentional tasks with faces (Monk et al., 2006; Monk et al., 2008), attentional tasks with affective images (Strawn et al., 2012) and peer evaluation (Guyer et al., 2008). Difficulties recruiting safety signalling regions to inhibit fear generative regions such as the amygdala may partly explain why anxious youth generalise threat.

Similarly to adult populations, children and adolescents have been shown to display activation patterns in amygdala-prefrontal circuitry that vary with anxious trait severity. For example, children and adolescent anxiety disorder patients with high intolerance of uncertainty (IU) scores (i.e. those who find uncertainty fearful and anxiety provoking) show hyperactivity of both the amygdala and prefrontal cortex during decision making, compared to children and adolescent patients with low IU scores and healthy controls (Krain et al., 2008). These findings were specific to IU over and above other anxiety traits. Direct mapping between anxious traits and the contexts where they become relevant has many implications for identifying neural mechanisms and developing appropriate treatment strategies.

In paediatric samples and relative to adult samples (reviewed above), structural markers of anxiety are limited and inconsistent. Similar to the adult anxiety literature, amygdala volume size in anxious youth, compared to healthy controls, are found to be larger in the right and left amygdala, specifically the basolateral amygdala (De Bellis et al., 2000; Qin et al., 2014), smaller in the left amygdala (Blackmon et al., 2011; Milham et al., 2005; Mueller et al., 2013) or no different in the amygdala (Strawn, Chu, et al., 2013). For regions associated with fear extinction and emotion regulation, such as the vmPFC, anxious youth show decreased grey matter volume, compared to healthy youth (Blackmon et al., 2011; Mueller et al., 2013; Strawn et al., 2015). Furthermore, in longitudinal studies, cortical thickness and cortical surface area within the vmPFC is negatively associated with self-reported anxiety in children and adolescents (Ducharme et al., 2014; Newman et al., 2015). However, this effect is also reported to change with age, such that it diminishes with age (Newman et al.,

2015) or the negative relationship shifts to a positive relationship with age (late adolescence) (Ducharme et al., 2014). The extent to which these structural differences in youth: (1) predict functional impairment of amygdala-vmPFC circuitry, (2) are predisposing factors, and (3) are consequential to an anxiety disorder, remain open questions.

Overall, from the literature reviewed above, those with anxious dispositions and/or anxiety disorders are generally shown to exhibit compromised fear generative and regulatory processes. This effect is predominantly shown via heightened and sustained arousal to threat and safety cues that manifests in aberrant recruitment of amygdala-vmPFC circuitry. Moreover, there is some evidence for structural abnormalities in amygdala-vmPFC circuitry for anxious individuals that may underlie the functional recruitment patterns observed. Strikingly, there is also converging evidence for similar functional and structural markers within the amygdala-vmPFC circuit in anxious youth and adults. From these findings we can conclude that functional and structural risk markers may appear before anxiety disorder onset in subclinical populations with anxious dispositions. Furthermore, these data point to the potential emergence and formation of functional and structural risk markers during early adolescence, rendering this developmental period as a window of vulnerability to anxiety disorders.

Whilst these findings are promising, many questions still remain regarding the specificity of anxious traits and their relationship with fear generative and regulatory processes during particular contexts. For example, much of the literature has focused on Trait Anxiety, which measures general feelings of apprehension and nervousness (Spielberger, 2010). This measure,

however, does not specify the elicitor of fear and anxiety, making it difficult to infer when or in which context fearful or anxious feelings would arise. Notably, recent research has started to separate out particular facets of anxious disposition, such as IU, which may be more sensitive in identifying compromised fear generative and regulatory processes during uncertain contexts (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, In press; Krain et al., 2008). Furthermore, inconsistencies within the structural literature and limited longitudinal data make it difficult to infer what, when or how particular factors such as anxious traits during development predict structural and functional abnormalities in the amygdala-vmPFC circuit that may increase anxiety disorder risk (Ducharme et al., 2014; Newman et al., 2015; Pfeifer & Allen, 2012).

1.2.2 Adolescence as a developmental window of vulnerability to anxiety disorders

Typically, puberty (sexual reproducibility) starts between 8-14 years in females and 9-15 years in males, although there is substantial individual variability in puberty onset, developmental trajectories and completion (Abbassi, 1998; Parent et al., 2003; Tanner & Whitehouse, 1976). Adolescence and early adulthood are times of exploration, change and stress, as new priorities emerge outside of the realms of home and school (e.g. career, peer relationships, identity) (Blakemore, 2012; Choudhury, 2009; Crone & Dahl, 2012; Somerville & Casey, 2010; Spear, 2000b). Alongside pubertal development and changes in environmental demands, the brain undergoes marked structural changes in white and grey matter density (Crone & Dahl, 2012; Lourenco & Casey, 2013;

Pfeifer & Allen, 2012; Spear, 2000a). During adolescence, observed global linear increases in white matter density are thought to reflect the optimisation of neuronal processing speed (Asato, Terwilliger, Woo, & Luna, 2010; Giorgio et al., 2008; Lebel & Beaulieu, 2011; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Paus et al., 1999), through increases in axon diameter, axon myelination and concentrations of iron (Connor & Menzies, 1996). The observed non-linear decreases in global grey matter density have been reported to signify the pruning and refining of synapses, neuropil and glial cells which may no longer be needed (Giedd, 2004; Giedd et al., 1999; Giedd, Keshavan, & Paus, 2008; Gogtay et al., 2004; Sowell et al., 2003; Toga, Thompson, & Sowell, 2006). Notably, local grey matter density decreases across childhood and adolescence, with most regions showing a steady decline. However, parietal and frontal regions are characterised by a rapid decline across adolescence into early adulthood, whilst temporal and occipital lobes increase steadily across adolescence into early adulthood (Giedd et al., 1999; Østby et al., 2009; Sowell et al., 2003; Tamnes et al., 2010). Sex differences in brain maturation occur in tandem with pubertal and hormonal changes. For example, females typically reach peak cortical thickness across the cortex 1-2 years earlier than males, except for the occipital cortex, which appears to mature at the same time in males and females (Giedd et al., 1999; Tamnes et al., 2010). Subcortical regions such as the amygdala and hippocampus mature earlier, with variations in size related to sex and pubertal stage (Bramen et al., 2012; Bramen et al., 2011; Giedd, Snell, et al., 1996; Giedd, Vaituzis, et al., 1996; Herting, Maxwell, Irvine, & Nagel, 2012; Neufang et al., 2009).

Complementary to the structural findings outlined above, behavioural and functional magnetic resonance imaging (fMRI) studies have demonstrated adolescents to exhibit greater arousal towards affective information. For example, adolescents, relative to adults, show larger amygdala response and decreased or more diffuse activation in top down regulatory areas such as the vmPFC (Hare et al., 2008; Monk, McClure, et al., 2003) during passive viewing of fearful faces (Swartz, Carrasco, Wiggins, Thomason, & Monk, 2014; Thomas, Drevets, Whalen, et al., 2001) and during cognitive tasks with fearful faces embedded (Hare et al., 2008; Monk, McClure, et al., 2003). In studies examining neural correlates of emotion regulation, through intentional cognitive modification, adolescents have been found to recruit less vlPFC during attempted down regulation of negative affect (and other emotions), relative to adults (McRae et al., 2012; Vink, Derks, Hoogendam, Hillegers, & Kahn, 2014). Moreover, recent fear conditioning experiments point to increased propensity for fear learning and blunted fear extinction in adolescents, relative to adults (Baker & Richardson, 2015; Den & Richardson, 2013; Kim, Li, & Richardson, 2011; Lau et al., 2011; Pattwell et al., 2012). For example, Lau et al (2011) found adolescent participants, compared to adults, to report less discrimination between learned threat and safety cues, as well as increased amygdala and decreased dlPFC activity to learned threat vs. safety cues during fear learning. In addition, adolescent mice and humans show resistant fear extinction to learned threat cues, indexed by continuous freezing in adolescent mice and skin conductance responding in adolescent humans (Baker & Richardson, 2015; Kim, Li, et al., 2011; Pattwell et al., 2012). Rodent work has demonstrated this effect to originate from poor top down control of the vmPFC

on the amygdala (Kim, Li, et al., 2011; Pattwell et al., 2012). These findings suggest that adolescents may have difficulty inhibiting and updating learned threat associations to new safety associations because of competing fear conditioning and extinction memories (Baker & Richardson, 2015). It has been argued that this developmental effect may stem from brain maturation in structures vital for fear extinction, such as the vmPFC.

Changes in connectivity between the amygdala and medial prefrontal cortex (mPFC) have also been found with age (Gabard-Durnam et al., 2014; Gee et al., 2013). Gee et al. (2013) found a developmental shift in processing of fearful faces, where children exhibited positive coupling between the amygdala and mPFC, and adolescents switched to a negative coupling between the amygdala-mPFC like that of adults. In addition, functional connectivity between the amygdala-mPFC during resting state has been found to increase across childhood and adolescence into adulthood (Gabard-Durnam et al., 2014). Furthemore, age-related structural integrity of the uncinate fasciculus (e.g. weaker in adolescence), a tract that is thought to relay information between the amygdala and vmPFC, predicts larger amygdala activation to sad and happy faces in adolescents (Swartz et al., 2014).

Taken together these findings suggest that in healthy adolescents, substantial structural and functional changes occur, alongside alterations in affective and cognitive functioning, environmental demands and puberty. More specifically, the imbalance between earlier growth of fear-generative regions such as the amygdala, and later pruning of fear-regulatory regions, such as the vmPFC, in combination with other developmental factors (Blakemore, Burnett, & Dahl, 2010; Hare & Casey, 2005; Somerville & Casey, 2010), likely leave

adolescents vulnerable to psychopathology, particularly anxiety disorders (Kessler et al., 2005; Paus, Keshavan, & Giedd, 2008). However, as noted by Pfeifer and Allen (2012), dual systems models of early subcortical and late prefrontal development may not capture the complexity of changes that occur during adolescence. More recently, to elucidate age-related changes in behaviour and brain, researchers in the field have been focusing on longitudinal, network-based, or structure-function integration approaches (Gee et al., 2013; Kim et al., 2013; Roy, Shohamy, & Wager, 2012; Swartz et al., 2014; Vijayakumar et al., 2013).

1.3 Principle questions

This thesis expands research on individual differences in development (adolescence and early adulthood) and anxious disposition on fear-related neurobiology. From the review above, there remain questions regarding: (1) the extent to which age and age-related structural changes predict function of fear generative and regulatory processes, and (2) the specificity of anxious traits, such as IU, and their ability to predict function of fear generative and regulatory processes during adulthood and adolescence. This thesis aims to examine these questions by testing both adult and adolescent populations using a series of fear conditioning experiments in combination with measurements of behaviour, psychophysiology and functional/structural MRI.

The following sections will outline, firstly, the efficacy and rationale of using fear conditioning as a tool for assessing individual differences (e.g. developmental stage and anxious disposition) in fear generation and regulation,

and secondly, the motivation behind each fear conditioning experiment of the thesis.

1.3.1 Fear conditioning as a tool for assessing individual differences in fear generation and regulation

As highlighted above in the review, fear conditioning experiments can be used to break down fear generative and regulatory processes. For example, during fear extinction, fear generative processes can be measured and operationalised through behavioural, psychophysiological, and neural responses to learned threat and safety cues. Similarly, during fear extinction, fear regulatory processes can be captured by measuring the diminution of behavioural, psychophysiological, and neural responses to learned threat and safety cues over time.

Additionally, there are a few notable advantages of using fear conditioning paradigms over other fear regulation experiments. For example, fear extinction is uninstructed, implicit, controlled (simple with few stimuli), translational, and adaptable for different samples (e.g. developmental, animal, and human) (Graham & Milad, 2011; Milad & Quirk, 2012). Despite this, fear conditioning experiments have been criticised for lacking ecological validity because of the simplicity and unambiguity of the stimuli used (Balsam & Gallistel, 2009). However, fear conditioning stimuli aren't necessarily unambiguous, for example an unreinforced CS+ without instruction contains elements of ambiguity to the perceiver. Furthermore, the abstract and simplistic nature of fear conditioning experiments may still be comparable to real life and prove valuable for examining individual differences in fear generation and

regulation processes during the learning of new safety experiences, particularly for developmental and anxious populations. For example, simple changes to stimulus contingency (threat to safety associations) during fear extinction may be useful for understanding: (1) how developmental changes may impact the learning of new safety information and (2) how future threat uncertainty may maintain learned fear in those who find uncertainty anxiety provoking, such as those who score high in IU.

Furthermore, fear conditioning experiments are clinically relevant, given that current cognitive behavioural therapies for anxiety disorders in children and adults include aspects of exposure therapy that are based upon fear extinction principles (Cartwright-Hatton, Roberts, Chitsabesan, Fothergill, & Harrington, 2004; Luhmann, Ishida, & Hajcak, 2011). Substantial gains have been made in the treatment of anxiety disorders with cognitive behavioural therapies. For example, remission rates for anxiety disorder treatments is within the range of 60-90% for adults (Bystritsky, 2006) and is within the range of 55-65% for children and adolescents (Cartwright-Hatton et al., 2004; Gole, Schäfer, & Schienle, 2012). However, there still remain populations, particularly developmental, that are: (1) left without any treatment, (2) prone to relapse, (3) and treatment resistant (Bystritsky, 2006; Merikangas et al., 2010; Rapee, Schniering, & Hudson, 2009). Therefore, examining anxious disposition and developmental differences in fear generative and regulatory processes in healthy adolescent and adult populations may further our understanding of which anxiety disorder treatments to apply and when to apply them (Casey, Duhoux, & Cohen, 2010).

To summarise, the combination of advantages pointed out above outweigh, at present, the disadvantages and suggest that fear conditioning is an appropriate tool for examining individual differences in fear generative and regulatory processes, particularly within anxious disposition and development.

1.3.2 Function of fear extinction circuitry in adults

The aim of the first two studies of the thesis is to create a fear conditioning paradigm that can: (1) replicate past fear extinction work and is appropriate for testing on an adolescent population, and (2) capture individual differences in anxious disposition. In the first study, we measure behaviour and psychophysiology during fear acquisition and extinction outside the MRI environment. In the second study we measure behaviour, psychophysiology and neural circuitry responses during fear acquisition and extinction in the MRI environment. Firstly, we hypothesised our designed fear conditioning paradigm to replicate behavioural, psychophysiological, and neural findings from past fear extinction studies. More specifically, we would expect evidence of conditioned responses to the learned threat vs. safety cue and diminishment of conditioned responses to the learned threat cue over time. Secondly, we hypothesised IU to be a strong predictor of compromised fear extinction, compared to more general dispositional measures of anxiety. We argue that IU may be more closely and specifically aligned to underlying biases that disrupt fear extinction processes. For example, in the context of fear extinction, uncertainty surrounding learned contingency changes (i.e. CS-US pairings) may initiate threat generalization behaviour to both learned threat and safety cues in individuals who find uncertainty anxiety provoking, such as high IU individuals.

1.3.3 The role of age, structure and anxious disposition on fear extinction circuitry in adolescence and early adulthood

The third study of the thesis investigates how individual differences in developmental stage (adolescents through to early adulthood) and anxious disposition predict fear extinction. We will use the same experimental design for this study as the two previous studies, as the experimental design was made to extend to developmental populations. During the third study, we will measure behaviour, psychophysiology and neural responses in the MRI environment, whilst participants complete fear acquisition and extinction phases. Structural (sMRI, DTI) and anxious disposition (IU vs. STAI and PSWQ) data will also be collected from the third study. Based on the literature outlined above, we hypothesise that younger age and age-related structural changes in fear extinction circuitry will predict greater fear generation and poorer regulatory control during fear extinction. Lastly, we hypothesise that anxious disposition, specifically IU, to predict fear extinction ability. The relationship between anxious disposition and function of fear extinction circuitry may vary with age

2. Psychophysiological correlates of adult fear extinction and individual differences in intolerance of uncertainty

2.1 Abstract

In this chapter we sought to: (1) replicate past psychophysiological findings of fear extinction in a classic paradigm adapted for a developmental sample, and (2) assess whether individual differences in intolerance of uncertainty (IU), a potential risk factor for anxiety disorders, underlies compromised fear extinction. We tested these hypotheses by recording electrodermal activity in 38 healthy participants during fear acquisition and extinction. We assessed the temporality of fear extinction, by examining early and late extinction learning. Across fear extinction, participants had greater uneasiness ratings and skin conductance response to the learned threat vs. safety cue. However, the temporality of fear extinction varied substantially with individual differences in IU. During early extinction, low IU was associated with larger skin conductance responses to learned threat vs. safety cues, whereas high IU was associated with no skin conductance discrimination. During late extinction, low IU predicted no difference in skin conductance between learned threat and safety cues, whilst high IU predicted continued fear expression to learned threat, indexed by larger skin conductance to threat vs. safety cues. Overall, these findings

suggest that the designed experiment can induce successful fear conditioning and is sensitive to capturing individual differences in IU.

2.2 Introduction

The ability to discriminate between threat and safety is crucial for survival. Through fear conditioning, an organism can associate neutral cues (conditioned stimulus, e.g. a visual stimulus such as a shape) with aversive outcomes (unconditioned stimulus, e.g. shock, loud tone). Repeated presentations of a neutral cue with an aversive outcome can result in fearful responding to the neutral cue alone (conditioned response). This learned association can also be extinguished by repeatedly presenting the learned threat cue without the aversive outcome, a process known as fear extinction (LaBar et al., 1998; Milad & Quirk, 2002; Phelps et al., 2004). During fear extinction, reduction in reactivity to the learned threat cue over time is thought to reflect changes in contingency beliefs and harm expectancy (for review see, (Hofmann, 2008)).

Designing a fear conditioning experiment that can be applied to different samples (developmental, sub-clinical and clinical) has clear merit for a number of reasons. Firstly, fear extinction is clinically relevant, given that current exposure therapies for anxiety disorders are based on fear extinction models. Secondly, comparing fear extinction processes across different samples may help separate out risk factors and consequences of anxiety disorder onset that originate from changes in development and individual differences in anxious disposition. Thirdly, tying the two points above together, identification of when and how fear extinction processes become disrupted may inform future

treatment strategies and targets for anxiety disorders. In the following sections we review previous literature on developmentally appropriate fear conditioning designs and the role of adult intolerance of uncertainty in fear extinction. Based on this literature, we then define the design and predictions of the first study.

2.2.1 Fear conditioning designs for developmental samples

Typically, in adult fear conditioning studies a more aversive electric shock stimulus is paired with a neutral cue (Delgado et al., 2008; LaBar et al., 1998; Milad et al., 2009; Phelps et al., 2004). This has commonly been done because more aversive cues prolong the effects of conditioning (LeDoux, 1998). However, for ethical or practical reasons, a handful of adult studies and the majority of studies on children and adolescents have used less aversive stimuli (e.g. white noise, loud tones, air puffs, human screaming, fearful faces and predatory animals) (Barrett & Armony, 2009; Britton et al., 2013; Büchel et al., 1998; Glenn et al., 2012; Grillon, Dierker, & Merikangas, 1998; Haddad, Lissek, Pine, & Lau, 2011; Johnson & Casey, 2015; Lau et al., 2011; Lau et al., 2008a; Liberman et al., 2006; Monk, Grillon, et al., 2003; Neumann & Waters, 2006; Neumann, Waters, & Westbury, 2008; Pattwell, Bath, Casey, Ninan, & Lee, 2011; Pattwell et al., 2012). Notably, with these study designs successful effects of fear conditioning and extinction has been found across a number of psychophysiological indices, such as skin conductance, fear-potentiated startle and behavioural ratings.

2.2.2 Psychophysiological correlates of fear extinction and the role of anxious disposition

A large corpus of data suggests that fear extinction processes are disrupted in individuals with anxiety and trauma disorders, whom have been shown to display delayed fear extinction or even extinction-resistant fear (Graham & Milad, 2011; Milad & Quirk, 2012; Mineka & Oehlberg, 2008). For example, compared to non-anxious controls, anxious patients show elevated autonomic nervous system activity to both learned threat and safety cues at the start of extinction, and to learned threat cues across fear extinction (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007; Milad et al., 2008; Milad et al., 2009). In two recent meta-analyses, however, only small differences in fear extinction behaviour were found between anxious and non-anxious adult individuals (Duits et al., 2015; Lissek et al., 2005). Furthermore, findings have also been mixed from studies examining fear extinction behaviour and trait anxiety, as measured with the Spielberger State-Trait Anxiety Inventory (STAI; (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). For example, trait anxious individuals have been shown to display slower reductions in startle reactivity to both threat and safety cues (Gazendam et al., 2013), but not in skin conductance (Haaker et al., 2015) or expectancy ratings (Barrett & Armony, 2009; Gazendam et al., 2013). These equivocal findings may stem from a lack of alignment between the STAI measure and the underlying biases that disrupt fear extinction processes.

Only very recently has research begun to assess the role of intolerance of uncertainty (IU) in fear extinction (Dunsmoor et al., In press). IU is defined as

a dispositional tendency that affects how uncertain situations are perceived and interpreted. During uncertain situations, individuals with high IU scores may have difficulty accepting the possibility of future negative events, thus rendering ambiguous or even neutral cues as potentially threatening (Dugas, Buhr, & Ladouceur, 2004). Originally, IU was considered to be specifically related to Generalised Anxiety Disorder (Dugas et al., 2004). However, growing evidence suggests IU may be a transdiagnostic factor across many anxiety and mood disorders (Carleton, Fetzner, Hackl, & McEvoy, 2013; Gentes & Ruscio, 2011; McEvoy & Mahoney, 2012). Furthermore, the development of new disorder-specific IU scales (Thibodeau et al., 2015), suggests that IU may be applicable to disorders such as specific phobia and PTSD, which are associated with compromised fear extinction.

In the context of fear extinction, uncertainty surrounding learned contingency changes (i.e. CS-US pairings) may initiate generalised harm expectancy in high IU individuals, resulting in fearful responding to both learned threat and safety cues. Given the mixed findings above, it seems pertinent to further examine whether IU proves to be a more sensitive predictor of compromised fear extinction, over more general trait anxiety measures such as the STAI.

2.2.3 Design and predictions

Here we used cued fear conditioning to assess: (1) whether we can replicate psychophysiological findings from past fear extinction studies in a classic paradigm adapted for developmental populations, and (2) the relationship between individual differences in self-reported IU and

psychophysiological correlates of fear extinction. We measured skin conductance response (SCR) and ratings whilst participants performed the conditioning task. We used an aversive sound as an unconditioned stimulus and visual shapes as conditioned stimuli, similar to that of previous conditioning research in adults (Barrett & Armony, 2009; Büchel et al., 1998; Delgado et al., 2008; Neumann & Waters, 2006; Phelps et al., 2004) and adolescents (Haddad et al., 2011; Johnson & Casey, 2015; Lau et al., 2011; Neumann et al., 2008; Pattwell et al., 2011; Pattwell et al., 2012).

We hypothesised that, during fear extinction, participants would exhibit greater uneasiness ratings and skin conductance responses to the learned threat vs. safety cues, particularly during early extinction, compared to late extinction, evidencing successful conditioning and extinction respectively. Furthermore, we predicted that future threat uncertainty sensitivity would be associated with generalised fear expression to both learned threat and safety cues, and/or sustained fear expression to learned threat cues. Given that fear extinction paradigms are temporally sensitive (Gazendam et al., 2013; LaBar et al., 1998; Milad & Quirk, 2012; Phelps et al., 2004; Sehlmeyer et al., 2011), we expected this effect to be indexed by: (1) Larger responses in high IU individuals to both learned threat and safety cues in *early* fear extinction, across SCR and behavioural measurements, and (2) sustained responses in high IU individuals to learned threat cues vs. safety cues during *late* fear extinction, across SCR and behavioural measurements. We tested the specificity of the involvement of IU by comparing it with broader measures of anxiety, such as Spielberger State-Trait Anxiety Inventory, Trait Version (STAIX-2) (Spielberger et al., 1983) and Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990).

2.3 Methods

2.3.1 Participants

38 volunteers took part in this study (age range = 18-25 years; 32 females & 6 males). The sample size was based on a power analysis (effect size f = 0.2, power level = 0.8 and significance level = 0.05), which suggested using an N of 36 (GPower 3.0.10). We recruited a few more participants because of likely subject attrition due to non-responding. All participants had normal or corrected to normal vision. Participants provided written informed consent and received course credit for their participation. Participants were recruited through advertisements and the University of Reading Psychology Panel. The procedure was approved by the University of Reading Ethics Committee.

2.3.2 Conditioning task

The conditioning task was designed using E-Prime 2.0 software (Psychology Software Tools Ltd, Pittsburgh, PA). Visual stimuli were presented using a screen resolution of 800 x 600 with a 60 Hertz refresh rate. Participants sat at approximately 60 cm from the screen. Sound stimuli were presented through headphones.

Visual stimuli were light blue and yellow squares with 183 x 183 pixel dimensions that resulted in a visual angle of 5.78° × 9.73°. The aversive sound stimulus consisted of a fear inducing female scream (sound number 277) from the International Affective Digitised Sound battery (IADS-2) and which has been normatively rated as unpleasant (M= 1.63, SD = 1.13) and arousing (M= 7.79, SD = 1.13) (Bradley & Lang, 2007). We used Audacity 2.0.3 software

(http://audacity.sourceforge.net/) to shorten the female scream to 1000 ms in length and to amplify the sound by 15 db, resulting in a 90 db (~5 db) sound.

Acquisition and extinction phases were presented in two separate blocks (see Fig. 3). In acquisition, one of the squares (blue or yellow) was paired with the aversive 90 db scream (CS+), whilst the other square (yellow or blue) was presented alone (CS-). In extinction, both stimuli were unpaired (CS+, CS-). The third phase was a partial reacquisition, CS+ squares were paired with the sound 25% of the time, and the CS- remained unpaired (results not reported here).

Participants were instructed to attend and listen to the stimulus presentations, as well as respond to a rating scale that followed each trial. The rating scale asked how 'uneasy' the participant felt after each stimulus presentation, where the scale was 1 'not at all'- 9 'extremely'. Participants used pressed the keyboard with their dominant hand to respond.

The acquisition phase consisted of 24 trials (12 CS+, 12 CS-), the extinction phase 32 trials (16 CS+, 16 CS-) and the reacquisition 30 trials (16 CS+ (4 unpaired), 14 CS-; results not reported here). Experimental trials within the conditioning task were pseudo-randomised into an order, which resulted in no more than three presentations of the same stimulus in a row. Conditioning contingencies were counterbalanced, with half of the participants receiving the US with a blue square and the other half of participants receiving the US with a yellow square.

The presentation times of the task were: 1500 ms square, 1000 ms sound (played 500 ms after the onset of a CS+ square), 3000 - 6450 ms blank

screen, 4000 ms rating scale, and 1000-2500 ms blank screen (see Fig. 3).

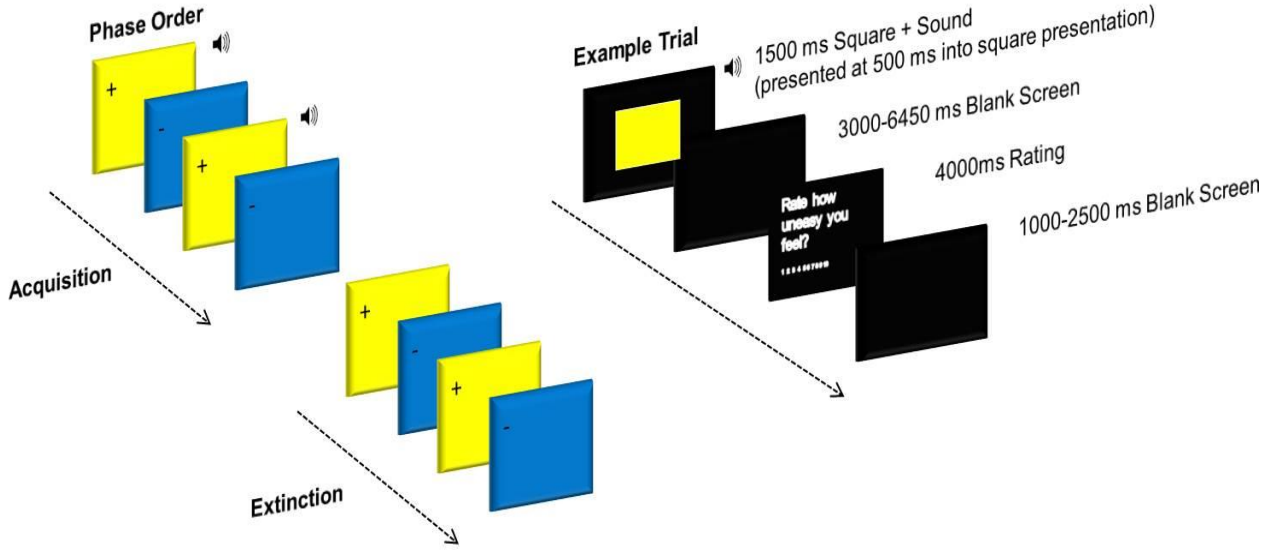

Figure 3. Conditioning task design

2.3.3 Procedure

Participants arrived at the laboratory and were informed on the procedures of the experiment. Firstly, participants were taken to the testing booth and given a consent form to sign as an agreement to take part in the study. Secondly, to assess emotional disposition we asked participants to complete a series of questionnaires presented on a computer in the testing booth. Next, physiological sensors were attached to the participants' non-dominant hand. Participants were simply instructed to: (1) maintain attention to the task by looking and listening to the coloured squares and sounds presented, (2) respond to the uneasiness scale using the keyboard and (3) to sit as still as possible. Participants were presented a conditioning task on the computer, whilst electrodermal activity, interbeat interval and behavioural ratings were recorded. After the task, subjects were asked to rate the valence and arousal of the sound stimulus using 9-point Likert scales ranging from 1 (Valence: very negative; Arousal: calm) to 9 (Valence: very positive; Arousal: excited).

2.3.4 Questionnaires

To assess emotional disposition, we presented the following six questionnaires on a computer: Two versions of the Positive and Negative Affect Scales (PANAS-NOW; PANAS-GEN) (Watson, Clark, & Tellegen, 1988), Spielberger State-Trait Anxiety Inventory, Trait Version (STAIX-2) (Spielberger et al., 1983), Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990), Intolerance of Uncertainty (IU) (Buhr & Dugas, 2002) and the Barratt Impulsiveness Scale (BIS-11) (Patton & Stanford, 1995). We focused on IU

because of the intrinsic uncertainty within conditioning paradigms. The IU measure consists of 27 items, example items include “I must get away from all uncertain situations” and “Uncertainty makes me uneasy, anxious, or stressed”. Similar distributions and internal reliability of scores were found for the anxiety measures, IU (M = 63.92; SD = 19.56; range = 31-116; α = .94), STAIX-2 (M = 44.02; SD = 9.33; range = 31-65; α = .90) and PSWQ (M = 51.60; SD = 11.56; range = 29-71; α = .88). Notably, the psychometric properties of the IU scale here match those presented in previous IU validation studies (Buhr & Dugas, 2002; Dugas et al., 2004). We collected the other questionnaires to check for correlational consistency and specificity across anxiety measures, as well as to check for outlying values on IU due to mood or impulsivity.

2.3.5 Behavioural data scoring and reduction

Rating data were reduced for each subject by calculating their average responses for each experimental condition using the E-Data Aid tool in E-Prime (Psychology Software Tools Ltd, Pittsburgh, PA).

2.3.6 Physiological data acquisition and reduction

Physiological recordings were obtained using AD Instruments (AD Instruments Ltd, Chalgrove, Oxfordshire) hardware and software. Electrodermal activity was measured with dry MLT116F silver/silver chloride bipolar finger electrodes that were attached to the distal phalanges of the index and middle fingers of the non-dominant hand. A constant voltage of $22mV_{rms}$ at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp. Interbeat Interval (IBI) was measured using a MLT1010 Electric Pulse

Transducer, which was connected to the participant's distal phalange of the ring finger. An ML138 Bio Amp connected to an ML870 PowerLab Unit Model 8/30 amplified the electrodermal and interbeat interval signals, which were digitised through a 16-bit A/D converter at 1000 Hz**.** IBI signal was used only to identify movement artefacts and was not analysed. The electrodermal signal was converted from volts to microSiemens using AD Instruments software (AD Instruments Ltd, Chalgrove, Oxfordshire).

Skin conductance responses (SCR) were scored when there was an increase of skin conductance level exceeding 0.03 microSiemens. The amplitude of each response was scored as the difference between the onset and the maximum deflection prior to the signal flattening out or decreasing. To be included, SCR onsets had to be within 7 seconds of CS onset. We used an extended SCR scoring window because the temporal signature of an aversive sound US may be more ambiguous than a traditional electric shock US, this SCR scoring window length is in line with previous fear conditioning studies that have used aversive sound stimuli as the US in both adults (Büchel et al., 1998; Soliman et al., 2010) and adolescents (Pattwell et al., 2012).

Trials with no discernible SCR's were scored as zero. The first trial of each experimental phase was excluded, to reduce contamination of averages from the unusually large SCR that typically occurs at the start of a session. SCR amplitudes were square root transformed to reduce skew. Trials with motion artefacts, as identified by distortions in both electrodermal and IBI signals, were discarded from the analysis. 1.3% (26 out of 1904) trials were removed from the analysis due to movement artefacts. SCR magnitudes were calculated from remaining trials by averaging SCR square root transformed

values and zeros for each condition. In acquisition, 33% of trials were scored as zero responses and in extinction 53% of trials were scored as zero responses

2.3.7 Learning assessment

To assess whether participants learned the association between the neutral

cue and aversive sound, we calculated a conditioned response score for behavioural ratings and SCR magnitude in extinction. The conditioned response score was the first 2 CS+ trials – the first 2 CS- trials, similar to previous work assessing conditioned responses in extinction (Dunsmoor et al., In press; Milad et al., 2009; Phelps et al., 2004). A positive score indicated a larger differential response for CS+ vs. CS-, indexing a conditioned response. Based on this criterion, only three participants out of the thirty-eight participants were considered non learners. However, as removing them did not change the results reported here[1], we decided to include these three participants for reasons of completeness.

2.3.8 Rating and SCR magnitude analysis

Effects of conditioning and IU differences across extinction were assessed by conducting a Condition (CS+, CS-) x Time (Early, Late) x IU repeated measures ANCOVA for behavioural ratings, and SCR magnitude, where IU was entered as a continuous mean centered predictor variable. The early part of extinction was defined as the first eight CS+ and eight CS- trials,

[1] Results do not change when non-learners are removed: The main effect of Condition for SCR magnitude during fear extinction, without non-learners $F(1,28) = 8.188$, $p = .008$. Condition x Time x IU interaction for SCR magnitude during fear extinction without non-learners, $F(1,28) = 4.204$, $p = .05$.

and the last part of extinction was defined as the last eight CS+ and eight CS- trials. We performed follow-up pairwise comparisons on the estimated marginal means, adjusted for IU. Any interaction with IU was followed up with pairwise comparisons of the means between the conditions for IU estimated at the specific values of + or - 1 SD of mean IU. These data are estimated from the ANCOVA of the entire sample, not unlike performing a simple slopes analysis in a multiple regression analysis. To check for specificity of findings with IU in extinction, we conducted a Condition (CS+, CS-) x IU repeated measures ANCOVA on behavioural ratings and SCR magnitude obtained in the acquisition phase.

We performed hierarchical regression analyses on the resulting significant SCR magnitude and rating difference scores (CS+ – CS- early; CS+ – CS- late; CS+ early – CS+ late; CS- early – CS- late) for extinction and the anxiety measures to test for IU-specific effects over and above the variance shared with trait anxiety. We entered STAIX-2 and PSWQ in the first step and then IU in the second step.

2.4 Results

2.4.1 Questionnaires

As expected, the anxiety measures were positively correlated with each other, suggesting shared variance, IU with PSWQ, $r(32) = .584$, $p < .001$, IU with STAIX-2, $r(32) = .815$, $p < .001$, and PSWQ with STAIX-2, $r(32) = .721$, $p < .001$.

2.4.2 Ratings

1 participant's task rating data were missing due to a recording error, leaving rating task data for 37 participants. All remaining participants rated the sound stimulus as aversive (M = 2.33, SD = 1.56) and moderately arousing (M = 6.97, SD = 1.48), in accordance with the normative data provided with the IADS-2 set (Bradley & Lang, 2007).

During acquisition participants significantly reported feeling more uneasy for the CS+ vs. CS- trials, $F(1,35) = 105.993$, $p < .001$ (see Table 1).

During extinction, participants significantly reported feeling more uneasy to the CS+ vs. CS- trials across extinction, $F(1,35) = 17.121$, $p < .001$. In addition, there was a significant interaction of Condition x Time, $F(1,35) = 6.146$, $p = .016$, revealing that the participants uneasiness ratings were larger to the CS+ vs. CS- during the early part of extinction, $p < .001$, and relative to the late part of extinction, $p = .007$ (for descriptive statistics of ratings, see Table 1). Furthermore, participants also reported feeling more uneasy at the start of extinction in general, compared to the end of extinction $F(1,35) = 36.492$, $p < .001$.

Contrary to predictions, results revealed no IU differences for uneasiness ratings in any of the experimental phases, p's >.3, F's <.1,5, max $F = 1.031$.

Table 1.
Summary of means (SD) for each dependent measure as a function of condition, separately for acquisition and extinction.

	Acquisition		Extinction		Early Extinction		Late Extinction	
Measure	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Physiological Square root transformed SCR magnitude (μS)	.79 (.30)[b]	.33 (.24)[a]	.31 (.24)[d]	.25 (.21)[c]	.32 (.24)	.28 (.25)	.29 (.27)	.22 (.20)
Behavioural Uneasiness rating (1-9)	6.14 (1.73)[b]	3.10 (1.73)[a]	2.70 (1.25)[d]	2.14 (1.09)[c]	3.12 (1.28)[f]	2.41 (1.30)[e]	2.28 (1.35)[h]	1.86 (.98)[g]

Note: SCR magnitude (μS), skin conductance magnitude measured in microSiemens. Superscripts indicate significant (p <.05) condition difference from: [a] Acquisition CS+, [b] Acquisition CS-, [c] Extinction CS+, [d] Extinction CS-, [e] Early Extinction CS+, [f] Early Extinction CS-, [g] Late Extinction CS+, [h] Late Extinction CS-.

2.4.3 SCR magnitude

4 subjects were removed from the SCR magnitude analysis due to 1 non-responding, 2 excessive movements, and 1 outlier on SCR magnitude from the early fear extinction CS+ vs. CS- difference score that was +6 SD from the group mean, leaving 34 participants.

As expected, CS+ stimuli elicited larger SCR magnitudes than CS- during acquisition, $F(1,32) = 121.995$, $p < .001$ (see, Table 1). There was no interaction between Condition x IU, $F(1,32) = .323$, $p = .574$.

During extinction, SCR magnitude was on average greater for the CS+ vs. CS-, suggesting participants learned the CS-US contingency, $F(1,32) = 9.145$, $p = .005$ (see Table 1). Additionally, SCR magnitude decreased as a function of time for both conditions, $F(1,32) = 5.446$, $p = .026$. However, no significant Condition x Time interaction was found, $F(1,32) = .524$, $p = .473$.

Taking into account individual differences in IU we found, as predicted, significant Condition x Time x IU interaction, $F(1,32) = 4.486$, $p = .042$ in extinction. Further inspection of follow-up pairwise comparisons for early vs. late extinction at IU ±1 SD from the mean on the regression line, showed that lower IU (1 SD below the mean) was associated with significantly greater SCR magnitude in early extinction to the CS+, relative to the CS-, $p = .029$, which dissipated over time (late extinction CS+ vs. CS-, $p = .459$) (see, Fig. 4). In contrast, higher IU (1 SD above the mean) was associated with no significant differences in early extinction between the CS+ and CS-, $p = .980$. In late extinction higher IU was associated with larger SCR magnitude to the CS+, relative to the CS-, $p = .008$ (see Fig. 4). Furthermore, high IU predicted a significant reduction in SCR magnitude to CS- in late extinction, relative

to CS- in early extinction, $p < .001$. This 3-way interaction qualified a significant Time x IU interaction, $F(1, 32) = 4.304$, $p = .046$. No other significant main effects or interactions were found with IU, p's > .1, Max $F = .985$.

We conducted hierarchical regression analyses on the effects that were significant in the ANCOVA above. Hierarchical regression analyses of early and late SCR magnitude difference scores in extinction revealed mixed specificity with IU over the STAIX-2 and PSWQ measures. We found no specificity of IU, over STAI and PSWQ measures for the CS+ vs. CS- early and late extinction difference scores (see Table 2). However, we did find specificity for IU, over and above the STAIX-2 and PSWQ measures for CS- early – CS – late extinction difference scores (see Table 2).

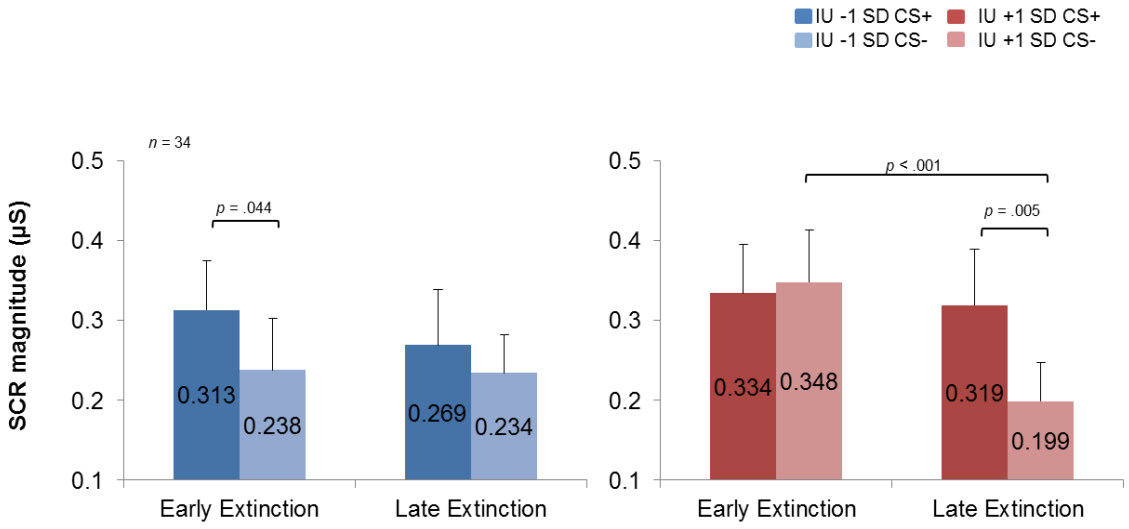

Figure 4. Bar graphs depicting IU differences at ±1 SD from the estimated means during early and late fear extinction. Low IU scores were associated with significantly greater SCR magnitude responses to CS+ vs. CS- in early extinction, and no differences between stimuli in late extinction, suggesting typical fear expression and extinction respectively. High IU scores were associated with no SCR magnitude discrimination between CS+ and CS- in early extinction, but did show SCR magnitude discrimination between CS+ and CS- in late extinction, as well as a reduction in SCR magnitude to CS- in early vs. late extinction, suggesting threat generalization and compromised safety learning.

Table 2.

Summary of hierarchical regression analysis of anxiety measures predicting extinction difference scores

	CS+ - CS- Early Extinction						CS+ - CS- Late Extinction						CS- Early Extinction - CS- Late Extinction					
Predictors	*B*	*SE B*	β	R^2	*F*	ΔR^2	*B*	*SE B*	β	R^2	*F*	ΔR^2	*B*	*SE B*	β	R^2	*F*	ΔR^2
Step 1				0.074	1.232	0.074				0.061	1.008	0.061				0.089	1.7	0.089
STAI	0.003	0.003	-0.39				0.006	0.004	0.322				0.007	0.004	0.439			
PSWQ	-0.006	0.004	0.258				-0.002	0.004	-0.125				-0.005	0.003	-0.377			
Step 2				0.12	1.571	0.046				0.073	0.386	0.012				0.298	10.124	0.209*
STAI	-0.001	0.006	-0.086				0.003	0.006	0.167				-0.002	0.005	-0.123			
PSWQ	0.003	0.003	0.255				-0.002	0.004	-0.124				-0.006	0.003	-0.45			
IU	-0.003	0.002	-0.371				0.002	0.003	0.188				0.006	0.002	0.77			

Note: * $p < .01$; ** p > .001

2.5 Discussion

In the present study, we show: (1) successful fear conditioning using a developmentally appropriate design and, (2) that self-reported IU, a personality trait implicated in the maintenance of anxiety and depression (Carleton et al., 2013; Gentes & Ruscio, 2011; Grupe & Nitschke, 2013; McEvoy & Mahoney, 2012; Whalen, 2007), predicts elevated fear expression to both learned threat and safety cues.

2.5.1 Comparison of findings with the existing fear extinction literature

Consistent with previous research (Delgado et al., 2008; Gazendam et al., 2013; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2009; Soliman et al., 2010), we found a general effect of conditioning for participants, as shown by greater uneasiness ratings and SCR magnitude to the learned threat vs. safety cues during fear extinction. These results suggest that successful conditioning can be achieved when using developmentally appropriate design (e.g. a less aversive CS such as a sound stimulus), similar to that of past fear conditioning work in developmental samples (Haddad et al., 2011; Lau et al., 2011; Neumann et al., 2008; Pattwell et al., 2011; Pattwell et al., 2012).

Expanding previous research on individual differences in anxious disposition (Barrett & Armony, 2009; Dunsmoor et al., In press; Gazendam et al., 2013; Indovina et al., 2011; Sehlmeyer et al., 2011), we found the temporality of fear extinction to vary substantially, depending on individual differences in IU. Low IU was associated with larger SCR magnitude to learned threat cues, relative to safety cues during early extinction, and no differences in SCR magnitude between learned threat and

safety cues during late extinction, suggesting successful fear extinction. However, high IU was associated with increased SCR magnitude to both learned threat and safety cues during early extinction and larger SCR magnitude to learned threat cues, relative to safety cues in late extinction. Furthermore, high IU was uniquely associated with a reduction in SCR magnitude to learned safety cues from early to late extinction. This latter effect was specific to IU, over STAIX-2 and PSWQ measures. Taken together, these results suggest that intolerance of uncertainty may play an important role in disrupting fear extinction processes.

2.5.2 Limitations

Self-reported uneasiness ratings were not found to reflect individual differences in IU in our sample. Differences between self-reported and psychophysiological measures are often reported, perhaps due to lack of sensitivity of self-report metrics to capture such individual differences. Psychophysiological indices during fear extinction were better predicted by IU, over self-reported uneasiness ratings. Such findings suggest IU as a more suitable predictor of psychophysiological responses during fear extinction than moment-to-moment subjective ratings of uneasiness which only capture felt changes in state. However, the lack of relationship between psychophysiological and subjective ratings may be simply due to the time between phasic cue events and rating periods, suggesting that these measures may be related to different processes.

We found no evidence of IU predicting differential psychophysiological responses during fear acquisition for the threat and safety cues. However, we used a 100% reinforcement schedule in the acquisition phase, where the CS+ and US are

confounded. Furthermore, the 100% reinforcement schedule is very certain and unambiguous. Therefore, high IU individuals are not generally more aroused to the US and do not generalise fear to CS- cues during acquisition, at least during 100% reinforcement.

2.5.3 Conclusions

In conclusion, the findings from this study inform us that the adapted classic fear conditioning experiment for developmental samples can induce successful conditioning and capture individual differences in IU. With regards to anxious disposition and fear extinction in adults, high IU was associated with elevated fear expression to both threat and safety cues during early extinction, and continued fear expression to threat cues during late extinction. Moreover, IU was the best predictor of compromised fear extinction over other general measures of anxiety such as the STAI and PSWQ. These findings suggest a critical role of uncertainty-based mechanisms in the maintenance of learned fear.

3. Neural and psychophysiological correlates of adult fear extinction and individual differences in intolerance of uncertainty

3.1 Abstract

Extending Chapter 2, we assessed whether past psychophysiological and neural findings of fear extinction could be replicated in adults within the scanning environment. In addition, we sought to determine whether individual differences in IU underlie compromised recruitment of fear extinction circuitry. 22 healthy subjects completed a cued fear conditioning task with acquisition and extinction phases. During the task, pupil dilation, skin conductance response, and functional magnetic resonance imaging were acquired. We assessed the temporality of fear extinction, by splitting the extinction phase into early and late extinction. Threat uncertainty sensitivity was measured using self-reported intolerance of uncertainty (IU). Across fear extinction, participants had greater skin conductance response and activated right amygdala and vmPFC activity subthreshold to the learned threat vs. safety cue. Similarly to Chapter 2, the temporality of fear extinction varied substantially with individual differences in IU. During early extinction, we found that low IU scores were associated with larger skin conductance responses and right amygdala activity to

threat vs safety cues, whereas high IU scores were associated with no skin conductance discrimination and greater activity within the right amygdala to previously learned safety cues. In late extinction, low IU scores were associated with successful inhibition of threat cues, reflected in comparable skin conductance response and right amgydala activity to threat vs. safety cues, whilst high IU scores were associated with continued fear expression to learned threat, indexed by larger skin conductance and amygdala activity to threat vs. safety cues. In addition, high IU scores were associated with greater vmPFC activity to threat vs. safety cues in late extinction. Similar patterns of IU and extinction learning were found for pupil dilation. The results were specific for IU and did not generalise to self-reported STAI or PSWQ. Overall, the neural and psychophysiological patterns observed here suggest that the designed experiment can capture adult fear extinction behaviour and individual differences in anxious disposition, particularly IU. Furthermore, these results suggest that high IU is associated with the generalization of threat during uncertainty, which subsequently compromises fear extinction. These findings are in line with psychophysiological results from Chapter 2, further highlighting the importance of uncertainty-based mechanisms in the maintenance of learned fear.

3.2 Introduction

In Chapter 2, we demonstrated that: (1) common psychophysiological findings of fear extinction could be replicated in adults and, (2) psychophysiological indices of fear extinction were predicted by individual differences in IU. More specifically, high IU was characterised by elevated fear expression to both threat and safety cues during early extinction, and continued fear expression to threat cues during late

extinction. Taken together these findings suggest that high IU individuals may be prone to threat generalization and deficient safety learning during extinction. In the current Chapter, we aim to extend our understanding of previous findings in Chapter 2. Firstly, we aim to investigate whether common psychophysiological and neural findings of fear extinction can be replicated in the scanning environment on adults. Secondly, we aim to investigate whether individual differences in IU underlie compromised recruitment of fear extinction circuitry.

3.2.1 Critical neural circuitry underpinning fear extinction

Past animal and human research using classical fear conditioning paradigms has demonstrated an important role of the amygdala in fear acquisition and expression, and of the ventromedial prefrontal cortex (vmPFC) in fear extinction (Büchel et al., 1998; LaBar et al., 1998; Milad et al., 2007). During fear acquisition, heightened amygdala activity and increased skin conductance has been observed in response to previously neutral cues that, through conditioning, have come to be associated with aversive outcomes (conditioned stimulus, CS+, e.g. shock or tone) (Büchel et al., 1998; Knight, Smith, Cheng, Stein, & Helmstetter, 2004; Neumann et al., 2008). Subsequent extinction training, which involves repeated presentations of the CS+ without the aversive outcome, results in reduced amygdala and skin conductance responsivity over time (Gazendam et al., 2013; Knight et al., 2004; LaBar et al., 1998). The vmPFC is critical for the fear extinction process and the observed reduction in amygdala and skin conductance responses to the CS+ over time (Milad & Quirk, 2012). For example, stimulation of the infralimbic cortex in rats, an area homologous to the human vmPFC, reduces responsiveness of amygdala

neurons and defensive freezing behaviour to conditioned tones (Milad & Quirk, 2002). In both humans and animals, increased vmPFC activity to the CS+ has been observed in late extinction phases (Milad et al., 2009; Milad et al., 2007), and during subsequent extinction sessions, conducted a few days after initial fear acquisition (Kalisch, Korenfeld, et al., 2006; Phelps et al., 2004).

3.2.2 Psychophysiological and neural correlates of fear extinction and the role of anxious disposition

A large body of research using fear extinction paradigms has shown that individuals with anxiety/trauma disorders are prone to delayed fear extinction or even resistance to fear extinction (for reviews see, (Etkin & Wager, 2007; Graham & Milad, 2011; Milad & Quirk, 2012)). For example, compared to healthy controls, anxiety patients show elevated autonomic nervous system and amygdala responding and reduced recruitment of the vmPFC, to both threat and safety cues at the start of extinction, and to threat cues across fear extinction (Blechert et al., 2007; Michael et al., 2007; Milad et al., 2008; Milad et al., 2009). A series of recent studies has also shown that individuals with high trait anxiety and genetic predisposition for anxiety exhibit: (1) exaggerated autonomic nervous system responding to both threat and safety cues in the early phase of extinction learning (Gazendam et al., 2013), and (2) sustained autonomic nervous system responding, sustained amygdala activation and atypical activation in the medial prefrontal cortex to threat cues from the early to late phase of fear extinction (Barrett & Armony, 2009; Gazendam et al., 2013; Sehlmeyer et al., 2011; Soliman et al., 2010). Genetic evidence also points to similar temporal patterns of delayed fear extinction and increased risk for anxiety in both homozygote

and heterozygote Met allele carriers of the brain-derived neurotrophic factor (BDNF) Val66Met genotype in mice (Chen et al., 2006; Soliman et al., 2010; Yu et al., 2009) and humans (Felmingham, Dobson-Stone, Schofield, Quirk, & Bryant, 2013; Soliman et al., 2010). Furthermore, both the phenotypic and genetic results in mice and humans appear to be specific to fear extinction rather than fear acquisition (Barrett & Armony, 2009; Chen et al., 2006; Dunsmoor, Åhs, & LaBar, 2011; Felmingham et al., 2013; Indovina et al., 2011; Sehlmeyer et al., 2011; Soliman et al., 2010; Torrents-Rodas et al., 2013; Yu et al., 2009) but see (Gazendam et al., 2013; Indovina et al., 2011), suggesting that individuals prone to developing an anxiety disorder have difficulty inhibiting learned threat cues and have a tendency to generalise threat to safety cues, rather than being more readily or strongly conditioned (Dunsmoor, Åhs, et al., 2011; Lissek et al., 2005).

As noted in Chapter 2, simple changes to contingency during fear extinction are inherently uncertain and ambiguous. Despite this, the majority of fear extinction studies have focused predominantly on self-reported trait anxiety (Barrett & Armony, 2009; Gazendam et al., 2013; Sehlmeyer et al., 2011) rather than self-reported IU (Dunsmoor et al., In press). IU is defined as a difficulty in accepting the possibility of future negative events, rendering ambiguous, uncertain or even neutral cues as threatening. In the context of fear extinction, changes to contingency may exacerbate future threat uncertainty. Indeed, in Chapter 2, we found high IU scores to be associated with equally high skin conductance to learned threat and safety cues, suggesting generalization of learned threat to safety cues. Furthermore, in late extinction learning, high IU scores were associated with continued fear expression to learned threat, indexed by larger skin conductance responses to learned threat vs.

safety cues. In addition, we found some evidence of specificity for IU in predicting fear extinction, over and above other anxious disposition measures. Given the findings outlined above, it seems pertinent to further examine whether: (1) the IU psychophysiological findings in Chapter 2 are supported by aberrant recruitment of amygdala-vmPFC circuitry during fear extinction, and (2) IU continues to be a more sensitive measure of compromised fear extinction over other broader measures of trait anxiety.

3.2.3 Design and predictions

In this Chapter, we used cued fear conditioning with acquisition and extinction phases to assess: (1) whether psychophysiological and neural findings from past fear extinction studies can be replicated using a classic paradigm adapted for developmental populations within the scanning environment, and (2) the relationship between individual differences in self-reported IU and in psychophysiological and neural correlates of fear extinction (same experimental design as in Chapter 2). We measured event-related fMRI, skin conductance response (SCR), pupil dilation and behavioural ratings whilst participants performed the conditioning task.

Building upon the hypotheses from Chapter 2, we expected that during fear extinction, participants would exhibit greater uneasiness ratings, skin conductance, pupil dilation, amygdala and vmPFC activity to the learned threat vs. safety cues, evidencing successful conditioning and extinction respectively. We further hypothesised that, during fear extinction, threat uncertainty sensitivity would predict generalised fear expression to both learned threat and safety cues, and/or sustained fear expression to learned threat cues. Given our previous findings in Chapter 2, we

expected this effect to be indexed by: (1) Larger responses in high IU individuals to both learned threat and safety cues in *early* fear extinction, across our physiological and behavioural measurements, including relatively higher amygdala activation; (2) sustained larger responses across measures in high IU individuals to learned threat cues vs. safety cues during *late* fear extinction. We further predicted (3) an association between vmPFC activation and the management of responses to threat vs. safety cues during extinction in low IU individuals. We tested the specificity of the involvement of IU by comparing it with broader measures of anxiety, such as STAIX-2 (Spielberger et al., 1983) and PSWQ (Meyer et al., 1990).

3.3 Methods

3.3.1 Participants

Twenty-two right-handed volunteers were recruited from the University of Reading and local area through advertisements (M age = 23.59, SD age = 2.75; 12 females & 10 males). The sample size was based on previous fear extinction studies conducted in the MRI environment (Milad et al., 2007; Phelps et al., 2004) and power analysis guidelines for fMRI (Mumford, 2012). All participants had normal or corrected to normal vision and were medication-free. Participants provided written informed consent and received a picture of their brain and £20 for their participation. The University of Reading's Research Ethics Committee approved by the study protocol.

3.3.2 Conditioning task

We used the same fear conditioning experiment as in Chapter 2 (see Chapter 2, Methods, Conditioning task and Figure 3). Visual stimuli were presented through MRI-compatible VisualSystem head-coil mounted eye goggles (Nordic Neuro Lab, Bergen, Norway), which displayed stimuli at 60 Hz on an 800 × 600 pixel screen. Sound stimuli were presented through MRI-compatible AudioSystem headphones (Nordic Neuro Lab, Bergen, Norway). Participants used an MRI-compatible response box with their dominant right hand to respond.

3.3.3 Procedure

Participants arrived at the laboratory and were informed of the experimental procedures. First, participants completed a consent form as an agreement to take part in the study. Second, a hearing test was performed with an audiometer to check for normative hearing (e.g.500-8000 Hz, below 30 dB). Third, participants completed a battery of cognitive tasks (results not reported here) and questionnaires on a computer outside of the scanner. Next, participants were taken to the MRI unit. We used a conditioning task inside the scanner, whilst concurrently recording ratings, electrodermal activity and pupil dilation. Participants were simply instructed to: (1) maintain attention to the task by looking and listening to the coloured squares and sounds presented, (2) respond to the uneasiness scale using the button box and (3) to keep as still as possible. After scanning, participants rated the sound stimulus outside of the scanner.

3.3.4 Questionnaires

The same questionnaires were presented from Chapter 2 (see Chapter 2, Methods, Questionnaires). Again, we focused on IU because of the intrinsic uncertainty within conditioning paradigms. Similar distributions and internal reliability of scores were found for the anxiety measures, IU (M = 53.04; SD = 15.68; range 27-85; α = .90), STAIX-2 (M = 40.33; SD = 7.92; range = 27-53; α = .85) and PSWQ (M = 41.47; SD = 11.10; range = 20-65; α = .90).

3.3.5 Sound stimulus rating

Participants rated the valence and arousal of the sound stimulus using 9 point Likert scales ranging from 1 (Valence: negative; Arousal: calm) to 9 (Valence: positive; Arousal: excited).

3.3.6 Behavioural data scoring and reduction

Ratings data from the conditioning task were reduced for each participant by calculating their average responses for each experimental condition. Missing data points were excluded.

3.3.7 Physiological data acquisition and reduction

Electrodermal recordings were obtained using AD Instruments (AD Instruments Ltd, Chalgrove, Oxfordshire) hardware and software. An ML138 Bio Amp connected to an ML870 PowerLab Unit Model 8/30 amplified the EDA signal, which were digitised through a 16-bit A/D converter at 1000 Hz**.** EDA was measured during the scanning session with MRI-safe MLT117F Ag/AgCl bipolar finger

electrodes filled with NaCl electrolyte paste (Mansfield R & D, St Albans, Vermont, USA) that were attached to the distal phalanges of the index and middle fingers of the left hand. A constant voltage of 22mVms at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp. Skin conductance responses (SCR) were scored when there was an increase of skin conductance level exceeding 0.03 microSiemens. The amplitude of each response was scored as the difference between the onset and the maximum deflection prior to the signal flattening out or decreasing. SCR onsets had to be within 7 seconds following each trial to be included. We used an extended SCR scoring window because the temporal signature of an aversive sound US may be more ambiguous than a traditional electric shock US, this SCR scoring window length is in line with previous fear conditioning studies that have used aversive sound stimuli as the US in both adults (Büchel et al., 1998; Soliman et al., 2010) and adolescents (Pattwell et al., 2012).

Trials with no discernible SCRs were scored as zero. The first trial of each experimental phase was excluded, to reduce contamination of averages from the orienting response. SCR amplitudes were square root transformed to reduce skew. Trials with motion artefacts were discarded from the analysis. SCR magnitudes were calculated from remaining trials by averaging SCR square root transformed values for each condition.

Pupil dilation was recorded at a sample rate of 60 Hz through a built-in infrared camera on the head-coil mounted eye goggles (Nordic Neuro Lab, Bergen, Norway). Pupil dilation data was averaged for each 1000 ms window following stimulus onset, resulting in 5 windows of 1000 ms each. These data were baseline

corrected by subtracting 1000 ms preceding each stimulus onset from a blank screen. Trials were averaged per condition and time window for each participant.

3.3.8 Learning assessment

Similarly to Chapter 2, we assessed whether participants learned the association between the neutral cue and aversive sound, by calculating conditioned response scores for behavioural ratings, pupil dilation and SCR magnitude in extinction. The conditioned response score was the first 2 CS+ trials – the first 2 CS- trials. A positive score indicated a larger response for CS+ vs. CS-, indexing successful conditioning. This type of learning assessment procedure is commonly reported in the fear extinction literature (Dunsmoor et al., In press; Milad et al., 2009; Milad et al., 2007; Phelps et al., 2004). To reduce subject attrition, we labelled subjects as learners if they had a positive conditioned response score for any measure. Based on the learning assessment criterion, we identified four potential non-learners out of the 22 participants. Since removing the data of these 4 subjects did not change the results reported here[2], we retained the data of all participants.

3.3.9 Ratings and psychophysiology analysis

Conditioning effects and IU differences across extinction were assessed by conducting a Condition (CS+, CS-) x Time (Early, Late) x IU repeated measures ANCOVA for behavioural ratings, SCR magnitude and pupil dilation. IU was entered

[2] Results do not change when non-learners are removed: The main effect of Condition for SCR magnitude during fear extinction, without non-learners $F(1,10) = 7.624$, $p = .020$. Condition x Time x IU interaction for SCR magnitude during fear extinction without non-learners, $F(1,10) = 8.380$, $p = .016$. Extinction CS+ - CS- difference scores for early and late extinction in the right amygdala correlated with IU: Early extinction without non-learners, $r(15) = -.66$, $p = .003$,. Late extinction without non-learners, $r(15) = .71$, $p = .001$.

as a continuous mean centered predictor variable. The early part of extinction was defined as the first eight CS+ and eight CS- trials, and the last part of extinction was defined as the last eight CS+ and eight CS- trials. For pupil dilation, which was based on second-by-second averaging, we also included the factor Window with 5 levels representing seconds post-stimulus onset. We performed follow-up pairwise comparisons on the estimated marginal means, adjusted for IU. Any interaction with IU was followed up with pairwise comparisons of the means between the conditions for IU estimated at the specific values of + or - 1 SD of mean IU. These data are estimated from the ANCOVA of the entire sample, not unlike performing a simple slopes analysis in a multiple regression analysis. To check for specificity of findings with IU in extinction, we conducted a Condition (CS+, CS-) x Window x IU repeated measures ANCOVA on behavioural ratings, SCR magnitude and pupil dilation obtained in the acquisition phase.

We performed hierarchical regression analyses on the resulting significant SCR magnitude and pupil dilation difference scores (CS+ - CS- early; CS+ - CS- late; CS+ early – CS+ late; CS- early – CS- late) for extinction and the anxiety measures to test for IU-specific effects. We entered STAIX-2 and PSWQ in the first step and then IU in the second step.

3.3.10 MRI

Participants were scanned with a 3T Siemens Trio set up with a 12 channel head coil (Siemens Inc., Erlangen, Germany). Three T2*-weighted echo planar imaging (EPI) functional scans were acquired for each phase of the conditioning task consisting of 161, 208, and 380 volumes respectively (TR = 2000 ms, TE = 30 ms,

flip angle = 90°, FOV = 192 × 192 mm, 3 × 3 mm voxels, slice thickness 3 mm with an interslice gap of 1 mm, 30 axial slices, interleaved acquisition).

Following completion of the functional scans, fieldmap and structural scans were acquired, which comprised of a high-resolution T1-weighted anatomical scan (MP-RAGE, TR = 2020 ms, TE = 2.52 ms, flip angle = 90°, FOV = 256 × 256 mm, 1 x 1 x 1 mm voxels, slice thickness 1 mm, sagittal slices), two fieldmaps (TR = 488 ms, TE 1 = 4.98 ms, TE 2 = 7.38 ms, flip angle = 60°, FOV = 256 × 256 mm, slice thickness 4 mm with an interslice gap of 4 mm, 30 axial slices) and diffusion weighted images, which will not be further discussed here (TR = 6800ms, TE = 93 ms, flip angle = 60°, FOV = 192 × 192 mm, slice thickness 2 mm with an interslice gap of 2 mm, *b*-value =1000, 64 axial slices, 30 diffusion gradients).

3.3.11 fMRI analysis

FMRI analyses were carried out in Feat version 5.98 as part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Brains were extracted from their respective T1 images by using the FSL Brain Extraction Tool (BET) (Smith, 2002). Distortion, slice timing and motion correction were applied to all extracted EPI volumes using FUGUE and MCFLIRT tools. Gaussian smoothing (FWHM 5mm) and a 50 second high pass temporal filter were applied.

A first-level GLM analysis was carried out for each functional scan run from acquisition and extinction. Separate regressors were specified for the experimental conditions of primary interest in each learning phase (acquisition: CS+/CS-, extinction: CS+ /CS-) by convolving a binary boxcar function with an ideal haemodynamic response (HR), which corresponded to the length of each trial (1500

ms). Regressors for the uneasiness rating period and six motion parameters were included to model out brain activity that was unrelated to the conditions of interest.

We defined two main effect contrasts to reveal fear extinction-related activity. To examine temporal effects across extinction, we contrasted (CS+ vs. CS-)$_{EARLY}$> (CS+ vs. CS-)$_{LATE}$. We defined early extinction as the first eight trials for CS+ and CS- and the last eight trials for CS+ and CS-. Particular focus is given to the temporal effects across extinction, given our predictions. We also examined the overall effect of CS+ vs. CS- during extinction for comparison against the extant literature. All contrasts were normalised and registered to MNI standard space using FLIRT (Jenkinson, Bannister, Brady, & Smith, 2002). Second-level GLM analysis consisted of regressors for the group mean and demeaned IU scores using FSL's FLAME stage 1 + 2 procedure. Whole-brain analysis was carried out using cluster thresholding with a $z = 2.3$ and a corrected $p < 0.05$.

We were specifically interested in the extent to which IU scores would be associated with the BOLD response in the amygdala and vmPFC for early and late extinction phases. Therefore, we performed small volume corrections on the left amygdala, right amygdala and vmPFC using cluster thresholding with a $z = 2.3$ and a corrected $p < 0.05$ on the IU x (CS+ vs. CS-)$_{EARLY}$> (CS+ vs. CS-)$_{LATE}$ extinction contrast map. We used anatomically defined masks from the Harvard-Oxford cortical and subcortical structural atlases in FSL (Desikan et al., 2006). We selected the left amygdala, right amygdala and frontal medial cortex regions with a 50% probability threshold. For control purposes we also applied small volume corrections within the left amygdala, right amygdala and vmPFC on the IU x acquisition CS+ vs. CS-and the IU x extinction CS+ vs. CS- contrast maps.

To assess fear expression correspondence between the amygdala and psychophysiology measures, we correlated percent BOLD signal response from significant amygdala regions and SCR magnitude/pupil dilation.

We performed hierarchical regression analyses on the resulting statistical a priori regions of interest difference scores from extinction (CS+ - CS- early; CS+ - CS- late; CS+ early – CS+ late; CS- early – CS- late) and the anxiety measures to test for IU-specific effects, STAIX-2 and PSWQ in the first and then IU in the second step.

3.4 Results

1 participant's data were removed from all analyses due to having an extreme IU score that was +3 SD from the group mean.

3.4.1 Questionnaires

As expected, the anxiety measures were positively correlated with each other, suggesting shared variance, IU with PSWQ, $r(19) = .590$, $p = .005$, IU with STAIX-2, $r(19) = .619$, $p = .003$, and PSWQ with STAIX-2, $r(19) = .657$, $p = .001$.

3.4.2 Ratings

Participants rated the sound stimulus serving as US as negative ($M = 3.52$, $SD = 1.63$) and moderately arousing ($M = 5.23$, $SD = 2.14$). With respect to the uneasiness ratings (on a scale from 1-10), a main effect of Condition was found for acquisition across all individuals, $F(1,19) = 13.394$, $p = .002$. During acquisition, participants significantly reported feeling more uneasy for the CS+ relative to the CS-

trials, p = .002 (for descriptive statistics, see Table 3). We found no effect of Condition or Condition x Time for the uneasiness ratings during extinction, p's >.1, F's < 1 (see Table 3). Results revealed no IU differences for uneasiness ratings for any of the experimental phases, p's >.3, F's >.1, max F = 1.015.

Table 3.

Summary of means (SD) for each dependent measure as a function of condition and phase.

	Acquisition		Extinction		Early Extinction		Late Extinction	
Measure	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Physiological								
Square root transformed SCR magnitude (μS)	.27 (.17)[b]	.13 (.11)[a]	.16 (.13)[d]	.13 (.12)[c]	.20 (.17)	.14 (.11)	.13 (.14)	.11 (.14)
Pupil dilation (Δmm)	-.023 (.010)	-.024.(.010)	-.025 (.008)	-.024.(.013)	-.027.(.015)	-.026.(.018)	-.023.(.008)	-.023.(.022)
Behavioural								
Uneasiness rating (1-9)	3.61 (1.93)[b]	2.09 (1.50)[a]	1.67 (1.23)	1.75 (1.32)	1.84 (1.27)	1.88 (1.42)	1.49 (1.38)	1.41 (1.31)

Note: SCR magnitude (μS), skin conductance magnitude measured in microSiemens. Pupil dilation (Δmm) measured in delta millimeters. Significant comparisons are specified with * = p<.05, and ** = p < .01. Superscripts indicate significant (p <.05) condition difference from: [a] Acquisition CS+, [b] Acquisition CS-, [c] Extinction CS+, [d] Extinction CS-, [e] Early Extinction CS+, [f] Early Extinction CS-, [g] Late Extinction CS+, [h] Late Extinction CS-.

3.4.3 SCR magnitude

7 subjects were removed from the SCR magnitude analysis due to 6 subjects not responding, which is not uncommon when recorded in an MRI setting (see Methods), and 1 subject with a recording error.

As expected, larger SCR magnitudes were found for CS+ vs. CS- during acquisition, $F(1,12) = 14.376$, $p = .003$ (see Table 3) but there was no interaction between Condition x IU, $F(1,12) = .564$, $p = .467$.

During extinction, we found greater SCR magnitude for the CS+ vs. CS-, $F(1,12) = 5.369$, $p = .039$ (see Table 3), but no significant interaction effect between Condition and Time, $F(1,12) = 1.711$, $p = .215$. However, as predicted, we found a significant Condition x Time x IU interaction, $F(1,12) = 8.782$, $p = .012$. Further inspection of follow-up pairwise comparisons for early vs. late extinction at IU ±1 SD from the mean revealed that at the low IU end (1 SD below the IU mean) is associated with the commonly reported extinction pattern, including discrimination between CS+ and CS- in early extinction, $p = .026$, but no significant differences between CS+ and CS- in late extinction, $p = .139$ (see Fig. 5a). Furthermore, low IU is associated with a reduction in SCR magnitude to the CS+ from early to late extinction, $p = .006$, but not to the CS- from early to late extinction, $p = .425$. High IU (captured at 1 SD above the mean) is associated with the opposite pattern, with no significant differences between CS+ and CS- in early extinction, $p = .586$, but discrimination between CS+ and CS- in late extinction, $p = .014$ (see Fig. 5a). In addition, high IU is not associated with differences in SCR magnitude between CS+ from early to late extinction, $p = .525$, and CS- from early to late extinction, $p = .582$.

No other significant main effects or interactions were found with IU, max $F = 3.552$, p's >.08.

We conducted hierarchical regression analyses on the effects that were significant in the ANCOVA above, creating difference scores by subtracting response to CS- from CS+. Hierarchical regression analyses of early and late SCR magnitude difference scores in extinction revealed mixed specificity with IU over the STAIX-2 and PSWQ measures: (1) CS+ - CS- early extinction, first step: R^2=.409, $F(2,11) = 1.108$, p= .364, second step: ΔR^2=.419, $F(1,10) = .101$, p= .757, (2) CS+ - CS- late extinction, first step: R^2=.390, $F(2,11) = .986$, p= .404, second step: ΔR^2=.755, $F(1,10) = 9.737$ p= .011, and (3) CS+ early – CS+ late extinction, first step: R^2=.620, $F(2,11) = 3.426$, p= .70, second step: ΔR^2=.664, $F(1,10) = 1.023$, p= .336.

3.4.4 Pupil dilation

One subject was removed from the pupil dilation analysis due to a recording error, leaving 20 participants. No effect of acquisition or extinction was found for the whole sample, p's >.1, F's < .2, Max $F = 1.615$ (see Table 3). We found a significant Condition x Time x IU interaction for pupil dilation during extinction, $F(1,18) = 7.921$, $p = .011$. Follow-up pairwise comparisons for early vs late at IU ±1 SD from the mean showed this effect to be driven by high IU scores, which were associated with greater relative pupil constriction for CS- relative to CS+ at trend during early extinction, $p = .052$, but did not display significant differences between CS+ and CS- in late extinction, $p = .134$ (see Fig. 5b). Furthermore, high IU was characterised by an increase in pupil constriction to the CS+ from early to late extinction at trend, $p = .057$, but not to the CS- from early to late extinction, $p = .167$. Low IU scores (1 SD

below the mean) were not associated with significant differences between condition and time, *p*'s > .065 (see Fig. 5b). No other significant interactions were found with IU, *p*'s >.1, Max *F* = 1.817.

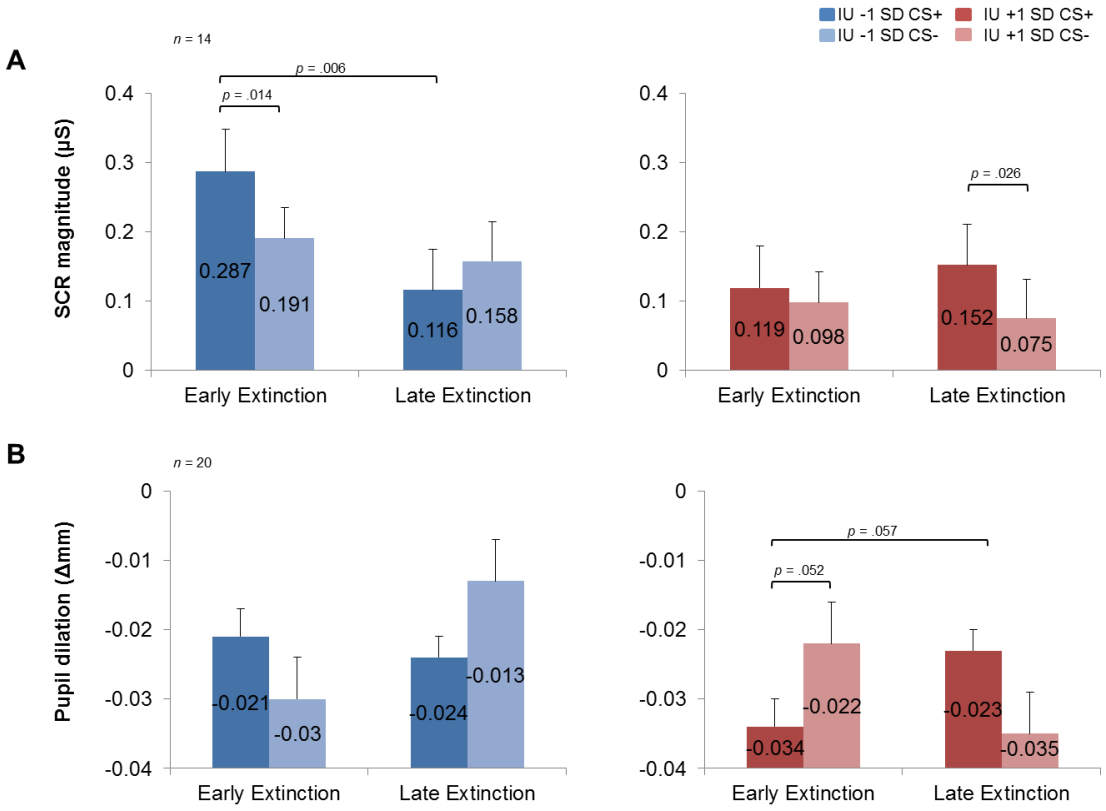

Figure 5. Bar graphs depicting IU differences ±1 SD from the estimated means during early and late extinction learning. (A) SCR magnitude and (B) pupil dilation. Low IU were associated with significantly greater SCR magnitude responses to CS+ vs. CS- in early extinction, and no differences between stimuli in late extinction. High IU scorers showed no differences in SCR magnitude to CS+ and CS- stimuli in early extinction, and delayed discrimination in SCR magnitude to CS+ vs. CS- in late extinction. The pupil dilation results followed a similar pattern to the SCR magnitude results, albeit at trend. SCR magnitude (µS), skin conductance magnitude measured in microSiemens; Pupil dilation (Δmm) measured in delta millimeters.

Following up on the significant effects from the ANCOVA above, hierarchical regression analyses of early and late pupil dilation difference scores in extinction revealed specificity for IU over the STAIX-2 and PSWQ measures: (1) CS+ - CS- early extinction, first step: R^2=.246, F(2,17) = .547, p= .589, second step: ΔR^2=.646, F(1,16) = 9.772, p= .007, (2) CS+ early – CS+ late extinction, first step: R^2=.075, F(2,17) = .048, p= .953, second step: ΔR^2=.476, F(1,16) = 4.565, p= .048.

3.4.5 fMRI

Likely because we had large individual variation in response patterns during extinction, our whole-brain analyses did not yield significant BOLD differences in our a-priori brain regions of interest often reported in the extinction literature (Büchel et al., 1998; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004). However, the CS+ > CS- contrast map revealed vmPFC (voxels = 21, max Z = 2.83, x = -2, y = 50, z = -10) and left amygdala (voxels = 3, max Z = 2.39, x = -16, y = -4, z = -12) clusters at sub-threshold, z = 2.0, p =.045. In addition, found greater lateral occipital cortex and parietal lobule activation across extinction for the CS+ > CS- (see Table 4), as well as greater occipital pole activation in early extinction for the CS+ > CS-, relative to late extinction for the CS+ > CS-, suggesting increased attention for the conditioned stimulus.

As expected, areas within the right amygdala and the vmPFC significantly correlated with IU scores during extinction (see Table 4, Fig. 6 & 7). We performed follow up correlations, to identify the source of the interaction effect from the significant IU x $(CS+ \text{ vs. } CS-)_{EARLY} > (CS+ \text{ vs. } CS-)_{LATE}$ contrast. During early extinction, higher IU predicted increased activation to the CS-, relative to CS+ for the

right amygdala cluster, $r(19) = -.58$, $p = .005$ (see Fig. 6). There were no significant effects of IU in the vmPFC cluster during early extinction however, $r(19) = -0.106$, $p = .646$. During late extinction, IU was positively associated with activation to the CS+ relative to the CS- for the right amygdala cluster, $r(19) = .47$, $p = .030$ (see Fig. 6), and, unexpectedly, for the vmPFC cluster, $r(19) = .62$, $p = .002$ (see Fig. 7). In addition, higher IU predicted relative higher right amygdala activity from CS- early to CS- late, $r(19) = .631$, $p = .002$, suggesting generalization of threat to the CS- at the start of extinction. All other condition and time difference scores were not significant for the right amygdala and vmPFC, *p's* > .125. Furthermore, the BOLD response in areas associated with vigilance, such as the opercularcortex, cingulate gyrus, lateral occipital cortex and precentral gyrus, significantly differed over time as a function of IU scores during extinction (see Table 4).

A hierarchical regression analysis confirmed the significant extinction difference scores from the right amygdala and vmPFC were specific to IU versus STAIX-2 and PSWQ; adding IU in the second step significantly improved the model: (1) right amygdala for CS+ - CS- early extinction, first step: $R^2 = .191$, $F(2,18) = .2.123$, $p = .149$, second step: $\Delta R^2 = .404$, $F(1,17) = 6.090$, $p = .025$, (2) right amygdala for CS+ - CS- late extinction, first step: $R^2 = .099$, $F(2,18) = .987$, $p = .392$, second step: $\Delta R^2 = .237$, $F(1,17) = 3.067$, $p = .098$, (3) right amygdala CS- early vs. CS- late extinction, first step: $R^2 = .334$, $F(2,18) = 1.127$, $p = .346$, second step: $\Delta R^2 = .642$, $F(1,17) = 8.692$, $p = .009$, and (4) vmPFC for CS+ vs. CS- late extinction, first step: $R^2 = .122$, $F(2,18) = 1.255$, $p = .309$, second step: $\Delta R^2 = .396$, $F(1,17) = 7.694$, $p = .013$.

We found no significant effects of IU during acquisition on a whole-brain basis or within the a-priori ROIs. Furthermore, we found no significant effects of IU across

the entire extinction phase (early and late collapsed) on a whole-brain basis, nor within the a-priori ROIs.

Table 4

Significant activation patterns in a priori regions of interest and other brain regions during extinction.

Extinction	Brain region	BA	Voxels (mm^3)	Max Z	Location of max Z		
					X	y	Z
A priori regions							
$(CS+ > CS-)_{EARLY} > (CS+ > CS-)_{LATE}$ x IU	R amygdala		33	2.96	26	-8	-12
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	R L vmPFC	10	40	2.92	-8	42	-16
Outside a priori regions							
CS+ > CS-	L lateral occipital cortex, inferior parietal lobule	7/39	439	3.31	-38	-60	44
$(CS+ > CS-)_{EARLY} > (CS+ > CS-)_{LATE}$	R occipital pole	18	643	3.88	34	-94	2
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$	R precentral gyrus, postcentral gyrus	3-4/6	504	3.49	38	-24	38
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	Cingulate gyrus, juxtapositional lobule, precentral gyrus, postcentral gyrus, parietal lobule	3-7/40	4267	3.99	-2	-8	60
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	R central opercular cortex	6	361	3.16	56	-2	6
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	L parietal operculum cortex	40	304	3.16	-52	-28	14
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	R parietal operculum cortex	40	292	3.33	56	-26	18
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	L cerebellum		274	3.29	12	-70	-18
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$ x IU	R lateral occipital cortex	37	259	3.23	46	-60	-8

Note: Clusters for small volume corrected a priori regions and whole brain corrected regions outside a priori regions corrected for multiple comparisons at $p < 0.05$. BA = Brodmann Areas. Location of cluster's maximum Z are in MNI space. R = right; L = left.

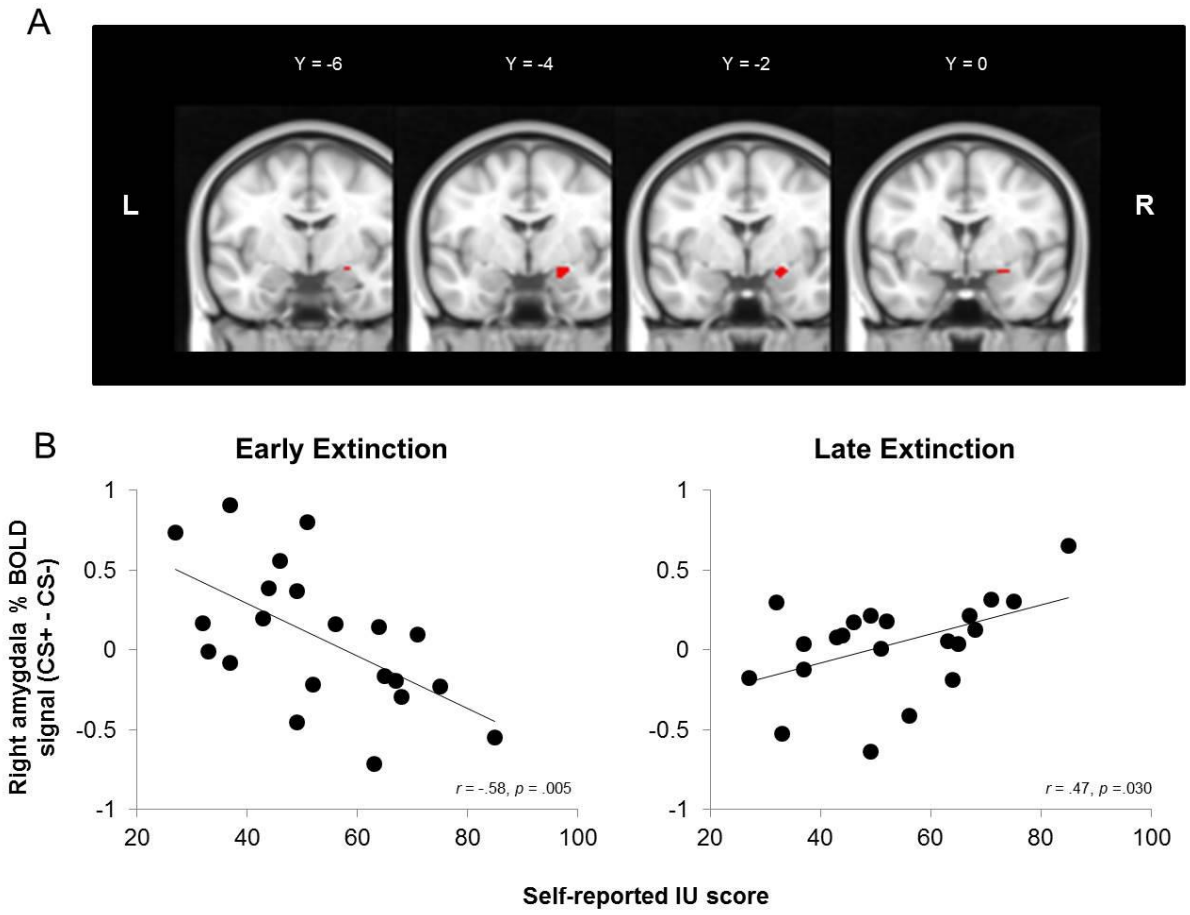

Figure 6. A: Right amygdala small volume correction from the (CS- > CS+) $_{EARLY}$ > (CS- > CS+) $_{LATE}$ x IU contrast in extinction. B: Significant correlations between percent signal change in the right amygdala for CS+ – CS- and IU scores during early and late extinction. High IU was associated with threat-like responses in the amygdala to CS- in early extinction and to CS+ in late extinction. These findings suggest high IU scorers generalise threat when faced with uncertainty, resulting in compromised safety learning. MNI coordinates; R, right; L, left.

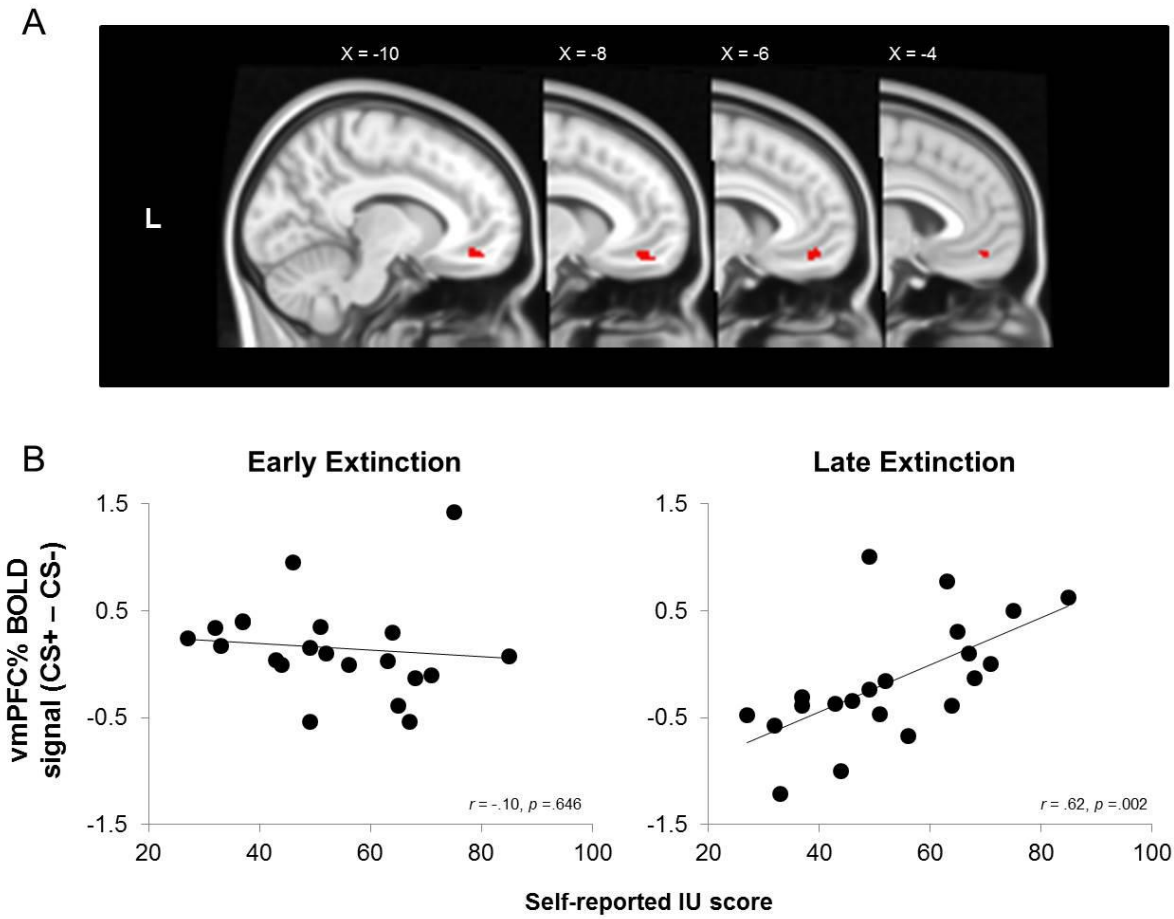

Figure 7. A: vmPFC small volume correction from the (CS- > CS+) $_{EARLY}$ > (CS- > CS+) $_{LATE}$ x IU contrast in extinction. B: Significant correlations between percent signal change in the vmPFC for CS+ – CS- and IU scores during early and late extinction. During late extinction, high IU scores were associated with increased recruitment of the vmPFC to the CS+, relative to the CS-, suggesting attempts to down regulate fearful associations. MNI coordinates; R, right; L, left.

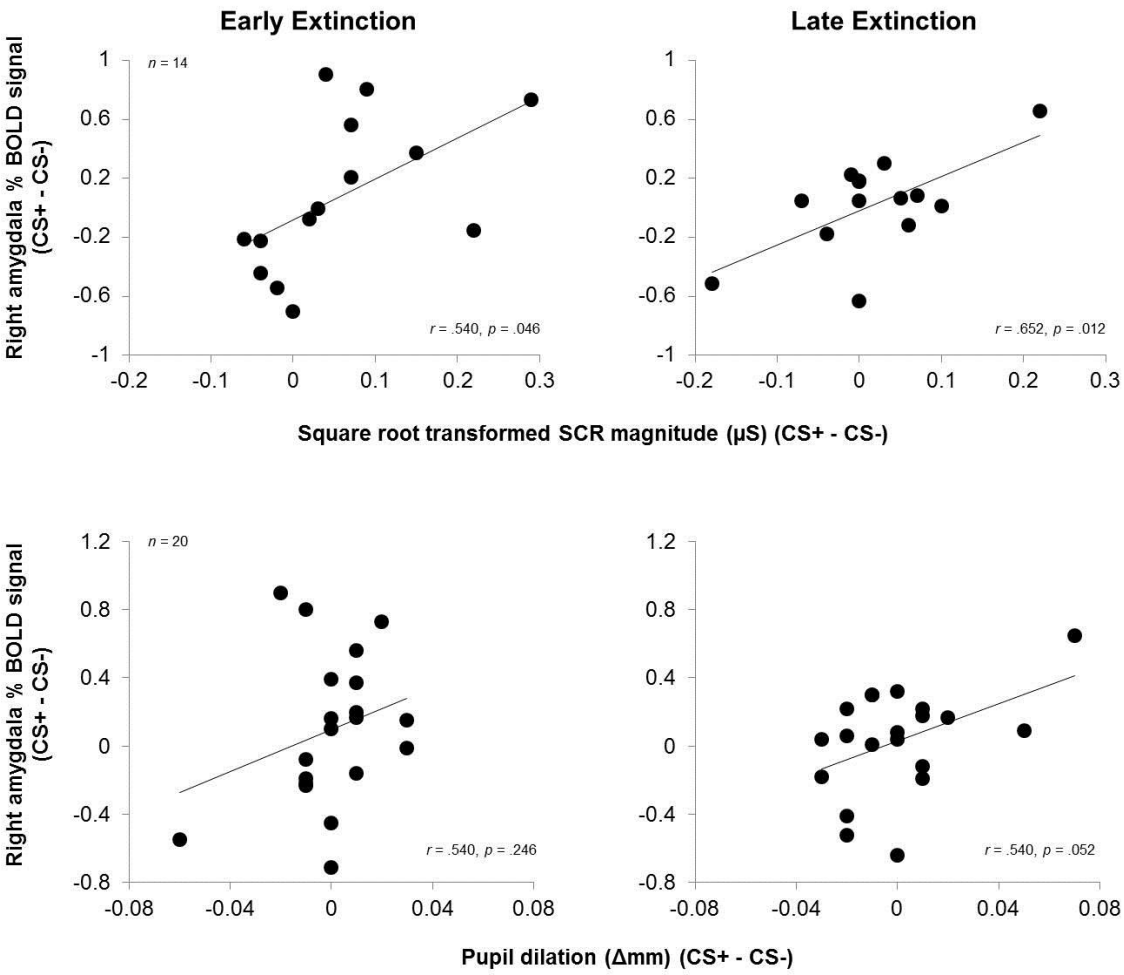

Figure 8. Correlations between percent signal change in the right amygdala and psychophysiology measures. The response in the right amygdala is significantly correlated with SCR magnitude and at trend with pupil dilation, suggesting correspondence between measures of fear expression. SCR magnitude (µS), skin conductance magnitude measured in microSiemens; Pupil dilation (Δmm) measured in delta millimeters.

3.4.6 Relationships between right amygdala and psychophysiology

Percent BOLD signal difference (CS+ vs. CS-) in the right amygdala correlated positively with SCR magnitude during early, $r(12) = .540$, $p = .046$, and late extinction, $r(12) = .652$, $p = .012$.(see Fig. 8). Percent BOLD signal in the right amygdala was not correlated with pupil dilation during early extinction, $r(18) = .540$, $p = .246$, but did correlate positively during late extinction, $r(18) = .540$, $p = .052$ (see, Fig. 8).

3.4.7 Relationships between a-priori ROIs and ratings

Uneasiness rating difference scores for early and late fear extinction did not significantly correlate with percent BOLD signal difference scores for early and late extinction in the a-priori ROIs, *p*'s > .3.

3.5 Discussion

Here, we show: (1) successful fear conditioning in psychophysiological and neural indices using a developmentally appropriate design in the scanning environment on adult participants, (2) and self-reported IU, a personality trait implicated in the maintenance of anxiety and depressive disorders (Grupe & Nitschke, 2013; McEvoy & Mahoney, 2012; Whalen, 2007), predicts psychophysiological and neural recruitment during fear extinction. Importantly, these data replicate and extend our findings from Chapter 2, further suggesting IU to be associated with threat generalization and deficient safety learning. Furthermore, these results were specific to an association between extinction

and IU, and did not generalise to other anxiety measures (STAIX-2, PSWQ), or associative learning phases (acquisition).

3.5.1 Comparison of findings with the existing fear extinction literature

In line with past fear extinction studies within the scanning environment (Delgado et al., 2008; Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2009; Soliman et al., 2010), we found greater SCR magnitude to the learned threat vs. safety cues during fear extinction across participants. Similar patterns were observed in the amygdala and vmPFC subthreshold. However, we did not find such patterns for the uneasiness ratings or pupil dilation data. In general, these results suggest successful conditioning can be achieved in adults when using a developmentally adapted design with a milder CS+ (e.g. a sound stimulus), similar to that of past developmental research (Haddad et al., 2011; Lau et al., 2011; Neumann et al., 2008; Pattwell et al., 2011; Pattwell et al., 2012).

Similarly, to Chapter 2, we found the temporality of fear extinction to vary substantially with individual differences in IU. In early extinction low IU was characterised by discrimination of threat and safety cues, where SCR magnitude and right amygdala response was larger to threat cues, relative to safety cues. High IU, however, was associated with fear expression to both learned threat and safety cues in early extinction, indexed by indiscriminate SCR magnitude. Furthermore, high IU was associated with larger pupil dilation (at trend) and right amygdala activity to safety vs. threat cues in early extinction. During late extinction, low IU predicted reduced SCR magnitude and right

amygdala activity to threat vs. safety cues, suggesting successful fear extinction, in line with previous extinction research (Milad et al., 2009; Milad et al., 2007; Phelps et al., 2004). High IU predicted larger SCR magnitude, pupil dilation (at trend) and right amygdala to threat vs. safety cues during late extinction, suggesting sustained fear expression to learned threat cues. Taken together, these results replicate the psychophysiological results from Chapter 2.

We found that high IU was associated with increased vmPFC activation in response to threat vs. safety cues in late extinction. Whilst this pattern was not predicted, it is similar to previous studies that report hyperactivity of the prefrontal cortex during fear extinction for trait anxious individuals (Barrett & Armony, 2009) and during emotion regulation tasks for depressed patients (Johnstone et al., 2007). Overall, these findings suggest that high IU is associated with slower discrimination of threat from safety cues, which subsequently compromises fear extinction.

3.5.2 Specificity of intolerance of uncertainty

Notably, we found the fear extinction results to be specific to IU, over other broader measures of trait anxiety and worry (STAIX-X2 and PSWQ). The specificity of IU was strongly supported by neural indices, and partially supported in SCR magnitude and pupil dilation. Crucially, these results suggest uncertainty to be an important factor in maintaining learned fearful associations and hindering the formation of new safety associations. Furthermore, these data provide initial evidence that uncertainty may be the driver behind previous trait anxiety and fear extinction findings (Barrett & Armony, 2009; Gazendam et

al., 2013; Sehlmeyer et al., 2011; Soliman et al., 2010). These results call for further study of the neural basis underlying uncertainty-based maintenance of anxiety, which may prove useful for clinicians in improving and developing therapies.

3.5.3 Limitations

We were unable to show main effects of fear extinction in the behavioural ratings and pupil dilation data, and only subthreshold in amygdala-vmPFC circuitry. This could have occurred for a few different reasons. Firstly, the aversiveness of the CS (sound) would likely be reduced in the scanning environment due to scanner noise, which may have weakened effects of conditioning in these measures. Secondly, variation in pupil dilation and brain indices was strongly associated with IU, suggesting that perhaps high IU individuals are more sensitive to conditioning than low individuals, even when the CS is only mildly aversive.

We found no evidence of IU predicting differential recruitment of brain regions or psychophysiological reactivity to threat and safety cues during fear acquisition. However, we used a 100% reinforcement schedule in the acquisition phase, where the CS+ and US are confounded. Similarly to Chapter 2, we can conclude that high IU individuals are not generally more aroused to the US and do not generalise fear to CS- cues during acquisition, at least during 100% reinforcement.

Individual differences in IU were reflected in physiological and brain indices during extinction. However, self-reported arousal ratings did not reflect

individual differences in IU in our sample. Interestingly, psychophysiological and neural indices during fear extinction were better predicted by IU, over self-reported uneasiness ratings. Such findings suggest IU to be a more suitable predictor of psychophysiological and neutral activity during fear extinction than moment-to-moment subjective ratings of uneasiness. However, as noted in Chapter 2, the lack of relationship between neural activity and subjective ratings may be simply due to the time between phasic cue events and rating periods.

3.5.4 Conclusions

In conclusion, the findings from this study confirm that the adapted fear conditioning experiment for developmental samples can induce successful conditioning and capture individual differences in IU within an adult sample in the scanning environment. Furthermore, we found individual differences in IU to specifically predict fear extinction capacity and associated responsivity in psychophysiology and amygdala-vmPFC circuitry. These findings suggest reduced flexibility in amygdala-vmPFC circuitry for high IU individuals. Importantly, converging evidence from Chapter 2 and this Chapter, suggest a critical role of uncertainty-based mechanisms in the maintenance of learned fear.

4. A multimodal brain imaging investigation of fear extinction across adolescence and early adulthood

4.1 Abstract

Previous research in rodents and humans points to an evolutionarily conserved profile of blunted fear extinction during adolescence. Building upon work from Chapters 2 & 3, we sought to examine the developmental effects of age (n = 55; age = 12-28 yrs) and IU upon fear extinction circuitry using functional and structural magnetic resonance imaging. We used a developmentally appropriate fear conditioning paradigm that was designed and tested previously in Chapters 2 & 3. During fear extinction, we found that: (1) younger age was linearly associated with greater activity in the amygdala to learned threat vs. safety cues, and (2) (mid-) adolescents was associated with reduced recruitment of the vmPFC to learned threat vs. safety cues. Furthermore, less age-related thinning of grey matter probability within the vmPFC was associated with continued responding in the amygdala to learned threat vs. safety cues during fear extinction. However, we found no significant relationships between IU and functional and structural correlates of fear

extinction circuitry. Overall, these findings suggest both age and age-related changes in the structure and function of amygdala-vmPFC circuitry may underlie fear dysregulation, rendering (mid-)adolescents vulnerable to anxiety disorders. Further longitudinal work is needed to establish how individual differences in anxious disposition shape the function and structure of fear extinction circuitry across adolescence and into early adulthood.

4.2 Introduction

Adolescence and early adulthood are times of great change, exploration, and stress, with the emergence of puberty and new priorities outside of the realms of home and school (Blakemore, 2012; Choudhury, 2009; Crone & Dahl, 2012; Somerville & Casey, 2010; Spear, 2000b). Alongside these changes, the brain undergoes marked structural developmental in growth and pruning (Crone & Dahl, 2012; Lourenco & Casey, 2013; Nelson, Lau, & Jarcho, 2014; Pfeifer & Allen, 2012; Spear, 2000a), particularly in those regions critical for fear extinction such as the vmPFC. Unfortunately, anxiety disorders are also frequently reported to emerge during adolescence (Kessler et al., 2005). Given ongoing development, adolescent populations may be less responsive to traditional forms of anxiety disorder treatment such as exposure therapy (Cartwright-Hatton et al., 2004; Rapee et al., 2009). Therefore, examining the function and structure of fear extinction circuitry in healthy adolescents may further our understanding of how fear extinction processes develop, and highlight which anxiety disorder treatments to apply and when to apply them during this sensitive period (Casey, Glatt, & Lee, 2015). In the following

sections we outline previous literature on the impact of individual differences in developmental stage and anxious disposition upon function and structure of fear extinction circuitry. Based on this literature, we then outline the design and predictions of the final study of thesis.

4.2.1 The impact of development upon function and structure of fear extinction circuitry

The brain circuitry that is at the core of cued fear learning and fear extinction processes includes the amygdala and ventromedial prefrontal cortex (vmPFC) (Milad & Quirk, 2012). During fear acquisition, heightened amygdala activity is observed in response to previously neutral cues that, through conditioning, have come to be associated with aversive outcomes. Subsequent fear extinction training, which involves repeated presentations of the conditioned stimulus without the aversive outcome, results in reduced amygdala activity over time (Büchel et al., 1998; LaBar et al., 1998). The vmPFC is critical for the extinction process and the observed reduction in amygdala responses to the conditioned stimulus (Kalisch, Korenfeld, et al., 2006; Milad & Quirk, 2002, 2012; Milad et al., 2007; Phelps et al., 2004).

Importantly, recent research in rodents and human samples has shown distinctive developmental profiles of blunted fear extinction in adolescents (Kim, Hamlin, & Richardson, 2009; Kim, Li, et al., 2011; McCallum, Kim, & Richardson, 2010; Pattwell et al., 2011; Pattwell et al., 2012), such that adolescents continue to show signs of defensive responding (freezing in rodents and skin conductance response in humans) to previously learned threat

cues during fear extinction, suggesting a failure to update previous threat associations as safe (Kim, Li, et al., 2011; Pattwell et al., 2012). Immunohistochemical evidence in rodents points to reduced synaptic plasticity in the vmPFC during adolescence (Kim, Li, et al., 2011; Pattwell et al., 2012). Whilst blunted fear extinction has been shown to be evolutionarily conserved across species behaviourally in rodents and humans (Kim, Li, et al., 2011; Pattwell et al., 2012), the neural recruitment of fear extinction in adolescent humans has yet to be examined.

Immature amygdala-prefrontal cortical interactions are thought to be responsible for this blunted fear extinction seen in adolescence (Hare & Casey, 2005; Nelson et al., 2014; Pfeifer & Allen, 2012). A large body of research has shown fear extinction circuitry such as the amygdala and vmPFC to undergo substantial developmental change in structure. The amygdala shows steady linear increases of grey matter growth across late childhood and adolescence, whilst the vmPFC is characterised by quadratic change, such that substantial grey matter growth occurs across childhood and grey matter pruning across adolescence (Gogtay et al., 2004; Østby et al., 2009; Shaw et al., 2008; Wierenga et al., 2014). Furthermore, the uncinate fasciculus, a white matter tract that connects the amygdala and vmPFC, has a protracted growth across adolescence and into early adulthood (Giorgio et al., 2008; Tamnes et al., 2010). No study to date has examined the relationship between age-related structural changes and functioning of fear extinction circuitry in human adolescents.

4.2.2 Individual differences in anxious disposition upon function and structure of fear extinction circuitry

Questions also remain on how individual differences in anxious disposition shape the function and structure of fear extinction circuitry during development. A few cross-sectional studies of fear acquisition in adolescence have shown trait anxious youth to display more amygdala activation and startle responsivity to safety cues (Haddad et al., 2015; Haddad et al., 2012; Kadosh et al., 2015). No fMRI findings have yet been reported on the role of anxious disposition and development during fear extinction. In Chapters 2 & 3, we showed that adult individual differences in IU specifically predicted fear extinction capacity, over trait anxiety and worry. More specifically, high IU was associated with exaggerated fear responding to both threat and safety cues during extinction. It is unknown whether high IU during adolescence predicts similar fear extinction outcomes to that of high IU during adulthood.

Structural abnormalities in fear extinction circuitry, such as reduced grey matter volume in the vmPFC and weaker integrity of the uncinate fasciculus have been frequently reported in adults with anxious disposition and anxiety disorders (Baur et al., 2012; Kim & Whalen, 2009; Phan et al., 2009; Shang et al., 2014; Soliman et al., 2010; Tromp et al., 2012). While the number of studies examining structural changes in anxious vs. non-anxious adolescents is limited, a handful of studies have suggested similar structural abnormalities in ventral portions of the prefrontal cortex and the uncinate fasciculus for anxious youth (Liao et al., 2014; Mueller et al., 2013; Newman et al., 2015; Strawn et al., 2015; Strawn, Wehry, et al., 2013). Findings in the amygdala are less clear,

with some studies reporting larger volumes in the right and left amygdala, specifically the basolateral amygdala (De Bellis et al., 2000; Qin et al., 2014), smaller volumes in the left amygdala (Blackmon et al., 2011; Milham et al., 2005; Mueller et al., 2013) or no difference in volume of the amygdala (Strawn, Chu, et al., 2013). There is still debate over when structural abnormalities emerge, either prior anxiety disorder onset or as a consequence of anxiety disorder onset.

4.2.3 Design and predictions

The age-related functional and structural evidence outlined above, combined with the frequently reported onset of anxiety (Kessler et al., 2005), suggest adolescence to be an important and vulnerable window of fear extinction circuitry development. Given on-going development of fear extinction circuitry, adolescent populations may be less responsive to traditional forms of anxiety disorder treatment that are based upon fear extinction models such as exposure therapy (Cartwright-Hatton et al., 2004; Johnson & Casey, 2015; Rapee et al., 2009). Therefore, examining how individual differences in developmental stage and anxious disposition (particularly IU) predict function and structure of fear extinction circuitry may further our understanding of how fear extinction processes develop. To address these questions, in the current study, we used a cued conditioning paradigm with event-related functional magnetic resonance imaging (fMRI) and behavioural measures (identical to that previously used in Chapters 2 & 3). In addition, we collected structural magnetic resonance imaging (sMRI) and diffusion tensor imaging (DTI) data. We used an

aversive sound as an unconditioned stimulus and visual shapes as conditioned stimuli. CS-US pairings were 100% reinforced during fear acquisition and extinction to match that of developmental animal literature (Kim et al., 2009; Kim, Li, et al., 2011; McCallum et al., 2010; Pattwell et al., 2011; Pattwell et al., 2012).

We hypothesised that younger age would predict blunted extinction. We expected this effect to be indexed by greater amygdala activation and reduced vmPFC recruitment during fear extinction, accompanied by elevated psychophysiology and behavioural responses to learned threat vs. safety cues. In addition, based on our previous findings in Chapters 2 & 3, we hypothesised that high IU would be associated with generalised fear expression to both learned threat and safety cues, and/or sustained fear expression to learned threat cues. We predicted larger responses in behaviour, psychophysiology and amygdala, as well as reduced vmPFC activation to learned threat vs. safety cues during extinction in high IU individuals. Furthermore, we expected age-related changes in: (1) structural integrity of the uncinate fasciculus and, (2) grey matter probability in the amygdala and vmPFC to predict amygdala activation during fear extinction to threat vs. safety cues. We also examined relationships between structural changes in the uncinate fasciculus, amygdala and vmPFC in relation to IU. In line with our prior work (Chapters 2 & 3), we also examined the time course (early vs late) of extinction, but we did not have specific predictions regarding the temporality of effects during fear extinction in this developmental sample.

4.3 Method

4.3.1 Participants

55 right-handed volunteers took part in this study (M age = 17.75yrs, SD age = 3.65yrs, range = 12-28yrs; 35 females & 20 males). The sample size was based on previous fMRI studies conducted on developmental samples (Hare et al., 2008; Swartz et al., 2014) and power analysis guidelines for fMRI (Mumford, 2012). All participants had normal or corrected to normal vision. Adult participants provided written informed consent, adolescent participants provided written informed assent and parental/guardian consent, and received a picture of their brain and £20 for their participation. The procedure was approved by the University of Reading Ethics Committee.

4.3.2 Conditioning task

We used the same fear conditioning experiment as in Chapter 2 (Please see Chapter 2, Methods, Conditioning task and Figure 3). Visual stimuli were presented through MRI-compatible VisualSystem head-coil mounted eye goggles (Nordic Neuro Lab, Bergen, Norway), which displayed stimuli at 60 Hz on an 800 × 600 pixel screen. Sound stimuli were presented through MRI-compatible AudioSystem headphones (Nordic Neuro Lab, Bergen, Norway). Participants used an MRI-compatible response box with their dominant right hand to respond.

4.3.3 Procedure

Participants arrived at the laboratory and were informed of the experimental procedures. First, participants (and parents/guardians) completed consent forms as an agreement to take part in the study. Second, a hearing test was performed with an audiometer to check for normative hearing (e.g.500-8000 Hz, below 30 dB). Third, participants completed a battery of cognitive tasks (results not reported here) and questionnaires on a computer outside of the scanner. Next, participants were taken to the MRI unit. We used a conditioning task inside the scanner, whilst concurrently recording ratings, electrodermal activity and pupil dilation. Participants were simply instructed to: (1) maintain attention to the task by looking and listening to the coloured squares and sounds presented, (2) respond to the uneasiness scale using the button box and (3) to keep as still as possible. After scanning, participants rated the sound stimulus outside of the scanner.

4.3.4 Questionnaires

The same questionnaires were presented from Chapter 2 (see Chapter 2, Methods, Questionnaires). We focused on IU because of previous findings from Chapters 2 & 3. Similar distributions and internal reliability of scores were found for the anxiety measures, IU (M = 59.56; SD = 18.35; range = 31-105; α = .93), STAIX-2 (M = 43.15; SD = 9.73; range = 26-75; α = .90) and PSWQ (M = 46.27; SD = 11.91; range = 23-71; α = .90).

4.3.5 Sound stimulus rating

Participants rated the valence and arousal of the sound stimulus using 9 point Likert scales ranging from 1 (Valence: negative; Arousal: calm) to 9 (Valence: positive; Arousal: excited).

4.3.6. Behavioural data scoring and reduction

Rating data were reduced for each subject by calculating their average responses for each experimental condition using the E-Data Aid tool in E-Prime (Psychology Software Tools Ltd, Pittsburgh, PA).

4.3.7 Physiological data acquisition and reduction

Skin conductance recordings were obtained using AD Instruments (AD Instruments Ltd, Chalgrove, Oxfordshire) hardware and software. An ML138 Bio Amp connected to an ML870 PowerLab Unit Model 8/30 amplified the electrodermal activity signal, which were digitised through a 16-bit A/D converter at 1000 Hz. Skin conductance was measured during the fMRI scanning with MRI-compatible MLT117F silver/silver chloride bipolar finger electrodes that were attached to the distal phalanges of the index and middle fingers of the left hand. A constant voltage of 22mVms at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp.

Skin conductance responses (SCR) were scored when there was an increase of skin conductance level exceeding 0.03 microSiemens. The amplitude of each response was scored as the difference between the onset and the maximum deflection prior to the signal flattening out or decreasing.

SCR onsets had to be within 7 seconds following each trial to be included. We used an extended SCR scoring window because the temporal signature of an aversive sound US may be more ambiguous than a traditional electric shock US, this SCR scoring window length is in line with previous fear conditioning studies that have used aversive sound stimuli as the US in both adults (Büchel et al., 1998; Soliman et al., 2010) and adolescents (Pattwell et al., 2012).

Trials with no discernible SCRs were scored as zero. The first trial of each experimental phase was excluded, to reduce contamination of averages from the orienting response typically seen at the start of a session. SCR amplitudes were square root transformed to reduce skew. Trials with motion artefacts were discarded from the analysis. SCR magnitudes were calculated from remaining trials by averaging SCR square root transformed values and zeros for each condition.

Pupil dilation was recorded at a sample rate of 60 Hz through a built-in infrared camera on the head-coil mounted eye goggles (Nordic Neuro Lab, Bergen, Norway). Pupil dilation data was averaged for each 1000 ms window following stimulus onset, resulting in 5 windows of 1000 ms each. These data were baseline corrected by subtracting 1000 ms preceding each stimulus onset from a blank screen. Trials were averaged per condition and time window for each participant.

4.3.8 Learning assessment

We used the same method as in Chapters 2 & 3. The conditioned response score was the first 2 CS+ trials – the first 2 CS- trials. A positive score

indicated a larger response for CS+ vs. CS-, indexing successful conditioning. To reduce subject attrition, we labelled subjects as learners if they had a positive conditioned response score for any measure (e.g. SCR, Pupil dilation and ratings). Based on the learning assessment criterion, we identified 11 potential non-learners out of the 52 participants. Since removing the data of these 11 subjects did not change the results reported here[3], we retained the data of all participants.

4.3.9 MRI

Participants were scanned with a 3T Siemens Trio set up with a 12 channel head coil (Siemens Inc., Erlangen, Germany). Three T2*-weighted echo planar imaging (EPI) functional scans were acquired for each phase of the conditioning task consisting of 161, 208, and 380 volumes respectively (TR = 2000 ms, TE = 30 ms, flip angle = 90°, FOV = 192 × 192 mm, 3 × 3 mm voxels, slice thickness 3 mm with an interslice gap of 1 mm, 30 axial slices, interleaved acquisition).

Following completion of the functional scans, fieldmap and structural scans were acquired, which comprised of a high-resolution T1-weighted anatomical scan (MP-RAGE, TR = 2020 ms, TE = 2.52 ms, flip angle = 90°, FOV = 256 × 256 mm, 1 x 1 x 1 mm voxels, slice thickness 1 mm, sagittal slices), two fieldmaps (TR = 488 ms, TE 1 = 4.98 ms, TE 2 = 7.38 ms, flip angle

[3] Age-related functional and structural findings did not change when non-learners were removed: Interaction between Condition x Age, $F(1,37) = 5.592$, $p = .023$ without non-learners. Significant quadratic correlation with early CS+ - CS- trials and age, $p = .008$ without non-learners. Significant correlation of vmPFC grey matter probability with age, $r(37) = -0.48$, $p = .002$. vmPFC grey matter probability significantly correlated with bilateral amygdala activity to the CS+ vs. CS- during late extinction, $r(37) = .327$ $p = .042$.

= 60°, FOV = 256 × 256 mm, slice thickness 4 mm with an interslice gap of 4 mm, 30 axial slices) and diffusion weighted images (TR = 6800ms, TE = 93 ms, flip angle = 60°, FOV = 192 × 192 mm, slice thickness 2 mm with an interslice gap of 2 mm, *b*-value =1000, 64 axial slices, 30 diffusion gradients (not analysed here).

4.3.10 fMRI data acquisition and analysis

FMRI analyses were carried out in Feat version 5.08 as part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Brains were extracted from their respective T1 images by using the FSL Brain Extraction Tool (BET) (Smith, 2002). Distortion, slice timing and motion correction were applied to all extracted EPI volumes using FUGUE and MCFLIRT tools (Jenkinson et al., 2002). Gaussian smoothing (FWHM 5mm) and a 50 second high pass temporal filter were applied.

A first-level GLM analysis was carried out for each functional scan run from each learning phase. Separate regressors were specified for the experimental conditions of primary interest in each learning phase (acquisition: CS+/CS-, extinction: CS+ /CS) by convolving a binary boxcar function with an ideal haemodynamic response (HR), which corresponded to the length of each trial (1500 ms). Regressors for the uneasiness rating period, six motion parameters and any head movements above 1mm were included to model out brain activity or movement artefacts that were unrelated to the conditions of interest.

We defined two main effect contrasts to reveal fear extinction-related activity. To examine temporal effects across extinction, we contrasted (CS+ vs. CS-)$_{EARLY}$> (CS+ vs. CS-)$_{LATE}$. We defined early extinction as the first eight trials for CS+ and CS- and the last eight trials for CS+ and CS-. We also examined the overall effect of CS+ vs. CS- during extinction. All contrasts were normalised and registered to MNI standard space using FLIRT (Jenkinson et al., 2002). Second-level GLM analysis consisted of regressors for the group mean and demeaned age (days) using FSL's FLAME stage 1 + 2 procedure. Whole-brain analysis was carried out using cluster thresholding with a $z = 2.3$ and a corrected $p < 0.05$.

We were specifically interested in the extent to which age would be associated with the BOLD response in our apriori regions of interest for fear extinction. Therefore, we extracted mean percent BOLD signal change across voxels from the left amygdala, right amygdala and vmPFC on the (CS+ vs. CS-)$_{EARLY}$> (CS+ vs. CS-)$_{LATE}$ and CS+ vs. CS- extinction contrast maps. We created cluster masks of the left amygdala, right amygdala and vmPFC using co-ordinate foci from past (n of studies = 9) fMRI and PET fear extinction studies (Barrett & Armony, 2009; Büchel et al., 1998; Knight et al., 2004; LaBar et al., 1998; Linnman et al., 2012; Milad et al., 2007; Phelps et al., 2004; Sehlmeyer et al., 2011; Soliman et al., 2010), as well as coordinates from Chapter 3. For each cluster mask, we specified previous study foci', a false discovery rate of $p < .05$ and minimum cluster size of 200mm^3 in GingerALE . Clusters were transformed to MNI standard space using FLIRT (Jenkinson et al., 2002). Resulting amygdala clusters (left: -23, -4, -20, right: 23, -4, -20) were

354mm^3. We expanded the vmPFC cluster to be more inclusive by taking the centre point (2, 38, -14) and applying a 10mm radius sphere (515 mm$^{3)}$. For control purposes we also extracted mean percent BOLD signal change in our apriori regions of interest on the CS+ vs. CS- acquisition contrast map.

4.3.11 White matter integrity of the uncinate fasciculus

Diffusion weighted image processing in FSL included corrections for motion, eddy currents and inhomogeneity's in the magnetic field. Then the tensor model was fitted using FDT (FMRIBS Diffusion Toolbox) in order to calculate FA values for each voxel, producing one FA image per subject. Voxels with FA values lower than 0.2 were removed. We created 25% probability masks of the left and right uncinate fasciculus (Swartz et al., 2014) from the JHU white-matter tractography atlas (Mori, Wakana, Van Zijl, & Nagae-Poetscher, 2005). These tract masks were transformed into diffusion space and applied to each subjects' FA image, resulting in an FA value for each tract per subject.

4.3.12 Grey matter probability in the vmPFC and amygdala

Processing of structural images was performed in FSL. First, structural images were brain-extracted using BET (Smith, 2002). Secondly, structural images were segmented based on tissue-type using FMRIB's Automated Segmentation Tool (FAST) (Zhang, Brady, & Smith, 2001). Thirdly, the left and right amygdala and vmPFC masks (same as outlined above) were transformed into structural space for each subject. Lastly, we extracted grey matter

probability estimates of the left and right amygdala and vmPFC from each subjects' segmented structural image.

4.3.13 Statistical analyses

Main effects of conditioning and interactions with age and IU during fear extinction were assessed by conducting a Condition (CS+, CS-) x Time (Early, Late) x Age (days) and a condition (CS+, CS-) x Time (Early, Late) x IU repeated measures ANCOVA on behavioural ratings, skin conductance, pupil dilation and percent BOLD signal change in the amygdala and vmPFC. The early part of extinction was defined as the first eight CS+ and eight CS- trials, and the last part of extinction was defined as the last eight CS+ and eight CS- trials. Because age effects during fear extinction may be non-linear, we conducted curve estimation using quadratic fits on fear extinction difference scores (CS+ - CS- early; CS+ - CS- late; CS+ - CS- across extinction) and age (days).

To check for specificity of findings in extinction, we conducted a Condition (CS+, CS-) x Age and a Condition (CS+, CS-) x IU repeated measure ANCOVA on behavioural ratings, skin conductance, pupil dilation and percent BOLD signal change in the amygdala and vmPFC in the acquisition phase.

We correlated grey matter probability values of the left and right amygdala and vmPFC, as well as the FA values in the uncinate fasciculus with age (days), IU and the left and right amygdala percent BOLD signal difference scores from extinction. To assess the specificity of age and structure predicting amygdala function during extinction, we performed hierarchical regression

analyses on the amygdala response difference scores during extinction that showed significant relationships with both age and structure. We entered age in the first step and structure in the second step.

4.4 Results

Three participants did not complete the scanning procedure and three participants were removed due to excessive head movements (> 3mm), leaving forty-nine participants for analysis (M age = 18.70yrs, SD age = 3.64yrs, range = 12-28yrs; 31 females & 18 males).

4.4.1 Questionnaires

The anxiety measures were positively correlated with each other, suggesting shared variance, IU with PSWQ, $r(47) = .635$, $p < .001$, IU with STAIX-2, $r(47) = .655$, $p < .001$, and PSWQ with STAIX-2, $r(47) = .772$, $p < .001$. There were no significant linear or quadratic relationships between IU and age, p's > .2. No significant linear or quadratic relationships between the anxiety measures and the dependant measures were found, p's > .1.

4.4.2 Ratings

All subjects rated the sound stimulus as aversive and moderately arousing. Sound arousal ratings negatively correlated with age, $r(47) = -.286$, $p = .047$, such that the youngest individuals rated the sound as more arousing than the older individuals. Sound valence ratings did not correlate with age, $r(47) = -.141$, $p = .333$.

During extinction, participants significantly reported feeling more uneasy to the CS+ vs. CS- trials across extinction, $F(1,47) = 5.094$, $p = .029$, suggesting the US-CS contingency had been learned (see Table 5). In addition, participants also reported feeling more uneasy at the start of extinction, compared to the end of extinction $F(1,47) = 6.875$, $p = .012$. Contrary to predictions, there was no interaction of Condition x Time, $F(1,47) = 1.004$, $p = .322$.

In the acquisition phase, participants significantly reported feeling more uneasy for the CS+ vs. CS- trials, $F(1,47) = 72.123$, $p < .001$ (see Table 5).

Results revealed no age or IU differences for uneasiness ratings in any of the experimental phases, max $F = 3.953$.

4.4.3 SCR magnitude

No significant main effects or interactions were found in extinction, max $F = 2.053$ (see Table 5).

During acquisition, SCR magnitude was significantly larger for the CS+ vs. CS- trials, $F(1,33) = 27.796$, $p < .001$ (see Table 5).

Results revealed no age or IU differences for SCR magnitude in any of the experimental phases, max $F = 2.353$.

4.4.4 Pupil dilation

Pupil dilation data could not be collected from twelve subjects due to problems calibrating the goggles with participants, leaving thirty-seven subjects

with usable pupil dilation data. No significant main effects or interactions were found in acquisition or extinction, max $F = 2.098$ (see Table 5).

Table 5.

Summary of means (SD) for each dependent measure as a function of condition and phase.

	Acquisition		Extinction		Early Extinction		Late Extinction	
Measure	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Physiological								
Square root transformed SCR magnitude (μS)	.27 (.21)[b]	.13 (.13)[a]	.15 (.11)	.13 (.14)	.14 (.12)	.12 (.15)	.13 (.13)	.12 (.14)
Pupil dilation (Δmm)	-.010 (.017)	-.014 (.026)	-.007 (.029)	-.011 (.022)	-.009 (.029)	-.014 (.029)	-.005 (.032)	-.007 (.022)
Behavioural								
Uneasiness rating (1-9)	3.36 (2.03)[b]	1.44 (1.58)[a]	1.04 (1.16)[d]	.92 (1.14)[c]	1.15 (1.26)	1.00 (1.21)	.93 (1.13)	.84 (1.12)

Note: SCR magnitude (μS), skin conductance magnitude measured in microSiemens. Pupil dilation (Δmm) measured in delta millimeters. Significant comparisons are specified with letters = Acquisition CS+, a; Acquisition CS-, b; Extinction CS+, c; Extinction CS-, d.

4.4.5 fMRI

The whole-brain analyses did not yield significant BOLD differences in our a-priori brain regions of interest often reported in the extinction literature (Büchel et al., 1998; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004). However, the CS+ > CS-)$_{EARLY}$ > (CS+ > CS-)$_{LATE}$ contrast map revealed a left amygdala cluster (voxels = 77, max Z = 3.1, x = -22, y = 0, z = -26) at subthreshold, z = 2.0, p =.045, suggesting a conditioned response to the CS+ vs. CS- during early extinction. In addition, we found greater occipital pole activation in early extinction for the CS+ > CS-, relative to late extinction for the CS+ > CS-, suggesting increased attention for the conditioned stimulus (see Table 6).

In the amygdala ROI during extinction, we found an interaction between Condition x Time, $F(1,47) = 3.498$, $p = .068$ (collapsed left and right amygdala as CS+ - CS- difference scores significantly correlated ,$r(47) = .45$ $p < .001$). Amygdala activation did not change as a function of time for the CS+, $p = .826$, but it did for the CS- at trend, $p = .083$. As expected, there was an interaction between Condition x Age, $F(1,47) = 5.519$, $p = .023$, such that younger age predicted greater amygdala activity to the CS+ vs. CS- (see Figure 9).[4] No other significant main effects or interactions were found for the amygdala during fear extinction, max $F = .666$.

In the vmPFC ROI during extinction, there was a significant negative quadratic correlation with early CS+ - CS- trials and age, $p = .006$, such that mid-

[4] Whole-brain analysis of the (CS+ > CS-) *decreasing age contrast map revealed bilateral amygdala clusters (Left amygdala, voxels = 133, max Z = 3.41, x = -30, y = -16, z = -6; Right amygdala, voxels = 133, max Z = 3.01, x = 30, y = -6, z = -8) at sub-threshold, z = 2.0, p =.045.

adolescents exhibited blunting of vmPFC activity to the CS+ (see Figure 10)[5]. In addition, we observed a significant linear interaction between Time x Age, $F(1,47) = 6.131$, $p = .017$, such that older age predicted greater vmPFC activation during late extinction. No other significant main effects or interactions were found for the vmPFC during fear extinction, max $F = 3.001$.

During fear acquisition, amygdala activation was significantly larger to the CS+ vs. CS-, $F(1,47) = 12.399$, $p = .001$. All other effects or interactions during fear acquisition in the amygdala and vmPFC were non-significant, max $F = 3.776$.

[5] Whole-brain analysis of the $(CS+ > CS-)_{EARLY}$ x $(CS+ > CS-)_{LATE}$ *increasing age contrast map revealed a dorsal medial prefrontal cortex cluster (voxels = 332, max Z = 3.63, x = -2, y = 56, z = 18 at subthreshold, z = 2.0, p =.045.

Table 6
Regional activation patterns in response to stimuli presented during fear extinction

Phase	Brain region	BA	Voxels (mm^3)	Max Z	Location of max Z		
					x	y	z
Extinction							
CS- > CS+	Cingulate Gyrus, Precuneus Cortex,	7,24	825	3.63	2	-48	12
$(CS+ > CS-)_{EARLY} > (CS+ > CS-)_{LATE}$	R Occipital Pole	17	315	4.34	28	-96	-4
$(CS- > CS+)_{EARLY} > (CS- > CS+)_{LATE}$	L Occipital Pole	17	260	3.91	-28	-96	-6

Note: Corrected cluster for multiple comparisons at $p < 0.05$. BA = Brodmann Areas. Location of cluster's maximum Z are in MNI space. R = right; L = left.

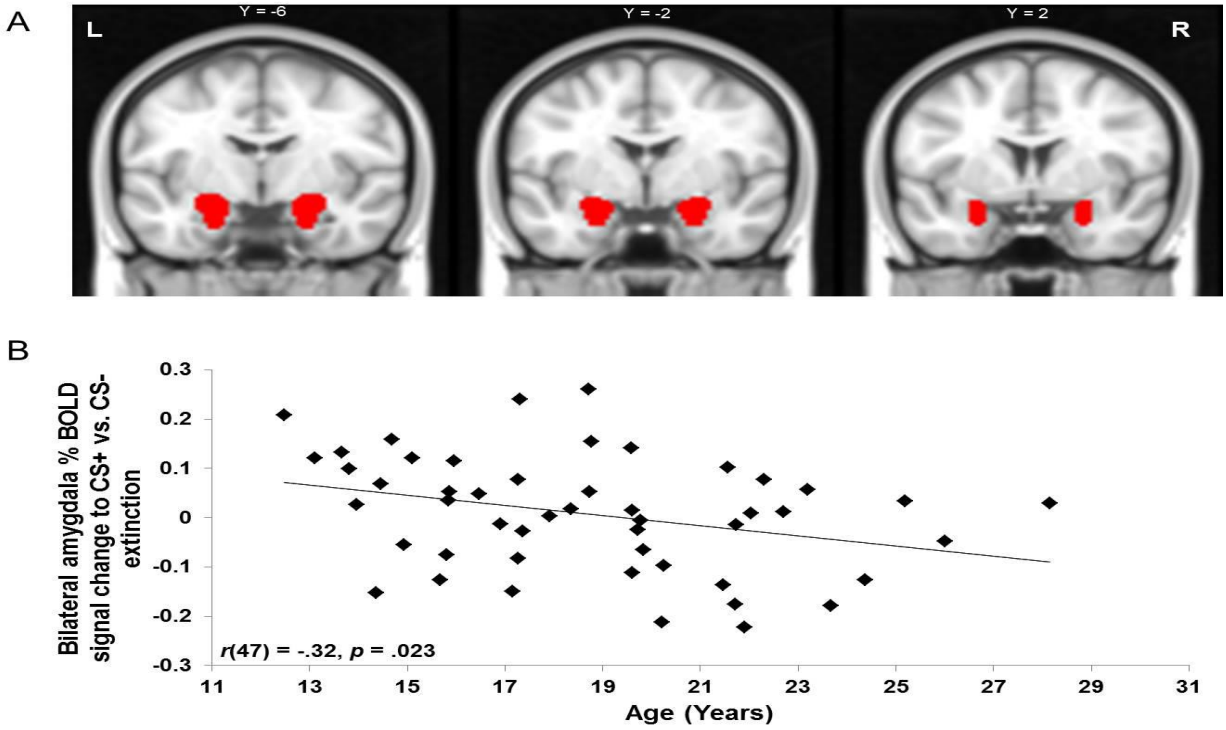

Figure 9. A: Bilateral amygdala region of interest. B: Younger age is significantly associated with greater bilateral amygdala activation to the CS+ vs. CS- across the fear extinction phase. R, right; L, left. Age, measured in days age is in years for display purposes only).

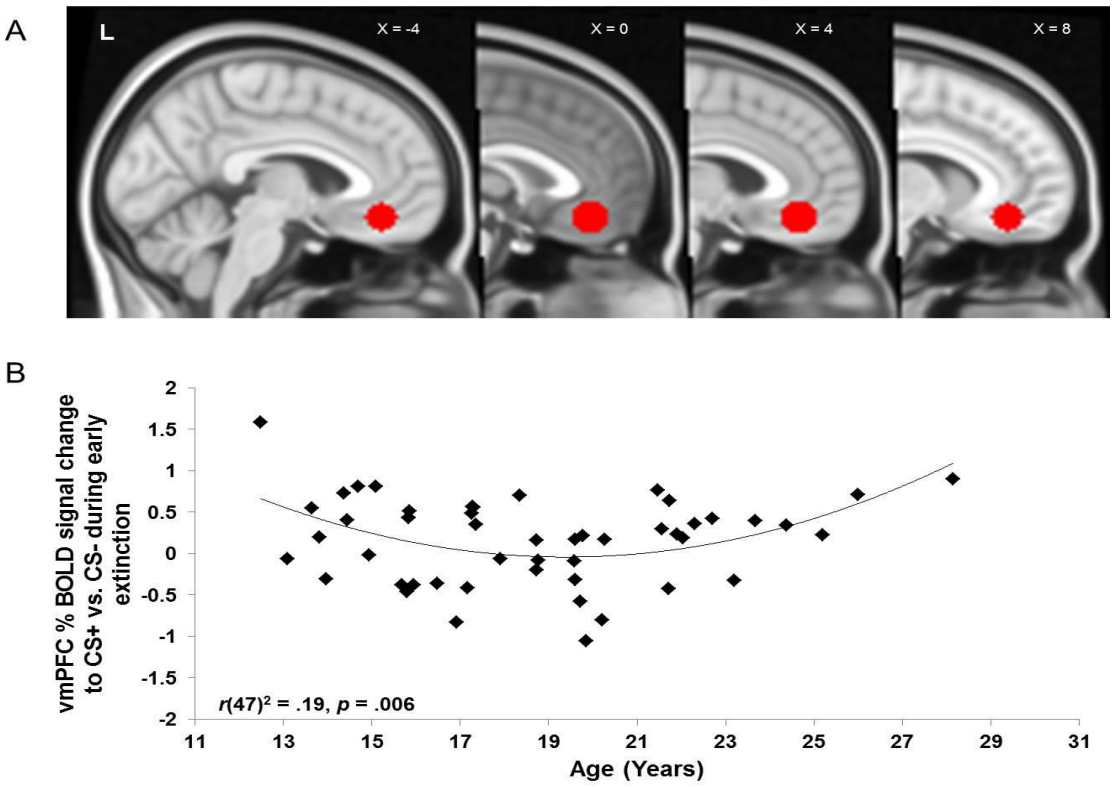

Figure 10. A: vmPFC region of interest. B: Significant quadratic correlation between age and vmPFC response to the CS+ vs. CS- during fear extinction, such that mid-adolescents was associated with reduced vmPFC activation to the CS+ vs. CS- during early fear extinction. R, right; L, left. Age, measured in days (age is in years for display purposes only).

4.4.6 White matter integrity of the uncinate fasciculus

Age effects. In line with predictions, we found positive correlations with age and structural integrity of the bilateral uncinate fasciculus (the left and right significantly correlated, $r(47) = .73$, $p < .001$, thus we collapsed across), $r(47) = .30$, $p = .035$, suggesting increased white matter integrity of this tract with age.

Structure-Function relationships. Greater structural integrity of the bilateral uncinate fasciculus predicted at trend increased bilateral amygdala activity to CS+ vs. CS- during early extinction, $r(47) = .24$ $p = .097$, and reduced bilateral amygdala activity to CS+ vs. CS- during late extinction, $r(47) = -.24$ $p = .084$.

Structure-IU relationships. IU did not significantly correlate with the bilateral uncinate fasciculus, $r(47) = -.012$, $p = .933$.

4.4.7 Grey matter probability in the vmPFC and amygdala

Age effects. As expected, we found a significant negative correlation of vmPFC grey matter probability with age, $r(47) = -0.54$, $p < .001$, suggesting greater grey matter thinning with age (see Figure 11). Furthermore, we found a significant positive correlation of bilateral amygdala (the left and right significantly correlated $r(47) = .71$, $p < .001$, thus we collapsed across left and right) grey matter probability with age, $r(47) = .29$, $p = .041$, suggesting steady linear growth with age.

Structure-Function relationships. We found no relationship between vmPFC grey matter probability and bilateral amygdala activity to the CS+ vs. CS- during early extinction, $r(46) = -.16$ $p = .271$. However, we found vmPFC grey matter probability to significantly predict bilateral amygdala activity to the

CS+ vs. CS- during late extinction, $r(47) = .34$ $p = .017$ (see Figure 11), suggesting that less vmPFC grey matter probability, indicative of grey matter thinning, is associated with reduced bilateral amygdala response to the CS+ vs. CS- during late extinction. Grey matter probability within the amygdala masks did not predict amygdala BOLD signal to CS+ vs. CS- in either early or late extinction, *p's* > .6.

Structure-IU relationships. IU did not significantly correlate with any of the grey matter ROI's, *p's* > .4.

4.4.8 Hierarchical regression of predictors of amygdala response

A hierarchical regression analysis on the predictors of amygdala response during late extinction suggested no specificity of age and vmPFC grey matter probability: step one age, R^2=.093, $F(1,47) = .4.822$, p= .033, and adding vmPFC grey matter probability in step two, ΔR^2=.136, $F(1,46) = 2.300$, p= .136.

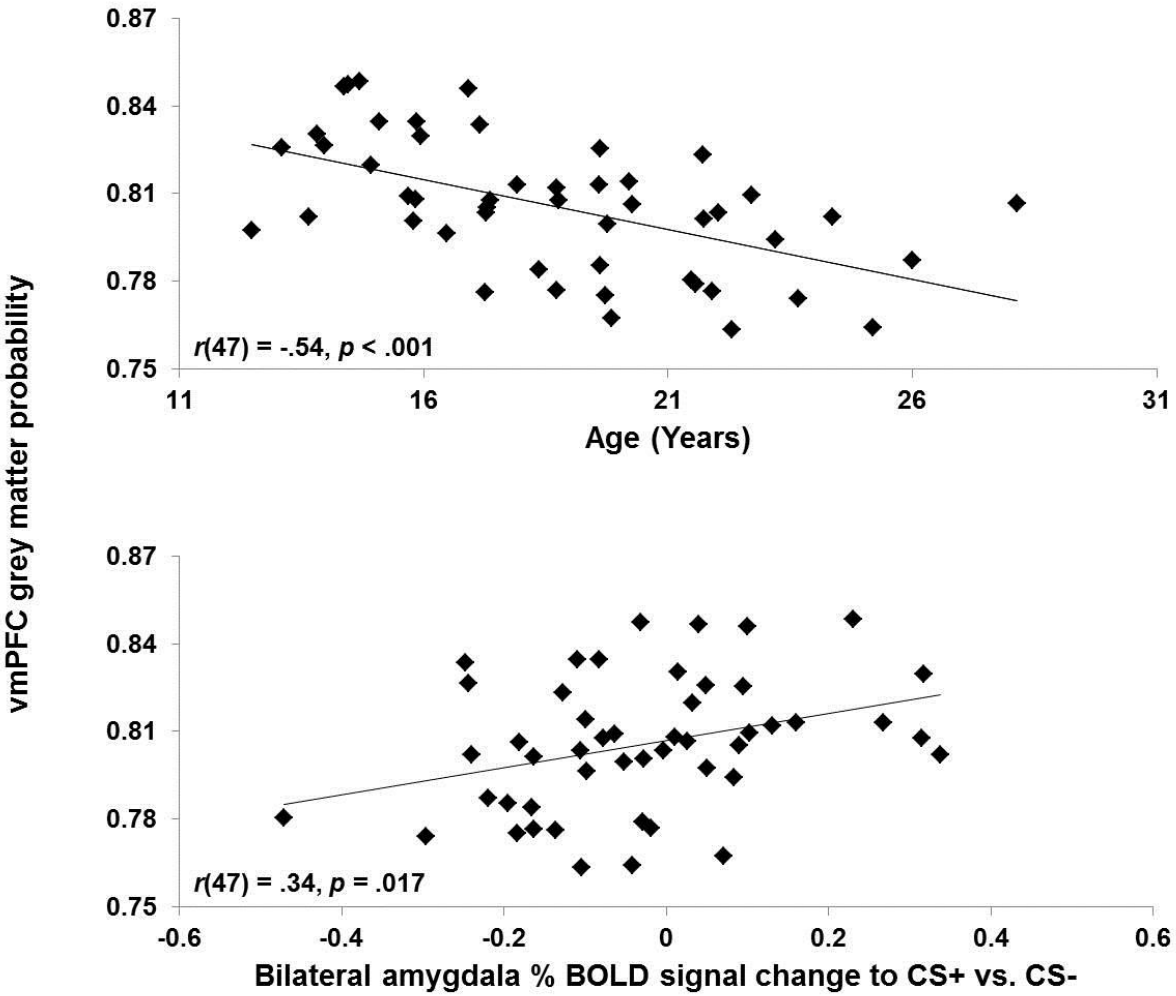

Figure 11. Top: Grey matter probability within the vmPFC significantly predicts age, such that older age is associated with reduced grey matter in the vmPFC, indicative of grey matter thinning. Bottom: reduced grey matter probability within the vmPFC significantly predicts less amygdala activity to CS+ vs. CS- during late fear extinction.

4.5 Discussion

In the current study, we show that human development during adolescence and early adulthood, predicts amygdala and vmPFC recruitment during fear extinction. Our data suggest that younger age, particularly mid-adolescence, is associated with blunted fear extinction, through reduced vmPFC and prolonged amygdala recruitment to learned threat vs. safety cues. Furthermore, as well as age, vmPFC grey matter probability was associated with continued responding in the amygdala to learned threat vs. safety cues during late extinction. However, we found no significant relationships between IU and functional and structural correlates of fear extinction circuitry.

4.5.1 Age-related changes in function and structure of fear extinction circuitry

Across fear extinction, younger age was characterised by increased amygdala activity to learned threat vs. safety cues, consistent with previous rodent and human fear extinction studies (Kim, Li, et al., 2011; Pattwell et al., 2011; Pattwell et al., 2012), suggesting exaggerated fear expression during the period of adolescence. Furthermore, younger age was quadratically associated with reduced vmPFC activity to learned threat vs. safety cues during early extinction, in line with previous rodent work (Kim, Li, et al., 2011; Pattwell et al., 2011; Pattwell et al., 2012), suggesting compromised fear inhibition during adolescence. Crucially, reduced vmPFC activity was observed during mid to late adolescence (e.g. 14-20 yrs), coinciding with the frequently reported age of anxiety disorder onset (Kessler et al., 2005). These data suggest that this group

of adolescents may be susceptible to impaired fear extinction, and perhaps the least responsive to current exposure-based therapies.

Age predicted structure of fear extinction circuitry. In line with prior structural work (Giedd, 2004; Gogtay et al., 2004; Lebel & Beaulieu, 2011; Lebel et al., 2008; Østby et al., 2009; Tamnes et al., 2010; Wierenga et al., 2014), younger age predicted less grey matter thinning in the vmPFC, steady grey matter growth within the bilateral amygdala and less thickening of the uncinate fasciculus. These structural data suggest our experimental sample were typical of a developmental cohort.

Alongside age, structural changes within the vmPFC and uncinate fasciculus both were associated with amygdala response during fear extinction. Notably, reduced grey matter probability in the vmPFC, indicative of grey matter thinning, is associated with reduced amygdala response to the learned threat vs. safety cues during late extinction. Similarly, stronger structural integrity of the uncinate fasciculus was associated with reduced amygdala response to learned threat vs. safety cues during late extinction, however this effect was at trend. We did not find grey matter probability in the amygdala masks to predict amygdala activity during extinction. From these results we can speculate that grey matter pruning in the vmPFC may be more predictive of blunted fear extinction during the adolescent period, over white matter thickening of the uncinate fasciculus and grey matter growth in the amygdala. However, both age and grey matter probability in the vmPFC was associated with amygdala response and there was no specificity for either measure. Future work with longitudinal designs may be able to elucidate the relationship between fear

extinction ability and vmPFC grey matter probability further by tracking the extent of thinning in the vmPFC across development.

4.5.2 Individual differences in IU and fear extinction

We did not find individual differences in IU (or STAI and PSWQ) to predict any dependent measure. The lack of IU effects could be due to: (1) weak statistical power i.e. too much variability within this sample due to the age range tested, and (2) developmental effects curbing or shielding IU-related fear extinction behaviour. Having said this, IU effects have been observed during adolescence in decision making tasks (Krain et al., 2008; Krain et al., 2006). However, decision making and fear conditioning tasks are not directly comparable and call upon different neural circuitry (with some overlap in the amygdala and vmPFC). Questions remain on how threat generalisation and deficient safety learning behaviours associated with high IU emerge during development. This question may be better assessed with: (1) large cross-sectional designs with less developmental variability (e.g. smaller age ranges), or (2) with longitudinal designs where participants are first tested as adolescents and followed up as adults, or tested repeatedly across adolescence.

4.5.3 Limitations

We found no evidence of age predicting differential recruitment of brain regions involved in fear acquisition for the learned threat and safety cues. However, we used a 100% reinforcement schedule in the acquisition phase,

similar to previous rodent work (Kim, Li, et al., 2011; Pattwell et al., 2011; Pattwell et al., 2012), where the CS+ and US are confounded.

We were unable to demonstrate main effects of conditioning in psychophysiological measures during extinction. This is most likely due to the scanning environment, which induces: (1) distortions upon psychophysiological signals, increasing the number of non-responders and decreasing the sensitivity of the measures, and (2) noise that may reduce the aversiveness of the CS (sound stimulus).

4.5.4 Conclusions

To conclude, we found that younger age was associated with blunted fear extinction. Younger age was associated with exaggerated amygdala response to learned threat vs. safety cues during fear extinction. Mid to late adolescence was associated with reduced vmPFC activity during early extinction. Furthermore, more grey matter probability within the vmPFC, is associated with continued responding in the amygdala to learned threat vs. safety cues during late extinction. These findings suggest that compromised amygdala-vmPFC recruitment during adolescence (particularly in mid-adolescence) may be explained by both age and age-related changes in structure of fear extinction circuitry.

5. General discussion

5.1 Overview

This body of work examined individual differences in development and anxious disposition on fear generative and regulatory processes during fear extinction. To assess these processes, we used an adapted fear conditioning experiment in combination with measurements of behaviour, psychophysiology and functional/structural MRI in adult and adolescent participants. We focused on a crucial but simplified brain model of fear extinction circuitry, comprising the coupling of the amygdala and vmPFC. We selected fear conditioning as our main experimental paradigm on the basis of it being adaptable for developmental samples and clinically relevant. During fear extinction, we measured and operationalised: (1) fear generative processes through behavioural, psychophysiological, and neural responses to learned threat and safety cues, and (2) fear regulatory processes by quantifying the reduction of behavioural, psychophysiological, and neural responses to learned threat and safety cues over time.

Firstly, we hypothesised that we could achieve successful fear conditioning in both adults and adolescents using a developmentally appropriate design with an aversive sound as the US (rather than a traditional shock as the US), similar to other developmental fear conditioning studies (Johnson & Casey, 2015; Lau et al., 2011; Lau et al., 2008b; Neumann et al., 2008; Pattwell et al., 2012). Secondly, based on the developmental literature, we hypothesised that age and age-related structural changes in fear extinction

circuitry would predict fear extinction ability, such that younger individuals would exhibit greater fear generation and reduced regulatory control to learned threat cues, resulting in poorer fear extinction. Thirdly, we hypothesised self-reported intolerance of uncertainty (IU) to predict fear extinction ability in adult and adolescent populations, over and above other general measures of anxious disposition. We argue that in the context of fear extinction, uncertainty surrounding learned contingency changes (i.e. CS-US pairings) may initiate greater fear generation to both learned threat and safety cues in individuals who find uncertainty anxiety provoking (high IU), subsequently compromising regulatory control, and resulting in poorer fear extinction.

In Chapters 2 and 3, we demonstrated in adult participants: (1) successful fear conditioning using a developmentally appropriate design, and (2) that IU predicted compromised fear extinction in psychophysiological and neural measures (amygdala, vmPFC), over and above other anxiety measures. In Chapter 4, we observed younger age to be associated with blunted fear extinction in neural measures (amygdala, vmPFC). Furthermore, we found age-related structural change in the vmPFC to predict responding in the amygdala during fear extinction. However, in this developmental sample, we did not observe an impact of individual differences in IU on function or structure of fear extinction circuitry.

5.2 Review of studies

5.2.1 Chapter 2

In Chapter 2, we sought to: (1) replicate past psychophysiological findings of fear extinction in a classic paradigm adapted for a developmental

sample, and (2) assess whether individual differences in intolerance of uncertainty (IU), a potential risk factor for anxiety disorders, underlies compromised fear extinction. We investigated these questions by using classical conditioning of learned threat and safety cues, whilst recording SCR and behavioural ratings. Coloured squares and an aversive sound served as conditioned stimuli (Delgado et al., 2008; LaBar et al., 1998; Neumann & Waters, 2006; Phelps et al., 2004). The extinction phase was split into early and late, in order to capture the temporality of fear generative and regulatory processes. This experimental design was created on the basis of it being: (1) appropriate for both adult and adolescent populations, and (2) extendable to the scanning environment.

Consistent with previous research examining fear extinction (Delgado et al., 2008; Gazendam et al., 2013; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2009; Soliman et al., 2010), we found a general effect of conditioning for participants, as shown by greater SCR magnitude and behavioural ratings to learned threat vs. safety cues during fear extinction. These results confirmed this developmentally appropriate paradigm to induce successful fear conditioning in adult participants.

Furthermore, the results revealed that self-reported IU is associated with elevated fear expression to both learned threat and safety cues during fear extinction. Our data suggest that when contingencies are uncertain during extinction, high IU is associated with threat generalization, as shown by elevated SCR magnitude to both threat and safety cues during early extinction and threat cues in late extinction. Furthermore, IU was uniquely associated with a reduction in SCR magnitude to learned safety cues from early to late

extinction. From these results we concluded that individual differences in IU predicted variability in fear extinction behaviour.

5.2.2 Chapter 3

In Chapter 3, we assessed whether: (1) past psychophysiological and neural findings of fear extinction could be replicated in adults within the scanning environment, and (2) individual differences in IU underlie compromised recruitment of fear extinction circuitry. Adult participants underwent the same classical conditioning procedure as in Chapter 2, whilst skin conductance, pupil dilation, behavioural ratings and fMRI were recorded.

Similarly to Chapter 2 and consistent with previous research (Delgado et al., 2008; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2009; Soliman et al., 2010), we found a general (albeit statistically subthreshold) effect of conditioning for participants in skin conductance and in amygdala/vmPFC regions. However, we did not show such effects in pupil dilation and behavioural measures.

Findings from this study showed that self-reported IU is associated with psychophysiological and neural recruitment during fear extinction. These data further suggest that high IU is associated with generalising threat during early extinction, which subsequently delays fear inhibition of conditioned responses to threat cues during late extinction, as indexed by heightened psychophysiology and amygdala/vmPFC function during this extinction phase. Furthermore, the psychophysiology (partially) and fMRI results were specific to an association between extinction and IU, and not STAIX or PSWQ. Importantly, this experiment yielded strong individual difference effects,

suggesting the paradigm would be potentially useful for capturing individual differences due to developmental stage.

5.2.3 Chapter 4

Chapter 4 aimed to examine the psychophysiological and neural correlates of fear extinction during development. In addition, Chapter 4 aimed to examine the impact of age-related structural change and individual differences in IU on the function of fear extinction circuitry. Participants underwent the same experimental procedure as those outlined in Chapters 2 & 3.

Results from this study showed that age is associated amygdala and vmPFC recruitment during fear extinction. More specifically, we observed younger age to be characterised by increased amygdala activity to learned threat vs. safety cues during fear extinction, consistent with previous rodent and human fear studies (Kim, Li, et al., 2011; Pattwell et al., 2011; Pattwell et al., 2012; Strawn, Wehry, et al., 2013), suggesting exaggerated fear expression during development. Furthermore, younger age was quadratically associated (positive direction) with vmPFC activity to learned threat vs. safety cues during early extinction. More specifically, mid-adolescents displayed the least activation in the vmPFC to learned threat vs. safety cues during early extinction, in line with previous rodent work (Kim, Li, et al., 2011; Pattwell et al., 2011; Pattwell et al., 2012), suggesting compromised fear inhibition during this stage of development.

Age was associated with the grey matter probability of structures related to fear extinction ability. In line with prior structural work (Giedd, 2004; Gogtay et al., 2004; Lebel & Beaulieu, 2011; Lebel et al., 2008; Østby et al., 2009;

Tamnes et al., 2010; Wierenga et al., 2014), younger age was associated with: (1) greater grey matter probability in the vmPFC, suggesting less age-related grey matter thinning, (2) reduced grey matter probability in the bilateral amygdala, suggesting steady grey matter growth in this region with age, and (3) reduced structural integrity of the uncinate fasciculus, suggesting thickening of this white matter tract with age. Crucially, age-related structural differences in grey matter probability within the vmPFC was associated with reduced amygdala response to the learned threat vs. safety cues during late extinction. Similarly, stronger structural integrity of the uncinate fasciculus was associated with reduced amygdala response to learned threat vs. safety cues during late extinction, however this effect was at trend. We did not find an association between amygdala grey matter and amygdala activity during extinction. There was no specificity of age or vmPFC grey matter predicting amygdala response during late extinction.

We did not find any significant associations between individual differences in IU and the dependent measures. The lack of effects with IU may be simply related to weak statistical power or developmental phenomena (see below for further discussion).

Whilst we showed main effects of fear extinction in the amygdala and behavioural ratings, we did not show main effects of conditioning in the vmPFC, SCR magnitude, and pupil dilation. In addition, we did not observe developmental effects on psychophysiology and behaviour (Johnson & Casey, 2015; Pattwell et al., 2012), however developmental effects on these measures are not always found (Britton et al., 2013).

To summarise, these data suggest age and age-related structural change in the vmPFC may underlie the blunted fear extinction profile observed during adolescence.

5.3 Comparison with previous fear extinction studies

Consistent with previous research, we show evidence of successful conditioning in both adult and adolescent samples in Chapters 2-4, indexed by conditioned responses in psychophysiological and behavioural measurements to learned threat cues vs. safety cues during fear extinction (Milad et al., 2007; Pattwell et al., 2012; Phelps et al., 2004). Furthermore, in the two fMRI studies, we observed differential activity in regions associated with fear extinction, such as the amygdala and vmPFC (Barrett & Armony, 2009; Kalisch, Korenfeld, et al., 2006; LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004; Sehlmeyer et al., 2011; Soliman et al., 2010). Importantly, we found psychophysiological and neural responding during fear extinction to vary substantially, depending on individual differences in developmental stage and IU.

In Chapters 2 and 3, we show that self-reported IU in adult samples was associated with psychophysiological and neural recruitment during fear extinction. In both Chapters 2 & 3, lower IU was associated with earlier discrimination of threat and safety cues during fear extinction, consistent with previous fear extinction studies (Milad et al., 2007; Phelps et al., 2004): SCR magnitude and right amygdala response was larger to threat cues, relative to safety cues during early extinction. Expanding previous research on individual differences in trait anxiety (Barrett & Armony, 2009; Gazendam et al., 2013; Indovina et al., 2011; Sehlmeyer et al., 2011; Soliman et al., 2010; Torrents-

Rodas et al., 2013) and IU (Dunsmoor et al., In press), higher IU was associated with fear expression to both learned threat and safety cues in early extinction. More specifically, in Chapters 2 & 3 we found that higher IU scores were associated with indiscriminate SCR magnitude to both threat and safety cues during early extinction. In Chapter 3, we also found that higher IU is associated greater pupil dilation (at trend) and right amygdala activity to safety vs. threat cues in early extinction. In general, these results suggest that high IU individuals are prone to overestimating the value of potential threat when contingencies are uncertain.

In Chapters 2 & 3, low IU was associated with reduced SCR magnitude (and reduced right amygdala activity in Chapter 3) to threat vs. safety cues in late extinction, suggesting successful fear extinction (LaBar et al., 1998; Milad et al., 2007; Phelps et al., 2004). However, high IU was associated with increased SCR magnitude (in both Chapters 2 & 3), pupil dilation (at trend) and right amygdala to threat vs. safety cues during late extinction, suggesting slower discrimination of threat and safety cues, and sustained fear expression to learned threat cues. Contrary to predictions, high IU was associated with increased vmPFC activation in response to threat cues in late extinction, similar to that shown in previous studies examining trait anxiety (Barrett & Armony, 2009). Overall, findings from Chapters 2 and 3 suggest that high IU adults are slower to discriminate threat from safety cues, which subsequently compromises fear extinction. Given that we found specificity of IU over and above other measures of trait anxiety in Chapters 2 & 3, we argue that IU may be more closely aligned (than STAI and PSWQ) with the underlying cognitive biases that disrupt fear extinction processes. Importantly, these results suggest

that threat generalization and deficient safety learning may be strong candidate markers of IU based maintenance of fear and anxiety.

Individual differences in IU (or STAI and PSWQ) were not associated with fear extinction behaviour in any dependent measure in Chapter 4. The lack of IU effects in this study could be due to experimental limitations, such as weak statistical power, or the choice of cohort sampling (across adolescence and early adulthood). Alternatively, non-linear developmental effects may curb or shield IU-related fear extinction behaviour during adolescence. However, this latter proposal is debatable: Firstly, a number of adolescent fMRI studies have found that trait anxiety and IU are associated with neural activity during fear conditioning (Haddad et al., 2015) and decision making (Krain et al., 2008; Krain et al., 2006), despite the task differences between fear extinction and decision making, these results still suggest that individual differences in anxious disposition (and specifically IU) can be captured during adolescence. Secondly, longitudinal survey research has shown that IU is highest at the start and end of secondary school education, suggesting that IU may have a unique developmental trajectory across adolescence (Dugas, Laugesen, & Bukowski, 2012). Assessing the relationship between function of fear extinction circuitry and anxious disposition (and specifically IU) across development may be better achieved by using large cross-sectional designs with less developmental variability (e.g. smaller age ranges) or using longitudinal designs where participants are first tested as adolescents and followed up as adults, or tested repeatedly across adolescence.

In Chapter 4, we found that younger age was associated with exaggerated amygdala activity and reduced vmPFC activity to learned threat

vs. safety cues across fear extinction, in line with previous rodent and human behavioural fear extinction studies (Den & Richardson, 2013; Johnson & Casey, 2015; Kim, Li, et al., 2011; McCallum et al., 2010; Pattwell et al., 2011; Pattwell et al., 2012). Alongside age, grey matter probability in the vmPFC, indicative of grey matter thinning (Giedd, 2004; Lebel & Beaulieu, 2011), was associated with reduced amygdala response to the learned threat vs. safety cues during late extinction. As far as we are aware, our study was the first to examine or report: (1) function of fear extinction circuitry during adolescence, and (2) how age-related structural changes in fear extinction circuitry are related to function of fear extinction circuitry during adolescence. On this basis there is a scarcity of literature to compare our imaging results against. However, our fear extinction findings in Chapter 4 complement previous imaging studies of fear acquisition in adolescence (Haddad et al., 2015; Lau et al., 2011), as adolescents and adults both display different patterns of neural recruitment during fear acquisition and extinction. More specifically, during acquisition and extinction, adolescents exhibit greater activation in limbic regions to threat vs. safety cues, whilst adults display greater activation in prefrontal regions to threat vs. safety cues. Notably, in fear acquisition, older adolescents and adults reveal greater activation in the dlPFC to threat vs. safety cues (Haddad et al., 2015; Lau et al., 2011), thought to reflect age-related differences in discrimination learning. In our fear extinction study, age was quadratically associated with vmPFC recruitment to threat vs. safety cues during early extinction, such that mid adolescents showed less vmPFC activity. These findings suggest that prefrontal regions with different developmental trajectories, such as the dlPFC and vmPFC, (Gogtay et al., 2004; Shaw et al.,

2008) may play important and interacting roles in fear acquisition and extinction processes across adolescence.

Taken together these results suggest that both age and age-related structural changes may underlie the difficulties that youth have in updating learned threat cues as safe. Future work with longitudinal designs that incorporate structure-function based approaches may be able to elucidate the relationship between fear extinction ability and vmPFC grey matter further by tracking the extent of thinning in the vmPFC (Newman et al., 2015; Pfeifer & Allen, 2012). Furthermore, following age-related structural and functional changes in other prefrontal regions involved in fear regulation may prove useful in separating out the different developmental trajectories of fear acquisition and extinction processes that contribute to the adolescent profile.

5.4 Comparison to other emotion regulation studies

Beyond fear extinction, this body of work is also in line with previous research examining more broadly the role of individual differences in developmental stage and anxious disposition in emotion regulation. For example, similar activation of amygdala-vmPFC circuitry underlying fear extinction is also observed during other types of emotion regulation, such as habituation, reappraisal-based regulation and attentional tasks with emotional distractors (Bishop, 2009; Blair et al., 2007; Delgado et al., 2008; Fisher et al., 2009; Ochsner et al., 2009; Urry et al., 2006).

In line with previous studies of anxious disposition that used a variety of emotion regulation tasks (Campbell-Sills et al., 2011; Etkin et al., 2004; Mujica-Parodi et al., 2009; Schienle et al., 2010; Somerville et al., 2013; Stein et al.,

2007), we observed similar aberrant recruitment of amygdala-vmPFC circuitry, such that anxious disposition was associated with hyperactivity in the amygdala and vmPFC to potential threat stimuli. In anxious populations, the vmPFC is more commonly reported as hypoactive (Mujica-Parodi et al., 2009; Sehlmeyer et al., 2011; Somerville et al., 2013; Xu et al., 2013), but hyperactivation has also been observed, and is thought to reflect effortful attempts in regulating emotions (Barrett & Armony, 2009; Campbell-Sills et al., 2011). Differences between previous studies of anxious disposition and emotion regulation, and the current fear extinction studies of the thesis lie in recruitment of other parts of the prefrontal cortex. For example, in previous studies of instructed emotion regulation, anxious individuals have also displayed increased activation in other parts of the prefrontal cortex when attempting to reduce responses to emotional stimuli (Campbell-Sills et al., 2011). As expected, we did not observe any differences in recruitment of these regions for anxious individuals during fear extinction.

Importantly, our results were specifically associated with IU, over and above other trait anxiety measures, suggesting a critical role of IU-based mechanisms in fear extinction, and potentially to other types of emotion regulation that rely on amygdala-vmPFC circuitry as well. For example, our findings support previous emotion regulation work showing a specificity of IU in contexts that manipulate uncertainty with a variety of unpleasant, pleasant, and neutral stimuli e.g. (Gole et al., 2012; Krain et al., 2008; Krain et al., 2006; Luhmann et al., 2011; Schienle et al., 2010; Somerville et al., 2013). Similar, to our findings, previous studies have shown that high IU is associated with heightened amygdala responses and disrupted recruitment of the vmPFC to

cues that signal uncertainty (Krain et al., 2008; Schienle et al., 2010; Somerville et al., 2013). Previous work has also reported high IU individuals to show differences in regions that we did observe in our studies, such as the insula, ACC, and other parts of the prefrontal cortex, suggesting that IU may impact a variety of emotion regulation processes (Krain et al., 2008; Krain et al., 2006; Schienle et al., 2010; Simmons, Matthews, Paulus, & Stein, 2008).

With regards to development, a key theme emerging from this thesis and the literature as a whole is that adolescents may have difficulty inhibiting emotional information more generally. There is ample evidence of adolescents exhibiting greater recruitment of the amygdala and reduced recruitment of top down control regions responsible for inhibition such as the vmPFC to affective information in a variety of contexts. For example, this has been observed in cognitive tasks with embedded faces (Hare et al., 2008; Monk, McClure, et al., 2003) passive viewing of fearful faces (Swartz et al., 2014; Thomas, Drevets, Whalen, et al., 2001) and instructed emotion regulation tasks (McRae et al., 2012; Vink et al., 2014). Based on this evidence, we can infer that adolescents may be drawn to highly arousing affective content, irrespective of valence.

5.5 Broader implications and remaining questions

Of note from the comparison of studies above is the different pattern of fear extinction behaviour that emerges as a function of individual differences in development and IU. Younger age is associated with compromised fear extinction through what appears to be a difficulty in updating the value of a learned threat cue to a safety cue. On the one hand this behaviour may be useful for driving adolescents away from potentially volatile situations when

they are beginning to establish independence (Somerville & Casey, 2010). On the other hand, this behaviour may become disruptive when the situation cannot be avoided and fear extinction is needed, leaving adolescence vulnerable to anxiety disorders. Interestingly, adults with high IU (over other trait measures), not only exhibit the difficulty in updating the value of a learned threat cue to a safety cue, but also generalise threat across cues. Importantly, these types of behaviours observed in the healthy cohort tested here are also commonly seen in both paediatric and adult samples with anxiety disorders (Lissek et al., 2005; Pine, 2007), suggesting that these behaviours may be apparent before any anxiety disorder diagnosis.

The distinction between the impact of individual differences in developmental stage and IU upon fear extinction processes poses a few important questions: (1) Do the mechanisms serving adolescent-based difficulties in updating the value of learned threat cues to safety cues continue into adulthood for high IU individuals? (2) During development is there a common time of emergence for mechanisms serving IU-based threat generalisation behaviour? (3) Can the mechanisms serving adolescent-based and IU-based fear extinction behaviour predict concrete outcomes (i.e. anxiety and stress-related pathophysiology)?

Overall, these findings suggest a critical role of age-related and uncertainty-based mechanisms in the maintenance of learned fear. Importantly, these results highlight an opportunity for further examination of structural and functional changes in amygdala-vmPFC circuitry during adolescence that relate to: (1) risk of anxiety disorder development, (2) effectiveness of current exposure based therapies, and (3) development of novel anxiety disorder

treatments that are age specific (Casey et al., 2015; Casey, Oliveri, & Insel, 2014; Johnson & Casey, 2015; Rapee et al., 2009). Similarly, this body of work shows promise for the further development of: (1) recently implemented focused forms of anxiety disorder treatment, such as intolerance of uncertainty therapy (van der Heiden, Muris, & van der Molen, 2012) and, (2) novel experimental models of targeted therapies (Dugas et al., 2004; Dunsmoor et al., In press) in those demonstrating IU-based symptomatology that could specifically help manage uncertainty-based maintenance of learned fear.

5.6 Conclusions

The current thesis adds to a growing body of neurobiological research examining individual differences in fear regulation processes, which are essential for maintaining health and wellbeing. Specifically, we examined individual differences in development and IU on fear generative and regulatory processes during fear extinction, as measured with behavioural, psychophysiological and neural correlates. We focused on a crucial but simplified brain model of fear extinction circuitry, comprising the coupling of the amygdala and vmPFC.

Importantly, we found a different pattern of fear extinction behaviour as a function of individual differences in development and IU. In our developmental sample, we found younger age and age-related structural changes in the vmPFC to be important predictors of continued responding in the amygdala to learned threat vs. safety cues during fear extinction. These findings suggest that development is associated with compromised fear extinction through a difficulty in updating the value of a learned threat cue to a safety cue. In our

adult samples, however, we found IU, over and above other general measures of anxious disposition, to specifically predict elevated responses to both learned threat and safety cues in psychophysiological correlates and the amygdala during fear extinction. These findings suggest that high IU adults are prone to both a difficulty in updating the value of a learned threat cue to a safety cue and in threat generalisation across cues. These developmental and IU effects on the functioning of the amygdala and vmPFC may extend to other types of fear generation and regulation that rely on this circuitry. In addition, such effects may extend to other relevant fear extinction circuitry and fear regulation circuitry more broadly, such as the hippocampus and other regions of the prefrontal cortex.

However, as highlighted throughout the discussion, a number of outstanding issues remain regarding the emergence, timing and permanency of these effects on fear regulatory processes. Future work may be able to address these issues, and in particular the onset of IU-based mechanisms in adolescence, by using longitudinal designs with larger samples where development of fear extinction ability (and other forms of fear regulation) can be followed across adolescence into early adulthood. Furthermore, tracking the development of fear extinction circuitry (and other fear regulation circuitry) through structure-function and network based approaches, are likely to tease apart some of the nuances in the field and advance our understanding of individual differences in development. Alongside previous work, the current thesis highlights the relevance and potential of developmental and IU-based mechanisms to help understand pathological fear in anxiety disorders and inform future treatment targets.

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Appendix

Executive function in adolescents

Behavioural and functional magnetic resonance imaging (fMRI) studies have demonstrated adolescents, relative to adults, to show a variety of deficits in cognitive control tasks, such as Anti-Saccade (Hwang, Velanova, & Luna, 2010), Stroop (Adleman et al., 2002), Stop-Signal (Rubia, Smith, Taylor, & Brammer, 2007), Simon and Go/No Go (Rubia et al., 2006), as well as Visual Spatial Working Memory (Conklin, Luciana, Hooper, & Yarger, 2007; Luciana, Conklin, Hooper, & Yarger, 2005) and Switch tasks (Rubia et al., 2006). In such tasks, adolescents are more susceptible to false alarms, display poorer error monitoring, and retain less information in memory, compared to adults. Additionally, fMRI data from these experiments show either decreased or more diffuse activation in regions of the prefrontal cortex that are associated with cognitive control, such as the dlPFC, vlPFC and ACC.

To assess that our sample of adolescents in the third study displayed normative developmental trajectories of cognitive functioning, we asked them to complete a Stroop task, switch task and letter memory task.

Participants

52 right-handed volunteers were taken from Chapter 4 (M age = 17.75yrs, SD age = 3.65yrs, range = 12-28yrs; 32 females & 20 males). Please refer to the participants and procedure sections from Chapter 4.

Cognitive tasks

Participants completed three computerised cognitive tasks outside of the scanner. These tasks have been used previously with both adult and adolescent samples (Luna, Padmanabhan, & O'Hearn, 2010). Each cognitive task tests one of three aspects of executive control: inhibition, switching and updating (Miyake et al., 2000).

We used the Stroop task to measure inhibition ability (Adleman et al., 2002). Stimuli for this task consist of words printed in one of four colours (red, blue, green or purple) to form three conditions: (1) in the Congruent condition coloured words printed in the same colour as the semantic meaning of the word (e.g. ''blue'' in blue ink). (2) In the Incongruent condition, colour words are printed in a colour different from the meaning of the word (e.g. ''red'' printed in blue ink). The task consisted of 8 practice trials, 12 congruent test trials and 12 incongruent test trials. Each word was presented for 1250 ms, followed by a blank screen for 750 ms. Participants responded using coloured keys on the keyboard.

We used a modified version of the Meiran task to measure switching ability (Christakou et al., 2009; Rubia et al., 2006). In this task participants are presented with a grid of four squares and fixation cross in the middle. Then, a red dot appears in one of the squares. A bi-directional arrow that points vertically or horizontally sits in the middle of the grid, which prompts the participant to respond whether the red dot is in the horizontal (left/right) or vertical (up/down) plane. In the switch trials the arrow in the middle of the grid changes directional plane. This occurs approx. every 4-6 trials. The task consisted of 8 practice trials, 180 repeat trials and 36 switch trials. The fixation

is presented for 800 ms, followed by an arrow for 200 ms and a red dot for 1400 ms. Participants used the arrow keys on the keyboard to denote the location of the dot e.g. right arrow for when the arrow is horizontal and the dot is in either one of the right squares.

To measure updating ability, we used the Letter Memory task (St Clair-Thompson & Gathercole, 2006), which consists of presenting letters serially (e.g. 5, 7, 9 or 11 letter strings). Participants are required to recall the last four letters presented in each list. To ensure continuous updating, participants must rehearse the last four letters out loud throughout the task. The task consisted of 2 practice trials and 12 testing trials. Each letter was presented for 2000 ms, followed by a 150 ms blank screen. The experimenter recorded the answers using pencil and paper.

Cognitive task data reduction and analysis

Reaction time and accuracy data for the Stroop and Meiran tasks were reduced for each subject by calculating the average responses for each experimental condition using the E-Data Aid tool in E-Prime (Psychology Software Tools Ltd, Pittsburgh, PA). For both the Stroop and Meiran tasks, reaction time responses were considered to be valid if they were correct and were above 250 ms. Accuracy scores consisted of a correct score percentage.

Responses to the Letter Memory task were coded using a point system for each trial. Participants could receive a maximum of 6 points per trial. A point for each correct letter (4 letters per answer), a point for letters in the correct order, and a point for completing the string with no hesitation or missing recall. A total score for all 12 trials was given to each participant.

Age differences for the Stroop and Meiran tasks were assessed by conducting a Condition (Stroop: Congruent, Incongruent; Meiran: Repeat, Switch) x Age repeated measures ANCOVA for the reaction times and accuracy scores. Age was entered as a continuous mean centered predictor variable. For the Letter Memory task we correlated the total Letter Memory score per participant against age.

Results of cognitive tasks and age

For the Stroop task, participants were significantly more accurate, $F(1,50) = 30.660$, $p < .001$, and faster, $F(1,50) = 189.950$, $p < .001$, to respond to congruent vs. incongruent trials. There was an effect of age at trend on the accuracy of congruent vs. incongruent trials, $F(1,50) = 3.419$, $p = .070$, in that older age was associated with better accuracy on incongruent trials. However, there was no age effect on reaction times for the congruent vs. incongruent trials in the Stroop task, $F(1,50) = 1.687$, $p = .2$.

As expected, participants were generally slower to respond to switch vs. repeat trials, $F(1,50) = 89.063$, $p < .001$. Furthermore, younger age was associated with slower reaction times to the switch vs. repeat trials, $F(1,50) = 6.076$, $p = .017$. The ANCOVA results for the Switch task revealed no main effects of condition or age x condition interaction on the accuracy scores, F's < 2.773.

As predicted, age significantly correlated with Letter Memory task scores, $r(50) = .440$, $p = .001$, such that increasing age was associated with better updating ability.