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Neural signatures of emotion processing in adolescence

Samantha Rhianne Linton

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Thesis abstract

Adolescence is associated with high levels of emotionality and an increased risk of developing anxiety disorders. To date, neurobiological models of adolescence have sought to explain these adolescent-specific behaviours and vulnerabilities as a result of developmental changes to subcortical (affective) and cortical (cognitive control) brain regions. However, very little work has examined adolescents' perceptual processing of emotional stimuli, especially negative, and how this processing may change in the transition from adolescence to adulthood.

To begin to address this, this doctoral work started by assessing the suitability of two tasks for use with adolescent and adult populations. The first study (Chapter 2) examined the effect of threat-related facial expressions on temporal recalibration in a late adolescent sample, and reported an enhanced temporal recalibration transference effect for fearful, relative to neutral, facial expressions. The second study (Chapter 3) used EEG to examine the effect of emotional vocalisations (laughter or crying) on the early visual processing of congruent and incongruent emotional faces (happy or sad), in a sample of late adolescents. This study reported a valence-dependent enhancement of emotional congruency on early visual P1, but not N170, responses to emotional faces.

The second half of this doctoral work took a Pavlovian fear conditioning approach to provide a more highly controlled measure of adolescent emotion processing. The third study (Chapter 4) consisted of a systematic review of the Pavlovian fear conditioning literature during adolescence, revealing that reported developmental differences in fear acquisition and extinction are based on very few studies, containing numerous methodological weaknesses. The fourth study (Chapter 5) was designed to address these weaknesses, using a fear conditioning and extinction task to examine the behavioural, physiological, and ERP correlates of fear conditioned cues, in a sample of mid-adolescents (13-14 years) and adults (25-26 years). The results demonstrated successful conditioning and immediate extinction of explicit behavioural measures, whilst also demonstrating both age and gender-dependent differences in the early perceptual processing of threat-predicting cues. Together, this doctoral thesis provides evidence for the suitability of Pavlovian fear conditioning for assessing adolescent emotion processing, and suggests that current neurobiological models of adolescence should be extended to incorporate developmental changes in visual cortical regions during threat processing.

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Chapter 1. General introduction

1.1. Introduction

Adolescence is a transitionary phase between childhood and adulthood, that is often associated with higher levels of harmful risk-taking behaviours (S. Burnett, Bault, Coricelli, & Blakemore, 2010; L. Steinberg, 2008), and with higher levels of emotionality (Casey, Jones, et al., 2010). In addition, this period of development is also associated with an increased risk of developing an anxiety disorder (Beesdo, Pine, Lieb, & Wittchen, 2010; Kessler et al., 2007; Kessler, Berglund, Demler, Jin, & Walters, 2005; Lijster et al., 2017; McGorry, Purcell, Goldstone, & Amminger, 2011; Pine, Cohen, Gurley, Brook, & Ma, 1998). It has been suggested that adolescent-specific behaviours and vulnerabilities, such as increased emotionality and anxiety, result from an imbalance between early-maturing subcortical brain regions, responsible for emotion processing, and late-maturing cortical regions, responsible for cognitive control (Casey, Getz, & Galvan, 2008; Casey, Jones, et al., 2010). Current work on emotion processing in adolescence has tended to focus on adolescent responses to positive rewarding stimuli, which suggests adolescents are hyper-responsive to rewards (Casey, Getz, et al., 2008; Casey, Jones, & Hare, 2008; Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst, 2014; Ernst, Pine, & Hardin, 2006; Nelson, Leibenluft, McClure, & Pine, 2005; Somerville, Jones, & Casey, 2010; L. Steinberg, 2008). In comparison, however, work examining responses to negative or aversive stimuli is still in its infancy, with seminal non-human animal work from Spear and colleagues suggesting that adolescents may be resistant to the effects of aversive stimuli (Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Schramm-Sapyta, Morris, & Kuhn, 2006; Torres, Tejeda, Natividad, & O'Dell, 2008). Therefore, the aim of this thesis was to examine whether, as proposed by Spear and colleagues (Doremus-Fitzwater & Spear, 2016; Doremus-Fitzwater et al., 2010), adolescence is associated with a reduction in responses to aversive stimuli in humans, which recent studies suggest may not be the case (Howsley & Levita, 2017; Levita, Howsley, Jordan, & Johnston, 2015).

To that end, this doctoral work was designed to examine emotion processing, especially to negative stimuli, in the transition from adolescence to adulthood, using a variety of approaches. Initially, this doctoral work began by investigating which emotion processing paradigm would be most appropriate for use with both adolescents and adults. Therefore, I first examined the precise temporal processing of emotional faces in a sample of late adolescents as part of a behavioural study (Chapter 2). This study was

followed by an assessment of the impact of emotional vocalisations on the early visual processing of emotional faces, as indexed by behavioural and ERP correlates, in a late adolescent sample (Chapter 3). In light of a detailed discussion of the findings of these two studies, which suggests they may not be optimal for use with a younger adolescent sample, this doctoral work began to specifically examine fear learning during adolescence, and its potential association with increased levels of anxiety at this age, by assessing fear acquisition and extinction in adolescents compared to adults. Prior to commencing an empirical study, a systematic review of the developmental Pavlovian fear conditioning literature was conducted (Chapter 4). This revealed that few studies have examined how adolescents' process fear conditioned cues. This review also identified a number of significant methodological limitations in the current fear conditioning and extinction literature. In response to these limitations, a final study was designed to examine the behavioural, physiological and ERP correlates associated with Pavlovian fear conditioning and extinction in a sample of mid-adolescents and young adults (Chapter 5).

This introduction will start by outlining definitions of adolescence, according to traditional socio-cultural and more recent neurobiological accounts of when adolescence begins and ends. Next, I will discuss the "storm and stress" of adolescence (Hall, 1904), and how it relates to adolescent brain development. Following this, I will offer a definition of emotion, and discuss the impact of developmental changes on the processing of both rewarding and threatening stimuli in the context of key neurobiological models of adolescence. These neurobiological models will be scrutinized regarding how informative they are regarding adolescent emotion processing, with a later focus on threat processing in particular. Finally, I will introduce the emotion processing EEG/ERP literature that has so far been conducted with adolescents, to reveal current knowledge regarding how emotional stimuli are processed by the visual system in this age group.

1.2. Defining adolescence

1.2.1. Defining adolescence according to chronological age

1.2.1.1. A neurobiological account of adolescence

Historically adolescence has been defined using a combination of biological and socio-cultural factors – beginning with the onset of puberty and ending when an

individual achieves independence from their parents. Typical examples of independence can include leaving school, moving out of the family home, or gaining full-time employment. Consistent with this view, the world health organization originally defined adolescence as a period of development between 10 and 19 years of age (World Health Organisation, 2003), to encompass the wide range of biological and social changes experienced by individuals at this developmental stage.

However, research now suggests that such events which were once considered to be adult milestones now occur much later in life, and that defining adolescence as a period between 10-19 years may no longer be sufficient. For example, young people are now more likely to continue onto higher education courses beyond 18 years (Department for Education, 2018), and are also more likely to wait until their thirties to get married, have children, and buy their first home (Office for National Statistics, 2016, 2018; UK Finance, 2017). Instead, researchers propose that adolescence begins around age ten (World Health Organisation, 2003) and continues into an individual's third decade of life (Dahl, 2004; Mills, Goddings, Clasen, Giedd, & Blakemore, 2014; L. Steinberg, 2008). More specifically, recent discussions by Sawyer et al. (2018) suggested that an expansion of the adolescent period from 10-19 years to 10-24 years would be appropriate, in line with the neurobiological changes that are observed during this time period.

Employing a neurobiological account when defining adolescence is helpful, because this account encompasses changes to biological growth as well as brain maturation processes during this period. This is important, as biological changes associated with pubertal development have recently been shown to begin earlier in life, largely due to improvements in nutrition during childhood (Soliman, De Sanctis, & Elalaily, 2014). In addition, advances in neuroimaging techniques have improved the study of brain maturation processes, with evidence suggesting that many key brain regions continue to develop into the third decade of life. For example, structural and functional evidence suggests that the prefrontal cortex (PFC) is not fully mature until a person's late twenties (Casey, Giedd, & Thomas, 2000; Giedd et al., 1999; Huttenlocher, 1979; Pfefferbaum et al., 1994; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999; Sowell, Thompson, Tessner, & Toga, 2001). Maturation of the PFC has been associated with increased cognitive control (see Casey, Galvan, & Hare, 2005 for a review), and is discussed within key neurobiological models of adolescence as being necessary to maintain cognitive control in highly emotional contexts (see Section 1.4;

Casey, Jones, et al., 2010; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008, 2010). Together, these studies support the utility of a neurobiological account of adolescence, as they demonstrate that adolescents continue to undergo significant changes to the structure and function of their brains well into their twenties, which are likely to impact both cognitive and affective processes. As a result, considering adolescence as a period from 10-24 years may provide fruitful results when examining emotion processing in this age group.

Despite the strengths of a neurobiological account, there are also drawbacks which should also be considered. For example, this account overlooks traditional socio-cultural definitions of adolescence. A socio-cultural understanding of adolescence is also needed, as there are significant cross-cultural variations in the environment in which adolescents are raised, which can impact their behaviour irrespective of the neurobiological changes they are experiencing. For example, as discussed by Kapadia (2017), the menarche in females is considered to be a significant developmental marker of the transition into adulthood in agricultural societies, which encourages a large family dynamic and therefore values and celebrates fertility. In contrast, industrialized societies place less emphasis on pubertal development and greater emphasis on the completion of schooling to signal the transition adulthood, as academic attainment is considered to be necessary for career progression. These cross-cultural differences are likely to impact how adolescents are socialised, which will impact their behaviour in future scenarios. In addition, environmental factors such as ecological hardship can influence adolescent behaviour, as evidenced by a study of risk-taking across 77 different countries (Mata, Josef, & Hertwig, 2016). The results of that study found the propensity for risk-taking was significantly associated with the level of hardship in each country, with ecologies with scarce resources and heightened competition associated with greater risk-taking regardless of both age and gender. Taken together, these studies provide evidence of additional cross-cultural environmental factors that can impact adolescent behaviour, which are not accounted for by neurobiological accounts of adolescence.

1.2.1.2. Early, mid, and late adolescence

Adolescence encompasses a broad range of developmental changes between childhood and adulthood. Because of this broad range of changes, whilst the Sawyer et

al., (2018) definition conceives of all 10-24-year-olds as adolescents, this expansion of the age range is intended cover multiple different stages of adolescence, which can be subdivided into early, mid and late adolescence. It has been suggested that 10-14-year-olds be defined as early adolescents, 15-17-year-olds as mid adolescents, and 18-24-olds as late adolescents (Vetter-O'Hagen & Spear, 2012). These delineations are useful when conducting adolescent research, as they recognize that whilst a 12-year-old is different from a 19-year-old, both are still undergoing physical, emotional, and neurodevelopmental changes that distinguishes them from both children and adults.

Considering adolescence as an extended developmental period from around age 10 to 24 years, containing discrete substages, may significantly improve the study of emotion processing in this age group. For example, previous studies that have focused on adolescent threat processing using a Pavlovian fear conditioning model have tended to study early, mid, and late adolescents as one developmental group (E.g., 10-17 years in Lau et al., 2011; 12-17 years in Den et al., 2015; Pattwell et al., 2012; Johnson & Casey, 2015). Collapsing all adolescents into one age category is problematic, because key brain regions involved in threat processing (e.g., the PFC) continue to develop and change throughout adolescence (e.g., Casey et al., 2000; Giedd et al., 1999; Huttenlocher, 1979; Pfefferbaum et al., 1994; Sowell, Thompson, Holmes, Jernigan, et al., 1999; Sowell et al., 2001). As a result, it is possible that developmental differences between adolescent and adult threat processing may be masked by differences *within* the adolescent group, which limits a coherent understanding of adolescent threat processing.

Despite recent advances regarding neurobiological definitions of adolescence, which suggest early adolescence begins around age 10 with the onset of pubertal development, less attention has been paid to when adolescence ends. Studies examining brain maturation processes have provided evidence that a number of key regions continue to develop into an individual's late twenties (see section 1.4. for more detail), which suggests that individuals at this age may make suitable adult comparison groups when studying emotion processing in adolescence. However, although adulthood was once presumed to be a period of relative stability in terms of brain structure and function, recent empirical evidence suggests this may not be the case. For example, whilst longitudinal evidence suggests that cognitive decline is not evident until adults reach their sixties (Giambra, Arenberg, Zonderman, Kawas, & Costa Jr, 1995; Salthouse, 2010, 2016), some work suggests this decline may be a linear process which

begins earlier on in adulthood (Salthouse, 2009, 2016). Additional brain imaging data suggests that structural changes to the brain continue throughout early-mid adulthood (Coupé, Catheline, Lanuza, Manjón, & Alzheimer's Disease Neuroimaging, 2017; Tian & Ma, 2017; Vinke et al., 2018). For example, Tian and Ma (2017) used diffusion tensor tractography imaging to examine the relationship between age and the brain's white matter microstructure in participants aged 18-55 years. The authors observed micro-aging of the human brain from early to mid-adulthood, with such specificity that authors were able to estimate the age of each individual from their brain data alone. This is supported by additional MRI work by Coupé et al. (2017), who examined the developmental trajectory of brain volume across the lifespan (N = 2,944, age range = 9 months to 94 years). The authors reported a slow decline in white matter growth from 30-40 years of age onwards and a continual decrease in grey matter volume throughout life. Taken together, these studies suggest that, as in adolescence, adulthood may be best understood as a period of continued neurobiological change; albeit with less dramatic change as that observed in adolescence. Importantly, this work makes it difficult to truly define when adolescence ends, and highlights the significant issue faced by the majority of previous psychology studies which considered 18-year-old undergraduates to be adult participants. It is highly likely that the inclusion of 18-year-olds as adult participants has skewed our current understanding of many cognitive, emotional, and behavioural processes to date.

1.2.2. Defining adolescence according to pubertal development

Using age to define adolescence in scientific research studies can be advantageous over other methods, as it can be measured easily and precisely (Blakemore, Burnett, & Dahl, 2010). However, age-based definitions of adolescence are based on arbitrary cut-offs, which fail to consider additional biological changes associated with pubertal development. Puberty can be defined as a period of development associated with numerous biological changes that are necessary to attain sexual maturation and independence (Spear, 2000b). Puberty is believed to occur between 8-13 years for girls and 10-15 years for boys (Soliman et al., 2014) and can be broken down into a smaller number of key endocrine events, with males and females following separate trajectories. The first event is the adrenarche (occurring around 6-9 years of age) which activates the adrenal stress hormones, the second event is the

gonadarche, which triggers gonadal changes in females aged between 8 and 14 years and in males aged between 9 and 15 years, and the third event is the growth spurt which is triggered by activation of the growth axis in females aged around 12 years and males aged around 14 years (Blakemore et al., 2010). This series of events has been associated with increased sensitivity to emotional stimuli during adolescence (Spear, 2009), which highlights the importance of considering pubertal development when examining emotion processes during this phase of development.

Currently, very little research has examined the relationship between puberty and adolescent brain development. However, it has been suggested that pubertal development gives rise to a wealth of physical, hormonal and emotional changes which impact brain maturation processes during adolescence (Blakemore et al., 2010; Goddings et al., 2014; Spear, 2000b). Pubertal development has been associated with changes in adolescents' perceptual processing of socio-emotional cues (Scherf, Behrmann, & Dahl, 2012; Steinberg, 2008), as well as changes in brain activation patterns when responding to facial expressions (Forbes, Phillips, Silk, Ryan, & Dahl, 2011; Moore et al., 2012), rewarding stimuli (de Macks et al., 2011; Forbes et al., 2010), and social situations (Goddings, Burnett Heyes, Bird, Viner, & Blakemore, 2012; Klapwijk et al., 2013), independently of chronological age. Similarly, in previous behavioural work where adolescents (10-17 years) were asked to categorise facial emotions, their results demonstrated a 10-20% increase in reaction times when adolescents at the average age of puberty onset (12-13 years for boys and 10-11 years for girls) responded to the emotional faces, with this effect decreasing gradually between then and age 17 (McGivern, Andersen, Byrd, Mutter, & Reilly, 2002). Together, this work demonstrates the utility of considering pubertal development within current aged-based definitions of adolescence, as evidence suggests there may be relationship between puberty and emotional processes.

However, although measures of pubertal development may be useful for understanding emotion processing in adolescence, puberty is highly correlated with age, which makes it difficult to dissociate between the two, and in practice it is difficult to measure puberty precisely in the research laboratory. This is because, like adolescence, puberty is not a unitary event, and instead covers a significant period of change in hormonal patterns, as well as emotional and physical development. To examine changes associated with puberty, researchers may use the Tanner scale to track puberty via physical bodily changes in males and females (J. M. Tanner, 1971), or rely on

participants to document their own bodily changes using the pubertal development scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988). However, both measures can provide only crude estimates of pubertal development. Researchers could instead obtain hormonal assays from participants to examine a biological measure of pubertal development, but such assays are costly and time-consuming, and it is not currently clear whether these could be used to accurately categorise individuals without the need for additional self-report measures. Therefore, whilst it is important to consider pubertal development within the context of the adolescent emotion processing literature, for practical reasons this doctoral work will make use of an age-based neurobiological definition of adolescence.

1.3. The "storm and stress" of adolescence

Adolescence has been characterised by significant changes in behaviour, cognition, and emotion (Abe & Suzuki, 1986; S. Burnett et al., 2010; Spear, 2000a). These changes are coupled with the onset of physical as well as hormonal changes, and the continuation of brain maturation processes (Giedd et al., 1999; Gogtay et al., 2004; Sowell, Trauner, Gamst, & Jernigan, 2002). Our thinking about the adolescent period is still very much influenced by Hall's (1904) notion that these changes give rise to a heightened phase of "storm and stress". Thus, there is still a great prevalence in viewing adolescence as a time of inevitable chaos, where "storm" refers to a decrease in self-control, and "stress" refers to an increase in emotional sensitivity/reactivity during this developmental period. Consistent with this view, there are many studies that show adolescence is associated with higher levels of harmful risk-taking behaviours – the storm (S. Burnett et al., 2010; L. Steinberg, 2008) . In addition, evidence suggests that adolescents show higher levels of emotionality (Casey, Jones, et al., 2010) and face a greater risk of developing an anxiety disorder – the stress (Kessler et al., 2005; Pine et al., 1998).

A number of predominant neurobiological models of adolescence have attempted to explain the "storm" of the adolescent period, as seen by increased levels of risk-taking (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Nelson et al., 2005; L. Steinberg, 2008). However, less work has attempted to explain the "stress" of the adolescent period, as seen by increased levels of emotionality and anxiety in this age group. This is surprising, given that adolescence is believed to be

a core risk phase for developing symptoms of anxiety (Beesdo, Knappe, & Pine, 2009; Kessler et al., 2007; Kessler, Ruscio, Shear, & Wittchen, 2009; McGorry et al., 2011), with the majority of adult anxiety disorders developing during this phase of development (Kessler et al., 2005; Pine et al., 1998). In addition, older work has demonstrated a lifetime peak in self-reported symptoms of anxiety in adolescents (Abe & Suzuki, 1986), with a more recent study conducted in the US suggesting that as many as one in three adolescents meet the criteria for an anxiety disorder (Merikangas et al., 2010). Unfortunately, anxiety can adversely impact adolescents' social functioning and educational attainment (Kessler, Foster, Saunders, & Stang, 1995; Woodward & Fergusson, 2001), and has been associated with poorer health-related quality of life (Raknes et al., 2017), an increased rate of suicide attempts (Davidson, Hughes, George, & Blazer, 1993), as well as substance abuse (Comeau, Stewart, & Loba, 2001; Woodward & Fergusson, 2001). In a longitudinal study of participants who were interviewed periodically from adolescence to age 30, adolescent anxiety predicted income, unemployment, maladjustment, poorer coping skills, as well as increased chronic stress and a greater number of life events (Essau, Lewinsohn, Olaya, & Seeley, 2014). Additional work has also highlighted the negative impact of sub-clinical anxiety during adolescence, which has also been associated with functional impairment and suicide risk (Balazs et al., 2013). A better understanding of how adolescents process emotional stimuli could help to explain why adolescents show greater levels of anxiety in comparison to adults. Thus, the central aim of this doctoral work was to examine adolescent responses to negative stimuli, using behavioural, physiological and neural measures.

1.4. Brain maturation processes throughout adolescence

Past research has utilised advanced neuroimaging techniques to track the precise structural and functional brain changes that occur throughout adolescence. Specifically, structural magnetic resonance imaging (sMRI) studies have shown that, despite children's brains reaching 95% of their expected size by age six (Giedd & Rapoport, 2010), multiple regions and distributed networks undergo a dynamic cascade of changes in the transition to adulthood. For example, past research suggests that lower order motor and sensory regions mature first, whilst higher order regions, such as the frontal and temporal lobes, mature later (Giedd, 2004; Gogtay et al., 2004; Khundrakpam et al.,

2012; Sowell et al., 2004). In addition to differences in the maturation rates of lower order and higher order brain regions throughout development, the human brain also undergoes a substantial re-organization of a multitude of functional networks. This re-organisation results in a shift from local anatomical architecture in children, to a distributed anatomical architecture by the time individuals transition into young adulthood (Fair et al., 2009). Furthermore, the frontal lobes have been shown to mature first from the back to the front, starting with the primary motor cortex, then the superior and inferior frontal gyri, and ending with the prefrontal cortex (PFC) (Gogtay et al., 2004), with a wealth of structural and functional work demonstrating that the prefrontal regions continue to develop from childhood well into an individuals' late twenties (Casey, Giedd, & Thomas, 2000; Giedd et al., 1999; Huttenlocher, 1979; Pfefferbaum et al., 1994; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999; Sowell, Thompson, Tessner, & Toga, 2001). The impact of the protracted development of the PFC will be explored in more detail in Section 0, as part of the neurobiological models of adolescence.

Longitudinal developmental sMRI studies also show that the transition from childhood to adulthood is characterised by a steady reduction in gray matter. Gray matter consists of neuronal bodies, blood vessels, glial cells, extracellular space, dendrites, as well as unmyelinated and myelinated axons (Mills & Tamnes, 2014). Research has previously suggested that cortical gray matter volume develops according to a U-shaped trajectory, peaking at the onset of puberty (Giedd, 2004; Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2002). However, more recent work examining multiple longitudinal MRI datasets failed to observe this U-shaped trajectory, and instead suggested that cortical gray matter volume and thickness is highest in childhood, and decreases throughout adolescence (Mills et al., 2016; Tamnes et al., 2017). Importantly, cortical thinning was the main predictor of reductions in cortical volume during adolescence (Tamnes et al., 2017). This is supported by other work showing significant cortical gray matter thinning from childhood to adulthood (Gogtay et al., 2004; Squeglia, Jacobus, Sorg, Jernigan, & Tapert, 2013; Tamnes et al., 2010), with additional evidence suggesting that adolescence is marked by accelerated thinning of the cortex compared to childhood and young adulthood (Zhou, Lebel, Treit, Evans, & Beaulieu, 2015). This accelerated cortical thinning observed during adolescence is greater in associative cortical regions, which mediate efficient connectivity throughout the brain's structural network (Whitaker et al., 2016). Notably, a greater degree of

cortical thinning during adolescence has been associated with greater intellectual functioning (Shaw et al., 2008), and is believed to result from a combination of synaptic pruning and ongoing myelination (Huttenlocher & Dabholkar, 1997; Paus, 2005, 2010).

The relative decreases in gray matter in the transition from childhood to adulthood is also coupled with increases in white matter throughout development. White matter consists of glial cells, extracellular space, and myelinated axons (Mills & Tamnes, 2014). Past studies have reported a roughly linear increase in white matter volume, particularly from late adolescence to early adulthood (Giedd, 2004; Giedd et al., 1999; Tamnes et al., 2010). These results are consistent with work using diffusion tensor tractography imaging, which also showed white matter increases occurring from childhood to as late as young adulthood (Lebel & Beaulieu, 2011). Adolescents typically have a larger volume of white matter in frontal and parietal cortices compared with younger age groups (Barnea-Goraly et al., 2005; Reiss, Abrams, Singer, Ross, & Denckla, 1996; Sowell, Thompson, Holmes, Batth, et al., 1999), which is believed to result from increased axonal myelination (Barnea-Goraly et al., 2005; Klingberg, Vaidya, Gabrieli, Moseley, & Hedehus, 1999; Paus, 2005). Myelin is a lipid-rich structure that surrounds axons in both the central and peripheral nervous systems (D. L. Sherman & Brophy, 2005), forming an electrically insulating layer. As a result, myelinated axons can transmit information faster than unmyelinated axons. Therefore, the increase in white matter observed from childhood to adulthood, resulting from an increase in myelinated axons, has been associated with improved cognitive functions and a greater efficiency of information transmission (Ernst, 2014; Nagy, Westerberg, & Klingberg, 2004), enabling better integration between different brain regions.

Overall, longitudinal imaging work has revealed a developmental cascade of changes to key brain regions during adolescent development, including early maturation of sensory and motor regions which are followed by later maturation of frontal and temporal regions, in combination with relative reductions in gray matter and increases in white matter. These changes have been proposed to, in part, explain the "storm and stress" of this transitionary period, because they provide evidence that regions involved in emotionally-driven behaviours are relatively mature by adolescence, whilst regions involved in cognitive control and emotion regulation are still relatively immature at this stage.

1.5. Gender differences in adolescence

It should be noted that whilst previous adolescent studies have attempted to understand the cognitive, behavioural, and emotional changes that occur during this period of development, much of this seminal work has failed to consider the potential impact of gender differences (Doremus-Fitzwater & Spear, 2016; Spear, 2000b). This was surprising, given research which has reported significant gender differences in the rate of adolescent brain development. Specifically, in previous sMRI work males have exhibited evidence of later maturation of cortical and thalamic grey matter volume during adolescence (Lenroot & Giedd, 2010), with some brain changes occurring approximately 1-2 years later than females (Lenroot et al., 2007). Furthermore, in reward processing regions such as the striatum males reach peak striatal volume much later (around age 15) compared to females (around age 12) (Raznahan et al., 2014), which may help to explain why previous meta-analytic work has reported greater risk-taking behaviours in adolescent males relative to females (Byrnes, Miller, & Schafer, 1999). In addition to gender differences in the rate of brain maturation during adolescence, previous research suggests that adolescent females experience greater anxiety (Abe & Suzuki, 1986; Lewinsohn, Gotlib, Lewinsohn, Seeley, & Allen, 1998), as well as greater levels of depression (P. Cohen et al., 1993; Cyranowski, Frank, Young, & Shear, 2000; Twenge & Nolen-Hoeksema, 2002) compared with adolescent males. Taken together, this work suggests that any assessment of adolescent emotion processing should seek to account for differences associated with gender.

1.6. Defining emotion

In order to examine emotion processing in adolescence, it is important to first define what is meant by the term “emotion”. Emotions can be defined as valenced responses to external sources and/or internal mental representations which may involve experiential, peripheral, behavioural and physiological changes (Ochsner & Gross, 2005). According to Appraisal Theory, the experience of emotion is built from an individual’s motivation to continually evaluate (or appraise) their relationships with their environment (Lazarus, 1991). If a person appraises an event in their environment as having personal significance to them, then a specific emotion (tied to the appraisal) will result. Within this theory, emotions are treated as complex states which are distinguished from moods, because they can be triggered by a combination of subjective

affect (including the appraisal), physiological responses, and action tendencies (i.e. responding to fear by escaping a situation). For example, imagine a teenager entering a social scenario for the first time. If they perceived that their interactions in the scenario went poorly, the teenager may feel sadness, rejection, or anger, because they have appraised the event as having long-term negative effects for them personally. This may affect their thoughts and behaviours regarding whether or not to enter similar social scenarios in the future, through fear of experiencing the same rejection as before. This demonstrates the dynamic, interactive nature of emotions, motivations and cognitions, and can help to explain why individuals have different emotional reactions to the same event, and how different events may lead individuals to experience similar emotions (Fernando, Kashima, & Laham, 2017), because each individual is motivated by unique goals.

1.7. Neurobiological models of adolescence

In order to understand how structural and functional brain changes impact emotion processing in adolescence, a number of neurobiological models have been proposed. Each model explains adolescent behaviour in the context of increased risk-taking and reward seeking (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008, 2010), with Casey's model also attempting to understand the increased emotionality observed during adolescence. Each of these models will be reviewed in detail, to understand the similarities and differences in their explanations of adolescent behaviour, and to elucidate what is currently known about adolescent emotion processing.

1.7.1. Dual System Models

A dual systems perspective was originally proposed to understand adolescent risk-taking, sensation-seeking and decision making. Overall, dual systems models theorise that the peak in risk-taking observed in adolescents (e.g., S. Burnett et al., 2010; S. H. Mitchell, Schoel, & Stevens, 2008; L. Steinberg, 2010) results from early maturation of subcortical socioemotional-incentive processing regions, in combination with relatively late maturation of cognitive control regions (Shulman et al., 2016). As a result of this developmental mismatch, adolescents are believed to be more prone to increased risk-taking and sensation-seeking behaviours. This is because the socioemotional system amplifies the salience and affinity for such experiences, whilst

the cognitive control system results in a weaker ability to exercise cognitive control in situations which may be potentially dangerous. Several dual systems models have been proposed (Figure *1-1*), and the following sections will describe each of these models in detail, to understand what they can explain about emotion processing during adolescence.

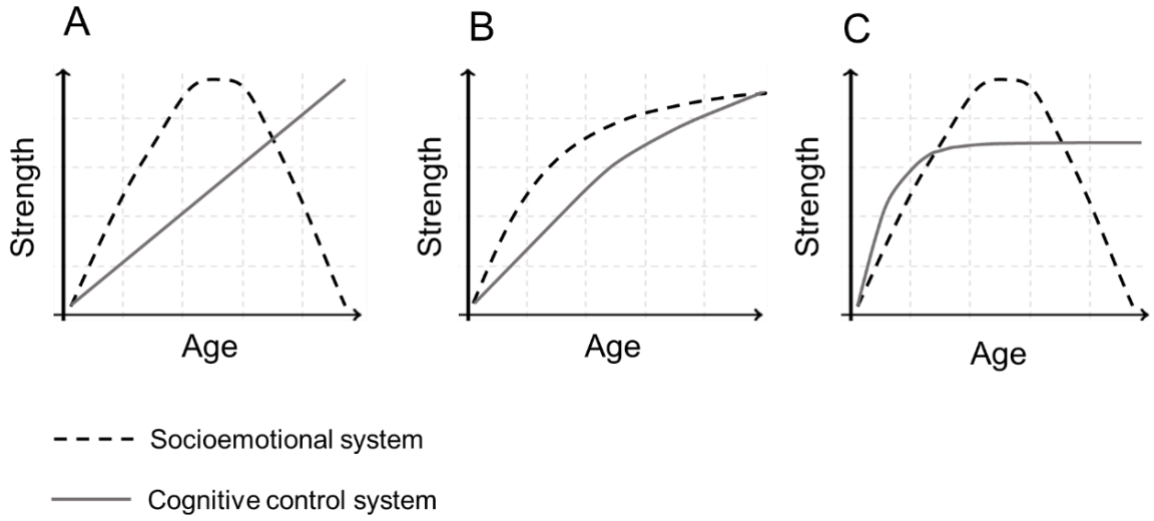

Figure 1-1: Schematic representations of the three key dual systems models of adolescence, adapted from (Shulman et al., 2016). Each model explains adolescent behaviour according to an early maturing subcortical socioemotional system, followed by a late maturing cortical cognitive control system. However, each model differs in its explanations of the precise developmental trajectory of these two regions. A: Steinberg's (2008) dual systems model. B: Casey's et al. (2010) imbalance model. C: Luna and Wright's (2016) driven dual systems model.

1.7.1.1. Steinberg's Dual Process Model

Steinberg's dual systems model (DSM; 2008, 2010) attempts to understand adolescence in the context of increased reward-seeking and risk-taking behaviours. As a result of the model's focus on rewarding stimuli only, this model is limited in its ability to explain threat processing in this age group. Steinberg's DSM stems from Metcalfe and Mischel's (1999) earlier DSM of willpower, which distinguishes between a "hot" and "cool" system, with self-control behaviours believed to result from a balance between these two systems. The hot system in this model is specialised for rapid emotional responding, driven by environmental triggers, whereas the cool system is a specialised complex cognitive system. Steinberg mapped these two systems to explain

differences in adolescent and adult risk-taking. Specifically, in this model increased risk-taking during adolescence is believed to result from a developmental mismatch between two key networks: the socio-emotional ("hot") system and the cognitive control ("cold") system.

The socio-emotional system consists of a number of key limbic and paralimbic brain regions, such as the superior temporal sulcus (STS), the medial PFC, as well as the orbitofrontal cortex (OFC), amygdala and the ventral striatum. This system follows a U-shaped developmental trajectory, whereby reward responsivity increases early on in adolescence and begins to decline in early adulthood (Figure 1A, Shulman et al., 2016). As this system of neuronal networks begins to mature, substantial remodelling of dopaminergic pathways occurs in projections from the limbic system to prefrontal regions. This is theorised to result in the increased reward-seeking behaviour that is observed during adolescence, because social and emotional information processing relies on affective and motivational regulation networks, which are heavily impacted by dopaminergic activity (L. Steinberg, 2008). These changes to dopaminergic pathways are coupled with the increase in gonadal hormones during puberty, which are believed to affect an individual's memory for social information and bonding (Nelson et al., 2005). The increase in gonadal hormones during puberty has been suggested to explain why adolescents, compared to children and adults, exhibited greater paralimbic, limbic, and medial PFC activation when responding to socio-emotional cues (L. Steinberg, 2008). Together, these brain and pubertal developmental changes are thought to drive increased risk-taking in adolescence, as increases in dopaminergic activity in regions within the socio-emotional system encourages adolescents to seek out rewarding stimuli.

Whilst the early maturation of the socioemotional system is argued to result in increased risk-taking during adolescence, Steinberg's DSM suggests that the slower maturation of the cognitive control system can explain the decrease in risk-taking in the transition from adolescence to adulthood. The cognitive control system consists of the lateral prefrontal cortex, the lateral parietal cortex, and the anterior cingulate cortex (ACC), which are involved in self-regulatory behaviours and cognitive control. Maturation of this system should result in a greater ability to regulate one's own behaviour and inhibit impulsive responses. This is because the increase in connections within different cortical areas, and also between cortical and subcortical areas, is likely to result in improved emotion regulation, due to increased connectivity between regions

which are important for emotional processing and regions which are important for cognitive control. This is supported by empirical work showing a linear decrease in impulsivity from around age ten onwards (Harden & Tucker-Drob, 2011; L. Steinberg, 2010), with a peak in sensation-seeking and risk-taking behaviours at mid-adolescence, that declines slowly into young adulthood (S. Burnett et al., 2010; S. H. Mitchell et al., 2008; L. Steinberg, 2010). This evidence is strengthened by fMRI work using a gambling task, which demonstrated increased sensitivity of reward-related regions (e.g., medial PFC and ventral striatum) during adolescence, and slower maturation of cognitive control-related regions (e.g., DLPFC, Van Leijenhorst et al., 2010). Similar fMRI work has also shown that adolescents engage cognitive control-related regions less than adults during a monetary decision-making task (Eshel, Nelson, Blair, Pine, & Ernst, 2007). Together, the maturation of the socio-emotional system and comparatively slower maturation of the cognitive control system is theorised to result in increased risk-taking and sensation seeking in mid-adolescence, as the relatively under-developed prefrontal regions struggle to execute sufficient cognitive control in emotionally salient situations.

However, whilst there is evidence to suggest that adolescents engage in greater risk-taking compared with adults (S. Burnett et al., 2010; S. H. Mitchell et al., 2008; L. Steinberg, 2010), there are inconsistencies in the literature, with some studies failing to observe any age effects on behavioural risk-taking between adolescents and adults (Bjork, Smith, Danube, & Hommer, 2007; Eshel et al., 2007). In response to these criticisms, recent clarifications of Steinberg's DSM posit that mid-adolescents do not necessarily demonstrate the highest degree of risk-taking across all possible activities, but that mid-adolescents demonstrate the greatest propensity for risk-taking, which is highly context-dependent (Shulman et al., 2016). Given the range of tasks that adolescents are asked to engage in (e.g., gambling tasks, risk-taking/impulsivity questionnaires, and driving simulations) it is likely that different studies capture different elements of risky behaviour, which could help to explain the mixed results reported here. Overall though, the weight of empirical evidence supports the theory that adolescent risk-taking can be at least partially explained by a developmental mismatch between socioemotional and cognitive control systems.

1.7.1.2. Casey's Imbalance Model

Casey's imbalance model (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010) is very similar to Steinberg's model, as it also posits that adolescent behaviour is modulated by a developmental mismatch between subcortical and cortical brain regions. However, unlike Steinberg's model, Casey's model seeks to explain adolescent behaviour in the context of responses to both rewarding and threatening stimuli. Specifically, Casey suggests that bottom-up, subcortical limbic regions responsible for emotional and motivational processes are relatively mature by adolescence, whilst the top-down prefrontal regions responsible for rational decision-making, emotional regulation and impulse control are relatively immature. This developmental mismatch leads to an "imbalance", and has been theorised to result in a stronger ability for adolescents to respond emotionally to their environment, with a weaker ability to exercise cognitive control over their reactions. Therefore, in emotional contexts, the more mature limbic system takes control of the situation, which may lead to a poor decision on the part of the adolescent. In this case, the adolescent may recognise that their action was inappropriate, but felt unable to exercise cognitive control in the situation, as it occurred in a highly emotive context. This imbalance is not observed in children, as both the limbic and prefrontal regions are both immature, and is not observed in adults, as both regions are thought to be fully mature. In contrast to Steinberg's model, which suggests that the socioemotional system develops according to a U-shaped trajectory, the imbalance model proposes that the socioemotional system peaks in reactivity at mid-adolescence, and remains at this level into adulthood, with the maturation of the cognitive control system causing the socioemotional system to become less reactive with age (Shulman et al., 2016; Figure 1B).

A number of fMRI studies provide support for Casey's model, demonstrating that changes to the differential recruitment of prefrontal cortical and limbic subcortical regions occurs throughout development. These changes can impact how adolescents process emotionally salient stimuli in their environment. For example, in the context of reward processing, studies have reported greater activation of the nucleus accumbens in response to rewards in adolescents compared to children or adults (Ernst et al., 2005; Galvan, Hare, Voss, Glover, & Casey, 2007; Galvan et al., 2006). In addition, work has shown impulsivity to rewarding stimuli to reduce in line with maturation of the ventral PFC, as an individual transitions from childhood to adulthood (Casey, Galvan, & Hare, 2005). Conversely, in the context of threat processing, a more limited body of work has

observed greater activity in the ventral striatum and amygdala in adolescents who were encountering or anticipating aversive stimuli, compared to children or adults (Galvan & McGlennen, 2013; Hare et al., 2008). These regions, as part of the limbic system, have been heavily implicated in emotional processing and motivational influences on behaviour (Cardinal, Parkinson, Hall, & Everitt, 2002; Everitt et al., 1999), with the striatum shown to have specific responsibilities regarding the processing of threatening or aversive stimuli (Jensen et al., 2003; Levita et al., 2009; Levita, Hoskin, & Champi, 2012; Pohlack, Nees, Ruttorf, Schad, & Flor, 2012; Seymour, Daw, Dayan, Singer, & Dolan, 2007). Taken together, the results from these studies suggest that adolescents may be exhibiting hyper-responsivity to both rewards and threats, as a result of a developmental mismatch between a late-maturing PFC and earlier-maturing subcortical regions.

1.7.1.3. Luna's Driven Dual Systems Model

Recently, an additional dual systems model has been proposed by (Luna & Wright, 2016), referred to as the driven dual systems model. This model was designed to address recent changes to the U.S. juvenile justice system, in which neuroscientific evidence of adolescent brain development is now used as part of juvenile appeal cases, to better understand serious criminal behaviours committed during adolescence. In doing so, this driven dual systems approach is focused on sensation seeking and reward processing, and so does not explain threat processing during adolescence. This model shares a core similarity with the other two duals systems models suggesting that hyperactivity of the reward system, resulting from the rise of dopamine activation during adolescence (L. Steinberg, 2008), can lead to greater risk-taking and sensation seeking behaviours. These sensation seeking behaviours are believed to be adaptive, as adolescents attempt to explore their environment and become more independent. In agreement with Steinberg's DSM, the driven dual systems model suggests that the subcortical socioemotional system follows a U-shaped developmental trajectory, making it more reactive during adolescence.

However, in contrast to both Steinberg (2008, 2010) and Casey's (2008; 2010) dual system models, which suggest that the cognitive control system continues to mature into young adulthood, the driven dual systems model suggests this system reaches adult levels by mid-adolescence. In support of this theory, Luna and Wright

(2016) provide evidence that adolescent executive functions that support cognitive control behaviours, such as inhibitory control and working memory skills, activate similar prefrontal cortical regions to adults, but that adolescents may be more prone to making errors during inhibitory control tasks, and were less able to monitor such errors (Ordaz, Foran, Velanova, & Luna, 2013). This suggests that whilst key prefrontal regions were adult-like, more specialised cognitive control functions were still not fully optimised. Furthermore, Hwang, Velanova, and Luna (2010) conducted an fMRI study with 8-27-year-olds, to examine inhibitory control using an anti-saccade task. In that task, participants are instructed to suppress an innate reflexive saccade towards a visual target which was presented on a screen, and attend to a point in an opposite location. Results from their functional connectivity analyses suggested that the top-down connections that support cognitive control during the anti-saccade task were present in both adolescents and adults, but in adulthood this connectivity was characterised by increased functional connections between more distributed networks, to support more efficient top-down executive control of behaviour. In summary, Luna and Wright (2016) argue that whilst specific processes may undergo increased specialisation during adolescence, many key aspects of prefrontal brain processes, such as executive functioning, are intact at this stage. However, they also argue that additional processes which strengthen executive performance may still be maturing, which leads to greater errors when task difficulty increases.

1.7.2. The Triadic Model

Like Steinberg's DSM, Ernst et al. (2006) proposed the triadic model to understand adolescent decision making in the context of risk-taking, as adolescent risk-taking is believed to be highly prevalent (Centers for disease control and prevention, 2006). However, like Casey's imbalance model, the triadic model also seeks to explain adolescent responses to both rewards and threats. The model describes three modules (Motivation/Approach, Emotion/Avoidance, and Regulation), and the relative neural systems which support them. Overall, the model puts forward: (1): a motivation system that is served by the ventral striatum and is associated with approach behaviours, (2): an emotion system that is served by the amygdala and is associated with avoidance behaviours, and (3): a regulatory system that is served by the PFC and is associated with cognitive and affective control (Ernst, 2014). In relation to specific behaviours observed

during adolescence, the model theorises that the ventral striatum is involved in risk-taking and cognitive impulsivity, whereas the amygdala is involved in the intensity and lability of emotional experiences. All three modules are involved in social reorientation, which is thought to represent a move from familial to peer relationships (Ernst, 2014).

In contrast to Casey's model (2008; 2010), which suggests adolescents show hyper-responsivity to both rewarding and threatening stimuli, the triadic model argues that adolescents have a stronger approach system in response to rewards and a weaker harm-avoidant system in response to threats - compared to adults who show equivalent responses to both approach and avoidance stimuli. This imbalance between approach and avoidance behaviours in adolescence is explained by differences in the developmental trajectories of the brain regions that support the approach, avoidance and regulatory systems. For example, the approach module is supported by the ventral striatum, particularly the nucleus accumbens, which has been heavily implicated in reward processing and approach behaviours (Balleine, Delgado, & Hikosaka, 2007; Delgado, 2007; Koepp et al., 1998). Ernst (2014) suggest that the development of this module follows a curvilinear developmental trajectory, due to MRI evidence that demonstrates a peak in responses to reward in the striatum during adolescence (Cohen et al., 2010; Galvan et al., 2006; Van Leijenhorst et al., 2010).

In comparison to the approach module, the avoidance module is supported by the amygdala, which has a well-established role in emotion, social and more specifically threat processing (Adolphs, Tranel, & Damasio, 1998; Davis & Whalen, 2001; J. E. LeDoux, 2000; Phelps & LeDoux, 2005). Ernst et al. (2006) link the development of this region to avoidance behaviours observed in adolescents, because of the amygdala's well-known role in the processing of negative emotional stimuli and responses. Like the approach module, this module follows a curvilinear developmental trajectory, in which the intensity and lability of negative emotional responses are said to peak during adolescence. However, the triadic model argues that adolescents may be less avoidant of risks because of the relative immaturity of the amygdala/avoidance system. This is supported by past work that examined adolescents and adults responses to risk-taking and reward-seeking in a monetary task, which showed weaker activation of the harm-avoidant amygdala, and greater activation of the approach-related nucleus accumbens in adolescents compared with adults (Ernst et al., 2005). Finally, the model suggests that this imbalance between approach and avoidance behaviours is exacerbated by the

immature, slowly developing PFC, which fails to effectively balance the approach and avoidance modules (Ernst et al., 2006).

However, this model is not supported by studies which suggest that the motivation/approach and emotion/avoidance systems, served by the ventral striatum and amygdala respectively, are not as discretely organised as originally described by the Triadic Model. For example, research into amygdala function has shown that, as opposed to its original conceptualisation as solely a fear module (Ohman & Mineka, 2001), the amygdala also processes appetitive stimuli (Baxter & Murray, 2002; Cardinal et al., 2002; Gottfried, O'doherty, & Dolan, 2003). Similarly, whilst the striatum has been previously conceptualised as a reward module (Balleine et al., 2007; Delgado, 2007; Koepp et al., 1998), it has since been shown to be involved in the processing of threatening and aversive stimuli (Jensen et al., 2003; Levita et al., 2009; Levita et al., 2012; Pohlack et al., 2012; Seymour et al., 2007). This evidence suggests that Ernst's model at the moment is an oversimplification of adolescent approach and avoidance behaviours, and moreover the suggestion that adolescents show hypo-responsivity to threats is not currently supported by scientific work in humans (e.g., Britton et al., 2013; Galvan & McGlennen, 2013; Hare et al., 2008).

1.7.3. Spear's Reward-Centricity and Attenuated Aversions Model

In an earlier model, Spear (2000b) initially theorised that adolescents may exhibit attenuated responses to rewarding stimuli, which would explain why adolescents choose to seek out additional rewards through increased risk- and sensation-seeking behaviours. More recently, however, Spear and colleagues now argue that adolescence is best characterised as a period of hyper-responsivity to rewarding stimuli, as well as resistance to their potentially aversive effects (Doremus-Fitzwater & Spear, 2016). As such, this reward-centric phenotype shares some similarities with each of the dual systems models described in Section 1.7.1, which also suggest adolescents are hyper-responsive to rewards, and shares some similarities with the triadic model, which has suggested that adolescents have stronger approach and a weaker harm-avoidant system than adults.

The majority of evidence used in support of Spear's model is based on non-human animal work, utilising methods such as the conditioned place preference (CPP) paradigm. In this task, a stimulus (e.g., rewarding or aversive) is paired with a specific

chamber in the animal's environment, whilst the absence of this stimulus is paired with a different chamber. Following this period of training, the rat undergoes an assessment phase, and can move freely between either chamber. During the test period, more time spent in the chamber that was paired with the stimulus indicates a preference for that stimulus, whereas more time spent in the other chamber is believed to indicate aversion for the stimulus (Doremus-Fitzwater et al., 2010). Studies of reward processing using CPP paradigms have demonstrated that adolescent rats may be more sensitive to the effects of rewarding stimuli than their adult counterparts. For example, studies have shown significantly stronger place preference conditioning in adolescents, relative to adults, using both nicotine (Torres et al., 2008; Vastola, Douglas, Varlinskaya, & Spear, 2002) and potential drugs of abuse (e.g., cocaine, Brenhouse & Andersen, 2008; Zakharova, Wade, & Izenwasser, 2009).

This enhanced reward seeking during adolescence has been proposed to result from a series of complex maturational changes to the reward neurocircuitry during this phase of development. Specifically, rodent work suggests that the dopamine systems innervating the nucleus accumbens (mesolimbic pathway), the prefrontal cortex (mesocortical pathway), and the striatum (nigrostriatal pathway) (Björklund & Dunnett, 2007) are different during adolescence (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Tarazi & Baldessarini, 2000), with enhanced activity of dopamine neurons observed in this age group, in comparison to younger and older rodents (for reviews, see Marinelli & McCutcheon, 2014; McCutcheon & Marinelli, 2009). This enhanced activity of dopamine neurons has been associated with increased drug intake during adolescence. For example, increased firing rates of dopamine neurons in the ventral tegmental area were shown to be associated with greater cocaine self-administration in adolescent rats, when compared with adults (W. C. Wong, Ford, Pagels, McCutcheon, & Marinelli, 2013). Furthermore, this increase in cocaine self-administration could be eliminated when adolescent rats were given quinpirole (D2/D3 agonist), a drug used to suppress the activity of these dopamine neurons.

Moreover, in addition to the work described above which suggests that adolescents are particularly sensitive to rewarding stimuli, as a result of enhanced activation of the DA system during this phase of development, additional work suggests adolescents are also resistant to the aversive effects of such stimuli. For example, administering potential drugs of abuse (e.g., cocaine), repeatedly or at higher doses can eventually lead to negative consequences such as nausea, anxiety, and/or motor

impairments. However, under these circumstances, studies have demonstrated that adolescent rats show reduced conditioned taste aversion (CTA) for nicotine (Shram, Funk, Li, & Lê, 2006), 4-methylenedioxymethamphetamine (MDMA, Cobuzzi et al., 2014), cocaine (Schramm-Sapyta et al., 2006), as well as the non-addictive substance lithium chloride (Schramm-Sapyta et al., 2006), compared to adult rats. This suggests that adolescent rats were less likely to avoid a taste which had been paired with a target drug in past experiments, indicating reduced sensitivity to the aversive properties of said drug. Additionally, adolescent rats have also been shown to be resistant to the toxic effects of high nicotine doses (Torres et al., 2008), acute ethanol intake (Ramirez & Spear, 2010), and negative effects associated with withdrawal from drugs (Brasser & Spear, 2002; Varlinskaya & Spear, 2004), compared to adult rats.

Importantly, however, some studies have reported no age differences in the degree of drug self-administration in adolescent and adult rats using cocaine (Harvey, Dembro, Rajagopalan, Mutebi, & Kantak, 2009; Kerstetter & Kantak, 2007). In addition, whilst there is a convincing amount of nonhuman animal evidence to suggest that adolescents are resistant to the effects of aversive stimuli, there is little conclusive human work to support this theory. Specifically, whilst some research has observed aversion-resistant behaviours in human adolescents (Moutsiana et al., 2013), other research shows no age differences at all (Barkley-Levenson, Van Leijenhorst, & Galván, 2013).

The debate regarding whether or not adolescents show reduced responses to aversive stimuli could be explained, in part, by the methodological considerations made by researchers. For example, whilst Spear has argued that adolescents do show hypo-responsivity to aversive stimuli (Doremus-Fitzwater & Spear, 2016; Doremus-Fitzwater et al., 2010), evidence for this view is based on non-human animal work, which has focused on the aversive effects of drugs of abuse which have both positive and negative reinforcing properties. Although Spear and colleagues acknowledge that these stimuli contain a mixture of appetitive and aversive properties (Doremus-Fitzwater & Spear, 2016), they do not discuss this in detail as part of their model, and they also do not discuss how adolescent responses may differ in response to the purely aversive properties of a stimulus. However, when stimuli with solely aversive properties (e.g., white noise burst, screams) are used, evidence from human work suggests that adolescents are hyper-responsive to these threatening stimuli, compared with adults. For example, recent human EEG work has reported enhanced potentiation of visual sensory

responses to learned danger signals in an instrumental avoidance paradigm, using an aversive loud tone that participants learned to avoid (Levita et al., 2015). Furthermore, fMRI work has shown greater activation of the amygdala and ventral striatum in adolescents, when presented with aversive stimuli such as loud screams or aversive liquids (e.g., sodium chloride) (Britton et al., 2013; Galvan & McGlennen, 2013). Therefore, in these scenarios rather than adolescents being hypo-responsive to aversive stimuli/outcomes, hyper-responsivity is observed, supporting Casey's imbalance model, which posits that adolescents are hyper-responsive to both rewards and threats.

1.7.4. The Social Information Processing Network

The Social Information Processing Network (SIPN; Nelson et al., 2005), was proposed to explain the emotional and cognitive changes in human adolescents' processing of socially-relevant stimuli. The SIPN consists of three primary nodes: the detection node, the affective node, and the cognitive-regulatory node. The *detection* node comprises visual processing brain regions such as the fusiform face area, as well as the anterior temporal cortex and superior temporal sulcus, and is primarily used to categorise and identify a stimulus based on its social properties. The *affective* node comprises the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens and the hypothalamus. These regions are integral to the processing of both rewards and threats, and ultimately determine whether to approach or avoid a social stimulus, and modulate the autonomic and cognitive processes in response to such stimuli. Finally, the *cognitive-regulatory* node comprises the dorsomedial PFC and the ventral PFC (including the orbitofrontal cortex). These regions determine one's ability to perceive other people's perspectives (e.g., theory of mind), as well as generating goal-directed behaviours that may require the suppression of highly emotional behaviours, in favour of achieving a more important goal. Together, these nodes are theorised to function sequentially, but can also function as an interactive network.

According to the SIPN, a number of developmental changes to key brain regions can explain the changes in social processing observed during adolescence. For example, because the detection node consists of regions primarily involved in visual processing, which have largely matured by adolescence, Nelson et al. argue that this node is relatively unchanged during adolescent development. In contrast, the affective node is argued to be heavily influenced by the development of gonadal hormones during

adolescence. For example, regions of the affective node receive innervations from gonadal steroid receptors, which develop during puberty (McEwen, 2001; Romeo, Richardson, & Sisk, 2002). The effect of gonadal hormones on social processing within the affective node has been observed behaviourally, with an increase in gonadal steroids shown to be associated with the initiation of sexual activity in adolescent male and females (Halpern, Udry, & Suchindran, 1997; Halpern, Udry, & Suchindran, 1998), preference for sexual traits (Penton-Voak et al., 1999), social attachment (Insel, 1997) and increased emotional distance between adolescents and their parents (L. Steinberg, 1987). As a result, the development of gonadal steroids could influence how adolescents assign affective attributions to social stimuli within the SIPN. Taken together, this work suggests that adolescents may show hyper-responsivity to social stimuli within affective node regions due to the development of gonadal steroids, and that the continued development of gonadal steroids during adolescence could impact the processing of emotional responses to a range of other social stimuli in this age group.

In contrast to the affective node, which is heavily influenced by the development of gonadal steroids during adolescence, the cognitive-regulatory node is heavily influenced by the protracted maturation of neural networks, such as the dorsomedial and ventral PFC. Specifically, these regions undergo increased myelination and synaptic pruning during adolescence. Because these changes are believed to occur at a protracted rate in prefrontal regions (As outlined in Section 1.3, Casey et al., 2000; Gogtay et al., 2004), adolescents may have difficulties when attempting to regulate top-down responses to social stimuli. This was shown in a passive viewing task by (Monk et al., 2003) who found that adolescents struggled to engage and disengage the prefrontal OFC compared with adults, when required to engage in attentional switching between emotional ("How afraid does this face make you feel?") and non-emotional ("Rate the nose width of the face") properties of fearful face stimuli. This provides support for the protracted development prefrontal brain regions within the cognitive-regulatory node of the SIPN, and provides empirical support for the interactive nature of the affective and cognitive-regulatory nodes. In sum, Nelson et al. suggest that gonadal hormone changes occurring during puberty result in altered processing of stimuli which contain social properties, as part of the affective node, whilst slower maturational changes in PFC regions result in difficulties when regulating responses to social stimuli, as part of the cognitive-regulatory node.

1.7.5. Casey's Circuit-Based Model

Despite the utility of the models discussed in providing helpful heuristics for understanding how adolescents process rewarding and/or aversive stimuli, it is important to note that all of these models are over-simplified (Casey, 2015; Pfeifer & Allen, 2012), which may be hindering a much richer understanding of adolescent behaviour. In response to this, recent developments of Casey's model (Casey, 2015; Casey, Galvan, & Somerville, 2016; Casey, Heller, Gee, & Cohen, 2017) posit that, rather than a simpler developmental mismatch between subcortical and cortical brain regions during adolescence, a succession of developmental changes to the fine tuning of fronto-limbic circuit connections could impact emotionally-driven behaviours during adolescence. Specifically, the developmental changes in behaviour that occur during adolescence coincide with developmental changes across multiple neural systems, with connections being fine-tuned first between subcortical and limbic circuits, followed by the fine-tuning of connections between cortical and limbic circuits, and subsequently between cortico-cortical circuits (Casey et al., 2016; Casey et al., 2017). This suggests that the circuits associated with emotionally driven behaviours may be strengthened before the maturation of top-down control mechanisms (Casey, 2015), which could help to explain increased emotionality and increased risk-taking behaviours during adolescence.

Evidence in support of a circuit-based account of adolescent brain development comes from functional connectivity studies, which suggest local subcortical circuits are refined first, whereas distal cortical circuits are refined later (Fair et al., 2009; Satterthwaite et al., 2013). These functional connectivity changes have also been associated with changes in adolescent behaviour. For example, Somerville, Hare, & Casey (2011) conducted a go/nogo task using happy (appetitive) and neutral facial expressions on a sample of children (6-12 years), adolescents (13-17 years), and adults (18-29 years). Their results demonstrated linear improvements in impulse control to the neutral cues with increasing age, however, they also report a nonlinear trajectory of impulse control to the appetitive cues. More specifically, adolescents demonstrated reduced impulse control in response to appetitive cues, relative to children and adults. In addition, they also conducted a functional connectivity analysis on their data, and found that adolescents demonstrated strengthened local subcortical coupling, whereas adults demonstrated strengthened prefrontal cortico-subcortical coupling. These results support a circuit-based account of adolescent behaviour, in which the development of

circuit connectivity in cortical versus subcortical regions are associated with developmental changes in behaviour. Overall, whilst other models specify the relative differences in how adolescents' recruit discrete regions of the brain during threat and/or reward processing, Casey and colleagues are now focused on how circuit-based changes occur with age, and how the strengthening of connections between subcortical and cortical regions could explain the behavioural changes observed during adolescence (Casey, 2015). Overall, this latest development of Casey's neurobiological model of adolescence suggests that adolescent changes in behaviour may be indexed by relative strengthening of subcortical-cortical neurocircuitry, providing a focus for future work in this area.

1.7.6. Limitations of current neurobiological models of adolescence

One of the main strengths of these aforementioned neurobiological models of adolescence regards their collective agreement that changes to cognitive and affective behaviours observed in adolescence can be partially explained by a developmental mismatch between early maturing subcortical (i.e., affective) and late maturing cortical (i.e., cognitive) regions. This is supported by evidence presented in Section 1.4, which discussed a number of key structural and functional brain changes in the transition from adolescence to adulthood. Together, each of these six neurobiological models provide helpful heuristics that have guided the study of adolescent decision making, with a focus on risk-taking behaviours, and most share similarities in their explanations of how adolescents process rewarding stimuli. However, these models have a number of key limitations, which impacts the conclusions that can be drawn from them. This next section will highlight some of the weaknesses in these models, and the aim of the work in this thesis is to make a start in addressing these where possible, and to extend current models of adolescence.

The first limitation of these models regards the reductionist view that adolescent behaviours can be explained simply by making distinctions between a subcortical emotional "hot" system, and a cortical cognitive "cold" system. This distinction emerged from older behavioural models which suggested that the "hot" system encompasses subcortical brain structures which are specialised for emotional responding, whilst the "cold" system encompasses cortical brain structures which are specialised for cognitive control (e.g., Metcalfe and Mishel, 1999). In support of this

idea, plenty of evidence suggests that the activation of subcortical structures is associated with emotion processing. For example, greater activation of subcortical structures such as the amygdala and the striatum have been observed in response to aversive stimuli (e.g., Davis, 1992; Jensen et al., 2003; Ledoux, 1990; Levita et al., 2009; Levita et al., 2012; Maren & Fanselow, 1996; Pohlack et al., 2012; Seymour et al., 2007), as well as appetitive stimuli (Baxter & Murray, 2002; Cardinal et al., 2002; Gottfried et al., 2003). This evidence has been used in neurobiological models of adolescence as support for emotions being generated primarily within subcortical brain structures. Additionally, there is evidence to suggest that the development of cortical structures is associated with a variety of cognitive processes, such as improved cognitive control (e.g., Casey, Getz, et al., 2008; Casey, Jones, et al., 2010). Together, these studies have been used to simplify explanations of cognitive-affective processes, with emotional processes linked to subcortical activation and cognitive processes linked to cortical activation.

However, whilst these studies provide support for the view that emotions are associated with subcortical regions and cognitions are associated with cortical regions, it does not necessarily mean that emotional and cognitive processes exist within discrete systems in the brain. Rather, recent models discuss the dynamic interaction between cortical and subcortical systems during emotion processing. For example, Ledoux and colleagues have proposed a higher-order theory of emotional consciousness, whereby subcortical circuits provide lower-order non-conscious inputs that interact with cortical circuits to support conscious emotional experiences (Ledoux & Brown, 2017). According to their model, emotions are cognitive states themselves and would not exist without higher order cognitive experiences of emotion. This is supported by work which has distinguished between hot (emotion-laden) and cold (emotion-independent) cognition (Roiser & Sahakian, 2013), which suggests that emotions may drive certain cognitive processes by making them motivationally relevant to an individual's personal goals. Therefore, although neurobiological models of adolescence often make the distinction between a "cold" cognitive system and a "hot" emotion system, it is important to recognise emotions are complex states which are influenced by both motivational and cognitive factors. The protracted refinement of neural circuitry between these systems may help to explain the increase in emotionally-driven behaviours in adolescence.

The second limitation of most of these models regards their continued emphasis

on the study of reward processing in adolescence, which appears to have resulted in a lack of focus on threat processing in this age group. Specifically, three of the six models attempt to explain how adolescents process threatening stimuli (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Evs, 2015), with the remaining models focusing solely on adolescent sensitivity to rewards and risk-taking behaviours (Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008, 2010). Although recent research suggests that this lack of focus on adolescent threat processing is beginning to change (e.g., Britton et al., 2013; Den, Graham, Newall, & Richardson, 2015; Ganella, Barendse, Kim, & Whittle, 2017; Ganella, Drummond, Ganella, Whittle, & Kim, 2018; Hare et al., 2008; Waters, Theresiana, Neumann, & Craske, 2017), there is currently a much weaker understanding of how adolescents process threats, relative to rewards.

A third issue relates to models which do include explanations of adolescent threat processing, as at the moment they are provide differing theoretical perspectives regarding how threatening stimuli are processed. Ernst's model (2006) suggests that adolescents should show hypo-responsivity to threats, due to a weaker harm-avoidant system. Similarly, Spear's model (2016) argues that adolescents should show hypo-responsivity to aversive stimuli, as part of a reward-centric/aversion-resistant phenotype. In contrast, Casey's imbalance model (2008; 2010) suggests that adolescents should show hyper-responsivity to both threats and rewards. Therefore, this doctoral work was designed to examine whether adolescents are hyper- or hypo-responsive to aversive versus appetitive stimuli.

A fourth limitation regards each model's focus on labelling increased risk-taking/sensation-seeking behaviours as inappropriate, rather than considering whether these behaviours serve an adaptive purpose. The majority of these neurobiological models explain adolescent behaviour within the context of early maturing subcortical regions and late maturing cortical regions, and suggest that this mismatch leads to "inappropriate" increases in risk-taking and sensation-seeking behaviours. These viewpoints perpetuate stereotypes of adolescents which suggest that they act carelessly and dangerously. In contrast, in a review of adolescent brain development, Crone and Dahl (2012) have argued that adolescents exhibit goal flexibility, which enables them to adapt their motivations and priorities depending on the social and motivational context. For example, their goal flexibility in social contexts may lead them to exhibit behaviours which appear to be impulsive and maladaptive (e.g. an adolescent who is

motivated to engage in risky driving behaviours to impress peers, at the potential cost of causing an accident), but such flexibility also allows them to learn and problem solve in a way that fosters independence in subsequent social scenarios. Furthermore, increased risk-taking behaviours may enable adolescents to explore new peer and romantic relationships which are vital for the development of new socioemotional skills. This may be why adolescents appear to be more inclined to take risks in emotional ("hot") scenarios rather than in non-emotional ("cold") scenarios (Figner, Mackinlay, Wilkening, & Weber, 2009), as this may increase their opportunity for emotional learning and social bonding.

Support for adolescent goal flexibility comes from research demonstrating that although adolescents exhibit increased risk-taking behaviours, they also demonstrate increased learning in risk-taking tasks (Humphreys et al., 2016; McCormick & Telzer, 2017), which suggests that adolescents take risks in order to explore and learn more about their environment. This was shown recently in McCormick and Telzer (2017), who asked children and adolescents (8-17 years) to complete the Balloon Analogue Risk Task (BART). In that task, children and adolescents were asked pump up a series of balloons, with each pump of the balloon increasing the chances that the balloon will burst. Participants earned points for each pump of air that they added to the balloon, and could choose to stop pumping the balloon and cash their points at any time. As expected, the results of that study demonstrated age-related increases in risk-taking, with adolescents taking on the greatest risk relative to children. However, this increase in risk-taking observed in adolescents was explained by age-related increases in learning, such that adolescents successfully explored the task parameters and made behavioural adjustments in response to task feedback. The authors suggested that adolescents' increased exploration of their environment came at a cost of increased risk, but also supported adaptive behaviour. From this work, it could be argued that the increased risk-taking observed in adolescence is not "inappropriate", as suggested by current neurobiological models, but such behaviour enables adolescents to actively explore their environment and learn from these experiences.

A fifth limitation regards the lack of consideration for potential gender differences in adolescent development in each of these models. Research has demonstrated significant gender differences in the development of anxiety (Abe & Suzuki, 1986; Lewinsohn, Gotlib, Lewinsohn, Seeley, & Allen, 1998), the rate of brain maturation (Lenroot & Giedd, 2010; Lenroot et al., 2007), as well as gender differences

in the level of risk-taking behaviours (Byrnes, Miller, & Schafer, 1999). Therefore, it is important that these differences are considered, where possible, when considering neurobiological accounts of adolescent development, as these differences are highly likely to have an impact on adolescent behaviour.

A sixth important limitation to consider when evaluating these models regards whether the neurobiological differences observed between adolescents and adults can be explained by age differences in their perception of the emotional intensity of the stimuli employed. For example, the majority of the literature discussed so far in this chapter has proposed that when the emotional intensity of a stimulus is higher in adolescents, researchers have observed different behavior, relative to adults. This is particularly relevant in research that measures risk taking for social reward. For example, Steinberg's DSM describes a socioemotional system which matures early in adolescents and encourages them to seek out rewarding stimuli, leading to increased risk-taking behaviours. Support for this system comes from studies which show that adolescents exhibited greater paralimbic, limbic, and medial PFC activation when responding to socioemotional cues, relative to adults (Steinberg 2008). However, given past research which has characterized adolescence as a period of heightened sensitivity to social stimuli (see Foulkes & Blakemore, 2016 for a review), it is possible that adolescents perceive socioemotional stimuli to be more emotionally intense than adults do, which would also explain the changes in brain activation that have been reported in previous studies. This may help to explain why previous studies that measured risk-taking without a socioemotional component did not observe differences between adolescents and adults (e.g. monetary/gambling tasks, Bjork et al., 2007; Eshel et al., 2007). Further support for this argument comes from studies which have examined adolescent threat processing. For example, two studies reported enhanced activation of the amygdala and the ventral striatum in response to aversive stimuli in adolescents compared to adults (Galvan & McGlennen, 2013; Hare et al., 2008). Similarly, Britton et al. (2013) reported significantly greater autonomic SCRs in response to an aversive scream in youths (8-19 years) compared to adults (21-48 years). Again, however, these enhanced responses may have been due to adolescents perceiving the stimuli to be more emotionally intense relative to adults. Overall, these studies highlight a pervasive problem for each of the neurobiological models of adolescence, and the field of adolescent emotion processing research more widely, because they suggest that adolescents may be exhibiting enhanced subcortical brain responses to emotion-laden

stimuli because the emotional intensity of such stimuli is greater during adolescence relative to adulthood.

A final limitation of current models is that most neurobiological models of adolescence do not include the visual sensory areas within their model structure, instead focusing solely on the developing subcortical (socioemotional) and frontal cortical (cognitive control) systems to explain adolescent behaviour (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; L. Steinberg, 2008, 2010). Notably, the one exception to this is the social information processing network (Nelson et al., 2005), which does describe a visual detection node. This node includes face processing regions, such as the fusiform face area, as well as the posterior visual regions of the brain used to process the social properties of a stimulus. As yet, no other model has attempted to explain how social stimuli might be processed by adolescents. Unfortunately, however, despite including this visual detection node, Nelson et al. (2005) describe it only briefly, as they suggest that areas such as the fusiform face area and posterior visual regions are largely mature by adolescence, and so they do not expect to see developmental differences in visual responses to social stimuli. They support this suggestion with studies which demonstrate that new-born infants show face-specific neural responses, as measured by event-related components (ERPs) and positron emission tomography (PET) scans (Halit, De Haan, & Johnson, 2003; Tzourio-Mazoyer et al., 2002), and therefore argue that the ability to detect and categorise social stimuli matures years before adolescence. Alternatively, Nelson et al. (2005) suggest that, due to early maturation of brain regions involved when processing inherently social stimuli, changes in adolescent behaviour are best explained by the interaction between the affective and cognitive-regulatory nodes.

However, a wealth of evidence suggests that the face processing network of the brain, consisting of the fusiform gyrus, inferior occipital gyrus, and the superior temporal sulcus (Haxby, Hoffman, & Gobbini, 2000; Kadosh, Johnson, Henson, Dick, & Blakemore, 2013), undergoes significant maturational changes during adolescence. Despite some research suggesting that face cognition abilities, such as novel face encoding and holistic face processing, are fully mature by 3-5 years of age (reviewed by McKone, Crookes, Jeffery, & Dilks, 2012), additional work suggests that these abilities continue to mature throughout childhood and into adulthood. For example, results from an early study suggest a rapid improvement in face recognition abilities between ages 6 to 10, followed by a decline around age 12, with adolescents not reaching adult

proficiency levels until age 16 (Carey, Diamond, & Woods, 1980). This developmental disruption in face encoding abilities during adolescence has also been linked to pubertal changes, as girls in the midst of puberty were found to be worse at face encoding than both age-matched prepubescent and postpubescent controls (Diamond, Carey, & Back, 1983). Similar results were found by Fuhrmann et al. (2016), who observed poorer face memory and perception skills in younger (11–13 years) and mid-adolescents (13–16 years) compared with older adolescents (16–18 years) and adults (18–33 years). Additional work using fMRI has reported a gradual increase in the degree of recruitment of core face-responsive brain regions, including the fusiform face area and the inferior occipital gyrus, during face processing tasks in children, adolescents and adults (Golarai et al., 2007; Kadosh et al., 2013), which suggests chronological, age-dependent maturation of the face processing network. Together, this work suggests that primary face processing regions continue to develop throughout the first two decades of life, in disagreement with the SIPN, and are also dependent on pubertal development. As a result of this body of work, Chapters 2 and 3 focused on studying the behavioural and/or neutral correlates of early visual face processing, using positive and/or negative emotional expressions in a sample of late adolescents, to test the suitability of these task paradigms for use with younger adolescent groups.

In addition, despite early structural maturation of the visual cortex (Gogtay et al., 2004), there is now evidence to suggest that posterior visual brain regions are modulated by rewarding and threatening stimuli in the transition from adolescence to adulthood, which does not support SIPN model. For example, recent EEG work has demonstrated developmental differences in how adolescents process threat-predicting stimuli in early visual areas (Howsley & Levita, 2017; Levita et al., 2015). Specifically, they found that adolescents exhibited greater activation of early visual responses, as measured by the N170 event-related potential, to cues that predict danger in an instrumental avoidance task. These findings do not support the assumption of the SIPN, which suggests that the visual detection node in adolescence is the same as it is in adulthood. Therefore, one of the key aims of the work in this thesis is to examine responses of visual perceptual regions to affective stimuli using EEG. Consequently, the next section of this review will introduce how EEG, and more specifically event-related potentials (ERPs), can be used to assess emotion processing in the transition from adolescence to adulthood.

1.8. EEG correlates of emotion processing during adolescence

1.8.1. Event-related Potentials

The majority of brain imaging studies that have assessed emotion processing during adolescence have relied on fMRI (e.g., Britton et al., 2013; Ernst et al., 2005; Galvan et al., 2007; Galvan et al., 2006; Galvan & McGlennen, 2013; Hare et al., 2008; Lau et al., 2011; Monk et al., 2003). This fMRI work suggests there may be developmental differences in the recruitment of key subcortical and cortical brain regions when adolescents process emotional face stimuli, as well as rewarding and threatening stimuli, compared with children or adults (Ernst et al., 2005; Galvan et al., 2007; Galvan et al., 2006; Galvan & McGlennen, 2013; Hare et al., 2008; Lau et al., 2011), as outlined in section 1.7.1.2. However, despite the importance of these key studies in revealing significant and important differences in emotion processing in the transition from adolescence to adulthood, fMRI lacks the temporal resolution that would allow one to capture rapid, millisecond-level brain responses to emotional stimuli. This is significant because more precise developmental differences in emotion processing are likely being masked by the poor temporal resolution of fMRI.

In addition, this focus on fMRI has left the EEG correlates of emotion processing in adolescents largely unexplored. This lack of exploration is important to this doctoral work, because the high temporal resolution and relatively low cost of EEG methodology provides an additional brain imaging tool, which can be easily implemented in developmental emotion processing paradigms. Unlike fMRI, which requires participants to lay still in a scanner, EEG can be implemented within typical laboratory conditions with participants seated normally, causing little-to-no discomfort. Therefore, this next section will describe the utility of EEG, specifically of event-related potentials (ERPs), to assess developmental differences in emotion processing, and will describe the current literature base in relation to ERPs and adolescent emotion processing.

ERPs can be described most simply as electrical potentials that are generated by the brain, that are related to specific events – such as in response to a stimulus presentation, or a motor response (Luck, 2012). It has been suggested that when cortical pyramidal neurons of a similar orientation fire together during the process of neurotransmission, their collective activity travels through the brain to the scalp and can be picked up by electrodes at the surface of the skull (Luck, Woodman, & Vogel, 2000)

. Once an EEG session has been recorded, the resulting neural signals are time-locked to an event (e.g., the presentation of a face) and are averaged across a larger number of trials to form an averaged ERP signal (e.g., Figure *1-2*), which represents brain activity in response to a particular event. It is expected that brain activity that is not related to the event will become averaged out over many trials, leaving only the activity that is consistently time-locked to the stimulus (Luck et al., 2000). The averaged ERP signal consists of various positive and negative peaks that are believed to represent responses to various sensory, cognitive or motor events in the environment. Because of their high temporal precision and relative cost efficiency, in comparison to fMRI methodology, ERPs can be easily implemented in emotion processing paradigms with both typical and atypical developmental populations to study a variety of sensory, cognitive and motor processes. This doctoral work is focused on potential modulation of visual ERPs in response to various cues, such as emotional faces (Chapter 3) and fear conditioned stimuli (Chapter 5).

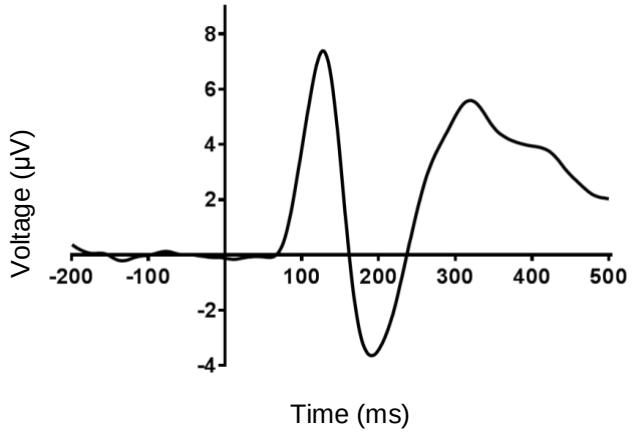

Figure 1-2: An example of a typical ERP waveform in response to a visual cue. The first positive wave is referred to as the P1 component, the first negative wave is referred to as the N1 component. It has been suggested that both the P1 and N1 are modulated by attention to the features of a visual stimulus (Paz-Caballero & Garcia-Austt, 1992), with research showing larger components when participants are attending to the stimuli, compared to when the stimuli are not attended to (see Mangun, 1995 for a review). Adapted using data from chapter 5 of this doctoral work.

1.8.2. Visual ERPs associated with emotion processing in adolescence

The earliest measurable visual ERP components (i.e., 100-250ms post-stimulus), such as the visual P1 and N1, are exogenous components generated by the visual system

in response to visual stimuli (Mangun & Hillyard, 1990). Notably, these components can also be modulated by the emotional salience of a stimulus (e.g., Olofsson, Nordin, Sequeira, & Polich, 2008). In contrast, later visual ERP endogenous components, such as the P300 or late positive potential (LPP), reflect higher level perceptual and cognitive processing of a stimulus. One study (Yuan et al., 2015) examined a series of early and late visual ERPs (N1, N2, P2, N2, P3) in adolescents (13-14 years) and adults (20-22 years), during a two-choice oddball task. In that task participants were either presented with the standard picture (a natural scene of a cup) or a deviant picture (affective picture), and were asked to correctly identify a standard or deviant picture as fast as possible, using one of two possible response buttons. Adolescents demonstrated enhanced N1, P2, N2, and P3 ERPs in response to highly negative compared to neutral images, as well as enhanced N1, P2, and N2 components in response to moderately negative compared to neutral images. However, in adults, they observed only an enhancement of the N2 and P3 visual ERPs for the highly negative pictures compared to the neutral pictures. From this, the authors suggested that adolescents may be more sensitive to negatively valenced emotional stimuli in comparison to adults, which can be indexed by visual ERPs. However, the authors did not collect self-report ratings of the stimuli, so it is unclear whether the enhanced ERP responses to the negative stimuli presented were simply due to adolescents finding them more aversive. Interestingly, from these results, one could argue that in adolescence both early and late visual ERPs are modulated by negative images, and in adulthood this modulation occurs only in late visual components. This developmental difference between the modulations of early and late visual components in response to negative stimuli could indicate potential age-related changes to neural networks which process valenced stimuli within the perceptual system.

The majority of other ERP studies which have examined changes in emotion processing during adolescence have focused the late positive potential (LPP) component. The LPP is observed approximately 300 ms post-stimulus onset, and is most prominent at centroparietal electrode sites. These studies have demonstrated greater LPP amplitudes in response to pleasant and/or unpleasant affective pictures compared to neutral pictures in adolescents ranging from 11 to 18 years (Desatnik et al., 2017; Zhang et al., 2014; Zhang et al., 2012; Zhang et al., 2013). Although these studies neglected to include adult or child comparison groups, their findings of LPP modulation by affective visual stimuli is consistent with previous work conducted in adults (Hajcak,

Dunning, & Foti, 2009; Hajcak, MacNamara, & Olvet, 2010; Hajcak & Olvet, 2008), which suggests that the LPP provides a stable electrophysiological index of emotion processing throughout development.

However, in addition two recent EEG studies have provided evidence to suggest that both early (N170) and late (LPP) components, evoked in response to discriminative visual stimuli that predict threat and reward in an instrumental task, are greater in adolescents relative to adults (Howsley & Levita, 2017; Levita et al., 2015). In their first study, Levita et al. (2015) conducted an instrumental avoidance task, in which both adolescents (12-15 years) and adults (18-32 years) learnt how to actively and passively avoid an aversive outcome (a loud tone) by making or withholding a motor response (a button press). Their results demonstrated greater learning-dependent potentiation of the N170 component to the threat-predicting discriminative stimuli in adolescents compared to adults. This work suggests that adolescents may show hyper-responsivity to cues that predict threat, not just reward, consistent with Casey's imbalance model (see section 1.7.1.2).

In a follow up study, Howsley and Levita (2017) conducted a similar instrumental task, but this task not only examined responses to cues that predict a negative outcome, but also reward, indexed by the N170 and LPP components. In that instrumental task, pre-adolescents (9-12 years), mid-adolescents (13-17 years), and late adolescents (18-23 years) were required to either make or withhold a motor action to gain rewards and avoid making losses. Specifically, participants were presented with two S^D and two control visual cues as part of a reward block, followed by an avoidance block. In the reward block, participants learnt that one S^D required them to make a motor response to win 10 points, whilst the second S^D required them to withhold a motor response to win 10 points. In the avoidance block, participants learnt that one S^D required them to make a motor response to avoid losing 10 points, whilst the second S^D required them to withhold a motor response to avoid losing 10 points. Notably, they found significant developmental differences in LPP amplitudes, which were greater to the avoidance cues in pre-, mid- and late-adolescents, whereas only the pre-adolescents showed greater potentiation to the cues signalling reward.

However, it is interesting to note that in their second study (Howsley & Levita, 2017), the authors did not observe reinforcement-dependent potentiation of the N170 to either rewarding or threatening discriminative cues, inconsistent with the findings

reported in their first study (Levita et al., 2015). The authors suggest this could be task-related, as their first study utilised a primary reinforcer (an aversive tone), whereas their second study utilised a secondary reinforcer (loss or gain of points in game). Whilst primary reinforcers are evolutionarily significant to an organism and elicit an innate biological response, secondary reinforcers will acquire their aversive properties by becoming associated with the aversive properties of a primary reinforcer (Delgado, Jou, & Phelps, 2011). This suggests that secondary reinforcers may not activate innate fear networks, which may explain the lack of reinforcement-dependent potentiation of early visual ERP responses in Howsley and Levita (2017). Notably, previous fMRI work directly comparing the degree of Pavlovian fear conditioning when using a primary (i.e., mild shock) or secondary reinforcer (i.e., loss of money) as the unconditioned stimulus found that both reinforcers led to successful expression of fear conditioned responses, as measured by skin conductance responses (SCR), but that the amygdala exhibited the greatest activation when a primary reinforcer was used (Delgado et al., 2011). These results suggest that loss of money/points in a task is less likely to activate more innate fear networks, and could explain the inconsistencies relating to N170 modulation in past studies (Howsley & Levita, 2017; Levita et al., 2015). In order to investigate this further, Chapter 5 consists of a Pavlovian fear conditioning paradigm, which assesses the effect of a primary reinforcer (aversive sound) on the modulation of early ERP components (P1 and N1) in adolescents and adults.

1.9. Thesis outline

This doctoral work was designed to address a number of weaknesses of current neurobiological models of adolescence, already outlined in Section 0. The overarching aim of this work was to examine the behavioural, physiological, and neural correlates of emotion processing during adolescence, and to add to a growing literature regarding how adolescents respond to emotional stimuli. Consequently, the first aim of this doctoral work was to identify an appropriate emotion processing paradigm for use with both adolescents and adults.

To achieve this aim, Chapter 2 aimed to understand how late adolescents bound motor-sensory events together in time, and whether this process could be influenced by threat-related faces. As such, this experiment assessed the suitability of a temporal recalibration paradigm for use with both adolescents and adults.

Following this, Chapter 3 aimed to understand how emotional vocalisations (laughter and crying) influenced the subsequent visual processing of congruent and incongruent emotional faces (happy and sad). To do this, the potentiation of the P1 and N170 ERP components were examined while a sample of late adolescents (18-20 year olds) carried out an emotion categorisation task. For reasons discussed in both Chapter's 2 and 3, it was determined that these paradigms would not be optimal for use with a younger adolescent group.

Instead, however, potential developmental differences in Pavlovian fear conditioning were examined, as this paradigm would provide a more highly controlled measure of emotion processing. Therefore, Chapter 4 took the form of a systematic review of the Pavlovian fear conditioning literature during adolescence, to determine what is already known about conditioning and extinction in this age group.

Chapter 5 aimed to account for the main criticisms identified by the systematic review, by examining reinforcement-dependent potentiation of early visual responses (P1 and N1) in a sample of mid-adolescents (13-14 years) and adults (25-26 years). As part of this study, I also sought to quantify changes in fear conditioning using explicit behavioural (evaluative ratings, contingency awareness) and implicit physiological (skin conductance responses) measures.

Chapter 6 summarises the results from this doctoral work, and offers a discussion of the findings in light of past research, describes the key strengths and limitations of the research presented, and concludes with some considerations for future research.

Chapter 2. The effect of threat-related facial expressions on temporal recalibration

Abstract

The main aim of this study was to examine the utility of a motor-sensory temporal recalibration paradigm for assessing emotion processing in adolescence. Temporal recalibration refers to a process by which the brain compresses the temporal delay between actions and sensory events which occur out of synchronisation with one another. As a result, when individuals experience a delay in visual feedback (e.g., a flash) following an action (e.g., a button press), the perception of subsequent flashes is accelerated, as a way of temporally realigning feedback, which helps to maintain a coherent visual percept. However, to date, the mechanisms of temporal recalibration remain poorly understood. Therefore, the current study investigated how stimulus-driven modulations of attention may interact with the temporal recalibration process, in a late adolescent population. To that end, I assessed whether adaptation to delayed visual feedback using a neutral oval stimulus would be differentially transferred between fearful faces and neutral faces during a testing phase. Results demonstrated that fearful faces were judged as appearing significantly earlier in time when compared with neutral faces during a temporal recalibration task. It can be argued that increased attention to the fearful faces produced this acceleration effect, due to their biological significance for communicating threat. The implications of this effect will be discussed in the context of other temporal order perception tasks, timing theories, and sense of agency mechanisms. However, due to issues in relation to high task difficulty and small condition effects, it was determined that an extension of this paradigm would not be optimal for use with a younger adolescent population.

2.1. Introduction

The overarching goal of this thesis was to examine emotion processing during adolescence. Therefore, the first aim of this doctoral work was to identify an appropriate emotion processing paradigm that would suitable for both adolescents and adults. Given past research (discussed in Chapter 1) which suggests that face processing regions undergo continued maturation throughout adolescence (e.g., Carey et al., 1980; Diamond et al., 1983; Fuhrmann et al., 2016; Golarai et al., 2007; Kadosh et al., 2013), and the social information processing network model of adolescence, which suggests adolescents process socioemotional stimuli differently to adults (Monk et al., 2003; Nelson et al., 2005), the present study aimed to examine threat-related face processing in a sample of late adolescents. To that end, the study reported here used a motor-sensory temporal recalibration paradigm which, if works, could then be extended for use with a younger adolescent and an adult sample. This chapter will begin by introducing motor-sensory recalibration, and will describe previous developmental work which has utilised this paradigm (Section 2.1.1). Current knowledge of the mechanisms underlying temporal recalibration will then be discussed, which so far has largely focused on potential top-down modulations of temporal processes, and an alternative focus on bottom-up, stimulus-driven modulation of temporal processes will be proposed (Section 2.1.2). Finally, previous experimental work conducted on temporal recalibration mechanisms by the author will be introduced, and the aims and hypotheses of the current study will be outlined (Section 2.1.3).

2.1.1. Motor-sensory temporal recalibration

The central nervous system continuously receives a barrage of signals from a range of modalities (e.g., sight, sound, touch, olfaction, self-motion), each of which are processed at different speeds. However, to maintain a coherent understanding of the world the brain must temporally align these signals and bind them to correct events (Eagleman, 2010). This is achieved through a process of temporal recalibration, in which the brain adapts to temporal asynchronies (Fujisaki, Shimojo, Kashino, & Nishida, 2004; Vroomen, Keetels, de Gelder, & Bertelson, 2004), for temporal order perception. The temporal recalibration effect (TRE) carries out this temporal adjustment by producing a subjective temporal compression between sensory events which occur out of synchronisation with each other. For example, when holding a conversation with

someone, one must be able to temporally align a person's speech and facial signals, to follow what they are saying with ease. This compensatory mechanism is necessary because there are natural asynchronies between sensory signals, due to differences in neural transmission time as well as differences in signal transduction time through the air (Keetels & Vroomen, 2007). The process of temporal recalibration is not limited to audio-visual signals, but is also used to realign an action and its sensory feedback. For example, when typing words on a keyboard that has a lagged response, visual processing could be adjusted until the presentation of letters on the computer screen were perceived as synchronous with the typing. This highlights the importance of temporal recalibration in enabling humans to speak coherently, to evade danger (i.e., avoiding a speeding car) and in determining the sensory consequences of their own actions from those that are generated by other external sources (i.e., "Did I do that"?). As such, temporal recalibration shares a core similarity with intentional binding, which is used to test for sense of agency (i.e., the feeling that I am in control of my own actions and outcomes). This intentional binding effect refers to the subjective temporal compression between an individual's intended actions and their subsequent external sensory outcomes (Haggard, Clark, & Kalogeras, 2002). Currently however, despite the importance of temporal recalibration for determining causality between events, its mechanisms remain poorly understood.

Past work has attempted to better understand the mechanisms of temporal recalibration, by investigating the interaction between motor actions and their subsequent sensory consequences. For example, one study presented participants with delayed visual feedback (a flash) 100 ms after each of a series of button presses, which caused participants to become adapted to this delay (Stetson, Cui, Montague, & Eagleman, 2006). Following a number of these adaptation trials, the point of subjective simultaneity (PSS: the point when participants perceive both stimuli as occurring simultaneously) increased significantly during test trials, using the method of constant stimuli. This suggests that the visual stimulus underwent accelerated perception, due to a TRE (Aytemur, Almeida, & Lee, 2017). In other words, being exposed to delayed feedback (i.e., a flash) following an action (i.e., a button press) accelerated the perception of subsequent flashes, as a way of temporally realigning feedback, in order to maintain a coherent visual percept. As a result, following this period of adaptation to delayed visual feedback, when a visual stimulus is presented instantaneously with a button press, participants experience a reversal of temporal order between the two

stimuli, and participants' incorrectly report that the visual stimulus occurred prior to their button press (Figure *2-1*). Past research has shown that this perceived reversal of temporal order occurs even when a variety of tactile, auditory, or visual stimuli are used as feedback, and that temporal recalibration can be transferred between modalities (Hanson, Heron, & Whitaker, 2008; Heron, Hanson, & Whitaker, 2009), which suggests that a central mechanism may be involved in recalibrating all motor-sensory, motor-auditory and motor-tactile information.

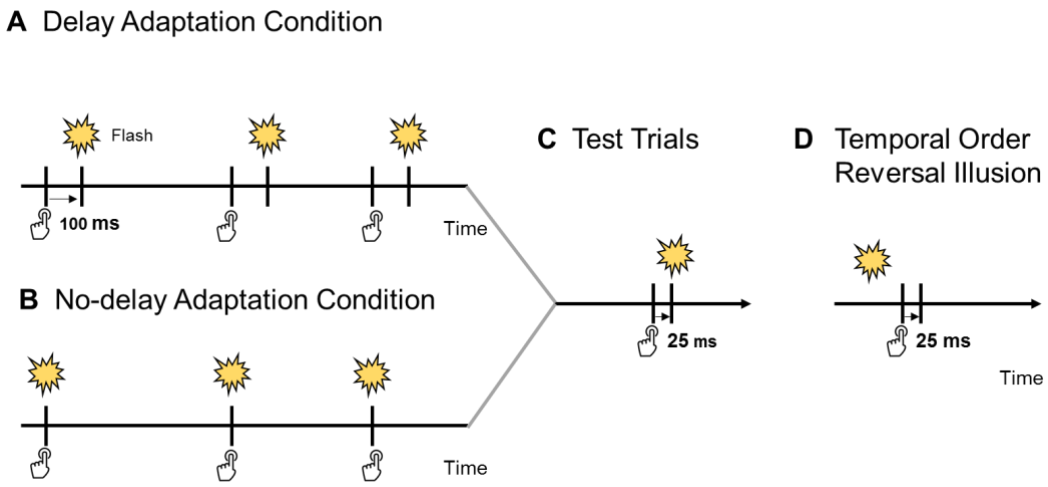

Figure 2-1: A standard temporal recalibration procedure. This process can be examined using two experimental phases, a delay adaptation condition (A), and a no-delay adaptation condition (B). Participants take part in both the delay adaptation and no-delay adaptation conditions, with the task order counterbalanced across participants. In this task, for each trial, participants are instructed to perform a series of button presses (e.g., six) in a regular rhythm. **A:** In this phase (Delay adaptation condition), there is a constant delay (e.g., 100ms) between participants' regular button presses and their external sensory consequence, in this example, visual feedback (a flash), **B:** In this phase (No-delay adaptation condition), there is no delay between participants button press and the visual feedback, **C:** Example of a test trial. During the participants' regular button pressing, on their 6th button press the visual feedback is presented earlier than the regular delay (e.g., 25 ms). **D:** Following a delay adaptation phase, the participant is more likely to report that the visual feedback presented on their 6th button press occurred before their button press, due to a temporal recalibration effect. Adapted from (Cai, Ogawa, Kochiyama, Tanaka, & Imamizu, 2018).

To the author's knowledge, to date no studies have examined motor-sensory temporal recalibration during adolescence. This is surprising, given research which suggests that developmental changes to the temporal binding of multisensory stimuli continue to mature during adolescence (Hillock, Powers, & Wallace, 2011; Hillock-

Dunn & Wallace, 2012), demonstrating a role for sensory experience in shaping individual's perception of cause and effect. However, one study has examined the development of motor-sensory TRE in children, and suggested there may be developmental differences in this type of temporal processing. For example, Vercillo, Burr, Sandini, and Gori (2015) examined motor-sensory temporal recalibration in children (8-11 years) and adults (mean age = 27.3, age range not provided). They employed a similar paradigm to Stetson et al. (2006), asking participants to make a temporal order judgment regarding whether an auditory tone came before or after their button press. Adults demonstrated the expected motor-sensory TRE as reported in previous studies, whereby participants reported that a sensory event occurred before their action, following a period of delayed adaptation between their action and the subsequent sensory event (as in Heron et al., 2009; Stekelenburg, Sugano, & Vroomen, 2011; Stetson et al., 2006; Yoshimori Sugano, Keetels, & Vroomen, 2010). However, children demonstrated no measureable TRE. The absence of a TRE in children was observed even when the adaptation delay was increased from 200 ms to 500 ms, which suggests the lack of recalibration in children was not due to them having a larger motor-sensory temporal binding window, compared to adults. Instead, Vercillo et al. (2015) suggested that the absence of temporal recalibration in children was associated with poorer temporal precision when making temporal order judgments about whether the auditory tone occurred before or after their action. This suggests that the motor-sensory temporal recalibration mechanism may mature relatively late in development.

Although there has been no work examining motor-sensory temporal recalibration in adolescents, one study has examined audio-visual temporal recalibration throughout the lifespan (Noel, De Niear, Van der Burg, & Wallace, 2016), which could provide some insight into the development of temporal recalibration effects, albeit in different modalities. In that study, a large sample of participants ranging in age from 7 to 86 years (N = 220) were presented with simple (a beep and flash) and complex (a face and voice) pairs of stimuli at various stimulus onset asynchronies, and judged whether the pairs were synchronous or asynchronous with each other. Their results suggest a protracted, U-shaped development of temporal recalibration across the lifespan, whereby the magnitude of the TRE decreases from childhood to young adulthood, and begins to increase again in participants in their sixties. This maturational time course suggests that children and older adults are more susceptible to the effects of delayed adaptation, with the developmental trajectory of these effects also occurring

earlier for simple (beep-flash) compared to complex (voice-face) stimuli. Together, in line with Vercillo et al. (2015), these results suggest that the TRE mechanism continues to develop from childhood, improving through adolescence and on to adulthood, followed by a decline in aging populations, and that TRE development takes longer to mature for voice-face stimuli.

2.1.2. Valence and the temporal recalibration effect

To date, the underlying mechanisms of temporal recalibration have not been fully elucidated. A number of studies have examined how top-down factors may influence the degree of temporal recalibration. This work has demonstrated that, despite the well-known effects of directed attention on temporal perception, which leads to accelerated perceptual processing of a sensory stimulus (i.e., a prior entry effect Spence, Shore, & Klein, 2001; Titchener, 1904), temporal recalibration effects cannot be simply explained by this top-down modulation of attention. This was evidenced in two studies, in which participants were asked to direct their attention to one of two modalities (visual or auditory) during an audio-visual temporal recalibration task (Fujisaki et al., 2004; Ikumi & Soto-Faraco, 2014). Both studies found that neither of their observed TREs could be modulated by directing participants' attention to either the visual or auditory stimulus. However, it has instead been shown that the magnitude of audio-visual TRE was significantly increased when participants were directed to attend to the temporal order of the two sensory stimuli, compared to when participants were directed to attend to non-temporal features of the same stimuli (e.g., visual stimulus size or sound location) (Heron, Roach, Whitaker, & Hanson, 2010). Similar evidence was demonstrated by Tsujita & Ichikawa (2015), who observed a motor-sensory TRE when participants were made explicitly aware of the adaptation delay (200 ms) between their button press and the subsequent flash, but not when participants were unaware of the adaptation delay in a second experiment, when a variable adaptation delay was used (40-200 ms). Together, this examination of the effect of top-down modulations of attention on temporal recalibration suggests that TREs are not modulated by modality-general enhancements of attention, but are instead affected by directed attention to temporal relationships.

However, as yet, no studies have investigated how stimulus-driven, bottom-up modulations of attention may interact with the temporal recalibration process. Stimulus properties, emotional valence for example, would also accelerate perception. Emotional

faces have often been used in the timing literature to measure changes in temporal perception. This is because faces are special, in as much as humans can rapidly detect face stimuli compared with non-face stimuli (Purcell & Stewart, 1988; Theeuwes & Van der Stigchel, 2006; Vuilleumier & Schwartz, 2001). This may be because face processing is supported by an extensive network of dedicated brain regions (e.g., the fusiform face area and superior temporal sulcus) that are modulated by attention (Vuilleumier, Armony, Driver, & Dolan, 2001), and also overlap with regions that are important for social information processing (see the Social Information Processing Network, Chapter 1 Section 1.4). It has also been argued that threat-related face processing in particular could be further facilitated automatically (e.g., increased autonomic arousal), subserved by the amygdala for survival (Ledoux, 2000). It has been suggested that face processing provides an ideal model to study developmental changes in emotion processing in the transition from adolescence to adulthood (Scherf, Behrmann, & Dahl, 2012), due to maturational changes to face processing brain regions that occur throughout adolescence (outlined in Chapter 1, section 1.4). Past research suggests that adolescents process emotional face stimuli differently to adults. For example, fMRI work has shown exaggerated amygdala activity in adolescents (13-18 years) in response to both emotional (fearful, happy and calm expressions) target and non-target face stimuli as part of an emotional go/no-go paradigm, relative to children (7-12 years), and adults (19-32 years) (Hare et al., 2008). In addition, Yurgelun-Todd & Killgore (2006) observed increasing activity in PFC regions, such as the inferior, middle, superior, and superior medial frontal gyri for girls (bilaterally) and boys (right-laterally), with increasing age when participants (8-15 years) passively viewed fearful faces, which suggests that the development of PFC regions play a key role in the development of threat-related face processing during adolescence. Research has even highlighted differences in emotional face processing between different stages of adolescence, with younger adolescents (11-12 years) found to be slower to disengage attention from fearful faces relative to happy or neutral expressions, compared with older adolescents (17-18 years) (Cohen Kadosh, Heathcote, & Lau, 2014). Together, this work suggests that the maturation of emotional face processing may be associated with changes in the recruitment of subcortical (e.g., amygdala) and cortical (e.g., PFC) brain regions. However, although face processing has been proposed to provide an ideal model to investigate adolescent-specific emotional development (Scherf et al., 2012), as yet, no studies have examined how emotional face processing may interact with the process of temporal recalibration.

Despite a dearth of evidence investigating the potential effects of emotional face processing on temporal recalibration, a substantial amount of evidence from various time perception tasks (e.g., temporal bisection, temporal order judgments) has provided evidence that threat-related facial expressions (i.e., anger or fear) result in significantly greater modulations of perceived time compared to other emotional expressions, and to neutral faces (e.g., Droit-Volet, Brunot, & Niedenthal, 2004; Fayolle & Droit-Volet, 2014; J Tipples, 2011; G. West, Anderson, Bedwell, & Pratt, 2010; G. West, Anderson, & Pratt, 2009). For example, using classic temporal order judgment tasks, in which participants are asked to report which of two emotional faces were presented first, some evidence has suggested that angry and fearful faces are prioritised by the visual system and so are perceived as occurring earlier in time, when compared with neutral faces (G. West et al., 2010; G. West et al., 2009). From this evidence, it is plausible to suggest that threat-related faces may modulate attention in a temporal recalibration paradigm, compared to stimuli with less salient stimulus properties. Therefore, the present study aimed to investigate whether the TRE could be modulated by stimulus-driven properties (i.e., the emotion of the face). If there is a greater transference of temporal recalibration using threat-related faces from delayed adaptation to test phases, this would suggest that temporal recalibration is affected by increased attention and/or arousal to biologically-significant stimuli, and not just via top-down modulations of temporal awareness.

Notably, although much of the time perception literature has focused on the processing of angry faces, neuroimaging research has demonstrated greater amygdala activation in response to fearful relative to angry faces (Blair, Morris, Frith, Perrett, & Dolan, 1999; Davis & Whalen, 2001; Whalen et al., 2001). Behavioural work supports this, with participants demonstrating an attentional bias towards, and faster detection of, fearful faces compared with neutral distractors (Fox, 2002; Ishai, Pessoa, Bikle, & Ungerleider, 2004). Moreover, Davis and Whalen (2001) posit that fearful faces may be a more potent threat signal for capturing participants' attention and increasing vigilance, because they are more ambiguous than angry faces. For example, whilst angry faces can inform us about both the existence of a threat and its likely source, fearful faces can inform us about the existence of a threat, whilst communicating very little about its source. This ambiguity could result in increased vigilance, which in turn may lead to increased amygdala activation, as this system attempts to obtain more information about the source of the threat (Whalen, 1998). As a result of this work, the current study utilised fearful faces in a temporal recalibration paradigm.

2.1.3. Past experimental work using temporal recalibration

Initial validation and work on the temporal recalibration of emotional face stimuli was investigated in a sample of late adolescents (22-24 years) as part of an earlier M.Sc dissertation project (Linton, 2015), which was comprised of two experiments. The aim of that work was to first examine whether the study by Stetson et al. (2006), which demonstrated a temporal recalibration effect using motor-sensory stimuli, could be replicated using a grey oval stimulus as visual feedback during both the adaptation and test phases of the task (Experiment 1). And second, to examine whether the TRE would be greater with fearful faces as visual feedback during the test phase, compared with oval stimuli (Experiment 2).

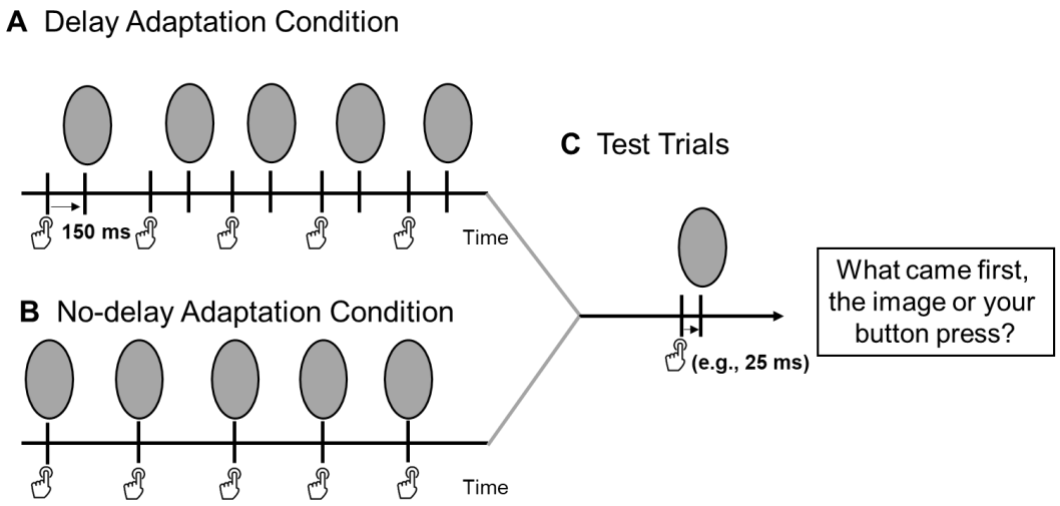

Figure 2-2: Experiment 1 temporal recalibration procedure. **A:** Delay adaptation condition, there is a regular delay (e.g., 150ms) between participants' first five button presses and the visual feedback (e.g., a grey oval), **B:** No-delay adaptation condition, there is no delay between participants first five button presses and the visual feedback, **C:** Example of a test trial, following the delay or no-delay adaptation phase, the visual feedback was presented at one of seven possible test delays (-70, 0, 25, 50, 75, 100, 125 ms). Participants were asked to decide what came first, the image or their sixth button press.

To that end, experiment 1 aimed to establish whether a TRE could be measured using the current experimental set-up, by comparing the difference in PSS scores between delay (150 ms) and no-delay (0 ms) adaptation conditions using an oval stimulus (Figure *2-2*). The results of Experiment 1 showed that, following a delay

during adaptation, participants showed significantly greater PSS values, signalling a greater TRE, compared with no-delay, during adaptation (Figure *2-3*). Specifically, participants perceived the image and their button press as occurring simultaneously if the image was presented approximately 42.6 ms after their button press. This result replicated past work, as the TRE was similar when compared to previous temporal recalibration studies (44 ms in Stetson et al., 2006; 39.9 ms in Timm, Schonwiesner, SanMiguel, & Schroger, 2014). Therefore, the results of Experiment 1 suggested that a TRE was being induced in participants using the current experimental set-up, thereby allowing follow-up investigations to assess whether fearful faces could impact the temporal recalibration process.

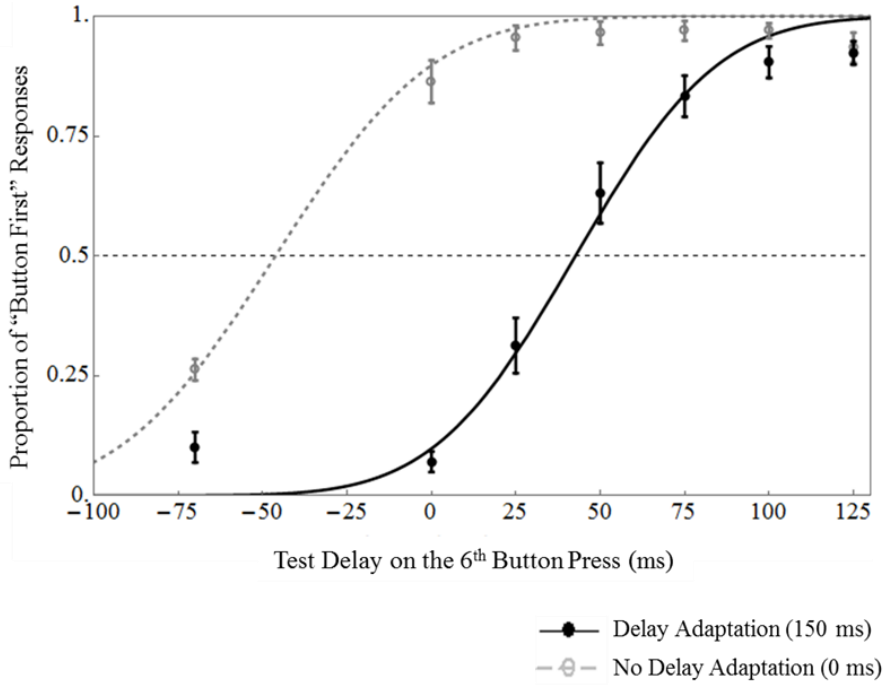

Figure 2-3: Experiment 1 findings. The mean proportion of "button first" responses provided by participants at each of the seven possible test delays, across the delay and no-delay adaptation conditions, as part of a previous study (Experiment 1). Participants exhibited significantly greater temporal recalibration effects following a delay (150 ms) during adaptation, relative to no-delay (0 ms) during adaptation ($p < .001$). For each condition, the average PSS and SD values were calculated (i.e., the average over the fitted curves for the sample) and were used to plot a cumulative curve over the data points. The dashed line intersects the 50% point of each curve. Error bars = SEM of each proportion for each condition. Adapted from (Linton, 2015).

Experiment 2 investigated whether this transference of TRE from adaptation to test phases could be modulated by valence, as examined by the presentation of fearful

faces. The delay adaptation phase from experiment 1 was repeated using the grey oval stimulus, whilst now manipulating the stimuli presented during the test phase (oval vs. fearful face, Figure *2-4*). It was predicted that greater PSS values would be observed, indicating a greater transference effect, when participants were presented with fearful faces compared with ovals.

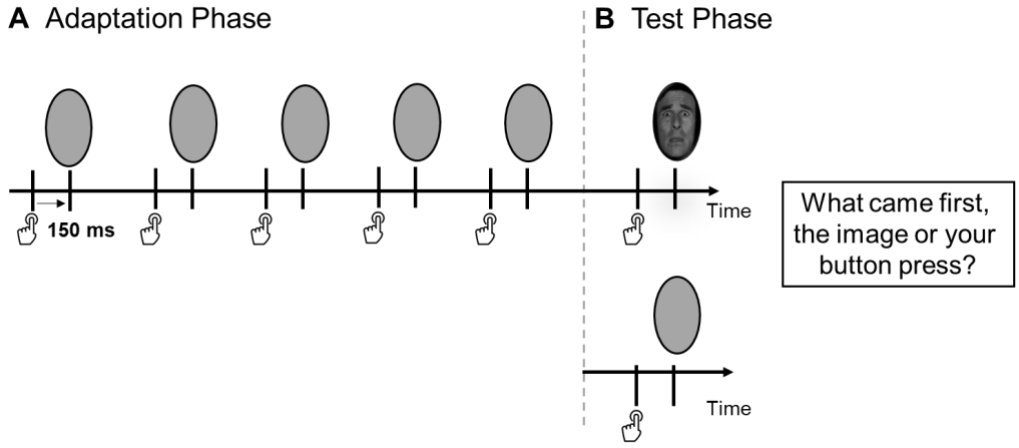

Figure 2-4: Experiment 2 temporal recalibration procedure. **A:** Delay adaptation condition, there was a regular delay (e.g., 150ms) between participants' first five button presses and the visual feedback (e.g., a grey oval), **B:** Example of a test trial, following the delay adaptation phase, the visual feedback (either a fearful face or non-face stimulus) was presented at one of seven possible test delays (-70, 0, 25, 50, 75, 100, 125 ms). Participants were asked to decide what came first, the image or their sixth button press.

In line with this hypothesis, participants demonstrated a greater transference effect when presented with fearful faces compared with ovals, following delay adaptation to oval stimuli (Figure *2-5*). This suggests that fearful faces undergo enhanced perceptual processing, as participants were more likely to report that the fearful face occurred before their button press, compared with oval stimuli.

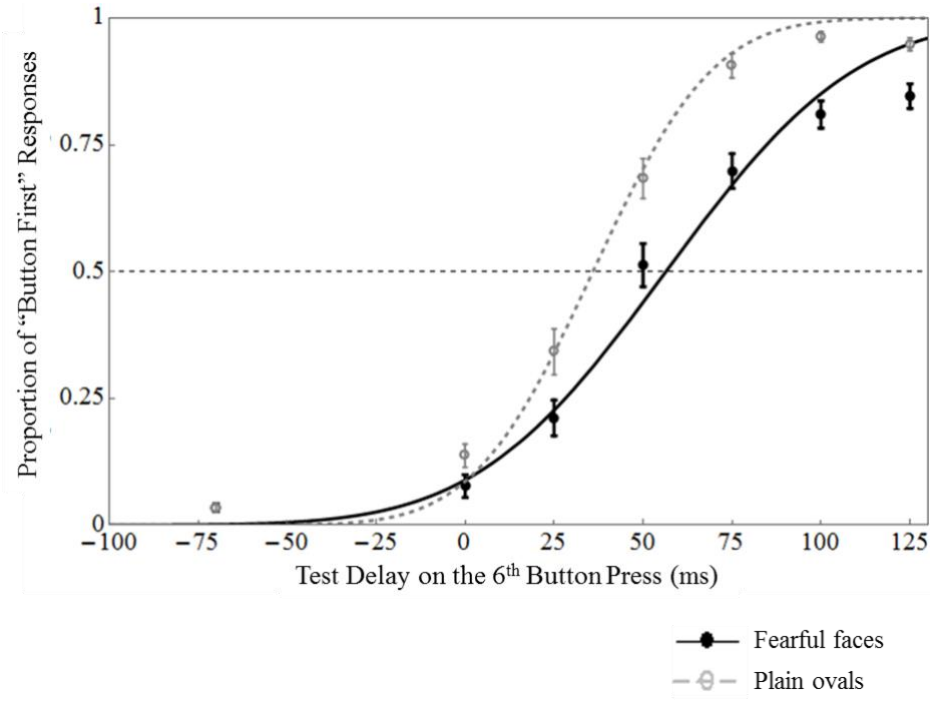

Figure 2-5: Experiment 2 findings.The mean proportion of "button first" responses provided by participants at each of the seven possible test delays, across the fearful face and plain oval conditions, as part of a previous study (Experiment 2). Participants exhibited significantly greater temporal recalibration transference effects in response to fearful faces compared to plain ovals at the testing phase ($p < .001$). For each condition, the average PSS and SD values were calculated (i.e., the average over the fitted curves for the sample) and were used to plot a cumulative curve over the data points. The dashed line intersects the 50% point of each condition. Error bars = SEM of each proportion for each condition. Adapted from (Linton, 2015).

One might have expected a greater transference effect in the oval condition, as the same stimuli are used in both adaptation and testing phases – but this was not the case. This indicates that TRE is not based on object identity. In fact, TRE can also be transferred between sensory modalities, as past work has shown a TRE when participants adapt to an auditory stimulus but make TOJs in response to a visual stimulus during the testing phase (Heron et al., 2009). This might be because actions typically produce consequences in multiple sensory modalities. However, it does not explain the greater TR transference effect observed for fearful faces compared to the oval stimulus. Instead, it is highly likely that modulation of the emotional properties of the visual feedback stimulus increased TRE. This is interesting, given previous work showing that modulation of other properties of the visual feedback stimulus did not give rise to an increase in TRE (e.g., using different colours for adaptation and test phases, Stetson et al., 2006). However, as this experiment compared fearful faces with non-face oval stimuli, it could not be concluded whether the differences in TR transference

effects were due to face-specific or emotion-specific properties of the stimulus. Therefore, the study reported in this chapter was designed to address this weakness, by examining the difference in TR transference effects using fearful compared with neutral faces as test stimuli. It was hypothesised that PSS values would be significantly greater when participants were presented with fearful compared with neutral faces as visual feedback in the current TR set-up. It was also hypothesised that participants would demonstrate faster reaction times (RT) when making TOJs in response to fearful versus neutral face stimuli, supporting the notion that fear-relevant stimuli undergo accelerated processing when compared to fear-irrelevant stimuli (Ohman, Flykt, & Esteves, 2001), and past work demonstrating faster RTs to fearful compared to neutral faces during an emotion categorisation task (Vlamings, Goffaux, & Kemner, 2009).

2.2. Methods

A formal power analysis was conducted in G*Power (Version 3.1) to assess the suitability of the sample size in this study. As no previous studies have examined the potential modulation of TRE according to emotional facial expressions, a power analysis was conducted using effect sizes derived from previous temporal judgement studies which compared threat-related and neutral face stimuli (Droit-Volet et al., 2004; Fayolle & Droit-Volet, 2014; J Tipples, 2011). The average effect size from these studies was 0.49. On this basis, ideally it would be necessary to recruit at least 35 participants to detect an effect size of $d = 0.49$, with power set at 0.8 and a two-tailed alpha set at 0.05.

2.2.1. Participants

Forty-two healthy students from the University of Sheffield participated. When compared with previously published studies with similar designs (Hanson et al., 2008; Heron et al., 2009; Timm et al., 2014), the current sample size was determined to be sufficient to detect a TRE. The data of 11 participants were excluded due to them being unable to reach at least 60% of "button first" responses at the largest (125ms) test delay. The data of the remaining 31 participants (24 females, mean age = 20.10, $SD = 1.54$) were analysed. As the final sample is lower than intended, it is possible that the study lacks sufficient power. Therefore, the reported results should be interpreted with

caution. The Department of Psychology Research Ethics Committee at the University of Sheffield approved this study.

2.2.2. Apparatus and Stimuli

In order to precisely measure a TRE in this study, potential timing delays had to be addressed within the experimental set-up. To that end, task stimuli were presented on PC running in-house Python scripts using PsychoPy (Peirce, 2008), with a 144 Hz monitor (24", Illyama, ProLite GB2488HSU-B1). An Arduino USB button box recorded participants' responses (response latency < 2 ms, Schubert, D'Ausilio, & Canto, 2013). The system delay was under 9ms from a button press to screen display (system delay of past studies: 25 ms, Heron et al., 2009; 35 ms, Stetson et al., 2006). To reduce the volume of the operational button press noises, which participants may use to influence their responses, participants wore ear-plugs and tightly-fitting headphones (Sennheiser, HD 265 Linear).

During this temporal recalibration task, the adaptation stimulus used was a grey oval, whilst the test stimuli were either fearful or neutral facial expressions. I selected black-and-white photographs of six actors (three female) from the Pictures of Facial Affect database (POFA; Ekman & Friesen, 1976), each displaying a neutral or a fearful expression. Their clothing and hair were concealed, and the grey oval stimulus was adjusted to have the same shape and average luminance of the facial stimuli, in line with previous work (Tsoi et al., 2008). The stimuli were presented centrally to participants, subtending 12.3° x 9° of visual angle at a 60cm viewing distance. The duration of each stimulus was 21ms (3 screen frames).

2.2.3. Procedure

In a within-subjects design, participants were asked to regularly tap a button six times, with each tap occurring approximately once per second. After each of the first five button presses (the adaptation phase), participants saw a grey oval stimulus after a 150 ms delay. The 150 ms adaptation delay was selected based on work demonstrating that larger adapting delays (>200 ms) gradually reduced participants' TRE (Heron et al., 2009). On the sixth button press (i.e., testing phase), either a fearful or a neutral facial expression was presented at a time point derived from one of seven test delay conditions ("physically before", 0, 25, 50, 75, 100, 125 ms). Participants were asked to decide

which came first, their 6th button press or the face stimulus, using the button box to record their decision (see Figure *2-6*).

For each trial, the computer calculated the running average of the 4 intervals between a participant's first five button presses, to predict the participants 6th button press. This made it possible to present the stimulus just before the 6th keypress on a subset of trials, which was necessary to prove to participants that stimuli can appear before a button press (Stetson et al., 2006; Timm et al., 2014). During these trials, a random test delay, which could range from 1-140 ms, was subtracted from the participant's running average for that specific trial. This resulted in 18 "physically before" negative test delay trials per condition, which averaged -70 ms (hereafter referred to as -70 ms condition). The inter-trial interval was 500 ms. Each condition (fearful vs. neutral face) consisted of 18 trials per test delay (126 pseudo-randomised trials). By pseudo-randomised, I mean the trial order of test delays and stimuli was random, but this random order was the same for all participants. Overall, this resulted in 252 trials overall, taking approximately 15 minutes to complete. Before the main test participants were asked to try 10 practice trials, receiving feedback from the experimenter, in order to become comfortable with the task.

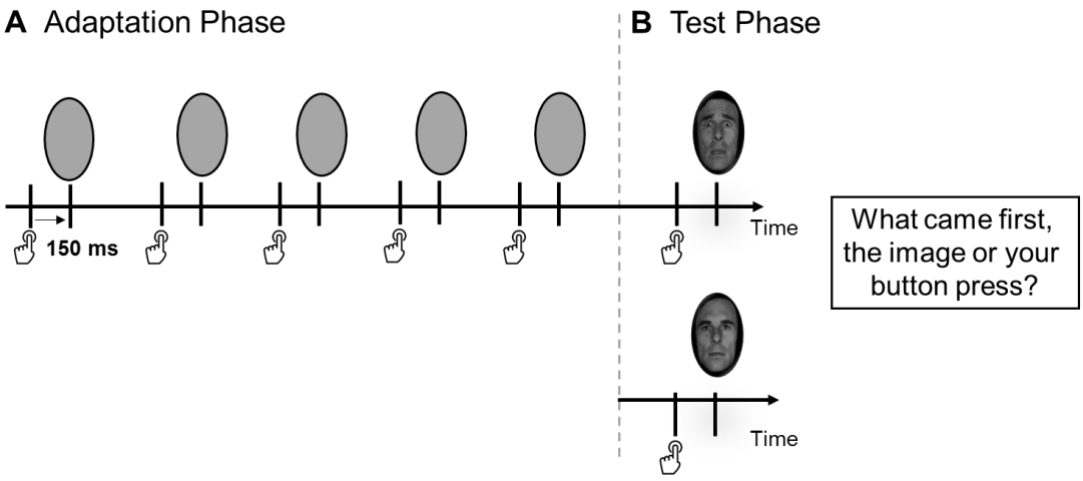

Figure 2-6: A schematic representation of a temporal recalibration trial this study. The presentation of the plain oval stimulus 150ms after the first five button presses comprises the adaptation phase, whilst the presentation of either a fearful or a neutral face at the sixth button press comprises the test phase. At the sixth button press, participants were either presented with a fearful or neutral facial expression at one of a range of possible test delays (-70, 0, 25, 50, 75, 100, 125 ms).

2.2.4. Statistical analyses

In each experimental condition for each participant (fearful vs. neutral face), I calculated the proportion of "button first" responses for each test delay, and used a psychometric cumulative Gaussian function to estimate the responses of each participant for each condition, using a maximum likelihood estimation based on a previous study (Stone et al., 2001). According to this estimation, a Gaussian function is defined by three parameters: the mean, standard deviation and the maximum amplitude of the Gaussian function. For each participant, the Maximum-Likelihood estimate was calculated by maximising the likelihood function L with regards to these parameters. The -70 ms data point conditions represent the average of the negative test delay trials for display purposes only, as the analysis was performed at individual trial level. PSS and SD values were calculated according to the range of all test delays using individual trial data were used to fit a cumulative curve over the data points in each condition. Using the fitted psychometric function, the PSS was estimated as the stimulus onset asynchrony (SOA) at which participants reported their button press occurring before the image presentation at 50% probability.

In order to compare the PSS scores and reaction times (RT) obtained when fearful or neutral faces were used as test stimuli during the temporal recalibration task, the data were first assessed for normality. Both PSS scores and RTs for both the fearful and neutral face conditions were found to be normally distributed, as determined by Shapiro-Wilk's test of normality (p's > .05). However, for the RT data, the assumption of Sphericity was violated (Mauchley's test, $p > .05$). Therefore, where needed, results are reported using the Greenhouse Geisser correction for degrees of freedom. The alpha level was set to $p < .05$ for all analyses. If post-hoc tests were conducted, Bonferroni corrections were used to control for the increased risk of Type I error associated with making multiple comparisons.

2.3. Results

2.3.1. Point of subjective simultaneity (PSS) scores

The PSS scores were analysed using a two-tailed, paired-samples t-test. The results demonstrated that the PSS scores resulting from the fearful face stimuli (M = 57.6 ms, SEM = 4.17) were significantly larger than the PSS scores resulting from the neutral face stimuli (M = 51.6 ms, SEM = 4.48), $t(30) = 2.40$, 95% CI [0.88, 11.11], p =

.023, *d* = 0.43 (Figure *2-7*). The slopes (standard deviation of the fitted Gaussians) were not significantly different between fearful (*M* = 55.04, *SEM* = 5.23) and neutral (*M* = 58.00, *SEM* = 4.35) face conditions, *t*(30) = 0.70, 95% CI [-5.63, 11.56], *p* = .487, which suggests there was no significant difference in task difficulty between the two conditions Furthermore, at the largest (125 ms) test delay, the mean proportion of "button first" responses in the neutral face condition (*M* = 0.86, *SEM* = 0.02) was significantly larger than the fearful face condition (*M* = 0.82, *SEM* = 0.02), *t*(30) = 2.70, *p* = .011, *d* = 0.48. Together, these results suggest a greater temporal recalibration transference effect when using fearful compared with neutral facial expressions, which suggests the emotion of the face impacted the degree of temporal recalibration.

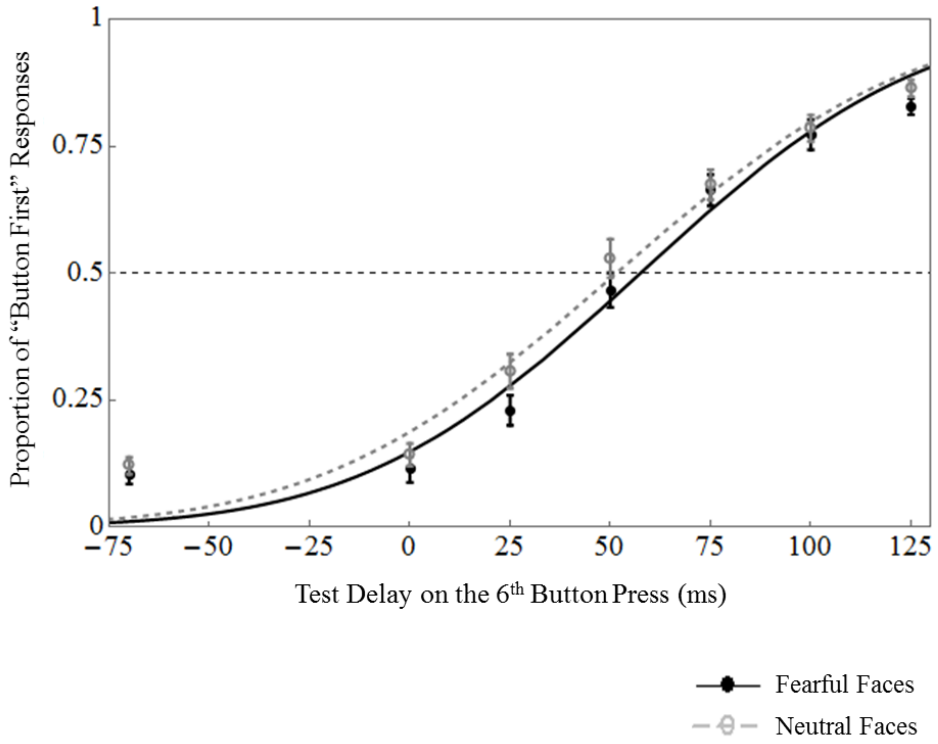

Figure 2-7: The temporal recalibration transference effect for fearful faces.The mean proportion of "button first" responses provided by participants at each of the seven possible test delays, across the fearful and neutral face conditions. Participants exhibited significantly greater temporal recalibration transference effects when responding to fearful faces, relative to neutral facial expressions (*p* = .023). For each condition the average PSS and SD values (i.e., the average over the fitted curves for the sample) were calculated and used to plot a cumulative curve over the data points. The dashed line intersects the 50% point of each curve. Error bars = SEM of each proportion for each condition.

2.3.2. TOJ reaction times

Following a comparison of the PSS values for fearful and neutral face conditions, this study also hypothesised that participants would make faster TOJs in response to fearful compared to neutral faces. Therefore, a 2 (*Stimulus-Type*) x7 (*Test Delay*) within-subjects ANOVA was conducted on the reaction times recorded at the TOJ button press. For this data, the assumption of Sphericity was violated (Mauchley's test, $p > .05$). Therefore, where needed, results are reported using the Greenhouse Geisser correction.

This analysis demonstrated a significant main effect of *Stimulus-Type*, $F(1, 30) = 4.45$, $p = .043$, $\eta_p^2 = .13$, with faster TOJs made in response to fearful faces, and a significant main effect of *Test Delay*, $F(3.69, 110.58) = 14.16$, $p < .001$, $\eta_p^2 = .32$ with faster TOJs made in response to "physically before" test delay trials (-70 ms), compared to the *0 ms* test delay, Mdiff = 134.52 ms, 95% CI [71.43, 197.60], $p < .001$, *25 ms* test delay, Mdiff = 168.07 ms, 95% CI [106.53, 229.60], $p < .001$, *50 ms* test delay, Mdiff = 230.32 ms, 95% CI [147.48, 313.17], $p < .001$, *75 ms* test delay, Mdiff = 252.74 ms, 95% CI [182.60, 322.89], $p < .001$, *100 ms* test delay, Mdiff = 163.39 ms, 95% CI [105.87, 220.90], $p < .001$, and the *125 ms* test delay, Mdiff = 169.19 ms, 95% CI [107.29, 231.09], $p < .001$. In addition, faster TOJs were made in response to 0 ms test delay trials compared to the *50 ms* test delay, Mdiff = 95.81 ms, 95% CI [52.00, 139.62], $p < .001$, and *75 ms* test delay, Mdiff = 118.23 ms, 95% CI [48.66, 187.80], $p = .002$. These results suggest that participants TOJs were fastest when both fearful and neutral faces were presented physically before the sixth button press, and when both faces immediately followed participants' sixth button press (0 ms test delay).

However, there was also a significant *Stimulus-Type* by *Test Delay* interaction effect, $F(4.64, 139.07) = 4.14$, MSE = 69141.78, $p = .001$ (Figure *2-8*). Follow-up pairwise comparisons showed participants made their TOJ faster when fearful faces were presented at the negative test delay, compared with when the stimulus was presented at compared to the *0 ms* test delay, Mdiff = 164.84 ms, 95% CI [96.62, 233.05], $p < .001$, *25 ms* test delay, Mdiff = 237.42 ms, 95% CI [161.15, 313.69], $p < .001$, *50 ms* test delay, Mdiff = 278.39 ms, 95% CI [167.34, 389.44], $p < .001$, *75 ms* test delay, Mdiff = 241.94 ms, 95% CI [141.16, 342.71], $p < .001$, *100 ms* test delay, Mdiff = 211.61 ms, 95% CI [134.78, 288.45], $p < .001$, and *125 ms* test delay, Mdiff = 247.42 ms, 95% CI [149.57, 345.27], $p < .001$. In addition, at the 75 ms delay, the analysis showed faster TOJs in response to fearful faces (M = 900.65 ms, SEM =

67.79), compared with neutral faces (M = 1030 ms, SEM = 73.00), Mdiff = 129.35 95% CI [60.97, 197.74], p = .001.

Conversely, participants made their TOJ faster when neutral faces were presented at the negative test delay, compared to the *50 ms* test delay, Mdiff = 182.26, 95% CI [94.09, 270.43], p < .001. In addition, participants made slower TOJs when neutral faces were presented at the *75 ms* test delay compared to the *negative* test delay, Mdiff = 263.55, 95% CI [193.25, 333.85], p < .001, the *0 ms* test delay, Mdiff = 159.36, 95% CI [87.97, 230.75], p < .001, the *25 ms* test delay, Mdiff = 164.84, 95% CI [83.90, 245.77], p < .001, the *100 ms* test delay, Mdiff = 148.39, 95% CI [67.71, 229.06], p = .001, and the *125 ms* test delay, Mdiff = 172.58, 95% CI [88.78, 256.38], p < .001. All other pairwise comparisons were non-significant (all p's > .05).

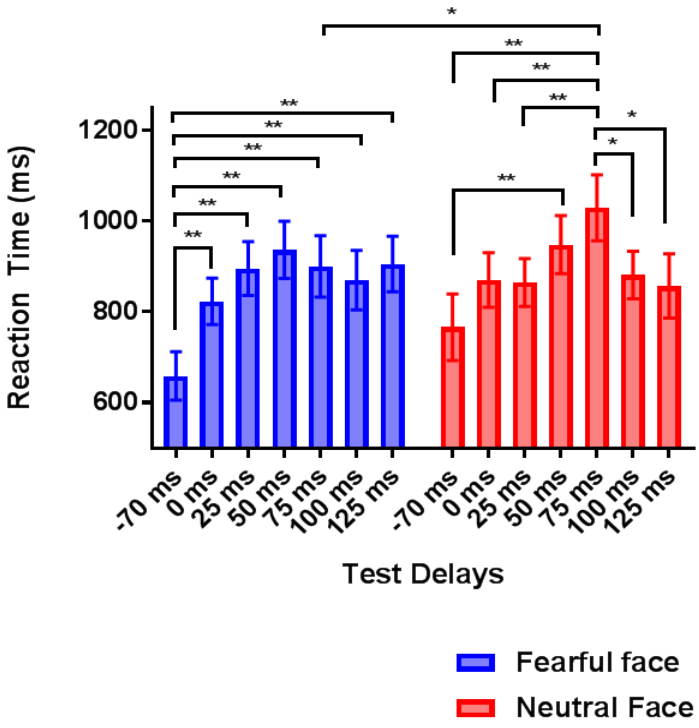

Figure 2-8: Reaction times during the temporal recalibration task. Participants made significantly faster TOJs in response to fearful faces presented during the negative test delay trials compared to all other test delays. Participants TOJs in response to neutral faces were significantly slower at the 75 ms test delay compared to all other test delays. **p < .001, *p < .05. Bar represent SEM.

Overall, these results suggest that participants were making significantly faster TOJs in response to the fearful, compared with neutral faces. Furthermore, there are differences in the speed of TOJs at different test delays, depending on whether participants were judging a fearful or a neutral face, and there was a reduction in

temporal sensitivity when making TOJs of fearful faces at the largest test delay (125 ms). Specifically, participants showed little difficulty in correctly judging that their button was first when presented with ovals at the largest test delay (125 ms following their sixth button press). However, when presented with faces at 125 ms, the proportion of "button first" responses were significantly reduced, which suggests participants still believed that the fearful face was presented before their button press, even though the fearful face was presented just 25 ms earlier than the adaptation delay. This provides support for the theory that the fearful faces were undergoing a greater degree of accelerated processing when compared with the neutral faces.

2.4. Discussion

2.4.1. General overview and theoretical discussion

The current chapter presented an investigation of the impact of threat-related face processing in a temporal recalibration paradigm of action and perception. The results of this study demonstrated greater PSS scores when fearful faces were used as testing stimuli, compared with neutral faces, in a sample of late adolescent participants. This finding suggests that facial emotion can modulate the transference effect from adaptation to test trials, as a greater transference effect for fearful compared with neutral faces was observed. Overall, the results presented in this chapter have, for the first time, demonstrated that the facilitated perception of fearful faces observed in classic TOJ tasks can also be examined within the process of temporal recalibration, and also suggests that stimulus-driven attention during the visual processing of fearful faces interacts with the process of temporal recalibration.

In line with the main hypothesis, fearful facial expressions led to greater PSS scores when compared with neutral faces. Specifically, following delay adaptation, participants showed a stronger transference effect from adaptation to test trials when presented with fearful compared with neutral faces. Interestingly, participants gave "image first" responses at the largest test delays during fearful face presentations, which can be attributed to accelerated perception of fearful stimuli. The 6 ms difference in PSS scores across the fearful and neutral face conditions in this study appears small. However, this difference is comparable to West et al. (2009), who conducted a series of TOJ tasks examining an attention effect using emotional face stimuli. Specifically, they asked participants to make TOJs in response to angry and neutral faces presented together at a number of possible SOAs (12, 24, 48, 60, 108 ms). Their results demonstrated a 7.85 ms

PSS difference between angry and neutral schematic faces, and a 7.66 ms PSS difference between angry and neutral human faces. Thus, the present study has replicated the effects shown in previous experiments, and demonstrated how emotional stimuli can modulate temporal recalibration, by increasing the chance of changes in the perception of temporal order. This change in temporal order alters the perceived causal relationship between two events, which significantly alters one's sense of agency regarding an action and its sensory consequence (e.g., *"The image must have appeared before my action"*).

These findings have important implications, because current models of temporal recalibration cannot account for TRE differences that are dependent on the type of sensory stimulus that is used for feedback (e.g., a fearful versus a neutral face). This is based on seminal work by Stetson et al. (2006), who suggested that modulating properties of the visual feedback stimulus would not lead to an increase in TRE. In that study, they examined changes to TRE when different colours were used for adaptation and testing stimuli, and found no difference in the TRE, which suggested that motor-sensory temporal recalibration is not sensitive to stimulus-driven properties. In addition, previous work from Heron et al. (2008; 2009) examined the degree of temporal recalibration when participants were presented with audio-visual, motor-visual, and motor-auditory stimulus pairings and were asked to judge which came first (e.g. did sound appear before vision). Their results showed that the degree of TRE was not significantly different depending on the modality of the stimulus pairs, which suggests there is a comparable degree of flexibility across each of these sensory pairings. Using these results, Heron et al. argued for the existence of a centralised, supra-modal temporal recalibration mechanism which encodes all audio-visual, motor-visual, and motor-auditory information. If this theory holds, the authors argue that this mechanism should occur during later temporal and sensory encoding stages, beyond modality-specific brain regions. By this notion, one may not expect to see a change in the transference effects generated by fearful and neutral face stimuli.

However, given that my results did demonstrate this change, it is possible that the emotion-specific properties of the fearful faces can modulate temporal recalibration beyond what is expected from the recalibration mechanism itself, and provides evidence that stimulus-driven, bottom up processing also interacts with the process of TR. This suggests that current models of temporal recalibration may need to be extended to incorporate changes to the TRE which result from these stimulus-driven attentional

processes. Importantly, however, the results of the current Chapter cannot fully quantify the size of the interaction between fearful face processing and temporal recalibration. Future work could directly compare the impact of fearful faces on temporal recalibration versus classic TOJ tasks to examine this interaction further.

Recent evidence has suggested that both temporal recalibration and related intentional binding effects are closely associated with sense of agency (SoA), which refers to the sense that "I" am the one who is generating a thought or action (J. W. Moore & Obhi, 2012; Timm et al., 2014). Currently, very little is known about the neural mechanisms which underlie sense of agency, however, the results of the current study suggest that the attribution of agency can be modulated by emotional valence. This is because participants demonstrated less agency when judging the temporal order of their button press and a fearful relative to a neutral facial expression. In light of the findings of the current Chapter, future studies could use this paradigm to examine the timing mechanisms which perpetuate symptoms in patients who attribute their thoughts and actions to external forces, through a lack of sense of agency (e.g., schizophrenia), or in those for whom emotional flashbacks can be persistent and debilitating (e.g., post-traumatic stress disorder).

In this study, a TRE would mean that participants' perception of the time interval between their button press and the presentation of the fearful face was compressed. A number of explanations have been proposed to account for this compression effect. For example, this TRE could result from relative changes in sensory processing speed, leading to a temporal shift in the arrival times of motor and visual signals to a hypothetical comparator, with one modality accelerated relative to the other (Di Luca, Machulla, & Ernst, 2009; Y. Sugano, Keetels, & Vroomen, 2016; Yarrow, Minaei, & Arnold, 2015). The extent to which one is moved towards the other would be an important question. Past work has recently shown that motor-sensory TRE is a sensory phenomenon, as visual perception was accelerated in relation to the motor component following delay adaptation, and was altered following visual cortex transcranial direct current stimulation (tDCS) in a recent study (Aytemur et al., 2017). The current findings are consistent with this work, as well as with electrophysiological evidence of motor-sensory recalibration, using a visual flash as feedback stimuli. The results from that study showed that the visual P1 ERP component (occurring between 85 ms-150 ms, reflecting sensory attention) associated with the flash that was presented during the test phase was significantly lowered following the delay adaptation

condition, compared with that of the no-delay adaptation condition (Stekelenburg et al., 2011). To explain this result, the authors suggested that when the flash appeared at the expected time (i.e., following the no-delay the adaptation phase), early visual processing of the flash was enhanced, and reflected in greater P1 amplitudes. Conversely, when the flash appeared earlier than expected (i.e., following the delay adaptation phase), the P1 amplitude was reduced. Overall, this finding suggests that the perceived time of the flash was being shifted towards the perceived time of the motor action. These aforementioned studies will have important implications for future temporal recalibration studies. Temporal recalibration has exclusively been studied with simple stimuli (i.e., beep and flash) and there are no studies examining the possibility of stimulus-driven enhancement of TRE. When stimulus-driven attention is studied, the use of complex visual stimuli appears to produce more fruitful results relating to temporal recalibration mechanisms.

It is possible that the fearful faces rapidly captured participants attention and resulted in accelerated processing via a fear-specific module, consistent with past work demonstrating faster perception of fear-relevant compared to fear-irrelevant pictures (Ohman et al., 2001; Ohman & Mineka, 2001). This could help to explain the increased transference of temporal recalibration for fearful faces in this study, as the fearful faces rapidly captured attention and led participants to perceive these stimuli as occurring earlier in time, resulting in an increased TRE when compared with neutral faces. The present results are also supported more generally by previous temporal perception tasks which have reported the greatest modulations of perceived time when presenting participants with threat-related facial expressions compared to other emotional expressions, or neutral faces (e.g., Droit-Volet et al., 2004; Fayolle & Droit-Volet, 2014; Jason Tipples, 2008; J Tipples, 2011). This supports the suggestion that the emotional content of the faces had an impact on timing processes, as opposed to general facial features.

Researchers have suggested that this type of accelerated fear processing may be subserved by a specialized subcortical visual pathway via the amygdala, to process threat-related stimuli in a rapid and automatic way (G. West et al., 2010). This is supported by work by West and colleagues (2010), who conducted a series of TOJ experiments with fearful face stimuli, and found that the accelerated processing effect observed for fearful faces could be suppressed using red diffuse light. Because the retinal magnocellular (M) pathway, which is tuned to low-spatial-frequency information, has also been shown to be suppressed under red diffuse light (e.g., Wiesel

& Hubel, 1966), West and colleagues surmised that this M pathway may be involved in the coarse prioritized processing of fearful faces. Furthermore, (G. L. West, Anderson, Ferber, & Pratt, 2011) also found that information processing in the primary visual cortex is biased towards the perceptual representations of fearful faces. This bias occurs due to the emotional expression of the face, as opposed to low-level features, and requires upright facial orientations to occur. Taken together, this research suggests that a fear-specific module may be engaged in response to fearful facial expressions, and suggests that threat-related stimuli are prioritised at the earliest stages of visual processing, potentially via a specialised retinal M-pathway. Future work could examine the effect of different emotional expressions on the TR transference effect, to better understand the precise nature of this emotion-specific modulatory effect.

2.4.2. Study limitations and future directions

Some limitations should be considered in light of the current findings. Firstly, one may argue that response bias in this temporal recalibration paradigm could have affected the results, with participants favouring attended stimuli when they were maximally uncertain about which stimulus came first (Alais, Orchard-Mills, & Van der Burg, 2015; Shore, Spence, & Klein, 2001). However, inspection of the data (Figure *2-7*) demonstrates no specific increase in favour of one stimulus around the 50% midpoint of psychometric functions. A response bias, if at all, should occur similarly in both the fearful and neutral conditions. Secondly, it could be argued that the enhanced transference effect observed in this study resulted from differences in low-level features between the fearful and neutral faces. However, the faces were matched according to luminance and shape to minimise this effect, and whilst accelerated processing has been demonstrated when asking participants to make TOJs of upright faces compared with inverted faces, this effect remained significantly greater for angry human faces vs. neutral faces (G. West et al., 2009). Thus, despite differences in low-level features, emotional faces can still affect judgments of temporal order.

Thirdly, 11 participants were excluded from the data analysis (26.19% of the sample). This may be considered a high exclusion rate, however, it is similar to those of previous studies in this area, as Stone et al. (2001) excluded 26.09% of the participants in their study because of poor performance during the task, with another excluding 22% of their sample for the same reason (Rayner, Lee, & Woodruff, 2015). Some studies have utilised smaller numbers (e.g., N = 5) of well-trained observers within their

experimental design (Hanson et al., 2008; Heron et al., 2009). However, the results of these studies are less representative of the population, and it is possible that extensive training may serve to bias participants' judgements of temporal order. Although the final sample size for this study (n = 31) was larger than previous studies, it was significantly smaller than my intended sample size (n = 42), and my initial power analysis determined that this study required 35 participants in order to achieve 80% power to detect a true effect. As a result, it should also be considered whether the significant difference in TRE between fearful and neutral faces in the current chapter is robust, or whether it reflects a false positive result. A false positive result, or type I error, results when the null hypothesis is incorrectly rejected. Smaller sample sizes usually result in lower statistical power, because they increase the chance of a false positive result. It has previously been shown that the average statistical power of studies in the field of Neuroscience ranges between 8-31% (Simmons, Nelson, & Simonsohn, 2011), which suggests that the chance of reporting a false positive result is a significant issue throughout the research area. Therefore, the results reported above should be interpreted with some caution, and future research should seek to replicate and extend this finding using larger sample sizes.

It is important for this doctoral work to also consider whether the lack of previous studies examining the TRE in adolescence reflects a genuine lack of research in this developmental population, or whether studies have been conducted with "adult" populations that instead reflect a period of late adolescence (i.e., 18-24-year-olds). Whilst an assessment of the age ranges of participants recruited into the TRE studies outlined in this chapter suggests that many adult studies did consist of participants which were 25+ years of age (Aytemur et al., 2017; Ikumi & Soto-Faraco, 2014; Stekelenburg et al., 2011; Sugano et al., 2010; Timm et al., 2014), a larger number of TRE studies include no age information at all (Fujisaki et al., 2004; Hanson et al., 2008; Heron et al., 2009; Heron et al., 2010; Stetson et al., 2006; Tsujita & Ichikawa, 2015; Vroomen et al., 2004). This lack of age information makes it difficult to examine potential developmental differences in temporal recalibration effects that may already exist in the literature. Future research should seek to address this issue by including detailed age information in their TRE studies.

2.4.3. Suitability of a temporal recalibration paradigm to assess emotion processing from adolescence to adulthood

For two key reasons, this paradigm was not used in follow-up studies in this doctoral thesis to assess emotion processing in the transition from adolescence to adulthood. Firstly, despite participants undergoing a training session prior to the task, the task difficulty of this paradigm was high, which led to the exclusion of approximately one quarter of the participants in the current study, as they did not reach a high enough performance level to be included in the final analysis. This is not uncommon when using TOJ tasks (Rayner, Lee, & Woodruff, 2015; Stone et al., 2001), and many temporal recalibration studies attempt to overcome this weakness by including extensive training sessions prior to the main task (Stetson et al., 2006; Vercillo et al., 2015; Vroomen et al., 2004). If, during training, participants fail to meet the minimum accuracy requirements (e.g., less than 30% errors in Vercillo et al., 2015) then they do not progress to the main task. However, studies often neglect to report how many participants are removed following these training sessions. This is problematic, as a large proportion of the population may struggle to complete these tasks, which limits how well these results represent temporal recalibration processes more generally. For this reason, it is likely that the task difficulty would be greater in a younger adolescent age group. This would lead to an even greater exclusion rate than the rate reported in the current study, and would make it more challenging to isolate age-dependent differences in emotion processing.

Secondly, whilst a significant effect of fearful faces in the temporal recalibration task was observed, the difference in TRE was small, and equated to a 6 ms difference in PSS scores. This also creates difficulties when implementing the paradigm developmentally, as a much larger number of participants would be needed to have a suitable degree of power to detect a similar effect. Therefore, in the context of this task, it is unlikely that a large enough difference between groups would be observed to draw any informative conclusions about emotion processing throughout development. For these two reasons, it was determined that this paradigm may not be optimal for use with younger developmental populations.

2.5. Conclusions

In conclusion, this study reports, for the first time, an enhanced temporal recalibration transference effect for fearful faces compared with the transference effect

produced by neutral faces. The results reflect a stimulus-driven enhancement of attention for the visual processing of fearful faces, which interacted with the process of temporal recalibration. Future work should continue to investigate the degree to which emotional stimuli modulate motor-sensory temporal recalibration. However, the high exclusion rate resulting from task difficulty, in combination with small condition effects, suggests this paradigm would not be suitable to examine developmental differences in emotion processing in adolescents and adults.

Chapter 3. The effect of emotional vocalisations on early visual face processing

Abstract

This study examined the effect of emotional vocalisations on early visual face processing, on which there is a paucity of research, and assessed the suitability of this paradigm for use with a younger adolescent population. To that end, EEG was used to examine the modulation of the visual event-related potentials, P1 and N170, in response to happy and sad facial expressions when these were presented following either a congruent or incongruent emotional vocalisation, laughter or crying. An enhancement of P1 mean amplitudes were found in response to happy facial expressions which were presented following emotionally-congruent vocalisations (i.e., laughter) relative to happy facial expressions which were presented following emotionally-incongruent vocalisations (i.e., crying). No modulation of the N170 in response to voice-face congruency was observed, which demonstrates a functional dissociation between the visual P1 and N170 components. These results suggest that presenting happy facial expressions with congruent positive emotional vocalisations, specifically laughter, can modulate the early visual processing of such faces as early as 100 ms post-stimulus. These findings provide support for a valence-dependent enhancing effect of emotional auditory information on early visual face processing. However, when assessing the suitability of the current task paradigm for use with a younger adolescent population, the small condition effects observed, coupled with difficulties in isolating the emotion-specific effects of the task from other aspects of facial processing, suggest this paradigm may not be optimal for examining developmental differences in emotion processing in the transition from adolescence to adulthood.

3.1. Introduction

As in Chapter 2, the aim of the current chapter was to identify an appropriate emotion processing paradigm that would be suitable for both adolescents and adults. In response to the issues raised by the findings of Chapter 2, regarding high task difficulty and the small condition effects observed when assessing the impact of threat-related faces on temporal recalibration, the current chapter aimed to examine the suitability of an emotional voice-face categorisation task, for use with EEG. The use of EEG provided an additional tool to study the early visual processing of emotional cues, in combination with classic behavioural measures. To that end, the present chapter investigated the impact of emotional vocalisations (i.e., laugher and crying) on subsequent early (P1 and N170) visual event-related potential (ERP) responses to congruent and incongruent emotional faces (i.e., happy and sad), in a sample of late adolescents. In addition, this study aimed to explore potential functional differences between the P1 and N170 components, by examining the relationship between these ERP responses and participants reaction times in each of the emotionally congruent and incongruent voice-face conditions.

The introduction to this chapter will begin by outlining why emotional voice-face processing might provide a suitable method for assessing emotion processing developmentally (section 3.1.1). Next, this introduction will discuss the functional significance of the visual P1 and N170 components in response to emotional stimuli (section 3.1.2). Following this, past work with adults which has specifically examined ERP responses during emotional voice-face processing will be described (section 3.1.3), followed by past developmental work that has assessed emotional voice-face processing (section 3.1.4),. Lastly, this introduction will outline the aims and hypotheses of the current study (section 3.1.5), based on the literature reviewed.

3.1.1. Multisensory integration of visual and auditory emotion information

Distinct emotional facial expressions serve an important socio-communicative purpose, by signalling to others the presence of either danger or safety, or our own internal states such as sadness or joy (Ekman, 1992; Frith, 2009). To date, past work has tended to focus on the extent to which emotional face stimuli are prioritised by the visual system, when compared to neutral faces (e.g., Chapter 2; Eimer & Holmes, 2007; Paulmann & Pell, 2009; Posamentier & Abdi, 2003). However, emotional facial

expressions are rarely processed in isolation of other sensory signals, which also carry important valence information (Collignon et al., 2008; de Gelder, Bocker, Tuomainen, Hensen, & Vroomen, 1999; de Gelder, Morris, & Dolan, 2005). Multisensory integration refers to the process by which multiple sources of sensory information (e.g., vision, audition, motion, and touch) become integrated by the central nervous system (Stein, Stanford, & Rowland, 2009). Significantly, emotion has been shown to modulate the degree of multisensory integration when participants are presented with emotional faces and vocalisations (Collignon et al., 2008; de Gelder & Vroomen, 2000; Massaro & Egan, 1996; Paulmann & Pell, 2011; Vroomen, Driver, & de Gelder, 2001).

To date, behavioural studies which have investigated the effect of emotion on multisensory integration in adults have observed improved emotion recognition and faster reaction times to stimuli presented via multiple sensory sources (i.e., voice and face) compared to unisensory emotion processing (i.e., face-only or voice-only presentations). This effect has been observed following both the simultaneous presentation of voice-face pairs (Collignon et al., 2008; Massaro & Egan, 1996; Paulmann & Pell, 2011; Vroomen et al., 2001) and also when the emotional voice precedes the facial presentation and acts as a priming stimulus (Carroll & Young, 2005; de Gelder & Vroomen, 2000; Pell, 2005a, 2005b). Additional congruency effects have also been observed, with emotionally congruent voice-face pairs (e.g., happy face and laughing voice) resulting in faster and more accurate categorisation of emotional facial expressions compared with emotionally incongruent voice-face pairs. Again, this effect has been observed using both simultaneous voice-face presentations (Collignon et al., 2008; Massaro & Egan, 1996) as well as in auditory priming studies (de Gelder & Vroomen, 2000).

Notably, brain imaging studies (fMRI and PET) using emotional audio-visual stimuli have demonstrated enhanced activation of brain regions previously implicated as multisensory convergence zones, including the posterior superior and middle temporal gyri (Ethofer, Pourtois, & Wildgruber, 2006; Jeong et al., 2011; Kreifelts, Ethofer, Grodd, Erb, & Wildgruber, 2007; Park et al., 2010). For example, Kreifelt et al. (2007) presented participants with dynamic voice-face clips containing a range of emotional expressions, and found enhanced activation of the right thalamus and the bilateral posterior superior temporal gyrus (STG) according to the emotional content of the stimulus, compared to stimuli containing neutral content. Similar findings were also observed in a study that utilised bimodal sensory presentation of faces paired with

musical excerpts rather than voices, with Jeong et al. (2011) finding greater activity in the STG when happy and sad music was presented concurrently with congruent, compared to incongruent, facial expressions. In addition, greater activation in the STG was observed for happy congruent compared to sad congruent stimuli. Taken together, these findings propose that emotional information from both the visual and auditory sensory streams can modulate activity in multisensory convergence zones, in particular in the posterior superior temporal cortices (Ethofer et al., 2006; Pourtois, de Gelder, Bol, & Crommelinck, 2005; Pourtois, de Gelder, Vroomen, Rossion, & Crommelinck, 2000). However, despite some advances in understanding the brain regions involved in emotional voice-face integration, relatively little is known about the time-course of this type of modulation, specifically about the modulation of early sensory percepts.

3.1.2. The visual P1 and N170 components

In theory, emotional voice-face processing can be indexed by early ERP components, such as the P1 and N170, by examining the impact of emotional vocal information on early visual responses to face stimuli. The P1 is a positive ERP component that peaks around 80-130 ms (Mangun, 1995). This component shows maximal activity over the lateral occipital scalp and its source is believed to originate from extrastriate visual areas (Clark, Fan, & Hillyard, 1994; Di Russo, Martínez, Sereno, Pitzalis, & Hillyard, 2002). The P1 is considered to be an early marker of selective attention to visual stimuli (S. J. Luck et al., 1994; Mangun, Hillyard, & Luck, 1993), and notably has also been shown to be sensitive to faces (R. J. Itier & M. J. Taylor, 2004a; Wang, Guo, & Fu, 2016). The N170 is a negative ERP component that peaks around 170 ms, which is often right-lateralized and maximal over occipital-temporal regions (Duchaine & Yovel, 2015; Rossion, 2014), which is consistent with sources located at the fusiform and inferior-temporal gyri (Gauthier et al., 2000; Kanwisher, McDermott, & Chun, 1997), and the superior temporal sulcus (R. J. Itier & M. J. Taylor, 2004b; Nguyen & Cunnington, 2014). In addition, the N170 is strongly associated with the neural processing of faces (Eimer & Holmes, 2002; R. J. Itier & M. J. Taylor, 2004a; Jeffreys, 1989), and a number of studies have shown that the N170 may be sensitive to facial emotional expressions, with greater N170 amplitudes evoked by sad and happy compared to neutral facial expressions (Batty & Taylor, 2003; Blau, Maurer, Tottenham, & McCandliss, 2007). However, in terms of face-sensitivity, the P1 and N170 show key functional dissociations, with the P1 shown to be most sensitive to

low-level visual features (Rossion & Caharel, 2011), and the N170 being more sensitive to the structural features of faces (Eimer & Holmes, 2002, 2007). Together, these studies suggest that the P1 and N170 may provide a neural index of emotional face processsing. Therefore, the study presented here examined whether these neural indexes of emotional face processing could be modulated by the prior presentation of emotionally congruent and incongruent vocalisations.

3.1.3. Past adult EEG work examining emotional voice-face processing

Electrophysiological methods, such as EEG, are well-suited for revealing, with millisecond precision timing, the effect of emotion on auditory and visual neural processing. A small number of studies have used EEG to investigate the effect of emotional faces on the processing of emotional vocalisations in adults. This work has found an enhancement of auditory ERP components as early as 100 ms post-stimulus, when an emotional face is presented before an emotional auditory stimulus (de Gelder et al., 1999; Ho, Schroger, & Kotz, 2015; Kokinous, Kotz, Tavano, & Schroger, 2015; Kokinous, Tavano, Kotz, & Schroger, 2017; Pourtois et al., 2000). This suggests that an emotional visual stimulus can influence the processing of an auditory stimulus in primary sensory cortices, before each signal has been independently processed. In addition, some of this work has shown that emotionally congruent voice-face pairs are processed differently to incongruent voice-face pairs (De Gelder, Pourtois, & Weiskrantz, 2002; Pourtois et al., 2000; Pourtois, Debatisse, Despland, & de Gelder, 2002), when the face was presented prior to the vocal information. Specifically, greater auditory N1 amplitudes have been observed in response to congruent compared with incongruent angry and sad voice-face pairs (Pourtois et al., 2000), as well as for happy and fearful voice-face pairs (De Gelder et al., 2002), with a follow-up study reporting an earlier posterior auditory P2b component for congruent happy and fearful voice-face pairs compared with incongruent pairs (Pourtois et al., 2002). Similar results have been observed in later auditory components, with greater auditory N2 amplitudes observed in response to congruent relative to incongruent fearful voice-face pairs (Magnée, de Gelder, van Engeland, & Kemner, 2008). So far, these EEG studies have shown that the auditory processing of emotional vocal information can be modulated by the prior presentation of emotional faces, and that auditory responses may be impacted by the emotional congruency of the voice-face information.

However, despite the handful of EEG studies that have focused on the effect of emotional voice-face pairs on auditory processing, to date just two studies have examined how emotional voice-face pairs modulate visual processing (Liu et al., 2012; Müller, Kellermann, Seligman, Turetsky, & Eickhoff, 2012). In Liu et al. (2012), participants were presented with happy, angry, and neutral facial expressions simultaneously with congruent and incongruent emotional vocalisations (laughter, growls and neutral utterances) for 1500 ms. However, Liu et al. (2012) did not observe emotional modulation of face-evoked ERPs observed in parietal-occipital regions (P100, N170, and P270). In a similar study, patients with schizophrenia and healthy controls were presented with happy, fearful, and neutral facial expressions paired with simultaneous congruent or incongruent emotional vocalisations (laughter, screams, and yawning) for 1500 ms (Müller et al., 2012). Their results demonstrated no evidence of visual P1/P2 modulation according to voice-face congruency in either group.

Notably, there has been one study which has observed evidence of early visual modulation by emotional auditory information, when an affective sound was presented prior to a picture (Gerdes et al., 2013). In that study, participants were presented with an emotional sound (unpleasant, pleasant or neutral) for 2000 ms, with a congruent or incongruent emotional picture presented 500 ms after the sound onset. Their results demonstrated enhanced visual parietal P1 and P2 amplitudes to all emotional (pleasant and unpleasant) pictures which were presented after both pleasant and unpleasant sounds, compared with neutral sounds. This suggests that both positive and negative emotional sounds may act to non-specifically enhance early visual sensory processing independently of valence. However, as yet it is unclear whether this enhancing effect of emotional sounds on the early visual processing of emotional pictures can be extended to voice-face pairs. Examining the potential impact of emotional vocal information on subsequent face processing is an important and ecologically-valid experimental question. For example, the sound of a person approaching in the corridor crying or laughing before they reach your office door may impact subsequent visual processing once you see them.

3.1.4. Developmental work assessing emotional voice-face processing

A small body of work has assessed emotional voice-face processing throughout development. Importantly, voice-face emotion recognition has been observed as early as

7-months-old across a number of early studies (Soken & Pick, 1992, 1999; Walker-Andrews, 1986). In those studies, infants were presented with two video-recorded facial expressions (angry or happy) in combination with one emotionally congruent or incongruent vocal expression. The time spent attending to the facial expression that was emotionally congruent with the vocal expression was used to index a basic level of voice-face emotion processing. It was found that 7-month-old, but not 5-month-old, infants spent more time attending to the emotionally congruent happy/angry voice-face pairs compared to the incongruent voice-face pairs. In light of these findings, (Grossmann, Striano, & Friederici, 2006) used ERPs to examine the processing of emotionally congruent compared to incongruent voice-face pairs in 7-month-old infants. In that study, infants were presented with angry or happy facial expressions, and were presented with a congruent or incongruent vocalisations following a 400 ms delay. Their results suggested that emotionally congruent voice-face pairs resulted in a smaller negative component, and a larger subsequent positive component, compared to emotionally incongruent voice-face pairs. These two negative and positive components were proposed to be akin to the adult N400 and late positive components respectively, and modulation of these components supports the results of the aforementioned behavioural studies, which suggested that infants as young as 7-months-old can recognise emotion across multiple modalities (Soken & Pick, 1992, 1999; Walker-Andrews, 1986), and that emotional information from the face and voice undergoes multisensory integration and can be indexed by ERP components.

However, despite this early recognition of voice-face emotion observed in infants, further work suggests that specific aspects of this process continue to develop throughout childhood and into early adolescence. For example, Chronaki, Hadwin, Garner, Maurage, & Sonuga-Barke (2015) asked child (4-11 years) and adult (21+ years) participants to complete an emotion identification task, in which they were presented with angry, happy and sad voice-face pairs at three different emotional intensity levels (50% - mild, 75% - moderate, and 100% - high intensity). The intensity of the face and vocal stimuli were manipulated by morphing the emotional expressions/vocalisations with their respective neutral counterparts. The results showed that emotion recognition accuracy in children improved with age, with emotion recognition from faces reaching adult levels in that study by age 11. However, emotion recognition from voices remained significantly less accurate by age 11 compared with those in adulthood, which suggests that emotional voice recognition continues to

develop beyond 11 years and into adolescence. Furthermore, recognition of sadness, as compared with anger and happiness, took a protracted developmental trajectory, which suggests that developmental trajectories of emotional voice-face processing can be modulated by emotional content, as well as the modality in which the stimuli are presented. Taken together, the results of these studies suggest that emotional voice-face processing is present from early infancy, but may continue to develop into early adolescence. However, there is still only a limited understanding of the developmental trajectory of emotional voice-face processing, and, to my knowledge, there appear to be no studies examining emotional voice-face processing in adolescence.

3.1.5. The current study

The main aim of this study was to determine whether the prior presentation of emotional vocal information can influence the subsequent visual processing of emotional faces. To achieve this aim, this study will focus on the effect of emotional vocalisations on visual sensory processing in occipital-temporal brain regions, where face-evoked ERP activity would be expected to be most prominent (Eimer, 2000; R. J. Itier & M. J. Taylor, 2004a). Specifically, this study will examine whether emotional vocal information, laughing and crying respectively, results in enhanced visual processing of emotional faces, happy and sad, and whether both positive and negative emotional vocalisations enhance visual responses to a similar degree, in line with a general, valence-independent enhancing effect of emotion (as in Gerdes et al. 2013), or whether there are differences in the degree of early visual processing depending on whether the voice-face pairs display a positive or negative emotion. In addition, this study will examine the effect of emotional congruency. This is because, although there is evidence for an enhancing effect of emotional congruency on auditory processing following a face presentation (De Gelder et al., 2002; Pourtois et al., 2000; Pourtois et al., 2002), previous studies have not yet examined the potential congruency effects on visual face processing following a vocal presentation. It is important to examine how the emotional congruency of voice and face information may also affect early visual sensory processing, to understand precisely how complementary or conflicting emotional information modulates early visual sensory processing.

To that end, this study used EEG to examine the effect of congruent and incongruent vocalisations on the earliest measurable face-evoked components observed

in occipital-temporal scalp regions. Specifically, this study investigated how presentations of emotional non-verbal vocalisations, laughter and crying, before presentations of emotional faces, happy and sad, would affect early visual ERPs to these faces, namely the P1 and the N170 components. Given the results of previous work (De Gelder et al., 2002; Pourtois et al., 2000), which reported an enhancing effect of congruency on auditory N1 responses to happy, fearful, angry and sad voice-face pairs, and of work showing a similar enhancing effect of congruency on later auditory N2 responses for emotional voice-face pairs (Magnée et al., 2008), it was predicted that greater P1 and N170 amplitudes would be observed, reflecting enhanced visual processing of emotional faces when they were presented after congruent versus incongruent vocalisations. However, based on Gerdes et al. (2013) it was predicted that both congruent happy and sad voice-face pairs would lead to a similar degree of enhancement of early visual responses, which would suggest this enhancing effect of auditory information on visual face processing is valence-independent. In addition, given recent behavioural as well as EEG studies suggesting that congruent voice-face pairs result in faster and more accurate emotion recognition as well as enhanced processing compared to incongruent voice-face pairs (Collignon et al., 2008; de Gelder & Vroomen, 2000; Pourtois et al., 2000), faster reaction times were expected in response to the faces in congruent compared to incongruent voice-face trials. Finally, this study also explored the functional relationship between reaction times and P1 and N170 mean amplitudes. Based on research suggesting faster reaction times may be associated with greater early visual responses to facial stimuli (Caharel, Courtay, Bernard, Lalonde, & Rebaï, 2005; Lerner, McPartland, & Morris, 2013), it was predicted that greater P1 and N170 amplitudes would be associated with faster reaction times for each of the corresponding conditions.

3.2. Methods

A formal power analysis was conducted using G*Power (version 3.1) to assess the suitability of the sample size in this chapter. The effect size was estimated based on the partial eta squared values from the P1 ERP analysis in the study by Gerdes et al. (2013, n = 22), as they had a similar study design and also focused on early visual ERPs. The average effect size estimated from that study was f(U) = 0.5. On this basis, it would be necessary to recruit at least 17 participants in order to detect an effect size f(U) = 0.5 in a within-subjects ANOVA, with power set at 0.8 and an alpha set at 0.05.

3.2.1. Participants

Twenty-two healthy volunteers participated in this study. All were students from the University of Sheffield, had normal or corrected-to-normal vision, normal hearing, and were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Two participants were excluded from the data analysis due to excessive EEG artefacts. Therefore, the data of 20 participants are reported here (18 females, 2 males, mean age = 18.80 years, SD = 0.62, range = 18-20). The University of Sheffield, Department of Psychology Ethics Committee approved this study.

3.2.2. Apparatus and Stimuli

Experimental events were controlled on a PC running E-Prime 2.0 software, which was synchronised with the EEG recording (Schneider, Eschman, & Zuccolotto, 2002), with stimuli presented on a Viglen Omnino III monitor. The monitor had a 60 Hz refresh rate and a 1024 x 768 pixel resolution. During the task, participants were seated in a dimly lit room inside of a Faraday cage.

3.2.2.1. Vocal Stimuli

The auditory vocalisations consisted of 10 female laugher and 10 female crying clips which were 3 seconds in duration, matched for maximum volume, and were acquired from Audio Sparx, a high-quality commercial stock audio library (www.audiosparx.com). Initially, the author intended to use various positive and negatively valenced audio clips from the international affective digital sounds database (IADS; Stevenson & James, 2008). This was because the IADS offers a series of validated self-report arousal and valence ratings for each sound stimulus, which meant that the valence of the stimuli could be manipulated (e.g., positive versus negative), whilst controlling for their arousal levels. However, extensive behavioural piloting with IADS sound stimuli (N = 49, 38 females, mean age = 20.02 years, SD = 0.20, range = 18-26) revealed a lack of sound-face integration effects, which would typically be indexed by faster reaction times to emotionally congruent sound-face pairs. This may have resulted from the wide variety of sound stimuli available as part of IADS. Specifically, these sounds consisted of various human, object, and musical properties, with pilot participants commenting that the sounds felt very dissimilar to the face

stimuli. As a result, the author selected human vocalisations with distinct emotional properties (i.e., laughter and crying) for the main experimental task. This was because whilst laughter can positively influence the affective states of listeners by inducing positive mood states (Owren & Bachorowski, 2003), crying indicates suffering and will often elicit attention in the form of empathy and emotional support from others (Hendriks & Vingerhoets, 2006). Therefore, it was expected that the differential affective states evoked by these stimuli would result in differences in the subsequent attentional processing of the emotional facial expressions, indexed by the P1 and N170 components. These vocal stimuli were presented binaurally through a pair of EchoTubez earphones, designed with no metallic conductor to reduce electro-magnetic noise, at a sound pressure level which was estimated to be 72 dB.

3.2.2.2. Face Stimuli

Forty images of female faces were used, 20 happy (10 x open-mouth expression and 10 x closed-mouth expression) and 20 sad (10 x open-mouth expression and 10 closed-mouth expression). These visual stimuli were colour frontal photographs of faces of 10 female actors from the NimStim database (Tottenham et al., 2009), each displaying two happy (one open-mouth and one closed-mouth) and two sad (one open-mouth and one closed-mouth) facial expressions. Female faces were selected for this study to control for additional potential modulations in early visual ERP components by gender, and based on research suggesting that female facial expressions are better recognised than male facial expressions (Gregorić et al., 2014). Also, in an early study of emotion recognition from the facial expressions of male and female actors, males and females were both successful in expressing happiness, but females were significantly better at expressing sadness than males (Wallbott, 1988), consistent with work suggesting that females may be more emotionally expressive than males (McDuff, Kodra, el Kaliouby, & LaFrance, 2017).

Initially, I intended to use fearful and happy facial expressions in the current study. This was because happy and fearful emotions differ in terms of valence, but not arousal, as both happy and fear emotions are considered to be highly arousing. Therefore, the use of happy and fearful faces would allow for a stricter control of the emotion-specific aspects of the face stimuli. However, behavioural pilot testing (N = 14, age range = 19-20) indicated that fearful faces took longer to recognise, and were

associated with significantly more errors, compared to happy faces. This observed difference in emotional recognition ability for happy versus fearful faces consistent with researchers who have suggested that positive facial expressions are better recognised than other facial expressions (Johnston, Devir, & Karayanidis, 2006; Johnston, McCabe, & Schall, 2003), and was present during pilot testing even when using happy faces with 70% of their original emotional intensity. These results raised concerns that any differences observed in the main paradigm could be due to differences in participants' ability to recognise happy versus fearful facial expressions. As a result, the main experiment implemented happy and sad facial expressions, consistent with previous behavioural studies which have examined the impact of emotion on the attentional processing of facial expressions (Fenske & Eastwood, 2003; Srinivasan & Gupta, 2010; Srinivasan & Hanif, 2010). The visual stimuli were adjusted to ensure they were the same size, and had the same average luminance and contrast levels, and were presented centrally to participants, subtending 15° x 12° of visual angle at a 60 cm viewing distance.

3.2.3. Procedure

Participants were tested individually and received a standardised set of instructions from the experimenter. At the start of a trial (Figure *3-1*), a central fixation point was presented, which varied randomly between 900-1100 ms. Following this, the emotional vocalisation stimulus was presented for 3000 ms, and an emotional facial stimulus was presented during the final 200 ms of the voice presentation. During a response window limited to 1200 ms, participants had to categorise the face as happy or sad using one of two response buttons on a keyboard. The response keys were counterbalanced across participants. Apart from the image presentation period of 200 ms, the fixation point remained on screen throughout each trial.

There were four within-subjects conditions Happy Congruent ($Vocalisation^{Laughter}$-$Face^{Happy}$); Happy Incongruent ($Vocalisation^{Crying}$-$Face^{Happy}$); Sad Congruent ($Vocalisation^{Crying}$–$Face^{Sad}$) and Sad Incongruent ($Vocalisation^{Laughter}$–$Face^{Sad}$), with 160 trials each. These trials consisted of combinations of 160 pseudo-randomised vocalisation-face trials. These trials were made up of combinations of 10 emotional voices (repeated 8 times each) and 10 emotional faces (repeated 8 times each). This resulted in 640 trials, with the task taking approximately 55 minutes to

complete. To reduce possible fatigue, the trials were presented in eight pseudo-randomised blocks of 80 trials, with 30 second breaks in between. The pseudo-randomised blocks were designed so that there were no more than 2 presentations of the same actor's facial expression (e.g. actor with happy open facial expression, or actor with sad closed facial expression) in a row.

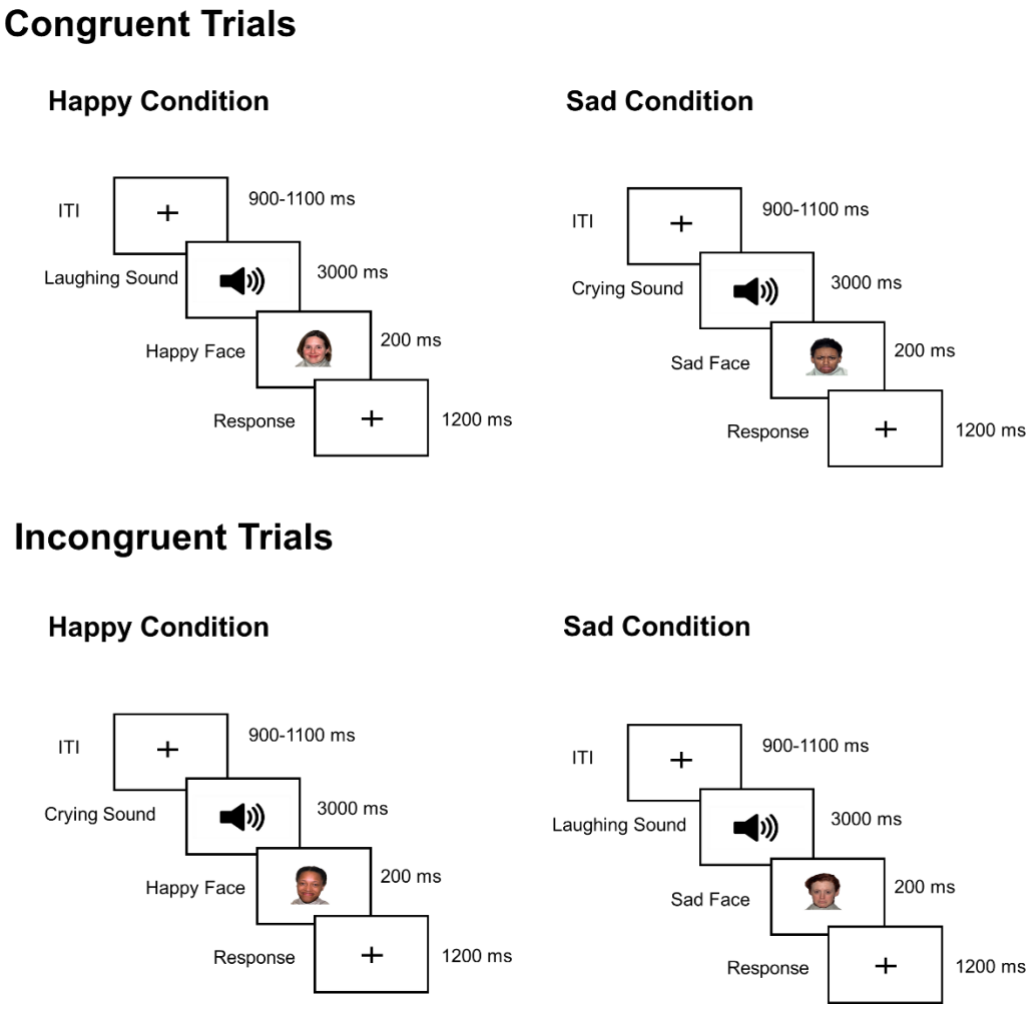

Figure 3-1: Experimental design of the voice-face task. Examples of congruent and incongruent happy and sad trials. For example, in the sad congruent condition (Vocalisation^{Crying}-Face^{Sad}), sad faces were presented following a crying vocalisation; in the incongruent condition (Vocalisation^{Laughter}–Face$^{Sad)}$, sad faces were presented following a laughing vocalisation. During the response window, participants had to categorise the face as happy or sad using one of two response buttons on the keyboard.

3.2.4. EEG recordings and data analyses

The EEG data were recorded using a Biosemi ActiveTwo 64-channel EEG System (Amsterdam, the Netherlands). Electrodes were placed upon the scalp and held

in place using a cap, with the electrodes fitted according to the 10-20 system. The EEG data were digitised by Biosemi ActiView software, at a sampling rate of 2048 Hz. A subset of recording channels monitored vertical and horizontal eye movements. Recordings were taken in an electrically shielded room, and direct current offset voltages were kept within +/- 25 mV, as recommended by the manufacturer. An air conditioner was used to keep the room cool and to reduce the onset of slow low-frequency drifts in the EEG recording due to sweating.

Once offline, the continuous data were subsequently down-sampled to 512Hz, using the Biosemi Decimator software. The EEG signals were processed and analysed using the EEGLAB 14.1.1b and ERPLAB 5.0 Matlab toolboxes (Delorme & Makeig, 2004; Lopez-Calderon & Luck, 2014). The data were re-referenced to the average reference, and a high-pass filter of 0.1 Hz was applied to the data to remove low-frequency drift such as sweating, which can cause slow and continued changes in the baseline voltage of an EEG signal (Luck, 2014). A 0.1 Hz filter was selected based on advice from key ERP researchers (Luck, 2014), as filters greater than 0.1 Hz have been shown to cause significant distortions of ERP waveforms (Acunzo, MacKenzie, & van Rossum, 2012; D. Tanner, Morgan-Short, & Luck, 2015). Following the application of a high-pass filter, The Cleanline Toolbox was used to reduce 50Hz line noise (Mullen, 2012). 50 Hz line noise stems from AC power line fluctuations, power suppliers (e.g., electrical equipment in the lab), fluorescent lights etc. The faraday cage used in the current chapter minimises line noise from contaminating the EEG signal, however, there are still some sources of 50 Hz line noise in the faraday cage that cannot be controlled for (e.g., computer monitors). This issue can be alleviated using Cleanline, a recommended cleaning tool implemented as part of the PREP pipeline (Bigdely-Shamlo, Mullen, Kothe, Su, & Robbins, 2015). This cleaning tool acts as an alternative to a notch filer – which cuts 50 Hz noise from your dataset and has been shown to create band-holds and distortion in other frequencies. For example, 60 Hz notch filters (e.g., line noise frequency in the USA) commonly have a notch width of 10Hz, which causes significant signal distortion in frequencies from 50-70 Hz (Bigdely-Shamlo et al., 2015). Instead, Cleanline runs a sliding window over the dataset, and uses a multi-taper fast Fourier transformation to transform the signal within each time window into the frequency domain. Cleanline then attempts to fit a 50 Hz sinusoid wave to the data, to estimate what 50 Hz line noise should look like, and subtracts this from the participant's EEG signal. This process is repeated for a maximum of 10 iterations until

the line noise has been significantly reduced. As such, Cleanline reduces the electrical noise without significantly distorting the EEG signal.

Following the use of Cleanline to reduce 50 Hz line noise, the artefact subspace reconstruction method (ASR) was applied to minimise artefacts associated with non-stationary high-variance signals from EEG (Mullen et al., 2013). ASR works by first finding 1 minute of "clean" EEG data from each participant, and uses this as a reference for the rest of the dataset. Statistics are computed on this clean section of data, and the function then runs a sliding window over the rest of the EEG data to identify portions of the EEG which are more than a set number of standard deviations away from the reference EEG. In this study, the function identifies "bad" sections of data that are more than 20 SD away from the reference EEG. Once this bad data has been identified, the data is treated as missing, and is reconstructed (or interpolated) using a mixing matrix that was initially calculated on the clean data. The use of ASR significantly improves the quality of ICA data, without introducing subjective biases that are inherent when researchers are manually removing bad sections of EEG data. Following ASR, visual inspection on all data was conducted to identify any bad channels which may have been missed by the ASR procedure using EEGLAB's Channel Statistics function, and remove them. After removing artefactual channels an average of 60.95 channels (SD = 2.28, range = 56-64) remained for each participant. Data were then decomposed into maximally temporally independent components using the extended infomax algorithm (Delorme & Makeig, 2004), and the ADJUST toolbox was used to identify and remove eyeblink and other eye movement components, based on artefact-specific spatial and temporal features (Mognon, Jovicich, Bruzzone, & Buiatti, 2011). Previously removed channels were then re-interpolated using a spherical spline interpolation (Perrin, Pernier, Bertrand, & Echallier, 1989).

From this pre-processed continuous data, face-locked ERP epochs were obtained for all conditions (-200 ms to 1400 ms time-locked to face presentation onset). Epochs were baseline corrected according to the average activity in the 200 ms window prior to the face onset. Epochs with voltage fluctuations greater than ± 100 μV were rejected from the study. Following this, the data from each condition were averaged to form 4 ERPs for statistical analysis and for creating brain maps. A 20th-order low-pass filter with a 30 Hz cut-off and a Hamming window was applied to the averaged epochs to remove high frequency noise, which may have been caused by muscle activity. Removing these artefacts improves the signal-to-noise ratio of the data, thereby

improving statistical power (Kappenman & Luck, 2010). Out of 160 trials for each condition, a very small number of trials were excluded on average: Happy Congruent ($vocalisation^{Laughter}$-$Face^{Happy}$) = 1.35 (SD = 2.23), Happy Incongruent ($vocalisation^{Crying}$-$Face^{Happy}$) = 1.40 (SD = 2.35), Sad Congruent ($vocalisation^{Crying}$-$Face^{Sad}$) = 1.40 (SD = 2.37), Sad Incongruent ($vocalisation^{Laughter}$–$Face^{Sad}$) = 1.20 (SD = 1.58). There were no significant differences in the mean number of excluded trials per condition (all p's > .05), suggesting no difference in signal-to-noise ratio across conditions.

Electrode clusters (Figure 3.3) were selected by creating one grand-averaged ERP waveform per electrode, containing ERP waveforms which were time-locked to the presentation of the visual face cues for all conditions together, forming a collapsed localizer (see Luck & Gaspelin, 2017). Visual inspection of this grand-averaged ERP for all conditions and participants demonstrated, for the visual P1, a positive peak at approximately 70-130ms post-stimulus at electrodes PO7/PO8 and O1/O2, and for the visual N1, a negative peak at approximately 130-190 ms at electrodes P7/P8. The selection of these electrodes are identical to previous work which has examined facial processing indexed by the P1 and N170 components (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010).

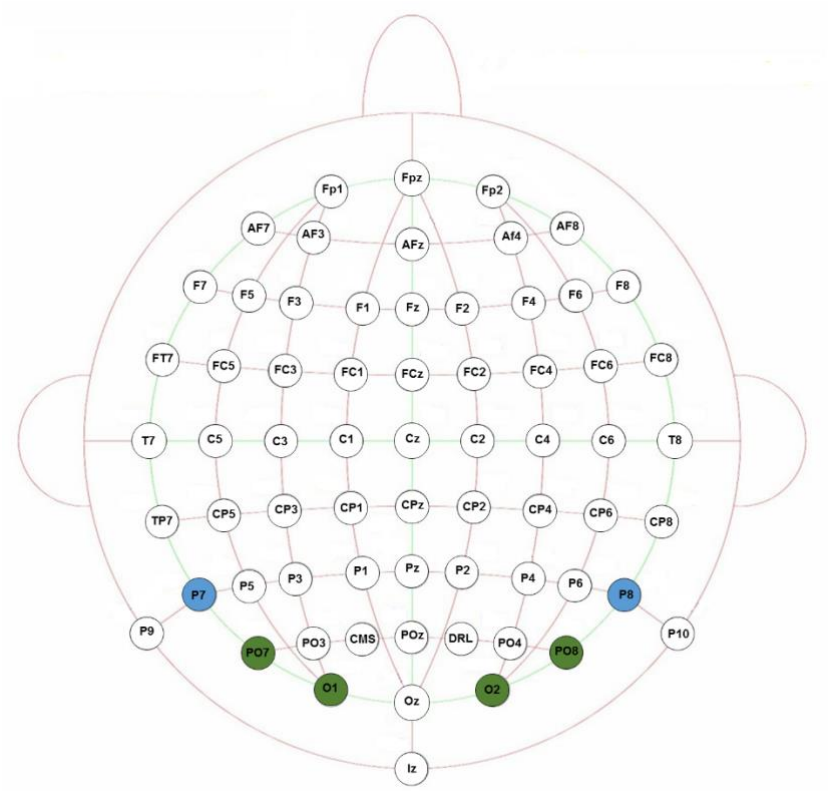

Figure 3-2: Electrode clusters for the visual P1 (green), and the visual N170 (blue) components.

3.2.5. Statistical analysis

The alpha level was set to $p < .05$ for all statistical analyses. The reaction time data, as well as P1 and N170 mean amplitudes for each condition, were all found to be normally distributed, determined by Shapiro-Wilk's test of normality (all p's > .05). Therefore, the data were analysed using a series of within-subjects ANOVAs, with additional exploratory Pearson's correlations used to examine the relationship between P1 and N170 mean amplitudes and the reaction times of the facial emotion categorisation task, for each of the corresponding conditions. If post-hoc tests were conducted, I applied a Bonferroni correction to control for the increased risk of Type I error that is associated with making multiple comparisons.

3.3. Results

3.3.1. Task performance

During the task participants were asked to indicate whether the faces they saw were either happy or sad. Performance was very high with errors occurring on average

in only 4.3 trials out of every 160 in each condition, with no significant differences between the errors made in each condition (Table 3.1). To examine the possible effects of vocalisation and face congruency on reaction times during the emotion recognition task, the average reaction times were calculated for each participant in each of the four conditions (Happy Congruent, Happy Incongruent, Sad Congruent, Sad Incongruent), for correct trials only.

Table 3.1 – *The mean (SD) number of incorrect trials excluded for each condition*.

Condition	**Mean errors (Trials)**	**SD**
Happy Congruent (Vocalisation^{Laughter} Face^{Happy})	4.93	4.11
Happy Incongruent (Vocalisation^{Crying} Face^{Happy})	4.60	3.42
Sad Congruent (Vocalisation^{Crying} Face^{Sad})	3.78	3.39
Sad Incongruent (Vocalisation^{Laughter} Face^{Sad})	3.90	3.84

There were non-significant differences in the mean number of excluded incorrect trials per condition (all p's > .005), suggesting no differences in task difficulty across the conditions.

Table 3.2 displays the mean reaction times (ms) for each of the four conditions. A within-subjects ANOVA was used to examine the effects of *Facial Emotion* (Happy and Sad) and *Congruency* (congruent and incongruent) on the reaction times measured when participants categorised the emotion of the face (Table 3.2). The analysis revealed non-significant main effects of *Facial Emotion*, $F(1, 19) = 0.99$, $p = .332$, $\eta_p^2 = .05$, and a non-significant main effect of *Congruency*, $F(1, 19) = 0.06$, $p = .812$, $\eta_p^2 = .00$. There was a significant *Facial Emotion* by *Congruency* interaction, $F(1, 19) = 5.94$, $p = .025$, $\eta_p^2 = .24$. However, follow-up pairwise comparisons were all non-significant (all p's > .05). From examining Table 3.2, it appears that a crossover interaction may have led to the non-significant pairwise comparisons. This is because Happy Congruent voice-face presentations led to faster reaction times than Happy Incongruent trials, but Sad

Congruent voice-face presentations led to slower reaction times than Sad Incongruent trials. From this crossover interaction, it is possible that both happy and sad faces were recognised faster when they were preceded by laughing vocalisations, as compared to crying vocalisations.

Table 3.2 – *Reaction times for each of the four conditions (n = 20).*

Condition	Mean Reaction time (ms)	SD
Happy Congruent (Vocalisation^{Laughter} Face^{Happy})	414.53	66.77
Happy Incongruent (Vocalisation^{Crying} Face^{Happy})	420.12	64.60
Sad Congruent (Vocalisation^{Crying} Face^{Sad})	425.39	73.17
Sad Incongruent (Vocalisation^{Laughter} Face^{Sad})	420.93	74.11

3.3.2. Event-related potentials

3.3.2.1. The visual P1 component

3.3.2.1.1 P1 mean amplitude

The P1 topographical maps revealed, as expected, bilateral positivity in posterior-occipital regions elicited by the face stimuli, and the topographical difference map shows the distribution of voltage elicited by Happy Congruent minus Incongruent conditions, as well as the Sad minus Happy Incongruent conditions (Figure *3-3*A). To examine the effect of emotional voice-face pairs on P1 mean amplitudes, a within-subjects ANOVA was conducted, with *Facial Emotion* (happy and sad), *Congruency* (congruent and incongruent), and *Hemisphere* (left and right) as the within-subjects variables. The analysis revealed a significant interaction between *Facial Emotion* and *Congruency*, F(1, 19) = 9.58, p = .006, η_p^2 = .34. Follow-up pairwise comparisons revealed significantly greater P1 amplitudes in response to Happy Congruent voice-face pairs (M = 3.12, SEM = 0.33) compared with Happy Incongruent voice-face pairs (M = 2.73, SEM = 0.36), Mdiff = 0.38, 95% CI [0.15, 0.61], p = .002. In addition, significantly greater P1 amplitudes were observed in response to Sad Incongruent voice-face pairs compared with Happy Incongruent voice-face pairs (M = 3.12, SEM = 0.39),

Mdiff = 0.42, 95% CI [0.20, 0.65], p = .001. There were non-significant main effects of *Facial Emotion*, F(1, 19) = 1.04, p = .321, η_p^2 = .05, *Congruency*, F(1, 19) = 0.80, p = .383, η_p^2 = .04, and *Hemisphere*, F(1, 19) = 0.15, p = .704, η_p^2 = .01. Overall, these results suggest that congruent laughing vocalisations led to enhanced P1 amplitudes in response to happy faces.

In order to test whether this P1 interaction is the result of mean amplitude differences in the pre-stimulus baseline (-200ms to 0ms), a within-subjects ANOVA was conducted, with *Facial Emotion* (happy and sad), and *Congruency* (congruent and incongruent) as the within-subjects variables. The analysis revealed a non-significant effect of *Facial Emotion*, F(1, 19) = 0.04, p = .839, η_p^2 = .00, *Congruency*, F(1, 19) = 0.30, p = .588, η_p^2 = .02, and *Face* by *Congruency* interaction, F(1, 19) = 0.89, p = .357, η_p^2 = .05, suggesting the current findings cannot be explained by differences in the pre-stimulus baseline.

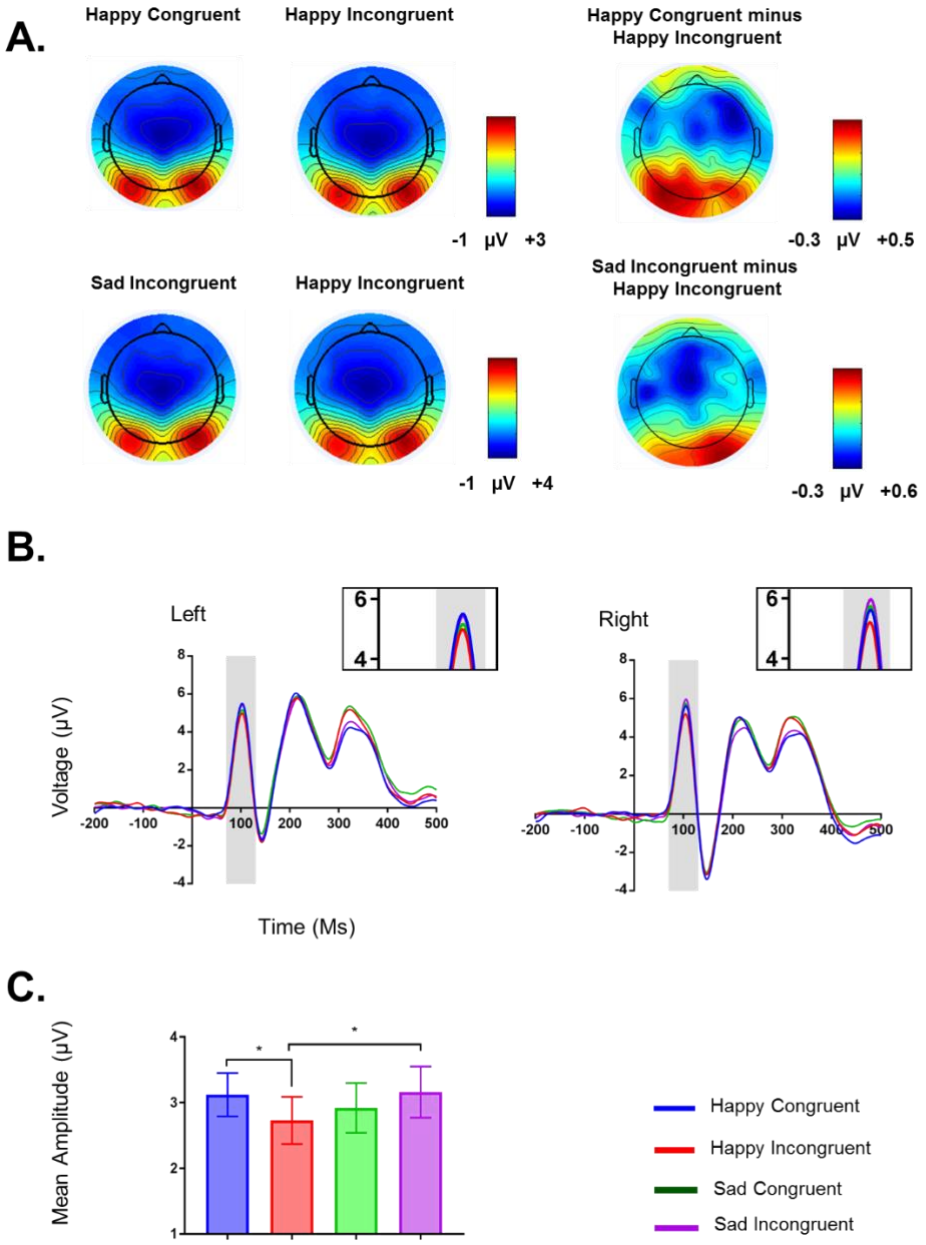

*Figure 3-3***:** The visual P1 component findings. Prior presentations of laughing vocalisations resulted in greater P1 amplitudes in response to congruent happy facial expressions, and sad faces presented following incongruent vocal information (laughter) resulted in greater P1 mean amplitudes relative to happy faces presented following incongruent vocal information (crying).**(A):** The scalp topography of the P1 (70-130 ms) for all faces that were preceded by either laughter or crying, and the difference of overall voltage between the congruent compared to incongruent conditions, for both happy and sad voice-face pairs. **(B):** Grand averaged ERP waveforms for each condition in the left and right hemisphere. **(C):** Bar chart to highlight the enhancing effect of Happy Congruent voice-face pairs versus Happy Incongruent voice-face pairs, and the enhancing effect of Sad Incongruent voice-face pairs versus Happy Incongruent voice-face pairs. *$p < .05$.

3.3.2.1.2 The relationship between P1 mean amplitudes and reaction times

To further examine the functional significance of the visual P1 component, a series of exploratory Pearson's correlations were conducted to assess whether P1 mean amplitudes were associated with reaction times in each of the corresponding conditions. There were non-significant correlations between P1 mean amplitudes and reaction times in response to the face stimuli in Happy Congruent, Happy Incongruent, Sad Congruent, and Sad Incongruent conditions (all *p*'s > .05). Please see Supplementary Table 3.1 for a full report of the correlation coefficients for each condition and hemisphere.

3.3.2.2. The N170 Component

3.3.2.2.1 N170 mean amplitude

Having shown an effect of congruent positive emotional vocalisations on P1 mean amplitudes in response to happy facial expressions, emotional voice-face effects on the N170 were examined. As expected, the N170 topographical maps revealed greater negativity over occipital-temporal electrode sites in response to the faces presented, and the topographical maps show the distribution of voltage elicited by Happy Congruent minus Incongruent conditions, as well as the Sad Congruent minus Incongruent conditions (Figure *3-4*).

To examine the effect of emotional voice-face pairings on N170 mean amplitudes, a within-subjects ANOVA was conducted, with *Facial Emotion* (happy and sad), *Congruency* (congruent and incongruent), and *Hemisphere* (left and right) as the within-subjects variables. There was a significant interaction between *Facial Emotion* and *Congruency*, F(1, 19) = 4.28, $p = .052$, $\eta_p^2 = 0.18$. However, all follow-up pairwise comparisons were non-significant. There were non-significant main effects of *Facial Emotion* F(1, 19) = 3.45, $p = .079$, $\eta_p^2 = 0.15$, *Congruency*, F(1, 19) = 0.11, $p = .744$, $\eta_p^2 = .01$, and *Hemisphere*, F(1, 19) = 2.44, $p = .135$, $\eta_p^2 = .11$, suggesting no modulation of the N170 component by emotion or congruency. All other interactions were non-significant (*p*'s > .005).

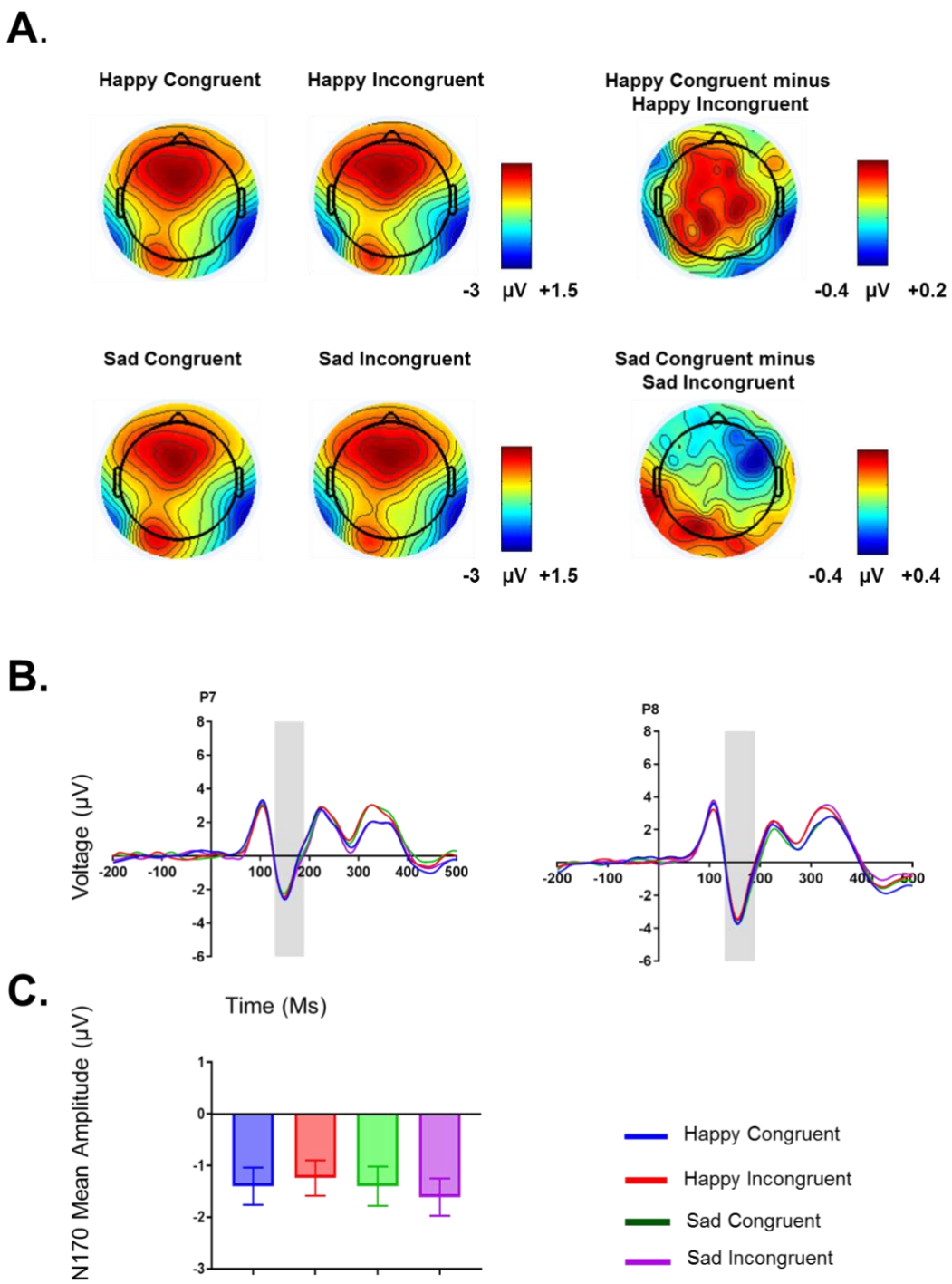

Figure 3-4: The visual N170 component findings.No modulation of the N170 by voice-face emotion was observed. **(A):** The grand averaged scalp topography of the N170 (130-190 ms) in response to each of the conditions, including the difference in overall voltage between congruent minus incongruent happy and sad voice-face pairs. **(B):** Grand averaged ERP waveforms for each condition at electrodes P7 and P8. **(C):** A bar chart displaying the mean N170 amplitudes in response to each of the conditions. *p < .05.

3.3.2.2.2 Exploring the relationship between N170 mean amplitudes and reaction times

As with the visual P1, the functional significance of the N170 was examined further through a series of exploratory Pearson's correlations, to determine whether N170 mean amplitudes were associated with reaction times in each of the corresponding conditions. These correlational analyses identified a significant positive correlation between N170 mean amplitude in the right hemisphere and reaction time in the happy congruent condition, $r(18) = 0.76$, $p < .001$, as well as in the happy incongruent condition, $r(18) = 0.65$, $p = .002$, the sad congruent condition, $r(18) = 0.61$, $p = .004$, and the sad incongruent condition, $r(18) = 0.61$, $p = .004$. Correlations between N170 mean amplitudes and reaction times in the left hemisphere were not significant (all *p's* > .05). Overall, these correlations suggest that faster reaction times were associated with greater N170 mean amplitudes in the right hemisphere only, for each of the corresponding conditions. Please see Supplementary Table 3.2 for details the correlation coefficients for each condition and hemisphere.

3.4. Discussion

3.4.1. Summary of key findings

The aim of this study was to examine the effect of emotional vocalisations on the early visual processing of emotional faces, as measured by the P1 and the N170. To that end, participants took part in a simple emotion categorisation task, in which an emotional vocalisation (laughter or crying) was presented prior to a congruent or incongruent facial expression (happy or sad). The results of this study provide partial support for the predictions outlined in Section 3.1.5. Regarding the P1, happy congruent voice-face pairs (laughter and a happy facial expression) led to enhanced visual P1 responses relative to happy incongruent voice-face pairs (crying and a happy facial expression). The observed lack of effect for sad congruent voice-face pairs (crying and a sad facial expression) relative to sad incongruent voice-face pairs (laughter and a sad facial expression), suggests that this congruency effect was specific to positively valenced voice-face pairs. In contrast, no modulation of the N170 in response to emotional voice-face pairs was observed. Regarding performance on the behavioural task, the present study did not observe the expected enhancing effect of emotionally congruent voice-face pairs on reaction times when categorising emotional facial expressions (Collignon et al., 2008; de Gelder & Vroomen, 2000; Massaro & Egan,

1996). However, the correlational analysis revealed that faster reaction times in the present task were associated with greater N170 mean amplitudes in the right hemisphere, for each of the conditions.

3.4.2. Potential mechanisms of P1 enhancement to happy congruent voice-face pairs

The observed enhancement of mean P1 amplitudes in posterior occipital-temporal regions, in response to happy congruent versus incongruent voice-face pairs, supports the theory that auditory information can modulate face processing early on in the visual processing stream (Collignon et al., 2008; de Gelder & Vroomen, 2000; Massaro & Egan, 1996; Paulmann & Pell, 2011; Vroomen et al., 2001), with the current study suggesting that this modulation can occur as soon as 100 ms post-stimulus. This result is consistent with previous work which assessed the impact of prior face presentations (angry, sad, happy, and fearful) on the subsequent early auditory processing of congruent and incongruent vocal information, which reported enhanced auditory N1 amplitudes in response to congruent versus incongruent voice-face pairs (De Gelder et al., 2002; Pourtois et al., 2000). Together, these findings suggest that congruent emotional voice-face pairs can lead to enhancements of early perceptual processes, regardless of whether the early auditory or visual responses are being examined.

One possible mechanism by which positively valenced auditory information could act to enhance early visual processing of the happy facial expressions, as in the present study, is by direct or indirect effects on attentional processes. Attention has been shown to play a substantial role in modulation of the P1 in response to visual stimuli. For example, in research using non-emotional stimuli, it has been shown that increases in P1 amplitudes to visual stimuli index larger sensory-evoked responses as a result of directing attention to the location of that stimulus (Hillyard, Vogel, & Luck, 1998; Posner & Dehaene, 1994). Therefore, the significant increase in P1 amplitudes observed when viewing happy faces that were presented following emotionally-congruent laughter, rather than crying vocalisations, could result from positive emotional vocalisations increasing attention to subsequent congruent face presentations. However, it should be considered why this congruency effect was not observed for sad voice-face pairs. In a separate study by Spreckelmeyer, Kutas, Urbach, Altenmuller, and Munte

(2006), participants were presented with happy, sad, and neutral affective pictures simultaneously with congruent and incongruent affectively sung tones. Their results demonstrated greater visual P2 components in response to happy, but not sad, picture-voice pairs. The authors suggested that this effect could have resulted from the specific physical structure of the vocal stimuli, as happy voice stimuli have a louder tone onset, and may be harder to ignore than sad vocal stimuli, resulting in their early integration with congruent visual stimuli. This is supported by behavioural work which has shown laughter to increase the perceived intensity of congruent happy facial expressions, relative to neutral or sad expressions (A. Sherman, Sweeny, Grabowecky, & Suzuki, 2012). Previous fMRI work has also demonstrated a difference in the processing of congruent happy and sad audio-visual information. Specifically, Jeong et al. (2011) presented participants with faces paired with musical excerpts rather than voices. Whilst they did observe greater activity in the superior temporal gyrus (STG) when both happy and sad music was presented concurrently with congruent, compared to incongruent, facial expressions, greater activation in the STG was observed for happy congruent compared to sad congruent stimuli, which suggests there may be inherent differences in the neural processing of happy and sad emotional information. As such, it is possible that positive emotional vocalisations (i.e., laughter) in the current study had a stronger attention-orienting effect, leading to enhanced P1 amplitudes when accompanied by a congruent facial expression.

The results of the present study, which provide evidence of a valence-dependent enhancing effect of positive emotional auditory information on the visual processing of congruent facial expressions as indexed by the visual P1, differs from previous work which reported a lack of emotional modulation in parietal-occipital visual components in response to voice-face pairs (P1, P270; Liu et al., 2012; P1, P2; Müller et al., 2012), and also differs from other work suggesting that both positive and negative emotional sounds can act to non-specifically modulate early visual sensory processing, independently of valence (Gerdes et al., 2013). In that study, Gerdes et al. (2013) found enhanced visual parietal P1 and P2 amplitudes to all emotional (pleasant and unpleasant) pictures which were presented after both pleasant and unpleasant sounds, relative to neutral sounds. However, these inconsistent results could be partially accounted for by methodological differences across studies. Firstly, there are differences between the studies reported here according to the type of audio-visual presentation employed, i.e., simultaneous (Liu et al., 2012; Müller et al., 2012) versus face-first (De

Gelder et al., 2002; Pourtois et al., 2000), versus vocal-first (the present study; Gerdes et al., 2013). Secondly, there are differences between studies in the type of emotional stimuli used, i.e., face-sound pairs (the present study; De Gelder et al., 2002; Liu et al., 2012; Müller et al., 2012; Pourtois et al., 2000) or picture-sound pairs (Gerdes et al., 2013; Spreckelmeyer, Kutas, Urbach, Altenmuller, & Munte, 2006). These differences suggest that the neural mechanisms that modulate the effect of emotional information on sensory processing may depend on the precise temporal relationships between the stimuli, the number of conditions used, and also on the type of emotional stimuli presented.

3.4.3. A lack of emotion-specific effects on the N170 component

In contrast to the results of the P1, which demonstrated a potentially enhancing effect of congruent relative to incongruent happy voice-face presentations on P1 mean amplitudes, no N170 modulation was observed in this study during emotional voice-face processing. This highlights a functional difference between the P1 and N170 components. Whilst the P1 is believed to originate from the extrastriate visual areas (Clark et al., 1994; Di Russo et al., 2002), and is primarily involved in selective attention to visual stimuli (S. J. Luck et al., 1994; Mangun et al., 1993), the source of the N170 originates largely from the superior temporal sulcus (STS) region (R. J. Itier & M. J. Taylor, 2004b; Nguyen & Cunnington, 2014) and has been primarily associated with the structural processing of faces (e.g., Eimer & Holmes, 2002; Eimer & Holmes, 2007). The lack of N170 modulation according to voice-face congruency is consistent with a study by (Liu et al., 2012), who observed no N170 modulation when participants were shown simultaneous presentations happy, angry, or neutral voice-face pairs. From the results of Liu et al. (2012) and the present study, it could be argued that the N170 solely indexed the structural encoding of facial features during the present task, and was therefore not sensitive to the prior presentation of emotionally-charged auditory information.

Notably, this study also did not observe any effect of emotional expression on the amplitude of the N170. This is consistent with other studies which have also found no effect of emotional facial expressions on N170 modulation (Eimer, Holmes, & McGlone, 2003; Pourtois, Dan, Grandjean, Sander, & Vuilleumier, 2005; Schacht & Sommer, 2009; Wronka & Walentowska, 2011), and supports the view that the N170 is

primarily involved in the encoding of face-specific structural features (Bentin & Deouell, 2000; Eimer, 2000; R. J. Itier & M. J. Taylor, 2004a). Although some studies have reported N170 modulation by emotional facial expression (Liu et al., 2013; Song et al., 2017), it has been suggested that the presence or lack of emotion-specific effects on the N170 may be dependent on the choice of EEG reference electrode (Rellecke, Sommer, & Schacht, 2012), rather than the emotion of the face per se. However, it must be noted that the emotional faces viewed in this study were always preceded by an emotional vocalisation, whereas the studies discussed above presented emotional faces in isolation. Therefore, it is possible that the conditions in this study generated ceiling levels of N170 activation, which would also explain the lack of facial emotion effects in this study. As a face-only condition was not included, the results of this study cannot fully address the question of whether the N170 face effect is emotion-specific.

3.4.4. Behavioural task performance

When examining participants' reaction times when categorising the emotional facial expressions in the current study, no effects of facial emotion or congruency were observed. It should be considered why the enhancing effect of positive congruent voice-face information at the electrophysiological level (observed at P1) was not mirrored in task performance. The lack of significant reaction time effects in this study is inconsistent with previous work showing faster reaction times and more accurate categorisation of emotionally congruent, relative to incongruent, voice-face pairs (Collignon et al., 2008; de Gelder & Vroomen, 2000; Massaro & Egan, 1996). For example, de Gelder and Vroomen (2000) presented participants with spoken utterances (happy or sad) and presented a congruent or incongruent facial expression at the onset of the final word in each utterance. Their behavioural results suggested that participants were faster to categorise congruent compared to incongruent voice-face pairs. There are a number of potential reasons for this discrepancy between the present study and previous work. Firstly, a crossover effect was observed in the reaction data, which showed faster reaction times for congruent happy voice-face pairs relative to incongruent happy voice-face pairs, but the opposite effect for congruent relative to incongruent sad voice-face pairs. This suggests that whilst there is no overall effect of congruency and no overall effect of facial emotion, reaction times were faster in response to both happy and sad faces which were preceded by laughing vocalisations – an effect which is not tested with the pairwise comparisons in the current statistical set-

up. Secondly, it is possible that the lack of behavioural effects resulted from participants not being sufficiently challenged by the task paradigm. More specifically, as a result of the simplicity of the task, participant responses could have reached ceiling performance levels when making decisions between two alternatives (happy face or sad face), indexed by very high performance rates (approximately 97% accuracy). Finally, participants were presented with 3000 ms vocal clips, with the face presented for the final 200 ms of the vocalisation. This was done to prevent auditory responses from contaminating the subsequent early visual responses to the faces. However, past research suggests the neural responses from two sensory stimuli are more likely to converge and be enhanced when the stimuli occur at approximately the same time (Holmes & Spence, 2005; King & Palmer, 1985; Stein & Wallace, 1996). Therefore, in this study, it is possible that any complementary/redundant information from the vocalisations were too temporally distant to influence participant's judgments of the facial expressions during the behavioural task.

However, when examining the relationship between P1 and N170 mean amplitudes and reaction times during the categorisation task, faster reaction times were associated with greater N170 mean amplitudes for each of the conditions. This demonstrates a functional dissociation between the P1 and N170 components, with previous work also reporting a positive association between reaction times and N170 responses to face stimuli (Carlson & Reinke, 2010; Lerner et al., 2013). Specifically, it could be argued that an enhanced N170 reflects greater attentional processing of the emotional faces, which subsequently influenced participants' behavioural performance. So, it could be argued that when participants paid greater attention to the facial stimuli, they exhibited greater N170 amplitudes, and made faster responses when categorising the emotion of the face that was presented. In contrast, the P1 is thought to be more reflective of initial attention-orienting to the low-level features of a visual stimulus (Rossion & Caharel, 2011), which may not be sensitive to reaction times in a task that requires participants to differentiate between emotional expressions. However, there are some inconsistencies across previous studies regarding the relationship between the N170 and reaction time, with one study reporting that greater N170 amplitudes were associated with slower reaction times (Calvo & Beltrán, 2013), and one study reporting no relationship at all when examining fearful face processing abilities in children (3-8-year-olds, Vlamings, Jonkman, & Kemner, 2010), so this effect would need to be explored in future work.

3.4.5. Study limitations and future directions

Notably, some limitations should be considered when evaluating the results of the current study. Firstly, whilst this study examined the effects of emotional vocalisations on subsequent processing of emotional faces, comparisons were not made with neutral, non-emotional conditions. A neutral condition would have enabled a more precise examination of the differing effects of positive and negative vocalisations on face processing. However, past research has questioned whether neutral faces really do express "neutrality" of emotion, or whether they would also be perceived as expressing a form of negative affect (Donegan et al., 2003; Thomas et al., 2001). Secondly, whilst the results of this study show increased visual ERP responses following the presentation of positive emotional vocal information, no comparisons were made with vocalisation- or face-only presentations. This means the degree of potentiation of visual facial processing that may have resulted from the prior presentation of emotional auditory information could not be fully quantified. For example, whilst enhanced P1 amplitudes were found in response to sad incongruent voice-face pairs relative to happy incongruent voice-face pairs, it is not possible to fully disentangle these effects. These choices were based on a need to reduce the number of trials presented during the experiment and the duration of the EEG recording. Thirdly, the comparison of happy and sad faces introduces some methodological issues, because these expressions differ in both valence and also in the arousal dimension. For example, whilst a happy face has a positive valence and is typically high in arousal, a sad face has a negative valence and is typically low in arousal. Although this issue would be solved by using happy and fearful faces, which differ in valence but are both highly arousing, an earlier pilot study suggested that participants struggled to identify the fearful face stimuli, even when happy faces were presented with 70% of their original emotional intensity. Therefore, the use of fearful faces instead of sad faces is likely to have introduced additional difficulties in the current study. However, future work could extend these results, by examining the effects of emotional vocalisations on early visual and behavioural responses to emotional and non-emotional visual stimuli, as well as examining differences between multisensory versus unisensory presentations.

3.4.6. Suitability of an emotion integration EEG paradigm to study emotion processing from adolescence to adulthood

Following an assessment of the findings from the current study, it was decided that this paradigm would not be optimal for use with a younger adolescent sample. Firstly, the observed modulation of the P1 ERP component by emotion from voice-face pairs in the current study was small, and no modulation of the N170 was observed. In addition, the expected modulation of emotion in the RT data during the emotion categorisation task was not observed. Because of these small/null effects in a late adolescent sample, it would be difficult to conduct a thorough analysis of potential developmental differences across multiple age groups, which suggests the current paradigm may not be suitable for studying emotion processing in the transition from adolescence to adulthood.

In addition, although some have argued that developmental changes in face processing abilities provides a useful model to study adolescent development (Scherf et al., 2012), the results from this chapter have highlighted a number of possible issues with this approach. Firstly, it is difficult to isolate the emotion-specific modulations of visual ERP responses to face stimuli from the impact of the inherent social properties of this type of stimuli. Similar issues have been highlighted by fMRI work, when attempting to dissociate the neural substrates of facial emotion identification from the neural substrates of social inference and emotional self-regulation (Burnett, Sebastian, & Kadosh, 2012) . Such issues most likely stem from significant overlap between cortical and subcortical regions that are involved in both face and social information processing (Blakemore, 2008; Nelson et al., 2005). Furthermore, adolescents undergo intense changes in the processing of socially-relevant stimuli (Nelson et al., 2005), with increased gonadal hormones during puberty likely to influence how adolescents process emotional face stimuli (Scherf et al., 2012). Together, this work suggests that any developmental difference observed in emotional face processing during adolescence could result from changes to a number of different neural networks. Based on this work, and the experimental work presented in so far in this doctoral thesis (Chapter 2; the present Chapter) I would argue that the use of emotional faces may introduce additional confounds which could negatively impact the study of emotion processing in adolescents compared to adults.

3.5. Conclusions

In conclusion, the present results suggest that positively valenced, rather than negative, vocal information can lead to an enhancement in the early visual processing of congruent facial expressions, as indexed by the visual P1 component in a sample of late adolescents. In line with other research, these findings suggest an enhancing effect of emotional congruency on the early sensory processing of voice-face pairs (De Gelder et al., 2002; Magnée et al., 2008; Pourtois et al., 2000; Pourtois et al., 2002), with the effects observed in the current study specific to positively valenced voice-face information. Additionally, the current study, together with others in the field (De Gelder et al., 2002; Gerdes et al., 2013; Liu et al., 2012; Müller et al., 2012; Pourtois et al., 2000), suggests that the modulation of visual and auditory sensory percepts depends on the type of audio and visual stimuli being presented, and whether these two sensory modalities are presented simultaneously, or whether one is presented before the other.

Chapter 4. Fear conditioning and extinction during adolescence: A systematic review

Abstract

Pavlovian fear conditioning is key to understanding of anxiety disorders, and it has been suggested that the increased risk for the development of anxiety disorders during adolescence could result from atypical acquisition and extinction of fear conditioned cues. Examining the mechanisms that underlie associative fear learning is important for understanding the processes which affect the development of typical threat responses as well as pathological fear and anxiety during adolescence. Hence, a systematic review was conducted, to synthesise and evaluate the strength of the existing literature on adolescent fear conditioning and extinction, and how these processes may differ from adulthood. Additionally, this review aimed to examine the methodological approaches of this research area, and propose a set of recommendations to guide the design and implementation of a fear conditioning and extinction task for use with adolescents and adults in Chapter 5. This review revealed a paucity of empirical data on the developmental trajectory of fear conditioning and extinction during adolescence. Despite this, when comparisons were made between adolescents and adults, both groups demonstrated equivalent levels of fear acquisition as indexed by their implicit autonomic conditioned fear responses. However, adolescents exhibited evidence of poorer CS+/CS- discrimination during acquisition when explicit self-report responses were examined, which was consistent with developmental differences observed in the activation of key cortical and subcortical brain regions. Furthermore, there is evidence for an impairment in extinction learning in adolescents relative to adults, which is highly dependent on the type of extinction task (e.g., delayed versus immediate) and the outcome measures employed (e.g., implicit versus explicit). However, there are some issues surrounding the interpretation of these extinction results, and notably, many of the studies reviewed do not compare their adolescent data with age-appropriate adult comparison groups, which precludes a more fine-grained analysis of the available adolescent data. Overall though, the work conducted so far suggests that implicit and explicit constructs of fear learning may mature at different rates, which provides insight into the development of fear conditioning processes in the transition from adolescence to adulthood.

4.1. Introduction

4.1.1. Background

So far, this doctoral work has assessed the suitability of two emotion processing paradigms for use with both adolescent and adult populations (Chapter 2; Chapter 3). These paradigms were deemed unsuitable for use with younger adolescent populations, for reasons relating to task difficulty, small condition effects, and issues regarding developmental differences in how adolescents and adults process social information from facial expressions. In response to these issues, a shift was made to make use of a Pavlovian fear conditioning model, to assess how threat processing develops in the transition from adolescence to adulthood. This shift was made because Pavlovian conditioning presents a highly-controllable and well-established model of emotion processing and learning (for reviews, see J. E. LeDoux, 2014; Maren, 2001; Shechner, Hong, Britton, Pine, & Fox, 2014; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). Before doing so, a systematic review of the fear conditioning and extinction literature will be conducted as it pertains to adolescents, to guide the design of an appropriate fear conditioning task for use with adolescents and adults (Chapter 5).

4.1.1.1. Pavlovian fear conditioning and anxiety

As outlined in Chapter 1 (Section 1.3) adolescence is an extended transitional phase of development, which is associated with significant changes in behaviour, cognition, and emotion (Abe & Suzuki, 1986; S. Burnett et al., 2010; Spear, 2000a), as well as physical and hormonal changes, and the continuation of brain maturation processes (Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2002). These changes make up some of the defining features of a heightened phase of storm and stress (Hall, 1904), where storm refers to a decreased level of self-control associated with higher levels of risk taking (S. Burnett et al., 2010; L. Steinberg, 2008), and stress refers to an increased level of emotionality (Casey, Jones, et al., 2010). Although storm and stress is not an inevitable part of every teenagers experience (Hollenstein & Lougheed, 2013), this increase in emotionality has been useful in understanding why adolescence is a high-risk period for the development of anxiety disorders, which often persist into adulthood (Beesdo et al., 2009; Costello, Egger, & Angold, 2005; Kessler et al., 2007; Kessler et al., 2005; Kessler et al., 2009; Kim-Cohen et al., 2003; McGorry et al., 2011; Pine et al., 1998).

Pavlovian fear conditioning is key to current theoretical explanations of anxiety disorders (Davis, 1992; Delgado, Olsson, & Phelps, 2006; Shin & Liberzon, 2010), and it has been suggested that the increased risk for the development of anxiety disorders during adolescence could result from atypical acquisition and/or extinction of fear conditioned cues (Den et al., 2015; Johnson & Casey, 2015; Lau et al., 2011; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012; Shechner et al., 2014). Revealing the developmental differences in associative threat learning is therefore important, to understand the processes which impact the development of typical fear responses, and how pathological fear and anxiety can emerge during adolescence. Therefore, the aim of this systematic review is to synthesise and evaluate the strength of the existing literature on fear conditioning and extinction processes during adolescence, and how these processes develop in the transition to adulthood. Given the relationship between fear conditioning and anxiety mechanisms, an additional aim was to examine studies which investigated fear conditioning and extinction in anxious adolescents.

Pavlovian fear conditioning has been implemented in numerous different paradigms with various different outcome measures. In Pavlovian, or classical fear conditioning (Pavlov, 1927), a previously neutral sensory stimulus (the conditioned stimulus, CS), acquires the ability to elicit conditioned fear responses (CR) after being paired with an aversive unconditioned stimulus (US), such as a loud tone or an aversive footstock. CRs can consist of various species-specific defensive responses (e.g., freezing, J. E. LeDoux, 2000). To date, a number of paradigms have been used to establish conditioned fear responses and they differ with respect to the number of CS presented: single cue (when one CS is presented), versus differential conditioning (when two or more CSs are presented). In addition, these paradigms often vary with respect to the temporal relationship between the CS and US. For example, in delay conditioning the US is presented at the end of the CS, whereas in trace conditioning a gap occurs between the end of the CS and the start of the US. Extinction of conditioned fear responses is more uniform, such that all paradigms examining extinction assess responses to fear conditioned cues in the absence of the aversive US.

Given the wide variety of fear conditioning protocols that have been employed in the literature, one of the aims of this review was to examine whether methodological differences between fear conditioning paradigms affect the strength of the fear conditioning and extinction observed (e.g., Cook, Hodes, & Lang, 1986; Glenn, Lieberman, & Hajcak, 2012; Grillon, Baas, Lissek, Smith, & Milstein, 2004; Lonsdorf

et al., 2017; Mineka & Öhman, 2002; Treviño, 2016), and also whether different outcome measures (e.g., explicit versus implicit measures) used to assess conditioning alter the interpretation of results (i.e., whether adults and adolescents differ in explicit but not implicit measures of conditioning, or vice versa). Together, these findings should enable a clearer understanding of fear conditioning and extinction processes during adolescence, before beginning my own study on this question (Chapter 5).

Notably, although studies focusing on fear learning during adolescence have been summarised in previous reviews (Baker, Den, Graham, & Richardson, 2014; Shechner et al., 2014), these reviews were not systematic, so they may have missed important studies in the area. In addition, these reviews had varied definitions regarding when adolescence begins and ends. For example, one review describes evidence as it relates to adolescents aged 12-17 years (Baker et al., 2014), and another as it relates to youths aged 2-19 years (Shechner et al., 2014). However, adolescence begins around age 10 (World Health Organisation, 2003), and continues into the third decade of life (Dahl, 2004; Mills et al., 2014; L. Steinberg, 2008). It has been suggested that the adolescence should be defined as a period from 10-24 years, as this better encompasses adolescent growth, relative to previous definitions (e.g., 10-19 years; Sawyer, Azzopardi, Wickremarathne, & Patton, 2018). Therefore, this systematic review will examine all fear conditioning and extinction studies that have been carried out on individuals aged 10-24 years. The results of this review will be used to inform current neurobiological models of adolescence, which are currently limited in their explanations of how adolescents respond to aversive or threatening stimuli, having largely focused on how adolescents respond to rewarding or appetitive stimuli (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008), as discussed previously (Chapter 1 section 0).

4.2. Methods

4.2.1. Literature search

The literature search was conducted on the 7th January 2019. Included in this review were peer-reviewed studies that were available in English, specified the use of a fear conditioning and/or extinction task, and made comparisons between an adolescent

(aged 10-24 years) and an adult sample. Studies which assessed clinical or sub-clinical anxious adolescent populations were also retained, because an understanding of how anxiety interacts with fear conditioning/extinction could explain adolescents' heightened risk for developing anxiety. Supplementary Table 4.1 shows the search terms used in three electronic databases: Web of Science core collection, PubMed, and PsychINFO, and all articles were screened regardless of the year of publication. The search strategy followed a four-step procedure (Figure *4-1*). Following the initial search (n = 14,456) and duplicate removal (n = 9028), the resulting articles were screened and reference lists of these studies were screened, to ensure a comprehensive search of the literature. The majority of references excluded at this stage had titles which were irrelevant, referred to threat response tasks that did not involve fear conditioning or extinction, or were conducted with non-human animals (n = 5696). The remaining abstracts (n = 3332) were then checked and those that stated the use of a fear conditioning or extinction task were retained. These abstracts were then assessed for suitability based on the exclusion criteria set out in the PRISMA flow diagram (Figure *4-1*). Following removal of the abstracts that met the exclusion criteria, the remaining empirical studies were read in full, and the studies that met the scope of this review were re-read (n = 8) before being deemed suitable for final inclusion.

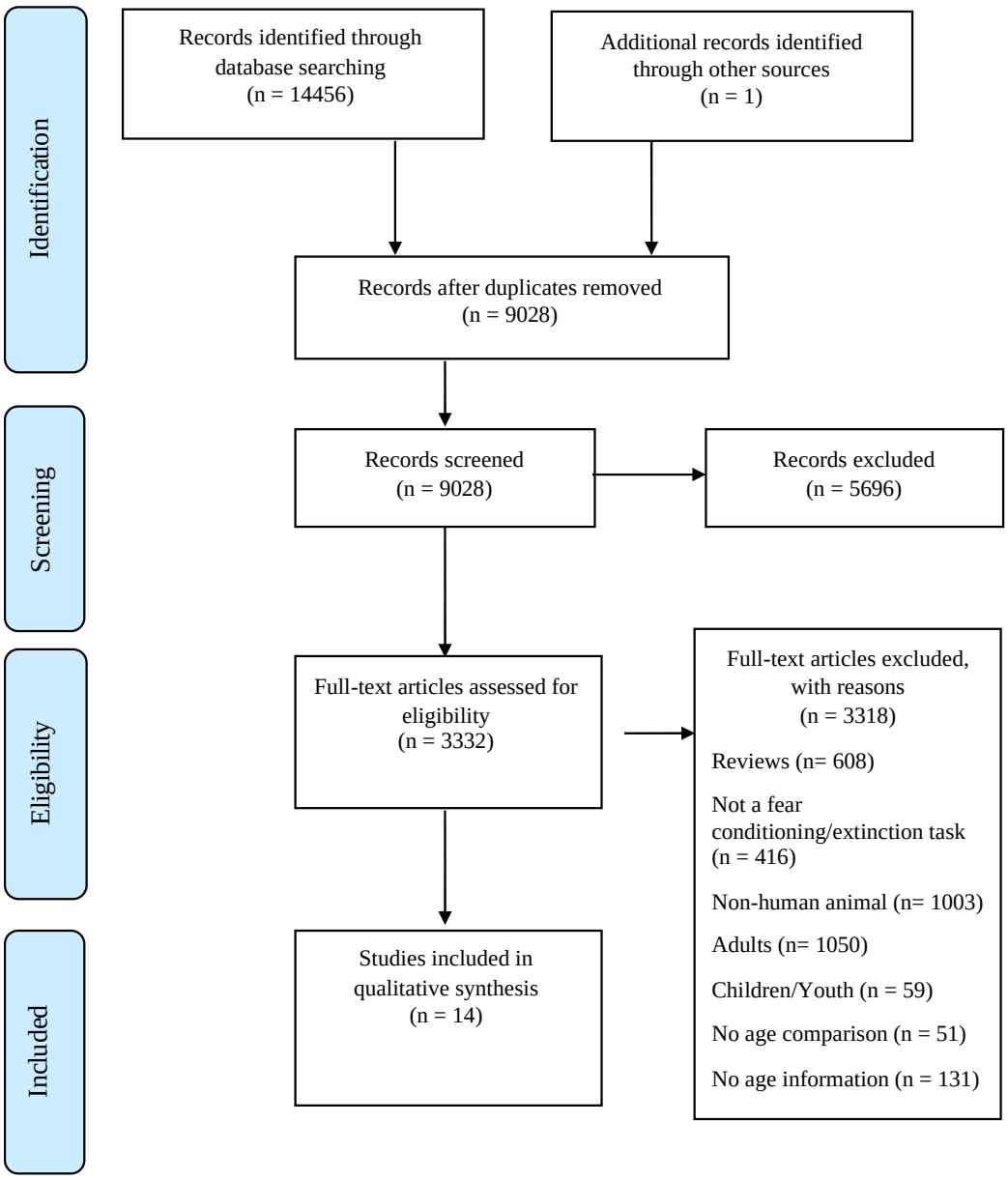

Figure 4-1: PRISMA Flow Diagram showing the systematic search process.

4.3. Results

4.3.1. Age groups

Just eight empirical studies included in this review (see Supplementary Table 4.2 for full details of these studies) included an adult comparison group within their design (Den et al., 2015; Ganella et al., 2017; Ganella et al., 2018; Johnson & Casey, 2015; Lau et al., 2011; Morrow, Boring, Keough, & Haesly, 1969; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012; Waters et al., 2017). Notably, however, three of these studies used adult groups that were also comprised of late adolescents

(Johnson & Casey, 2015; Lau et al., 2011; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), and so were not necessarily using an age-appropriate adult comparison group. Two of these studies (Ganella et al., 2017; Ganella et al., 2018) are based on the same data from 17 adolescents (14-16 years) and 14 adults (25-35 years). Two studies included a child comparison group, although like the adult comparison groups, one of the child comparison groups used an age range which included adolescents (5-11 years in Pattwell et al., 2012).

4.3.2. Experimental designs

As presented in Supplementary Table 4.2, most of the studies included in this review (n = 11) used a differential fear conditioning paradigm to examine fear conditioning in adolescents and three studies utilised a combined cue and context conditioning paradigm. During differential fear conditioning one stimulus (CS+) is paired with the presentation of an aversive US, whilst a second stimulus is unpaired (CS-). Conditioned responses (CRs) are quantified by examining differences between the CS+ and CS-. In combined cue and context conditioning studies, researchers examine conditioned fear responses to the conditioned stimulus (e.g., a fearful face) as well as responses to the context in which the CS cue was presented (e.g., an image of a room). In addition to different paradigms used in developmental studies examining fear conditioning and extinction, there is also great heterogeneity with respect to the type of stimuli that have been used as the CS and US (Supplementary Table 4.2). For example, the CSs presented were either neutral stimuli such as geometrical shapes (n = 3), lights (n = 1), coloured letters (n = 1), spoken sentences (n = 1), or more complex social stimuli such as facial expressions (n = 8). The unconditioned stimuli used were either physical stimuli such as electric shocks (n = 2) or muscle contractions (n = 1), or were visual stimuli such as faces paired with screams (n = 7), or pictures from the international affective picture system (IAPS; Lang, 2005) (n = 1), or auditory stimuli, such as high-pitched tones or white noise bursts (n = 3). Additionally, the studies reviewed utilised a variety of measures to assess fear conditioning and extinction, with the majority employing a multi-modal approach. Out of the 12 studies included, 10 of these assessed Pavlovian fear conditioning using behavioural measures (outcome expectancy or evaluative CS ratings), 10 studies used psychophysiological measures (SCR, EMG, eye tracking), and four studies used neuroimaging techniques (fMRI).

4.3.3. Fear conditioning and extinction during adolescence

4.3.3.1. Fear conditioning

A review of the literature revealed developmental differences between adolescent and adult fear acquisition, which depend on the fear outcome measures used. Of the seven studies which compared adolescent and adult differential responses to the CS+ and CS-, five studies utilised implicit autonomic measures and reported no age differences in fear acquisition. This was indexed by greater SCRs to the CS+ compared to the CS- in both adolescents and adults, with no apparent differences in the strength of acquisition (Ganella et al., 2018; Johnson & Casey, 2015; Experiment 1, Lau et al., 2011; Morrow et al., 1969; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). Similar results were reported in a study examining differential fear conditioning in youths (8-17 years) and adults (M age = 29.2-29.7 years, age range not reported), which demonstrated equivalent differential acquisition of SCRs to the CS+ compared to the CS- in both age groups (Shechner et al., 2015). However, when using explicit measures of conditioning, age differences emerge, with two studies suggesting that adolescents exhibited poorer discrimination between the CS+ and CS- (Den et al., 2015; Experiment 2, Lau et al., 2011). Both of these studies utilised the "screaming lady" paradigm, in which participants were presented with two female faces with neutral expressions. One face was used as the CS+ and was paired with a fearful expression and an aversive scream, whilst the other face was used as the CS- and was unpaired. Lau et al. (2011) examined trial-by-trial self-reported fear ratings of CS+ and CS- cues, using a nervousness rating on a scale of 0 to 100, and demonstrated poorer discrimination between CS+/CS- cues in adolescents when compared with adults. The authors suggested that adolescents may be overgeneralising fear from the CS+ (where the visual cue is reinforced by the aversive US) to the CS- (where the visual cue is not reinforced). Similarly, in Den et al. (2015) adolescents demonstrated poorer discrimination in their US expectancy scores at the end of conditioning, as they rated CS- as being significantly more likely to be followed by the US, relative to adults. Based on these results, similarly to Lau et al. (2011), the authors suggested that adolescents' propensity to overgeneralise their fear of the CS+ to the CS-, when discriminating between cues signalling safety and threat, could play a crucial role in the development of adolescent anxiety disorders (Den et al., 2015). Together, this pattern of results across studies does

suggest that differences in fear acquisition between adolescents and adults are dependent on the outcome measures used to assess conditioning, with both age groups exhibiting similar implicit autonomic responses, but with differences emerging in their explicit awareness of the CS-US contingencies.

Moreover, there is also evidence of developmental differences in fear acquisition measured at the neural level, as adolescents' poorer explicit discrimination between CS+ and CS- cues (Den et al., 2015; Lau et al., 2011) was consistent with age differences that emerged from the fMRI data in Lau et al. (2011). In that study, adolescents (10-17 years) and adults (18-50 years) underwent MRI during a differential conditioning protocol (80% reinforcement). Behaviourally, as discussed, they found poorer explicit discrimination between the CS+ and CS- cues in adolescents compared to adults, as measured by their self-reported fear. In addition, these results were associated with greater amygdala activation to CS+ versus CS- face cues during acquisition in adolescents, relative to adults. This result is supported by other work showing greater amygdala activation in adolescents in response to fearful faces, compared with children and adults (Hare et al., 2008), which suggests adolescents may recruit subcortical limbic regions to a greater degree in emotional situations (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010). In addition, Lau et al. (2011) found that in adults, but not adolescents, greater dorsolateral prefrontal cortex (dlPFC) activity was associated with more precise CS+/CS- discrimination. This is supported by similar work showing that the maturation of threat-related emotional processes results from increasing functional activity within PFC regions (Yurgelun-Todd & Killgore, 2006). Overall, the studies reviewed so far are supported by nonhuman animal models which suggest that subcortical fear neurocircuitry, in particular the amygdala, is important for the acquisition of fear conditioned responses (Davis, 1992; Ledoux, 1990; Maren & Fanselow, 1996), whilst later maturation of the PFC regions, such as the dlPFC, may be important for stronger explicit discrimination between the CS+ and CS- (Lau et al., 2011).

However, although Lau et al. (2011) measured autonomic SCRs in their first experiment, their second experiment only examined self-reported fear ratings in combination with fMRI. As a result, it is not possible to know whether differences in amygdala activity would have been associated with differences in the strength of differential SCRs to CS+/CS- cues. In adult studies, fear conditioned SCRs have been associated amygdala activation (Carter, O'doherty, Seymour, Koch, & Dolan, 2006;

Cheng, Knight, Smith, & Helmstetter, 2006; Wood, Ver Hoef, & Knight, 2012, 2014), with a greater magnitude of amygdala activation associated with greater differential SCRs to CS+ versus CS- cues during the early stages of fear acquisition (Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011). Therefore, it is possible that differences in amygdala activation may also be associated with differences in the strength of autonomic fear acquisition in adolescents compared with adults, an idea that needs to be examined in future work.

However, it is important to note that not all studies which use explicit measures of fear conditioning report age differences in fear acquisition between adolescents and adults. In contrast with Den et al. (2015) and Lau et al. (2011), there is a study which did not find poorer CS+/CS- discrimination in US expectancy scores or evaluative CS ratings in adolescents (Waters et al., 2017). In that study, participants were presented with geometrical shapes as CS cues and an aversive sound as the US, which differs from the social salience of the CS-US cues used in the screaming lady paradigm (e.g., facial expressions; Den et al., 2015; Lau et al., 2011). In addition, the two studies which did show evidence of poorer discrimination in their explicit measures of conditioning (Den et al., 2015; Lau et al., 2011) utilised adolescent groups which included early to late adolescents (12-17 and 10-17 years, respectively). In contrast, Waters et al. (2017) used an older and more discrete adolescent age group (15-18 years). It is therefore possible that poorer CS+/CS- discrimination during fear acquisition is more reflective of childhood/early adolescent fear conditioning processes. This idea is supported by additional findings from Waters et al., (2017) who also examined fear conditioning in children (7-10 years) in their study, and found that children were also more uncertain about the absence of the US on CS- trials, and gave less distinct evaluative ratings of the CS+ and CS- throughout the acquisition phase, rating the CS+ less negatively and the CS- less positively than both adolescents and adults. Together this suggests that, during fear acquisition, poorer CS discrimination observed in adolescents' explicit self-report measures may reflect younger child/early adolescent responses. If so, poorer discrimination would be expected to improve in line with the maturation of PFC brain regions that occurs throughout adolescence (Casey et al., 2000; Giedd et al., 1999; Huttenlocher, 1979; Pfefferbaum et al., 1994; Sowell, Thompson, Holmes, Jernigan, et al., 1999; Sowell et al., 2001).

Table 4.1 – *The seven studies that compared adolescent and adult fear acquisition.*

Fear acquisition?	How was fear acquisition quantified?	**Implicit**			**Explicit Self-Report**						**Neuroimaging**	
		Autonomic SCRs			Fear		US expectancy		CS Pleasantness ratings		FMRI	
		Adolescent		*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*
Johnson and Casey (2015)	CS+ > CS-	Yes 12-17 years	✓	Yes 18-32 years								
Pattwell et al. (2012)*	CS+ > CS-	Yes 12-17 years	✓	Yes 18-28 years								
Den et al. (2015)	CS+ > CS-				Yes 12-17 years	✓ Yes 38-57 years	Yes	✕ Yes Poorer discrimination in adolescents at end of conditioning				
Waters et al. (2017)	CS+ > CS-						Yes 15-18 years	✓ Yes 25+ years	Yes	✓ Yes		
Lau et al. (2011) Experiment 1	CS+ > CS-	Yes 10-17 years	✓	Yes 18-50 years	Yes	✓ Yes						
Experiment 2 (trial-by-trial ratings)					Yes	✕ Yes Poorer discrimination in adolescents					Greater right amygdala, and left and right hippocampal activation to CS+ vs CS-	✕ Greater right hippocampal activation to CS+ vs CS-
Morrow et al. (1969)	CS+ > CS-	Yes 19-21 years	✓	Yes 62-75 years								
Ganella et al. (2018)	CS+ > CS-	Yes 14-16 years	✓	Yes 25-35 years								

* Effects reported for the last of three acquisition runs, so it is not known whether there were age differences during early conditioning

✓ Equivalent fear acquisition between adolescents and adults. ✕ Strength of acquisition is different between adolescents and adults.

4.3.3.2. Fear extinction

Regarding fear extinction, a process in which threat-predicting fear conditioned cues are presented to the participant without the aversive US, the studies reviewed here suggest there may also be developmental differences in extinction when comparing adolescents and adults. However, these differences also appear to depend on the outcome measures used, as well as the type of extinction task employed. Overall, six studies (summarised in Table 4.2) have compared extinction learning in adolescents relative to children and/or adults (Den et al., 2015; Ganella et al., 2017; Ganella et al., 2018; Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012; Waters et al., 2017).

Of the six studies which compared adolescent and adult fear extinction, two studies reported successful immediate extinction of explicit self-report ratings in both adolescents and adults when examining fear ("*How scary is this face?")* (Den et al., 2015) and US expectancy (Den et al., 2015; Waters et al., 2017), which initially suggests similar extinction learning in adolescents and adults. However, when examining trial-by-trial evaluative ratings of the CS cues ("*Rate the degree of pleasantness of the shape you just saw*"), on a scale of -5 (very unpleasant) to 0 (neutral) to +5 (very pleasant), Waters et al. (2017) reported that adolescents maintained less pleasant ratings of both the CS+ and CS- from conditioning to extinction, when compared to adults, which were maintained during a re-test (following a reinstatement phase in which 3 unexpected US were presented). This would suggest that adolescents struggle to re-evaluate their negative perceptions of the CS even when the US is no longer present. However, this interpretation can be challenged when examining their trial-by-trial data (Figure 2, Waters et al., 2017) as although their statistical analysis suggests adolescent evaluative ratings to both CSs were more negative than adults, the figure indicates they were just closer to 0 (indicating neutral CS evaluations). This suggests that adolescents did not maintain negative perceptions of CS cues, but instead were expressing neutral responses, which would also be indicative of good extinction learning.

A different picture emerges when examining implicit autonomic measures of extinction, with two studies reporting evidence of blunted fear extinction in adolescents, when extinction was assessed 24 hrs after conditioning (Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). In Pattwell et al. (2012), children (5-11 years), adolescents (12-17 years), and adults (18-28 years) were

presented with geometrical shapes as CS cues and an aversive sound as the US. The authors reported blunted extinction learning in adolescents relative to adults and children. However, the findings reported by Pattwell et al. (2012) should be interpreted with caution, as extinction was assessed by comparing the first two and the last two CS+ trials presented during extinction training, so the reduction in SCR during extinction in adults and children could have simply reflected habituation in response to CS+, which can and often occurs naturally over time. Moreover, when all extinction trials were included in the analysis, their report of blunted fear extinction in adolescents relative to adults revealed only a trend for significance (p = .078, page 1).
This issue was resolved in a later study by Johnson and Casey (2015) who assessed extinction by comparing differential SCRs to the CS+ versus the CS- during delayed extinction. Similarly to Pattwell et al. (2012), they also reported blunted extinction learning in adolescents (12-17 years) relative to adults (18-32 years).

However, this blunted effect of extinction learning in adolescents as measured by autonomic responses is not ubiquitous. Ganella and colleagues (2018) examined differential fear conditioning (CS+ 100% reinforcement) between two age-appropriate adolescent (14-16 years) and adult (25-35 years) groups, and immediate extinction was assessed by examining SCRs to the CS+ and CS- without the aversive US, whilst participants underwent fMRI. Ten minutes after extinction, the authors examined extinction recall by presenting one CS+ (reinforced) and CS- trial each. Following this, participants were "re-conditioned" with another pair of CS+ (reinforced) and CS- trials, and underwent "re-extinction", which was the same as the original extinction phase. Consistent with other studies (Johnson & Casey, 2015; Experiment 1, Lau et al., 2011; Morrow et al., 1969; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012) they found no age differences in the degree of fear acquisition, but inconsistent with the findings of Johnson & Casey (2015) and Pattwell et al. (2012) they found no age differences in extinction using SCR when it was measured on the same day as acquisition. Similar findings were reported by Shechner et al. (2015), who reported successful immediate extinction of SCRs and fear-potentiated startle (FPS) in youth and adult participants in their differential fear conditioning task. Instead however, Ganella et al. (2018) reported an adolescent-specific failure to retain their extinction learning when examining extinction recall, which was characterised by greater SCR difference scores (CS+ minus CS-) in adolescents compared to adults. These results are similar to those reported in nonhuman animal work, as adolescent rodents have also shown an

impairment in extinction recall, relative to younger or older rodents (J. H. Kim, Li, & Richardson, 2011; McCallum, Kim, & Richardson, 2010; Zbukvic, Park, Ganella, Lawrence, & Kim, 2017). Together, these results suggest a differential pattern of adolescent-specific impairments in the extinction of autonomic SCRs, whereby immediate extinction is intact, but delayed (24 hr) extinction is attenuated when compared with adults, with recent work suggesting additional impairments in adolescents' extinction recall (Ganella et al., 2017; Ganella et al., 2018).

As in fear acquisition, developmental differences in extinction have also been observed at the neural level. Notably, despite observing equivalent immediate extinction of SCRs in adolescents and adults, Ganella et al. (2018) reported an association between the magnitude of the SCR difference scores and the degree of ventromedial prefrontal cortex (vmPFC) recruitment in adults. Specifically, during the immediate extinction phase, activation of the vmPFC in adults in response to the CS+ versus the CS- was negatively correlated with the magnitude of the differential SCRs observed during late extinction. This can be interpreted as a reduction in CS+/CS- discrimination towards the end of extinction in adults, with greater vmPFC activity associated with reduced SCRs to the CS+ versus the CS-. Importantly, adolescents did not show this relationship, which suggests that adolescence may be characterised by reduced recruitment of the vmPFC during immediate extinction. Moreover, a similar pattern between autonomic responses and brain activity emerged during extinction recall, whereby greater SCRs to the CS+ observed in adolescents during recall were associated with reduced vmPFC and dlPFC activity, relative to adults (Ganella et al., 2018). Overall, these results are consistent with work demonstrating that the vmPFC has an important role in extinction processes (Morgan, Romanski, & LeDoux, 1993; Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk, Russo, Barron, & Lebron, 2000) with activity in the vmPFC associated with the successful recall of their extinction learning in human adults (Milad et al., 2007; Phelps et al., 2004). Together, these studies provide evidence for a potential neural impairment in adolescents, which may impact the neural processes involved in immediate extinction and extinction recall (Ganella et al., 2017; Ganella et al., 2018) resulting in a failure to remember their extinction training.

Ganella et al. (2017) followed up their extinction recall findings with additional analyses, to examine functional connectivity between top-down prefrontal brain regions (vmPFC and dlPFC) and bottom-up subcortical brain regions (amygdala and hippocampus) during extinction recall in adolescents and adults. Their findings suggest

that adults exhibited significant negative vmPFC-amygdala connectivity during extinction recall, compared with adolescents, which suggests that adults may exhibit greater top-down cognitive control of amygdala reactivity during extinction recall. This could explain adults increased ability to recall that a fear-inducing CS+ had been extinguished. In contrast, extinction recall was not associated with vmPFC connectivity in adolescents, and instead they showed significant negative connectivity between the dlPFC and subcortical regions (i.e., the amygdala and hippocampus) during extinction recall, compared with adults. From this evidence, the authors speculate that this negative dlPFC connectivity could reflect an inefficient attempt at the top-down control necessary for extinction recall in adolescents. This is consistent with additional findings which demonstrated, unexpectedly, significant negative functional connectivity between adolescent dlPFC, and the posterior cingulate cortex, fusiform gyrus, thalamus, palladum, and the orbitofrontal cortex. Increased negative connectivity between these regions may reflect less refined dlPFC connectivity during adolescence, which leads to the engagement of more brain regions during extinction recall. Together their functional connectivity analysis supports neurobiological models of adolescence which propose an imbalance between early-maturing subcortical limbic regions and late-maturing prefrontal regions (Casey, Jones, et al., 2010; Somerville & Casey, 2010), which may affect the recruitment of brain regions that are key for extinction processes during adolescence.

So far, the extinction literature reviewed suggests that there may be differences in the activation of brain regions involved in immediate extinction and extinction recall (e.g., vmPFC activation and vmPFC-amygdala connectivity) between adolescents and adults. However, although (Ganella et al., 2018) reported a significant difference between adolescent and adult extinction recall, which they interpreted was due to adolescents exhibiting greater SCR difference scores (CS+ minus CS-) during recall trials, there are some issues with this interpretation. Specifically, when examining the mean SCR difference scores reported by the authors (Supplementary Table 1, Ganella et al., 2017), the adolescent SCR data shows a very small difference between the two cues ($M = 0.07$, $SD = 0.54$), whereas the adult data suggests a greater negative difference score ($M = -0.65$, $SD = 0.57$). From this data, it could be argued instead that the significant difference in the mean SCR difference scores between the two age groups may have resulted from adults' greater autonomic responses to the CS- during

extinction recall, rather than adolescents showing greater autonomic responses to the CS+ versus the CS-.

Overall, however, a consistent pattern is beginning to emerge in the extinction literature, which provides evidence for a potential impairment in adolescent extinction learning. Whether one observes this age difference is dependent on the outcome measures used, and whether delayed or immediate extinction is examined. This review of the literature provides evidence of successful immediate extinction learning in adolescents and adults when examining explicit outcome measures such as self-reported fear or outcome expectancy (Den et al., 2015; Waters et al., 2017), and when examining autonomic responses to CS+ versus CS- cues (Ganella et al., 2018). However, there is also evidence for an adolescent-specific impairment in the extinction of autonomic responses when extinction was examined after a 24 hr delay (Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Ruberry, et al., 2012), and when examining extinction recall (Ganella et al., 2018). Notably, fMRI data provides strong evidence for developmental differences in the brain regions that mediate both immediate extinction and extinction recall, with adults showing greater recruitment of the vmPFC during immediate extinction and greater connectivity between the vmPFC and the amygdala during extinction recall, relative to adolescents (Ganella et al., 2017; Ganella et al., 2018). However, these findings are not conclusive, as they are based on very few studies, and there are issues with some of the studies reviewed regarding the way in which extinction was quantified, and regarding the authors' interpretations of the data. Hence, it is possible that some of these analyses are biased in favour of observing an extinction impairment in adolescence. As a result, replication and extension of the studies reviewed here is required, using more stringent analyses, to fully delineate the developmental differences in fear extinction.

Table 4.2 – *The six studies that compared adolescent and adult fear extinction.*

Fear extinction?			**Implicit**		**Explicit Self-Report**						**Neuroimaging**	
		How was fear extinction quantified?	Autonomic SCRs		Fear		US expectancy		Evaluative CS ratings		FMRI	
Study	**Type of extinction**		*Adolescent*	*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*	*Adolescent*	*Adult*
Johnson and Casey (2015)	Delayed (24 hr)	CS+ = CS-	No ✗ 12-17 years	Yes 18-32 years								
Pattwell et al. (2012)	Delayed (24 hr)	CS+ = CS+	No ✗ 12-17 years	Yes 18-28 years								
Den et al. (2015)	Immediate	CS+ = CS-			Yes ✓ 12-17 years	Yes 38-57 years	Yes ✓	Yes				
Waters et al. (2017)	Immediate	CS+ = CS-					Yes ✓ 15-18 years	Yes 25+ years	No ✗ *Greater negative evaluations of both CS+ and CS- in adolescents relative to adults	No		
Ganella et al. (2017) & (2018)	Immediate	CS+ = CS-	Yes ✓ 14-16 years	Yes 25-35 years							No relationship between vmPFC and SCRs ✗	Greater vmPFC activity associated with reduced SCRs
	Recall		No ✗	Yes**							Greater SCRs to CS+ during recall associated with reduced vmPFC and dlPFC activity. ✗	Evidence of greater top-down cognitive control of amygdala reactivity during recall.

✓ Equivalent fear extinction between adolescents and adults. ✗ Strength of extinction is different between adolescents and adults.

* Based on authors' interpretation, however, their data appear to show adolescents' greater negative evaluations were instead more indicative of neutral evaluations.

** Based on authors' interpretation, however, their data suggest the difference between adolescent and adult recall resulted from adults exhibiting greater SCRs to the CS-.

4.3.4. Fear conditioning and anxiety during adolescence

As discussed, Pavlovian fear conditioning and extinction is a particularly useful model for understanding anxiety (Duits et al., 2015; Lissek et al., 2005) as anxiety disorders are driven by excessive or persistent fear (Shin & Liberzon, 2010). Notably, adolescents face an increased risk of developing an anxiety disorder (Beesdo et al., 2010; Kessler et al., 2007; Kessler et al., 2005; Lijster et al., 2017; McGorry et al., 2011; Pine et al., 1998), however, the mechanisms of adolescent anxiety its relationship to fear conditioning are not well understood. To date, six studies have examined the degree of fear conditioning in anxious adolescent populations (K. Cohen Kadosh et al., 2015; Dvorak-Bertsch, Curtin, Rubinstein, & Newman, 2007; Haddad, Bilderbeck, James, & Lau, 2015; Lau et al., 2008; Raes, De Raedt, Verschuere, & De Houwer, 2009; Waters et al., 2014), however, none of these studies included an adult comparison group.

Two studies compared clinically anxious and non-anxious adolescents using the "screaming lady" paradigm (Haddad et al., 2015; Lau et al., 2008). Following a fear acquisition phase, both studies demonstrated evidence of equivalent successful differential fear conditioning in both anxious and non-anxious adolescents, measured by greater self-reported fear or nervousness in response to the CS+ relative to CS- (Haddad et al., 2015; Lau et al., 2008). However, despite observing a similar degree of discrimination between the CS+ and the CS- in anxious and non-anxious adolescents, anxious adolescents demonstrated significantly greater levels of overall fear when fear/nervousness ratings were averaged across both CS+ and CS- cues. The authors suggested that anxious adolescents may be overgeneralising their fear from the threatening CS+ cue to a non-threatening CS- cue, resulting in greater fear when the CS+/CS- ratings were averaged together. These findings are similar to Shechner et al. (2015) who assessed conditioning and extinction in both anxious and non-anxious youth (8-17 years) and anxious and non-anxious adults (M age = 29.2-29.7 years, age range not reported). The results of that study revealed similar differential fear conditioning among all groups of participants, assessed by self-reported fear ratings, SCR, and fear-potentiated startle in response to the CS+ versus CS-. However, anxious youth and adults demonstrated greater overall self-reported fear throughout the task compared to non-anxious youth and adults, when fear ratings of the CS+ and CS- were averaged together. Overall, these results initially suggest that clinically anxious adolescents' exhibit similar differential fear conditioning compared to non-anxious adolescents, but

may also overgeneralise their fear from the CS+ to CS- when overall fear of both CSs is quantified.

The suggestion that adolescent anxiety may be characterised by an overgeneralisation of fear from the CS+ to the CS- during acquisition is consistent with past work conducted with adults. For example, in a meta-analysis of 44 studies that assessed fear conditioning in clinically anxious and non-anxious adults, Duits et al. (2015) observed greater fear responses to the CS- during acquisition in the anxious, relative to non-anxious, adults. The authors suggested that this pattern of results could reflect anxiety patients' tendency to generalise their learned fear responses from the CS+ to the CS-, also suggested by Haddad et al. (2015) and Lau et al. (2008), or it could reflect an impaired ability to inhibit their fear to a "safety" CS- cue, as it is being presented in a fearful context (Davis, Falls, & Gewirtz, 2000; Jovanovic, Kazama, Bachevalier, & Davis, 2012). This effect of greater fear could act to maintain adult anxiety disorders, such as panic disorder (Lissek et al., 2009), with the results of Haddad et al. (2015) and Lau et al. (2008) suggesting that this effect may already be present in adolescent anxiety.

However, it is possible that this effect of greater overall fear, observed in anxious adolescents and adults, could be driven by greater fear of the CS+, rather than an overgeneralisation of fear from the CS+ to the CS-, or failure to inhibit fear responses to the CS-. For example, in Lau et al. (2008), anxious adolescents reported greater fear of the CS+, which was positively associated with adolescents' anxiety symptoms (self- and parent-reported). Greater fear of the CS+ was previously reported in adults with anxiety disorders, relative to healthy controls, as part of an older meta-analysis (Lissek et al., 2005). However this effect was not replicated by their updated meta-analysis (Duits et al., 2015). The authors suggested that this lack of effect may be due to ceiling effects of the aversive US, which provoked similar conditioned fear responses in both anxious and non-anxious adults. However, it should also be noted that Lau et al. (2008) did not collect baseline measures of fear prior to the acquisition phase, so greater fear of the CS+ reported by anxious adolescents may have been present prior to the conditioning phase. This argument is strengthened by Haddad et al. (2015), who did assess baseline nervousness ratings, and found anxious adolescents gave greater nervousness ratings of the CS+ than the healthy adolescents even before conditioning. Overall, whilst it is possible that anxiety in adolescence is maintained through greater

fear responses to CS+ cues, relative to non-anxious adolescents, this effect needs to be further investigated and validated.

Haddad et al. (2015) also collected fMRI data whilst anxious and non-anxious adolescents took part in the screaming lady task, in which the CS+ (a neutral face) was paired with a US (a fearful face and a scream, 50% reinforcement schedule) and the CS- (a different neutral face) was unpaired. Their fMRI analysis made contrasts between the CS+ (unreinforced trials only) and a control cue (an oval) and between the CS- and a control cue. The results of that study showed non-anxious adolescents, in response to the aversive CS+, exhibited robust activation of the vmPFC, posterior cingulate cortex, bilateral amygdalae, and right hippocampus. Moreover, in response to the CS-, they demonstrated increasing activation of the dlPFC, bilateral insula, and left striatum with increasing age, relative to anxious adolescents. This was interpreted by the authors as reflecting typical developmental changes in non-anxious adolescents' processing of the safety CS- cue. Therefore, despite both anxious and non-anxious adolescents reporting similar differential fear ratings during acquisition, anxious adolescents may exhibit reduced differential activation of brain regions required for typical acquisition. This result contrasts with the results of a previous study that suggest elevated, as opposed to reduced, activity in similar regions (e.g., the vmPFC) in anxious relative to non-anxious youth/adolescents when responding to threat (Britton et al., 2013). However, importantly, the population studied by Britton et al (2013) consisted of much wider age ranges containing children and early adolescents (8-19 years), which differs from Haddad et al. who examined anxious and non-anxious 12-17-year-olds. Therefore, it is possible that the elevated activity in relevant brain regions during fear acquisition might be more typical of younger anxious paediatric populations.

Finally, in addition to anxious adolescents showing greater overall fear/nervousness ratings during the screaming lady paradigm, potentially resulting from overgeneralisation of fear from the CS+ to the CS- (Haddad et al., 2015; Lau et al., 2008), and reduced activity in relevant cortical/subcortical brain regions during acquisition (Haddad et al., 2015), one study suggests that fear responses to the CS+ could generalise to the wider context in which the US appears in highly anxious adolescents, relative to low anxious adolescents (K. Cohen Kadosh et al., 2015). This study utilised a cue and context version of the screaming lady paradigm, whereby CS+ and CS- face cues were presented in three different room contexts. The degree of CS-US contingency varied across each room: in the predictable room, the CS+ was

reinforced with the US on 100% of trials, in the unpredictable room the CS+ and the US were presented randomly and independently of each other, and in the neutral room, the CS+ was presented without the US. Their results mirrored Lau et al. (2008) and Haddad et al. (2015), in that high anxious adolescents demonstrated greater fear, as measured by increased fear-potentiated startle across all conditions, relative to low anxious adolescents. Furthermore, whilst low anxious adolescents came to understand the relationship between CS+ and US and more readily learned the association, high anxious adolescents generalised their fear from the CS+ cue (i.e., a face) to the wider context in which conditioning took place (i.e., an image of a room). Hence, whilst past research has found anxiety disorders to be characterised by overgeneralisation of conditioned fear responses from the CS+ to the CS- (Duits et al., 2015; Lissek & Grillon, 2015), Cohen Kadosh et al. (2015) found that fear responses towards the threatening CS+ could generalise to a much wider context in highly anxious adolescents.

4.4. Discussion

4.4.1. Summary of key findings

The overarching aim of this systematic review was to synthesise and evaluate the strength of evidence regarding developmental differences in fear conditioning and extinction in adolescents relative to adults. The studies reviewed suggest that, relative to adults, adolescents exhibit similar acquisition of conditioned fear responses when implicit autonomic responses to the CS are examined. However, adolescents show evidence of poorer discrimination between CS+ and CS- when examining explicit measures of conditioning, such as self-reported fear or US expectancy (Den et al., 2015; Lau et al., 2011), which may be driven by greater subcortical (e.g., amygdala) relative to cortical (e.g., PFC) activity (Lau et al., 2011), and which may be more prominent in child/early adolescent populations. Furthermore, studies assessing fear extinction provide tentative evidence of successful immediate extinction as measured by explicit self-report (Den et al., 2015; Waters et al., 2017) and of physiological responses to CS+ versus CS- cues (Ganella et al., 2018) in both adolescents and adults. However, developmental differences in immediate extinction do emerge when examining adolescent and adult brain activation patterns (Ganella et al., 2017; Ganella et al., 2018). In addition, recent work suggests that adolescents may show impairments in delayed (24 hr) extinction, with adolescents showing attenuated extinction of SCRs relative to adults

(Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), and also in extinction recall (Ganella et al., 2017; Ganella et al., 2018), which may result from the relative immaturity of adolescent PFC regions that are important for fear extinction. Notably, an examination of anxious and non-anxious adolescent responses to fear conditioned cues revealed that both groups exhibited similar differential fear acquisition in response to the CS+ versus the CS-, consistent with work conducted with anxious adults (Duits et al., 2015). However, clinically anxious adolescents demonstrated reduced differential activation of key brain regions during fear acquisition, and expressed greater overall fear when CS ratings were collapsed together, relative to non-anxious adolescents. This effect of greater fear is consistent with work conducted with anxious adults, and may result from anxious participants overgeneralising their fear from the CS+ to the CS- (Duits et al., 2015; Lissek et al., 2009) or from a failure to inhibit their fear responses to the CS- (Davis et al., 2000; Jovanovic et al., 2012).

Overall, these results support a number of neurobiological models of adolescence (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008). In particular, Casey's model purports an imbalance between the rapid development of subcortical limbic circuits and slower development of prefrontal circuits (Casey, Jones, et al., 2010; Somerville & Casey, 2010). Because activity in prefrontal regions has been shown to increase gradually during adolescent development (Rubia et al., 2000; Rubia et al., 2006; Tamm, Menon, & Reiss, 2002), it is possible that fear learning in adolescence is being driven by subcortical limbic activity, with gradual increases in the recruitment of top-down prefrontal cortical regions as adolescents' transition into adulthood. This provides some explanation for inconsistencies amongst studies when using different outcome measures (explicit versus implicit) to assess conditioning and extinction. For example, this review demonstrated equivalent fear acquisition in adolescents and adults when using implicit measures (i.e., autonomic SCR), which are strongly associated with subcortical amygdala activation (Carter et al., 2006; Cheng et al., 2006; Wood et al., 2012, 2014) whilst demonstrating developmental differences in other explicit self-report measures (i.e., US expectancy) which are associated with cortical activation of areas such as the PFC (Cardinal et al., 2002). However, as yet, a direct comparison between adolescents' brain activation patterns and their explicit and implicit fear responses is

currently missing, and is needed to better understand the relationship between brain development and threat processing in adolescence.

Importantly, the extinction results of this systematic review provide some support for the theory that adolescents may be hyper-responsive to aversive outcomes. Specifically, it could be argued that the attenuated extinction, or failure to "remember" extinction, indexed by autonomic responses in human studies (Ganella et al., 2017; Ganella et al., 2018; Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), could result from adolescents experiencing greater reactivity to the aversive US. This theory is based on previous research in which greater activation of the amygdala and ventral striatum was observed in youth/adolescents in response to aversive cues, compared to adults, in a threat processing task (Britton et al., 2013; Galvan & McGlennen, 2013). Specifically, Britton et al. (2013) showed significantly greater SCRs to an aversive scream in youths (8-19 years) compared to adults (21-48 years), and Galvan and McGlennen (2013) showed significantly greater unpleasantness ratings of an aversive liquid (i.e., sodium chloride) in adolescents (13-17 years) compared to adults (25-35 years). However, in this review just one study compared adolescent and adult physiological reactivity (SCR) to the aversive US, and reported no significant differences between their responses (Johnson & Casey, 2015). As such, this would need to be explored in future work. Overall though, impairments in adolescent fear extinction could provide a potential mechanism for the increased risk and maintenance of anxiety observed in adolescents (Beesdo et al., 2009; Costello et al., 2005; Kessler et al., 2007; Kessler et al., 2005; Kessler et al., 2009; Kim-Cohen et al., 2003; McGorry et al., 2011; Pine et al., 1998), as adolescents may struggle to completely extinguish conditioned fear responses, due to immature prefrontal neurocircuitry. As a result, these conditioned fear responses could return even in the absence of any threatening cue.

4.4.2. Limitations and Recommendations

4.4.2.1. Defining adolescence and adulthood

Having assessed the strength of current evidence regarding adolescent fear conditioning and extinction, it must also be acknowledged that the evidence to date consists of a very small number of developmental studies, the majority of which preclude the ability to examine developmental changes in adolescent fear conditioning

and extinction, due to a lack of age-appropriate control groups. For example, of the eight studies which do include an adult comparison group, three utilised adult age groups which also contained late adolescents (18-32 years in Johnson & Casey, 2015; 18-50 years in Lau et al., 2011; 18-28 years in Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), while the remaining five studies utilised appropriate adult groups (25+ years Den et al., 2015; Ganella et al., 2017; Ganella et al., 2018; Morrow et al., 1969; Waters et al., 2017). In addition to a lack of age-appropriate adult controls in the studies reviewed, researchers also examined fear conditioning and extinction processes using participants at different stages of adolescence. For example, all six fear extinction studies that include an adult comparison group utilise adolescents at early, mid, and late adolescence, often collapsing participants into one age category (e.g., 12-17 years in Den et al., 2015; Pattwell et al., 2012; Johnson & Casey, 2015; 15-18 years in Waters et al., 2017). This variation in the age of adolescents included in these studies highlights a pervasive problem in this field, as researchers have different definitions regarding when adolescence begins and ends. It is highly likely that these age differences are impacting the degree of acquisition and extinction, given the wealth of neurobiological evidence that suggests key brain regions involved in fear learning continue to develop throughout adolescence (e.g., Casey et al., 2000; Giedd et al., 1999; Huttenlocher, 1979; Pfefferbaum et al., 1994; Sowell, Thompson, Holmes, Jernigan, et al., 1999; Sowell et al., 2001).

Casey (2015) has highlighted the importance of assessing adolescent behaviour in the context of transitions into and out of adolescence. This is because increasing evidence supports the view that whilst some behaviours are non-specific to adolescence, emerging in childhood and steadily increasing/decreasing into adulthood (e.g., impulsivity; Harden & Tucker-Drob, 2011; L. Steinberg, 2010; Figure 17A), other behaviours are adolescent-emergent and so persist into adulthood, and may be characterised by rapid change or peak engagement during adolescence (e.g., PFC-mediated cognitive control; Dreyfuss et al., 2014; Somerville, 2013; Figure 17B). Similarly, other behaviours may be adolescent-specific, and so are maximally engaged in adolescents relative to children and adults (e.g., increased sensation-seeking behaviours; Dreyfuss et al., 2014; Harden & Tucker-Drob, 2011; Somerville et al., 2013; Figure 17C). This view proposes an increasingly complex picture of adolescent brain and behaviour, which creates difficulties when interpreting the results of

adolescent data without age-appropriate controls, as it cannot be assumed that each of the neural networks involved in fear learning mature in a linear fashion.

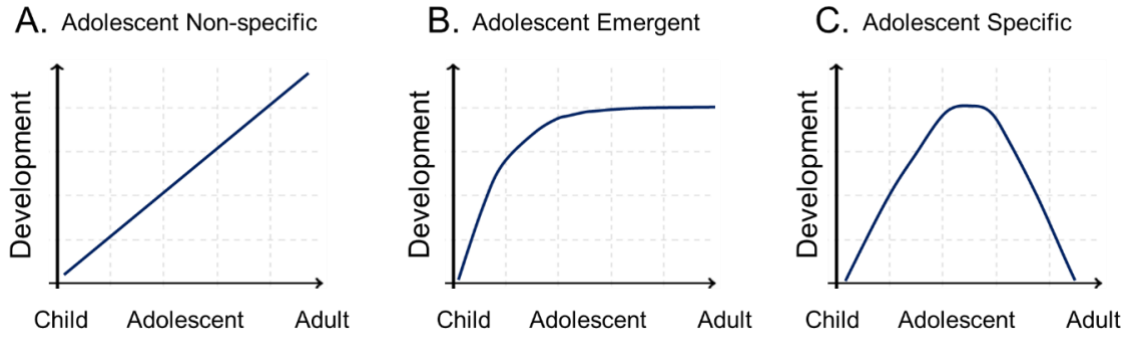

Figure 4-2: The potential developmental trajectory of brain and behaviour changes in the transition from childhood to adolescence and adulthood.**A**: Adolescent non-specific behaviours increase or decrease steadily throughout development. **B**: Adolescent emergent behaviours show rapid rate of change during adolescence and persist into adulthood. **C**: Adolescent specific behaviours peak in adolescence relative to childhood and adulthood. This figure was adapted from work by both Casey (2015) and Somerville et al. (2013).

4.4.2.2. Measuring different facets of fear learning

Whether or not developmental differences in acquisition and/or extinction are observed between adolescents and adults tends to vary with the outcome measures used by each study. For example, during fear acquisition, a developmental dissociation was observed between implicit autonomic responses, which were intact in both adolescents and adults, and explicit self-report responses, in which adolescents demonstrated poorer discrimination between CS+ and CS- cues. Conversely, during extinction, developmental differences were observed in implicit autonomic responses, with adolescents showing some impairment relative to adults, whilst no differences were observed according to explicit self-report responses. Notably, the dissociations observed between implicit and explicit measures in this review provide support for a dual process account of fear conditioning, which suggests that affective and expectancy learning are each represented by distinct learning mechanisms (Hamm & Vaitl, 1996; Hamm & Weike, 2005; Sevenster, Beckers, & Kindt, 2012), and that fear conditioning tasks can induce conditioned autonomic responses even in the absence of explicit CS-US contingency awareness, and vice versa (Bechara et al., 1995; Knight, Waters, & Bandettini, 2009; Schultz & Helmstetter, 2010). Support for this theory was proposed by an early study (Bechara et al., 1995), which examined patients with selective brain

lesions. This work demonstrated that amygdala damage could result in impaired implicit autonomic responses but unimpaired explicit CS knowledge, whilst hippocampal damage could result in impaired explicit CS knowledge but unimpaired implicit autonomic responses, and finally damage to both the amygdala and hippocampus could result in impairments of both implicit and explicit responses. Together, this review provides evidence for a dissociation between explicit and implicit measures of fear learning, inconsistent with single-process or propositional accounts of fear learning, which suggest instead that fear conditioned responses cannot be produced without explicit, propositional knowledge about CS contingencies (C. J. Mitchell, De Houwer, & Lovibond, 2009).

Notably, the findings of this review suggest there may be differences in the maturation rates of specific fear networks during adolescent fear learning, which may be associated with the development of key brain regions in the transition from adolescence to adulthood. Research regarding adolescent brain development suggests that subcortical brain regions responsible for emotional responding, such as the amygdala, are mature in adolescence, but cortical brain regions responsible for exerting cognitive control, such as the PFC, are immature at this stage (Casey, Jones, et al., 2010; Somerville & Casey, 2010). Research has consistently shown an association between threat-mediated amygdala activity and autonomic SCRs (Cheng et al., 2006; Wood et al., 2012, 2014) with top-down PFC activity believed to inhibit the amygdala-mediated emotional responses produced by a threat (Quirk, Likhtik, Pelletier, & Paré, 2003; Rosenkranz & Grace, 2001; Wood, Kuykendall, Ver Hoef, & Knight, 2013; Wood et al., 2012). This suggests that the successful acquisition of differential SCRs to the CS+ vs CS- observed in adolescents in this review may reflect early maturation of the amygdala, which is poised to respond in emotionally arousing situations. In contrast, failure to extinguish these responses 24 hrs later (Johnson & Casey, 2015), or failure to recall extinction training (Ganella et al., 2018) could reflect the immaturity of the PFC in mediating these responses in adolescents. However, with so little evidence, age differences between the engagements of each of these specific fear networks cannot be fully delineated. Therefore, an important recommendation for future work in this area would be to take a multi-modal approach to fear conditioning and extinction, to understand precisely how these different neural networks are represented in adolescents relative to adults.

4.4.2.3. Heterogeneity of experimental design and protocols

In addition to the small number of studies reviewed and coupled with the weaknesses associated with the selection of age appropriate participants, inconsistencies regarding age differences in acquisition and extinction could also be partially explained by the heterogeneity in experimental design and protocols employed throughout this literature. For example, studies that have assessed fear learning utilise different types of CS (e.g., faces, shapes, lights), and different types of US (e.g., shocks, screams, white noise bursts). This creates challenges when attempting to synthesise their results, as differences in the relative salience of CS cues and aversiveness of US cues will impact the strength of fear acquisition (Cook et al., 1986; Glenn et al., 2012; Grillon et al., 2004; Lonsdorf et al., 2017; Mineka & Öhman, 2002; Treviño, 2016), which in turn impacts the degree of extinction (Cook et al., 1986; Lonsdorf et al., 2017). For example, a number of studies in this review use emotional faces as CS. However, increased gonadal hormones influence how adolescents process social information from facial stimuli (Scherf et al., 2012), which could impact fear learning independently of acquisition and extinction processes. Similarly, past work suggests the degree of conditioning and extinction varies depending on the type of US cues used, with a shock US judged to be more aversive than a scream US, and a scream US judged to be more aversive than a white noise US (Glenn et al., 2012; Joos, Vansteenwegen, & Hermans, 2012). The lack of standardised experimental protocols reported in this review was also recently highlighted as a key issue for fear conditioning studies in general (Lonsdorf et al., 2017), and could be contributing to the contradictory results that were highlighted in the acquisition and extinction literature, as this research area currently lacks a standardised methodology for examining fear conditioning and extinction throughout development.

4.5. Conclusions and recommendations for future work

Based on the weaknesses highlighted in this systematic review, a number of key recommendations are proposed here. First, studies assessing acquisition and/or extinction in adolescents should include both adult and/or child comparison groups, to understand changes to these processes as individuals transition into and out of adolescence (Casey, 2015), and should also consider gender differences where possible, as there is currently little work which has examined how male and female adolescents

respond to learned fear cues. Second, researchers should either study adolescents within discrete age categories (i.e., 13-14 years), or treat age as a continuous variable in future analyses. This will enable a more precise examination of potential age-dependent differences in acquisition and extinction, and how these may develop through different stages of adolescence. Thirdly, researchers should seek to replicate simple fear conditioning experiments using emotionally-neutral CS-US cues that contain little social information, to first gain a basic understanding of conditioning and extinction during adolescence. Finally, studies should take a multi-modal approach to measuring fear conditioning and extinction, to understand the developmental trajectories of implicit and explicit fear networks throughout adolescence. Together, these recommendations will lead to advancements in the study of fear learning during adolescence. This may have important implications for the current understanding of adolescent behaviour and their responses to threat in normative development, and also in cases of non-normative development and the emergence of anxiety disorders in this age group.

Chapter 5. A multi-modal examination of fear conditioning and extinction in the transition from adolescence to adulthood: Behavioural, physiological, and ERP correlates

Abstract

A systematic review of the fear conditioning and extinction literature identified a potential impairment in adolescent extinction learning. To investigate this further, perceptual event-related potentials, the P1 and N1, were recorded from male and female adolescents (13-14 years) and adults (25-26 years) while they completed a differential Pavlovian fear conditioning task. In that task, a geometric shape conditioned stimulus (CS+) was paired with an aversive sound US (50% of the time) and another shape (CS-) was never paired with the US. An immediate extinction phase followed, in which both CSs were presented alone. During acquisition, only adolescent males showed significant potentiation of the P1 component in response to the CS+ compared to CS-, a potentiation which was attenuated during the extinction phase. At the level of the N1, both male and female adolescents, but not adults, showed greater visual N1 responses to the CS+ compared to CS-, a dissociation which remained during extinction. Both adolescents and adults exhibited successful acquisition of conditioned fear responses as measured by contingency awareness and evaluative valence CS ratings, albeit with adolescents taking longer to learn the CS+/CS- contingencies, with no evidence of age differences in extinction as indexed by these two self-report measures. Together, these findings provide initial evidence for age- and gender-dependent differences in the early perceptual processing of learned fear cues, as well as a dissociation between explicit (i.e., behavioural) and implicit (i.e., event-related potentials) measures of fear learning.

5.1. Introduction

Chapter 4 consisted of a systematic review which synthesised and evaluated the strength of evidence regarding Pavlovian fear conditioning and extinction in adolescence. The studies reviewed have been seminal in providing the first examinations of adolescent fear learning. However, my review revealed that much of what is currently understood about adolescent associative fear learning processes is based on a small number of developmental studies, with confounds that preclude a complete understanding of how adolescents acquire and extinguish learned fear responses, and how this differs in adults. In response to this, a set of key recommendations were proposed to guide the design and implementation of a Pavlovian fear conditioning and extinction paradigm, suitable for use with a mid-adolescent and adult population in the present study.

5.1.1. Designing a Pavlovian fear conditioning task for use with adolescents and adults

First and foremost, the current study aims to add to a paucity of studies that have examined adolescent fear learning, by investigating both age and gender-dependent differences in the early perceptual processing of Pavlovian fear conditioned cues. An examination of gender-related differences is particularly pertinent to the study of adolescent fear conditioning, given that significant gender differences have been observed in the development of anxiety disorders (Abe & Suzuki, 1986; Lewinsohn et al., 1998) as well as in brain maturation processes during adolescence (Lenroot & Giedd, 2010; Lenroot et al., 2007). Second, to overcome issues in relation to the use of "adult" groups that actually contain late adolescents (e.g., 18-28 years in Pattwell et al., 2012), and the use of adolescent groups that span wide age ranges (e.g., 12-17 years) which cover multiple stages of adolescent brain development (see Chapter 1 section 1.4), the study presented here utilised two developmentally distinct samples of adolescents (13-14 years) and adults (25-26 years). Third, Chapter 4 highlighted the heterogeneity of experimental protocols in the adolescent fear conditioning literature, which creates difficulties when synthesising the findings of key studies. Notably, it can be argued that the use of emotional faces as CS cues is particularly problematic, as the inherent salience of facial expressions could be impacting responses to the CS+ and CS-, over-and-above the effects of fear conditioning or extinction. Furthermore, there will

already be differences in how adolescents and adults process the social information from facial expressions (Blakemore, 2008; S Burnett et al., 2012; Nelson et al., 2005; Scherf et al., 2012) which could lead to differences in the strength of fear acquisition and/or extinction observed. Therefore, the current study used simple emotionally-neutral CS cues (coloured geometrical shapes) and a primary reinforcer as the US (aversive sound), containing little social information, to first gain a basic understanding of conditioning and extinction during adolescence. Fourth, the systematic review highlighted inconsistencies in the findings of different conditioning tasks, which were largely dependent on the outcome measures used to assess conditioning and/or extinction (i.e., implicit versus explicit). As a result of these differences, the current chapter took a multi-modal approach to the study of Pavlovian fear conditioning and extinction, by examining behavioural, physiological, and EEG correlates of visually-presented CS+ and CS- cues.

Overall, the studies reviewed in Chapter 4 provided tentative evidence to suggest that adolescents may exhibit an impairment in extinction learning, relative to adults. It has been suggested that adolescent sensitivity to anxiety could partially stem from age-specific reductions in fear extinction, when responding to conditioned stimuli that no longer predict danger. Following these findings, this study aimed to further investigate extinction mechanisms in adolescents and adults. To that end, both explicit and implicit measures of acquisition and extinction were collected, to examine whether differences in the sensitivity of different measures impacts the degree of extinction being reported. Although each of these measures will index the fear conditioned response, the use of multiple measures of conditioning and extinction will allow for an assessment of both implicit (ERP and SCR) and explicit (contingency awareness and evaluative CS ratings) conditioned responses. It has previously been argued that implicit and explicit measures of conditioning are dissociable (Bechara et al., 1995; Schultz & Helmstetter, 2010) and therefore reflect different neural processes. For example, evidence suggests that implicit autonomic conditioning may reflect amygdala activation, whereas explicit behavioural conditioning may reflect hippocampal activation (Knight et al., 2009). As a result, the use of multiple measures in this study enables an examination of whether adolescents and adults engage different fear networks during conditioning and extinction.

Importantly, it is possible that previous developmental differences observed between adolescent and adult extinction processes could have resulted from differences between adolescent and adult reactivity to the aversive US. For example, greater fear of

the aversive US may have impacted the strength of fear acquisition, leading to a resistance of subsequent extinction in adolescents, relative to adults. However, very little work has examined developmental differences in US reactivity. Johnson and Casey (2015) compared adolescent and adult SCRs to the aversive sound US used in their study, and observed no significant differences between their physiological reactivity to the sound. However, given that none of the other previous studies which compared adolescent and adult fear conditioning assessed participants' reactivity to the US (Den et al., 2015; Ganella et al., 2017; Ganella et al., 2018; Johnson & Casey, 2015; Lau et al., 2011; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012; Waters et al., 2017), this study was also designed to examine reactivity to the US in the adolescents and adults.

Despite evidence from two studies demonstrating attenuated SCRs following delayed extinction (Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), I opted to examine immediate extinction in the present study. This decision was made to enable stronger comparisons across the adolescent extinction literature, as a majority of studies in this area utilise an immediate extinction protocol. This is also consistent with much of the adult extinction literature, which has largely examined immediate extinction (for a review, see Lonsdorf et al., 2017). Moreover, whilst some studies have reported successful immediate extinction in both adolescents and adults when using self-report and SCRs (Den et al., 2015; Ganella et al., 2018; Waters et al., 2017), Ganella et al. (2018) also observed developmental differences in the activation of key brain regions that are involved in immediate extinction. These differences in brain activation patterns may also mediate early visual responses, which can be measured using EEG.

Notably, the systematic review also highlighted that previous studies examining the mechanisms that may underlie blunted extinction in adolescence focused on interactions between top-down prefrontal brain regions and subcortical amygdala activation (Ganella et al., 2017; Ganella et al., 2018; J. H. Kim et al., 2011; Morriss, Christakou, & van Reekum, 2018; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). This focus is consistent with neurobiological models of adolescence, which largely attempt to explain adolescent behaviour in the context of early-maturing subcortical and late-maturing cortical brain regions (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008). However, it is also important to examine whether

developmental differences in the potentiation of early perceptual responses to learned fear cues could also underlie differences in extinction processes during adolescence. Consequently, this study was designed with EEG to examine the responses of two perceptual ERPs, the P1 and N1, during Pavlovian fear conditioning and extinction in adolescents and adults.

5.1.2. Early perceptual processing of fear conditioned cues

EEG is a useful tool to examine the precise neural time course of the implicit perceptual processing of fear conditioned cues. Previous EEG studies with adults have reported potentiation of early visual sensory cortices as a consequence of Pavlovian fear conditioning, demonstrated by greater early visual ERP responses to CS+ compared to CS- cues (C1, P1, N1; Pizzagalli, Greischar, & Davidson, 2003; Stolarova, Keil, & Moratti, 2006; P. S. Wong, Bernat, Bunce, & Shevrin, 1997). It has been suggested that early visual ERPs, such as the P1 and N1 components, index early attentional processing, with larger P1/N1 amplitudes observed in response to attended versus unattended stimuli (see Mangun, 1995 for a review). The visual P1 is a positive ERP component, which is most prominent in occipital electrode sites and peaks between 80-130 ms post-stimulus (Mangun, 1995), and is believed to represent selective orienting of attention towards a visual stimulus (S. J. Luck et al., 1994; Mangun et al., 1993). The P1 is followed by the visual N1, which tends to peak between 150 to 200 ms post-stimulus (Luck, 2014), and is believed to reflect a process of visual discrimination between attended stimuli (Vogel & Luck, 2000). Modulation of similar components has also been reported in MEG work, showing early (50-80 ms post-stimulus) as well as mid-latency (130-190 ms post-stimulus) modulation of visually-evoked responses to aversive CS+ compared with CS- cues (Dolan, Heinze, Hurlemann, & Hinrichs, 2006; C. Steinberg et al., 2012). In contrast, less EEG work has examined extinction processes, but some evidence suggests that CS+/CS- discrimination in early visual responses can be extinguished following a delayed (24 hr) extinction procedure (C1 component, Stolarova et al., 2006), with another study observing reduced visual P1 amplitudes in response to CS+ cues which were extinguished 24 hr earlier, compared with CS+ cues which were not extinguished (Muench, Westermann, Pizzagalli, Hofmann, & Mueller, 2016). Together, this work suggests that initial sensory responses in the visual cortex can be modulated by threat-predicting CS+ cues, and can be indexed by early visual ERPs.

The modulation of these early perceptual responses to fear conditioned cues could reflect amplified attentional processing by the visual system in response to aversive cues. For example, when a CS+ is identified as a threat-predicting cue, relevant subcortical and cortical structures are rapidly engaged (e.g., the amygdala, frontal cortices, and insula), activating re-entrant bias signals, which influences ongoing visual processing across multiple levels of the visual cortex (Miskovic & Keil, 2012). This leads to enhanced sensory processing of CS+ compared to CS- cues, as indexed by visual P1 and N1 components. As a result, whilst early visual ERPs reflect some of the earliest measurable responses to conditioned stimuli, the P1 and N1 represent visual sensory responses that already include multiple iterations of feedback from rapid (< 30 ms) top-down interactions between cortical and subcortical brain regions (Foxe & Simpson, 2002). This notion is supported by fMRI work, which has shown emotion-related increases in BOLD signal change in the amygdala and infero-temporal visual cortex occurring approximately 1000 ms before the extrastriate occipital cortex (Sabatinelli, Lang, Bradley, Costa, & Keil, 2009), and by work demonstrating greater activation of both the amygdala and occipital brain regions in response to CS+ compared to CS- cues (Morris, Buchel, & Dolan, 2001; Morris, Öhman, & Dolan, 1999; Tabbert, Stark, Kirsch, & Vaitl, 2005). Together, these studies suggest that the amygdala evaluates the emotional significance of a stimulus and subsequently modulates visual cortex activation, which can be indexed by visual P1 and N1 ERPs.

However, as yet no study has assessed whether there are age differences in the reinforcement-dependent modulation of these early visual ERPs to conditioned fear stimuli in adolescents, and if they show this potentiation, does it abolish during fear extinction. While there have been no developmental Pavlovian fear conditioning studies using EEG to date, previous work has suggested that adolescents may show enhanced activation of early visual responses to learned danger cues (Howsley & Levita, 2017; Levita et al., 2015). Specifically, these studies investigated reinforcement-dependent potentiation of visual ERPs in response to discriminative stimuli (S^D) that predicted threatening outcomes in an instrumental avoidance paradigm, and found greater N170 and LPP components in response to cues associated with an aversive outcome in adolescents compared to adults. However, from this work, it is unclear whether adolescents' enhanced visual responses are primarily dependent on the response-outcome contingencies (i.e., acting to avoid an aversive outcome) or on the learned Pavlovian association between a once neutral stimulus and an aversive outcome.

5.1.3. The current study

To address the lack of studies examining how adolescents process fear conditioned cues in early perceptual areas, and to overcome the key issues identified by the systematic review in Chapter 4, the main aim of this study was to take a multi-modal approach to studying fear conditioning and extinction in the transition from adolescence to adulthood using both implicit (EEG & SCR) and explicit (contingency awareness & evaluative self-report ratings) measures of fear learning, whilst assessing US reactivity in adolescents and adults. Specifically, behavioural and physiological responses to fear-conditioned cues (CS+/CS-) were examined, as well as the early visual processing of such cues, indexed by visual P1 and N1 components, in a differential fear conditioning and extinction task. In this task, geometrical shapes served as the CSs and an aversive sound served as the US. A partial reinforcement protocol was used, where the CS+ was reinforced with the aversive US on only 50% of trials. This strategy was used to slow the rate of habituation to the aversive US that may occur over time (Lonsdorf et al., 2017), given that EEG requires large trial numbers to accurately capture ERP components and achieve sufficient signal-to-noise ratio.

Based on previous studies, it was predicted first that adolescents and adults would not show differences during fear acquisition, as measured by evaluative valence ratings of the CS and differential SCRs to CS+ versus CS- (Den et al., 2015; Ganella et al., 2018; Johnson & Casey, 2015; Lau et al., 2011; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012; Waters et al., 2017), but that developmental differences would be found in contingency awareness ratings during acquisition, with adolescents being slower to discriminate between CS+ and CS- cues compared with adults (Den et al., 2015). Second, it was predicted that similar immediate extinction of contingency awareness, evaluative CS ratings (Den et al., 2015; Waters et al., 2017) and SCRs (Ganella et al., 2018) would be observed in adolescents and adults. Third, it was predicted that reinforcement-dependent potentiation in both the P1 and N1 components in response to threat-predicting CS+ compared to the CS- cues would be found during fear acquisition in both adolescents and adults (Pizzagalli et al., 2003; Stolarova et al., 2006; P. S. Wong et al., 1997); but that this CS+/CS- differentiation would be greater in adolescents compared with adults (Levita et al., 2015) and as a result, adolescents may show blunted extinction of early perceptual responses compared with adults. However, it was predicted that this blunted fear extinction would not be explained by differences

in US reactivity between adolescents and adults (Johnson & Casey, 2015). Finally, based on studies showing significant differences between male and female brain development during adolescence (Lenroot & Giedd, 2010; Lenroot et al., 2007) the present study proposed an additional exploratory aim, to examine potential gender differences in Pavlovian fear conditioning and extinction. As yet, there appear to be no studies which have focused on gender differences in fear learning in adolescents compared with adults. However, based on research showing that females experience greater anxiety compared to males (Abe & Suzuki, 1986; Lewinsohn et al., 1998), and meta-analytic data showing a tendency for anxious individuals to exhibit greater fear during conditioning tasks (Duits et al., 2015), it was predicted that females would exhibit a similar degree of differential fear conditioning compared to males, but may exhibit greater fear of the US indexed by behavioural (unpleasantness ratings), physiological (SCR) and ERP (auditory N1) responses.

5.2. Methods

A formal power analysis was conducted using G*Power (version 3.1) to assess the suitability of the sample size in this chapter. As this is the first EEG fear conditioning study conducted with adolescents and adults, there is no direct literature from which the effect size for this study can be estimated. Furthermore, previous key studies that have assessed fear conditioning using behavioural and physiological measures did not include estimates of effect sizes in their studies (Ganella et al., 2017; 2018; Johnson & Casey, 2015; Lau et al., 2011; Pattwell et al., 2012; Waters et al., 2017) and did not include the relevant descriptive statistics (means, standard deviations) required to calculate the effect sizes by hand. Therefore, the current power analysis was based on an estimated medium effect size of $f = 0.25$. On this basis, it would be necessary to recruit 98 total participants in order to detect an effect size $f = 0.25$, with power set at 0.8 and an alpha set at 0.05. Due to time constraints and the difficulties associated with recruiting developmental populations, a total of 62 participants were recruited for this study. Therefore, the results of this study should be interpreted in the context of reduced statistical power.

5.2.1. Participants

Thirty adults (25-26 years) and 32 adolescents (13-14 years) participated in this study. One adolescent withdrew before starting the task due to high levels of anxiety, and the data of five adults and three adolescents were excluded from the analysis due to excessive EEG artefacts. Table 5.1 displays the participant demographics of the final sample. All participants were right-handed, and had normal or corrected-to-normal vision/hearing. All participants reported no current medical, psychiatric, or neurological conditions, based on their own self-reports or those provided by their parents. Full informed consent from all participants and from the parents of the adolescent participants was obtained. Participants received £10 for taking part. The University of Sheffield, Department of Psychology Ethics Committee approved this study.

Table 5.1 – *Participant characteristics.*

Age Group	Gender	*n*	Age *M* (SD)	STAI-S % *M* (SD)	STAI-T % *M* (SD)	PDS *M* (*SD*)	US Rating M_{adj} (SD)
Adolescents 13-14 years	Females	14	13.79 (0.43)	53.33 (8.67)	62.97 (11.91)	3.03 (0.41)	5.88 (0.37)
	Males	14	13.79 (0.43)	51.55 (8.58)	56.11 (11.61)	2.34 (0.62)	5.85 (0.37)
	All	28	13.79 (0.42)	52.44 (8.52)	59.58 (12.05)	2.71 (0.61)	5.86 (0.27)
Adults 25-26 years	Females	11	25.63 (0.50)	45.11 (5.87)	53.07 (12.25)	N/A	5.74 (0.37)
	Males	14	25.50 (0.52)	46.43 (16.73)	49.64 (15.62)	N/A	6.14 (0.42)
	All	25	25.56 (0.57)	45.85 (12.90)	51.15 (14.06)	N/A	5.94 (0.28)

Note: STAI = State Trait Anxiety Inventory; S = State; T = Trait; PDS = Pubertal Development Scale. US rating adjusted for state anxiety.

5.2.2. Stimuli

The conditioned stimuli (CS) consisted of two geometrical shapes (purple circle and a blue hexagon). Both the colour and shape of the stimuli used for the CS+ and CS-

were counterbalanced across participants, and were adjusted to have the same size and average luminance. It has been suggested that traditional US stimuli, such as mild electric shocks, may be unsuitable for children and adolescents. This is because the use of electric shock stimuli usually requires participants to select a shock intensity that is unpleasant but not painful, and younger participants may lack sufficient self-awareness to make that decision (Neumann 2008). Therefore, the unconditioned stimulus (US) was an unpleasant sound (1000 ms), based on a recording of a three-pronged garden fork being scraped over slate, which has been validated as an effective unconditioned stimulus in Pavlovian fear conditioning experiments involving children and adolescent participants (Neumann & Waters, 2006; Neumann, Waters, & Westbury, 2008; Neumann, Waters, Westbury, & Henry, 2008). In this study the sound was presented at a sound intensity level of 95 dB for all participants, and the duration of the original recoding was reduced from 3000 to 1000 ms, to decrease the length of each CS trial; allowing additional time to present more trials to participants. As part of a pilot study, 11 participants (5 males, age range = 20-28 years) rated this shortened version of the US on a scale from 1 to 7, where 1 = "Very unpleasant" and where 7 = "Very pleasant". The mean rating was 2 (SD = 0.77), demonstrating that the sound remained highly aversive, despite being shortened.

5.2.3. Task Procedure

Participants were tested individually and received a standardised set of instructions from the experimenter. Experimental events were controlled on a PC running a custom MATLAB script (Version 2014a, The Mathworks inc, USA) designed using Psychtoolbox 3 (Kleiner et al., 2007), the task stimuli were presented on a 24" Prolite (GB2488HSU-B1) monitor with a 1920 x 1080 pixel resolution and a 144 Hz refresh rate. The aversive US was delivered via a Kinden radiation-free headset, consisting of earbuds connected to a metal shielded wire and air tubes rather than electrical wires, to minimise potential electrical interference with the EEG signal. During the task, the stimuli were presented centrally to participants in a dimly lit room, subtending 9 x 9 ° of visual angle at a 50 cm viewing distance. The conditioning task consisted of two learning phases, acquisition and extinction (Figure *5-1*). In acquisition, one of the shapes (CS+, 500 ms) was paired with the aversive US (1000 ms) 50% of the time, with a 500 ms interval between the CS+ and US presentation, whilst the other shape (CS-, 500 ms) was presented alone. In extinction, both the CS+ and CS- cues

were presented alone. Throughout the task a red fixation dot remained on the screen, and the inter-trial interval for both phases was jittered (duration varied randomly between 1500-2200 ms). A partial reinforcement strategy was used, in which CS+ trials were reinforced with the US on 50% of trials. During acquisition, there were 80 CS+ trials, of which 40 were reinforced with the US, and 80 CS- trials. During extinction, there were 80 CS+ trials and 80 CS- trials, with no presentations of the aversive US. Early pilot work determined that 80 trials per condition would be sufficient to detect reliable visual P1 and N1 components, whilst keeping the duration of the experiment as short as possible. The task took approximately 30 minutes to complete and the trials within the conditioning task were pseudo-randomised, so no more than two presentations of the same stimulus occurred in a row. In order to reduce possible fatigue and keep participants attending to the task, each learning phase was split into four shorter, three minute runs, resulting in four acquisition and four extinction runs, with short breaks in between.

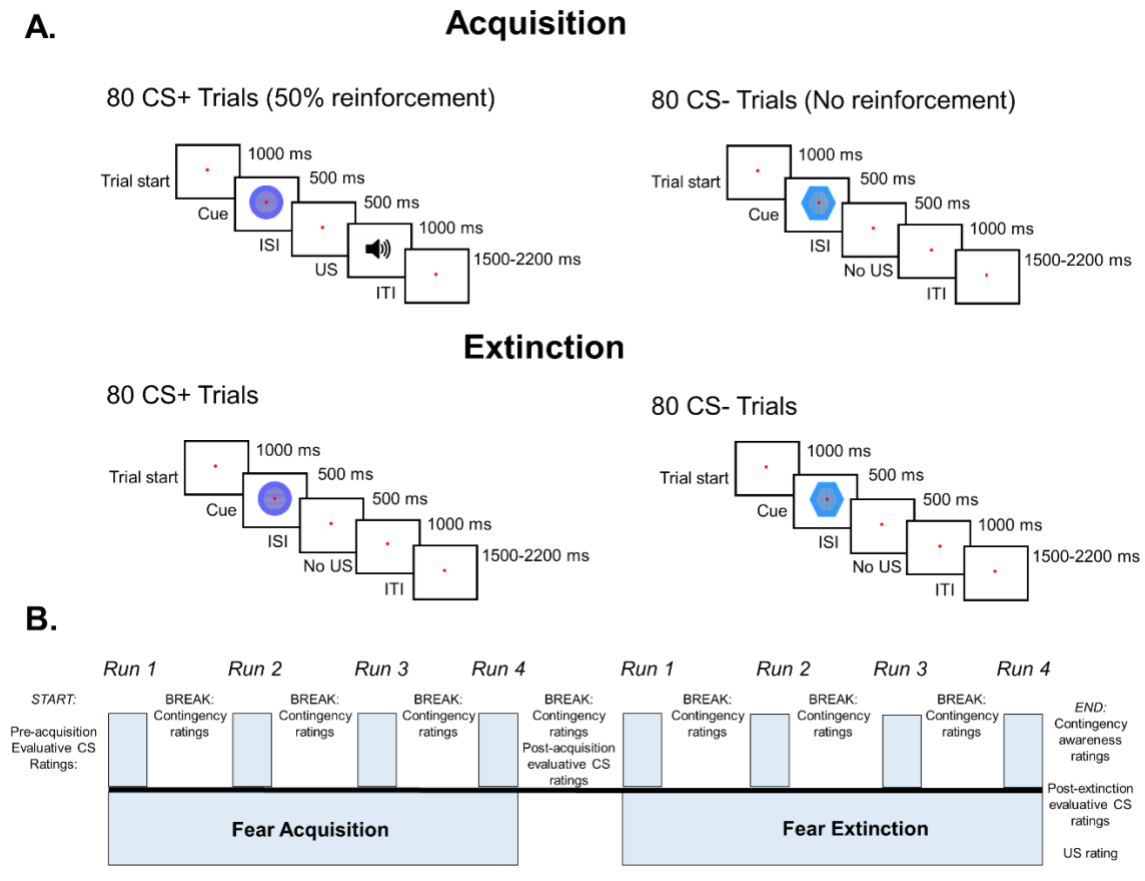

Figure 5-1: Experimental design of the fear conditioning task.**A:** During fear acquisition, 80 CS+ trials were presented, of which 50% were followed by the aversive US, and 80 CS- trials were presented without the aversive US. During fear extinction, the US is no longer presented following the CS+. **B:** Both the acquisition and extinction phases were made up of 4 shorter runs. During each run, 20 CS+ and CS- trials were

presented. Evaluative CS valence ratings were collected before acquisition, after acquisition, and finally after extinction. Participants rated the unpleasantness of the aversive US as soon as the experiment ended.

5.2.4. Outcome measures

5.2.4.1. CS-US contingency awareness

CS-US contingency awareness was recorded after every run during both acquisition and extinction learning phases. Following each run, the instruction: "*During the run you have just experienced, was this shape: 1) Always followed by the US, 2) sometimes followed by the US, or 3) never followed by the US*", was presented on the screen with the shape displayed in the centre. Participants could rate their expectation of the US following each CS using one of three possible response buttons.

5.2.4.2. Conditioned stimuli evaluative valence ratings

Participants rated the pleasantness of each conditioned stimulus three times, before the start of the conditioning task, and after the acquisition and extinction phases. The instruction: "*Please rate how this shape makes you feel on a scale of 1 to 7*" was presented on the screen with the CS shape displayed in the centre. Beneath the shape, a 7-point graphic Likert scale was shown, ranging from 1 = *"Very unpleasant"* to 7 = "*Very pleasant"*. To assess whether acquisition and/or extinction impacted participants affective evaluations of the CSs, difference scores were calculated based on the difference in ratings of each CS before and after acquisition, and before and after extinction. From this, the change in evaluative CS ratings throughout the task could be quantified.

5.2.4.3. US rating

At the end of the experiment, participants were instructed to "*Please rate the unpleasantness of the loud sound you heard during the study on a scale of 1 to 7*", on a 7-point Likert scale that was presented on the instruction screen, where 1 was "*Not at all unpleasant*" and 7 was "*Very unpleasant*". Participants used one of 7 possible response buttons to provide their answer.

5.2.4.4. Pubertal Development

At the end of the experiment, pubertal development was assessed using the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) in the adolescent group. An between-subjects t-test revealed significantly greater PDS scores in adolescent females (M = 3.03, SD = 0.41) compared with adolescent males (M = 2.34, SD = 0.62), t(22.33) = 3.23, 95% CI [0.23, 1.06], p = .004. This suggests that the adolescent females had reached a later stage of their pubertal development compared to adolescent males, consistent with other studies (e.g., Carskadon & Acebo, 1993; Howsley & Levita, 2018; J. M. Tanner, 1971).

5.2.4.5. State Trait Anxiety Inventory–Adult and Child versions

At the end of the experiment, the State-Trait Anxiety Inventory (STAI, Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) was used to measure participants' state and trait anxiety. In line with the STAI manual recommendations, adolescents completed the child version of the STAI and adults completed the adult version. The maximum score for the child version of the STAI is 60, whilst the maximum score for the adult version of the STAI is 80. Therefore, I converted the raw scores to a percentage score, to allow for statistical comparisons to be made between each age group. Adolescents were found to have significantly higher levels of state and trait anxiety compared to adults (Table 5.1). A mixed MANOVA conducted with *Age* (adolescent and adult) and *Gender* (male and female) on *State* and *Trait* A*nxiety*, revealed a significant main effect of *Age*, F(4, 46) = 3.34, p = .044, η_p^2 = .12. Pairwise comparisons revealed adolescents had significantly greater *state* (Mdiff = 6.67, 95% CI [0.57, 12.77], p = .033) and *trait anxiety* Mdiff = 8.23, 95% CI [1.01, 15.44], p = .026). All other main effects and interactions were non-significant. As adolescents reported greater state and trait anxiety levels, levels of state anxiety were controlled for in all subsequent behavioural, physiological and ERP analyses using a series of mixed ANCOVAS.

5.2.5. SCR and EEG data acquisition

The EEG data and skin conductance responses were recorded using a Biosemi ActiveTwo system (Amsterdam, the Netherlands), with 64 AgCl electrodes and two

SCR sensors. Electrodes were fitted according to the 10-20 system, and Signagel was applied to the participants scalp to maintain conductivity between the scalp and the EEG electrodes throughout the experiment. All data were sampled at 2048 Hz, and direct current offset voltages were kept within +/- 25mV, as recommended by the manufacturer. The SCR data was measured with an AC (16 Hz) constant current source with 1 µA amplitude, using two flat Nihon Kohden Ag/Agcl electrodes filled with a conductive electrolyte medium (Elefix), placed on the distal phalanges of the index and middle finger of each participant's non-dominant hand. All participants washed their hands with water and dried them before the SCR electrodes were attached. Participants were asked to take a sharp intake of breath before the main experiment began, to determine if they exhibited a measurable skin conductance response, or whether they were non-responsive.

5.2.6. Skin conductance response data analysis

Electrodermal activity (EDA) is an overarching term used to describe autonomic changes in the skin's electrical properties, caused by sweat secretion from eccrine glands. Skin conductance (SC) is one of the more widely studied properties of EDA, and is measured by passing a small current through two electrodes placed on the skin's surface. Ohm's law is used to calculate SC changes, which proposes skin resistance (R) to be equal to the voltage (V) that is applied between two electrodes attached to the skin, divided by the current (I) that is passed through the skin. If the voltage remains constant, then the current flow can be measured, which varies according to the reciprocal of the skin resistance, allowing changes in skin conductance to be measured non-invasively (Boucsein et al., 2012; Dawson, Schell, & Filion, 2007). The SC time series consists of tonic activity (skin conductance level, SL) which varies slowly, and of phasic activity (SCRs) which varies quickly (Benedek & Kaernbach, 2010). A typical SCR can be identified by a steep rise to the peak amplitude, followed by a slow fall towards the baseline response, which can typically occur between 1-3 or 1-4 seconds post-stimulus (Dawson et al., 2007; Levinson & Edelberg, 1985). Evidence suggests that SC is modulated by activity from the sympathetic nervous system, and so has been widely used in psychophysiology research to reflect changes in autonomic arousal produced by emotional or stressful events (Roy, Boucsein, Fowles, & Gruzelier, 2012). Arguably, SCRs are most commonly used as an index of fear conditioning (see

Supplementary Table 4.2), with conditioned fear responses quantified by an increase in skin conductance following the presentation of the CS+ compared to the CS-.

In the present study, skin conductance data were analysed using Ledalab (version 3.4.9), along with in-house Matlab scripts. First, data were downsampled to 16 Hz and a low-pass butterworth filter with a cut-off of 1 Hz was applied to the data. Ledalab was then used to run a continuous decomposition analysis (CDA), which separates skin conductance data into two continuous signals of tonic and phasic activity, to retrieve the signal characteristics of the underlying activity of the sudomotor nerves, with high temporal precision (Benedek & Kaernbach, 2010). A total of four optimisation phases were performed, and event-related changes in skin conductance were determined within a response window of 1000-4000ms (Benedek & Kaernbach, 2010) following the presentation of the conditioned stimuli (CS+/CS-) and the aversive unconditioned stimulus (US). Event-related SCRs were examined, which represent the average phasic driver within the response window (CDA.SCR, Benedek & Kaernbach, 2010). Trials which had no detectable SCR were scored as zero. Greater amplitude scores indicated greater sympathetic arousal to the stimulus presentation. One adult's SCRs were not recorded due to an equipment failure, two adult's SCRs were not included in the analysis as they were identified as SCR non-responders, exhibiting no measureable skin conductance level during an assessment before the experiment began, and the data of one adult was excluded due to excessive artefacts. Therefore, the SCR analysis was carried out on the remaining participants (21 adults, 28 adolescents). The SCR values were not normally distributed and were therefore log transformed ($\log_{10}$). Following this transformation, the data were normally distributed with no extreme outliers (all Z scores < +/-3).

5.2.7. EEG processing and analysis

The recorded continuous EEG data were down-sampled to 512Hz, using the BioSemi Decimator software, and processed and analysed using the EEGLAB 14.1.1b and ERPLAB 5.0 toolboxes (Delorme & Makeig, 2004; Lopez-Calderon & Luck, 2014). A high-pass filter of 1 Hz was applied to the data to remove low-frequency drifts, and the Cleanline Toolbox was used to reduce 50 Hz line noise (Mullen, 2012). Although the previous EEG study presented in this thesis (Chapter 3) used a 0.1 Hz filter to remove low-frequency drift, as recommended by a subset of ERP researchers (Luck, 2014), other research has suggested that a 1 Hz filter consistently produces better

ICA results in terms of signal-to-noise ratio (SNR) (Winkler, Debener, Müller, & Tangermann, 2015). Due to the nature of the fear conditioning and extinction paradigm used in the current study, in which an aversive US is presented to participants, I observed a greater degree of low-frequency drift caused by increased sweat responses. This artefactual drift contaminates the EEG signal and negatively impacts the quality of the ICA decomposition. As a result of the increase in sweat activity observed in the current chapter, a 0.1 Hz filter could not sufficiently correct for this drift activity, and a 1 Hz high-pass filter was shown to be optimal in this instance. Whilst high-pass filters above 0.3 Hz have been shown to distort later N400 and P600 components in a typical language processing paradigm (D. Tanner et al., 2015) such filter settings are unlikely to cause distortion of early ERPs of interest in the current study (the P1 and N1). This is because P1 and N1 components are expressed around 100 ms (i.e., 10 Hz), and are therefore unlikely to be significantly distorted by a 1 Hz filter. Following the application of a 1 Hz high-pass filter, artefact subspace reconstruction was applied to minimise the impact of additional artefacts that are associated with non-stationary high-variance signals from EEG (Mullen et al., 2013). Then, a visual inspection of the data was conducted and bad channels along with artefacts in the continuous EEG data (e.g., high-frequency noise) were removed. For each participant, an average of 4.02 channels were removed (SD = 3.03, range = 0-12). There were no group differences in the mean number of channels removed according to *Age*, $F(1, 49) = 3.29$, $p = .076$, $\eta_p^2 = .06$, or *Gender*, $F(1, 49) = 1.09$, $p = .303$, $\eta_p^2 = .02$, and there was no *Age* by *Gender* interaction, $F(1, 49) = 0.32$, $p = .576$, $\eta_p^2 = .01$. Following the removal of bad channels, the data were re-referenced according to the average reference, and were subjected to an independent components analysis, which decomposed the data into maximally temporally independent components, using an extended infomax technique (Delorme & Makeig, 2004), and the ADJUST toolbox was used to remove artefact components based on artefact-specific spatial and temporal features (Mognon et al., 2011). Finally, any channels that were previously removed were re-interpolated using a spherical spline interpolation (Perrin et al., 1989).

From the processed continuous data, CS-locked and US-locked ERP epochs were extracted for all conditions (-200 to 1200 ms). Epochs were baseline corrected according to the average activity in the 200 ms window prior to the CS or US onset. Any epochs with voltage fluctuations greater than ± 100 mV were rejected, and the remaining epochs were averaged to form five ERP waveforms (CS+ Acquisition, CS-

Acquisition, CS+ Extinction, CS- Extinction, and US Response) for statistical analysis. Finally, a 20th-order low-pass filter with a 30 Hz cut-off and a Hamming window was applied to the averaged ERP waveforms in order to remove any remaining high frequency noise. Overall, out of a maximum of 80 CS trials per condition, the following number of trials across all participants remained on average: CS+ Acquisition = 70.89 (SD = 5.58), CS- Acquisition = 69.11 (SD = 6.85), CS+ Extinction = 69.85 (SD = 7.30), CS- Extinction = 71.36 (SD = 6.66). For the CS+, all participants had 1.8 more trials during acquisition (M = 70.96, SEM = 0.72) compared with during extinction (M = 69.87, SEM = 0.92), Mdiff = 1.81, 95% CI [0.73, 2.89], p = .002. For the CS-, all participants had 1.5 fewer trials during acquisition (M = 69.16, SEM = 0.90) compared with during extinction (M = 71.39, SEM = 0.88), Mdiff = 1.52, 95% CI [0.44, 2.60], p = .007. On average adolescents (M = 68.10, SEM = 0.98) had 4.5 fewer CS epochs overall compared with adults (M = 72.59, SEM = 1.05), F(1, 49) = 9.82, p = .003, η_p^2 = .17. Out of a maximum of 40 US presentations, 35.06 (SD = 5.30) trials were retained on average per participant. Adolescents (M = 33.46, SEM = 0.95) had 3.2 fewer US epochs compared with adults (M = 36.64, SEM = 1.01), F(1, 49) = 5.29, p = .026, η_p^2 = .10.

Electrode clusters were selected by creating one grand-averaged ERP waveform per electrode, which was then time-locked to the presentation of the visual CS cues for all conditions and participants together, which formed a collapsed localizer (Luck & Gaspelin, 2017). Visual inspection of this grand-averaged ERP for all conditions and participants revealed a visual P1 which peaked between 65 and 160 ms post-stimulus onset and was most prominent at electrodes PO7/O1 in the left hemisphere and PO8/O2 in the right hemisphere, as well as a visual N1 which peaked between 140 and 250 ms post-stimulus onset and was most prominent at P7/P9 in the left hemisphere and P8/P10 in the right hemisphere. Electrodes were clustered for the left and right hemispheres by calculating the average of the channels for each hemisphere, for the P1 and N1 respectively, which allowed us to examine potential laterality effects. The selection of these electrodes is supported by previous studies that have assessed the effect of attention on the P1 (Doherty, Rao, Mesulam, & Nobre, 2005) and N1 (Doherty et al., 2005; Kissler, Herbert, Winkler, & Junghofer, 2009). In order to examine potential age and gender-dependent differences in early auditory responses to the US, a second grand-averaged ERP waveform per electrode was also created, which was time-locked to the presentation of the auditory US. Visual inspection of this grand-averaged ERP revealed an auditory N1 which peaked between 140 and 220 ms post-stimulus onset and was

most prominent at electrodes FCz and Cz. The selection of these electrodes is consistent with studies which have assessed early auditory N1 responses (De Pascalis, Cozzuto, & Russo, 2013; De Pascalis & Russo, 2013; J. Kim, Kim, Jung, Im, & Lee, 2016).

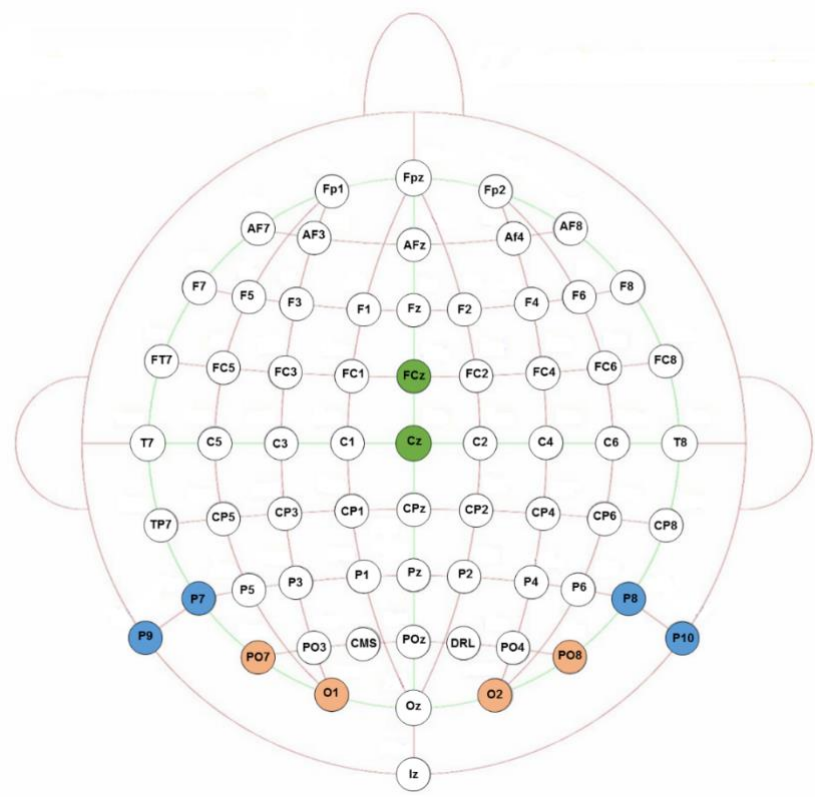

Figure 5-2: Electrode clusters for the visual P1 (orange), visual N1 (blue), and auditory N1 (green).

To overcome issues in relation to observed latency differences in the components between the adult and adolescent participants, the signed area amplitude of the visual P1/N1 and auditory N1 components were examined. The signed area amplitude represents a measure of the positive area above (i.e., P1) or negative area below (i.e., N1) the baseline. This measure reduces the inherent biases involved when selecting time measurement windows, because this measure is not influenced by potentially overlapping components (Luck, 2014). For example, when measuring the P1 wave, it takes into account the positive area amplitude of this component, and is not cancelled out by the subsequent N1 wave.

5.2.8. Statistical Analysis

All behavioural self-report measures were normally distributed, and Levene's test indicated equality of variance for each of these measures (all *p's* > .05). For the log-transformed SCR data, Levene's test also indicated equality of variance ($p > .05$). For the ERP data, Levene's test was significant for a minority of conditions, which suggests there may be a difference in the variance of a subset of conditions. However, ANOVA models are fairly robust to these violations if sample sizes are roughly equal (Tabachnick & Fidell, 2007), and there is currently no alternative, non-parametric test that would enable as detailed an analysis of age and gender-dependent differences, whilst controlling for state anxiety, as an ANCOVA model. Therefore, I proceeded to analyse the data using a series of mixed ANCOVAs. Given the relatively small sample sizes reported in this Chapter once groups were separated according to age and gender (Table 5.1), ANCOVA models were initially conducted without including gender as a factor, to preserve statistical power. Following this, exploratory analyses of gender differences were conducted using additional ANCOVA models. Any additional effects observed according to gender differences are described in separate sections (5.3.2.1.1, 5.3.2.2.1, 5.3.3.1.1, and 5.3.3.3.1). The Alpha level was set to $p < .05$ for all analyses, and the Greenhouse Geisser correction was used in situations where Sphericity was violated (Mauchley's test, $p > .05$). If post-hoc tests were conducted, Bonferroni corrections were used to control for the increased risk of making a Type I error due to running multiple comparisons.

5.3. Results

5.3.1. Behavioural results

5.3.1.1. US rating

Both adolescents and adults found the US to be equally aversive. A between-subjects ANCOVA, with *Age* (adolescent and adult) as the between-subjects variable and *State Anxiety* as the covariate, found no significant differences between self-reported unpleasantness ratings of the US by *Age*, $F(1, 49) = 0.02$, $p = .901$, $\eta_p^2 = .00$.

5.3.1.2. CS-US Contingency awareness

Mean contingency ratings for adolescent and adult participants, adjusted for state anxiety, are displayed in Figure 5-3A. The CS-US contingency ratings reveal that

adolescents were slower to discriminate between the CS+ and CS- compared with adults during the acquisition phase. In contrast, there were no age differences during extinction. Age-dependent differences in contingency awareness were examined using a mixed-design ANCOVA, with *Age* (Adolescent and Adult) as the between-subjects variable, and *Stimulus* (CS+ and CS-) and *Acquisition Run* (1-4) as the within-subjects variables, whilst controlling for *State Anxiety* as the covariate (see Supplementary Table 5.1 for mean contingency awareness ratings for each condition, split by age group and gender). A significant effect of *Stimulus* was observed, F(1, 50) = 23.31, $p < .001$, $\eta_p^2 = .32$, with participants more likely to correctly state that the CS- was never followed by the US ($M_{adj} = 2.76$, SEM = .03) compared to the CS+ ($M_{adj} = 1.81$, SEM = 0.04).

Furthermore, results showed a significant interaction between *Stimulus, Run* and *Age*, F(2.40, 120.22) = 6.00, $p = .002$, $\eta_p^2 = .10$. Pairwise comparisons revealed that adolescents were more likely to correctly state that the CS- was never followed by the US compared to the CS+, following acquisition *run 2*, Mdiff = 1.42, 95% CI [1.19, 1.66], $p < .001$, *run 3*, Mdiff = 1.32, 95% CI [1.07, 1.58], $p < .001$, and *run 4*, Mdiff = 1.22, 95% CI [1.02, 1.41], $p < .001$, whereas adults were more likely to correctly state that the CS- was never followed by the US compared to the CS+ following all four runs of acquisition (all *p's* < .001). This suggests that adults were correctly discriminating between the CS+ and the CS- throughout all acquisition runs, whereas adolescents were slower to discriminate, only starting to show significant CS+/CS- differentiation in *run 2* (Figure *5-3*A). In support of this, adolescents became more accurate with their contingency ratings over the course of the experiment, as they were more likely to correctly state that the CS+ was sometimes followed by the US during acquisition *run 2*, compared with *run 1*, Mdiff = 0.34, 95% CI [0.16, 0.52], and were more likely to correctly state that the CS- was never followed by the US during *run 2*, Mdiff = 0.88, 95% CI [0.64, 1.13], $p < .001$, *run 3*, Mdiff = 0.85, 95% CI [0.61, 1.09], $p < .001$, and *run 4*, Mdiff = 0.81, 95% CI [0.57, 1.04], $p < .001$, when compared with their contingency awareness during *run 1*. All remaining main effects and interactions were non-significant (all *p's > .05).*

5.3.1.3. Conditioned stimuli evaluative valence ratings

The mean change in evaluative ratings of the CS+ and CS- after fear acquisition and after extinction for adolescents and adults suggest they rated the CS+ more

negatively and the CS- more positively following the acquisition phase of the experiment – an effect which was reversed following extinction (Figure *5-3*B). Based on this measure, successful fear conditioning and extinction was observed for all participants, as revealed by a mixed-design ANCOVA, with *Stimulus* (CS+ and CS-) and *Phase* (post-acquisition and post-extinction) as the within-subjects variables, and *Age* (Adolescent and Adult) the between-participants variable, with *State Anxiety* as the covariate (see Supplementary Table 5.1 for mean evaluative ratings for each condition, split by age group and gender). A significant main effect of *Stimulus*, $F(1, 50) = 6.11$, $p = .017$, $\eta_p^2 = .11$ was found, with descriptive statistics showing that participants change ratings of the CS+ became significantly more negative from pre-acquisition to post-acquisition ($M_{adj} = -0.28$, SEM = 0.08) than change ratings of the CS- ($M_{adj} = 0.27$, SEM = 0.09) from pre-acquisition to post-extinction. There was a non-significant main effect of *Age*, $F(1, 50) = 1.82$ $p = .167$, $\eta_p^2 = .03$, suggesting no overall age differences in the change in evaluative ratings as participants worked their way through the acquisition and extinction procedure.

Prior to controlling for state anxiety, a significant interaction between *Stimulus* and *Phase* was observed, $F(1, 51) = 33.12$, $p < .001$, $\eta_p^2 = .40$, that was not modulated by age group. However, once controlling for state anxiety this effect was reduced, $F(1, 50) = 3.22$, $p = .079$, $\eta_p^2 = .06$, which suggests that this *Stimulus-Phase* interaction can be partially accounted for by the state anxiety reported by the participants. Follow-up pairwise comparisons were conducted to examine this interaction effect further, and showed that ratings of the CS+ were rated as significantly more negative at post-acquisition ($M_{adj} = -1.35$, SEM = 0.20) compared to post-extinction ($M_{adj} = 0.78$, SEM = 0.20)**,** Mdiff = -2.13, 95% CI (1.40, 2.86), $p < .001$, and ratings of the CS- were significantly more positive at post-acquisition ($M_{adj} = 0.79$, SEM = 0.19) compared to post-extinction ($M_{adj} = -0.25$, SEM = 0.18), Mdiff = 1.04, 95% CI [0.41, 1.68], $p = .002$. Further follow-up pairwise comparisons showed that at the post-acquisition phase of the study, participants ratings of the CS+ were significantly more negative compared to the CS-, Mdiff = 2.12, 95% CI [1.48, 2.80], $p < .001$, and at the post-extinction phase of the study this relationship was reversed, with participants ratings of the CS- being significantly more negative compared to the CS+, Mdiff = 1.03, 95% CI [0.52, 1.55], $p < .001$.

The reduction of statistical significance observed in the ANCOVA model was accompanied by a significant main effect of *State Anxiety*, F(1, 50) = 6.63, p = .013, η_p^2 = .12, along with a significant interaction between *State Anxiety* and *Stimulus*, F(1, 48) = 13.50, p = .001, η_p^2 = .21. Examination of the relationship between state anxiety and stimulus suggested there was a negative association between state anxiety and affective change scores across all conditions and participants: the greater the state anxiety, the more negative the affective change scores of the CS cues. When examining the relationship between state anxiety and each CS separately, for the CS+, participants with greater state anxiety gave greater negative evaluations of the cue, for the CS-, there was little to no association between state anxiety and the affective change scores of this cue. This suggests that state anxiety was primarily impacting participants' evaluative ratings of the CS+ cue. Please see Supplementary Data 5.2 for any additional interactions which were observed as part of this ANCOVA analysis.

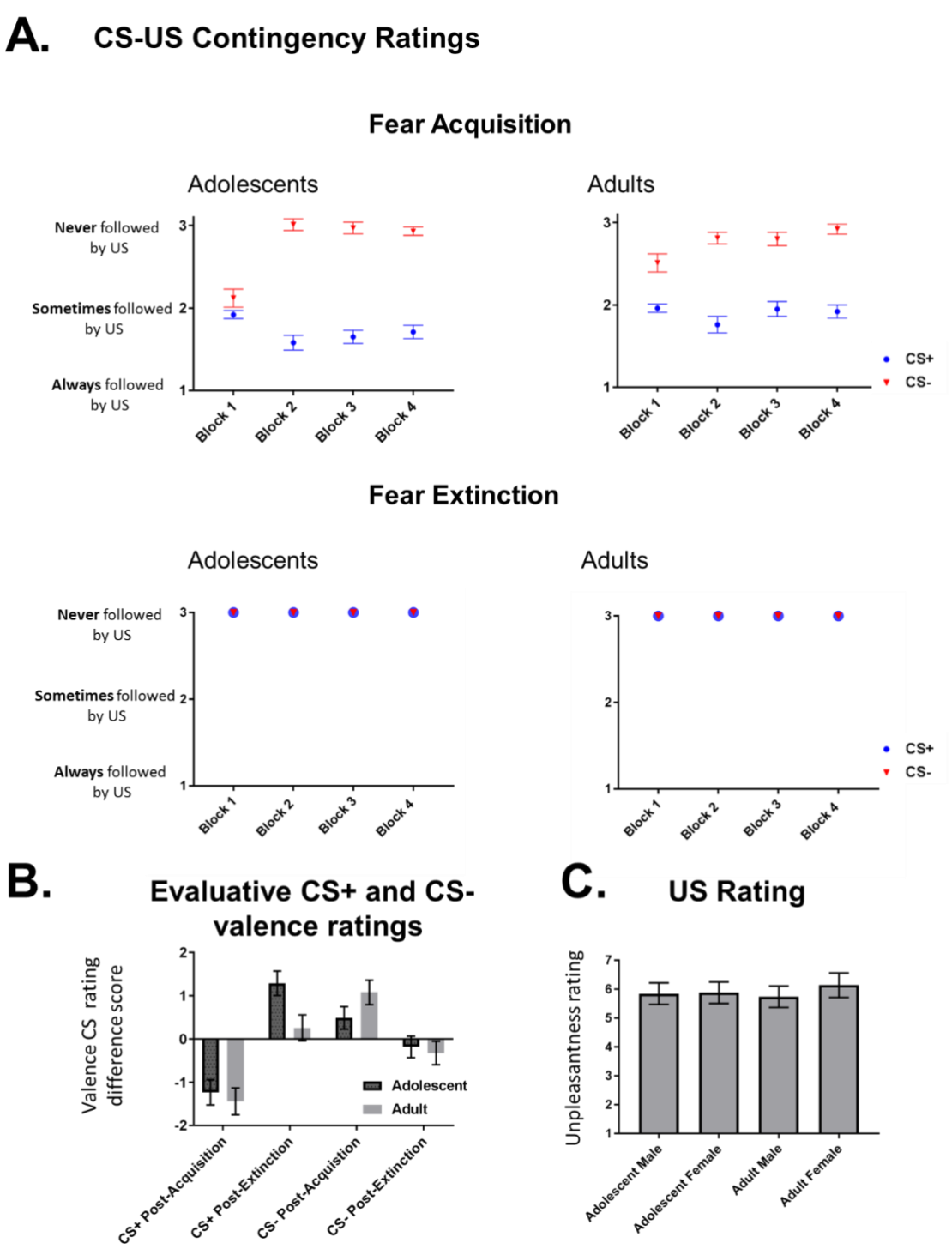

Figure 5-3: Behavioural evidence of successful Pavlovian fear conditioning and extinction. **A:** Graphs showing age differences in contingency awareness for adolescents and adults during fear acquisition and extinction. Contingency ratings ranged from 1 = Always followed by the aversive US, 2 = Sometimes followed by the aversive US, 3 = Never followed by the aversive US. Adolescents were slower to discriminate between CS+ and CS- cues during fear acquisition. Both adolescents and adults showed immediate extinction as measured by their contingency awareness. **B:** Evaluative CS difference scores, calculated by subtracting the pleasantness ratings given to CS cues after fear acquisition from the ratings given before acquisition, and by subtracting the pleasantness ratings given to CS cues after extinction from the ratings given after acquisition. Results suggest both adolescents and adults rated the CS+ more

negatively and the CS- more positively following fear acquisition; this effect reversed during the extinction phase. **C:** All participants rated the aversive US as equally unpleasant.

5.3.2. Physiological Results

5.3.2.1. Greater SCRs in adolescents compared with adults, but no differences between the CS+ and CS-

Overall, adolescent SCRs were greater in response to both CSs compared to adults, and were greater in males compared with females, however no differences between the CS+ and the CS- were found in either group. Age- and gender-dependent effects on the acquisition and extinction of autonomic responses were assessed using a mixed ANCOVA, with *Stimulus* (CS+ and CS-), *Task Phase* (Acquisition and Extinction) and *Run* (1-4) as the within-subjects' variables, *Age* (Adolescent and Adult) as the between-subjects' variable, and *State Anxiety* as the covariate. Table 5.2 displays the adjusted means and standard errors for the SCRs in each group and condition. The interaction between *Stimulus* and *Task Phase* was non-significant, $F(1, 46) = 0.01$, $p = .925$, $\eta_p^2 = .00$, suggesting no evidence of differential SCRs to the CS+ versus CS- during fear acquisition. However, a significant main effect of *Age* was observed, $F(1, 46) = 10.57$, $p = .002$, $\eta_p^2 = .19$, with adolescents exhibiting greater SCRs overall ($M_{adj} = 0.032$, SEM = 0.004), compared with adults ($M_{adj} = 0.015$, SEM = 0.004).

Despite not observing a significant stimulus-phase interaction, the results also revealed a significant interaction between *Task Phase* and *Run*, $F(3, 138) = 4.79$, $p = .003$, $\eta_p^2 = .09$. Pairwise comparisons revealed significantly greater SCRs during acquisition *run 1* ($M_{adj} = 0.041$ SEM = 0.005) compared with *run 2* ($M_{adj} = 0.020$, SEM = 0.003), Mdiff = 0.38, 95% CI [0.31, 0.44], $p < .001$, *run 3* ($M_{adj} = 0.019$, SEM = 0.003), Mdiff = 0.45, 95% CI [0.35, 0.54], $p < .001$, and *run 4* ($M_{adj} = 0.018$, SEM = 0.003), Mdiff = 0.46, 95% CI [0.35, 0.56], $p < .001$. In addition, SCRs were greater during acquisition *run 1* compared to extinction *run 1* ($M_{adj} = 0.021$, SEM =0.003), Mdiff = 0.46, 95% CI [0.33, 0.58], $p < .001$. These results suggest that both adolescents and adults' SCRs were greatest during the first run of fear acquisition, and subsequently habituated during the remaining acquisition runs. Please see Supplementary Data 5.3 for any additional interactions which were observed as part of this ANCOVA analysis.

5.3.2.1.1 Additional exploratory gender effects

When gender was included as an additional between-subjects factor in the ANCOVA model, a significant main effect of *Gender* was observed, F(1, 43) = 12.76, p = .001, η_p^2 = .23, with males showing greater SCRs overall (M_{adj} = 0.030, SEM = 0.004), compared with females (M_{adj} = 0.016, SEM = 0.004). This effect was strengthened by an additional significant interaction between *Stimulus* and *Gender*, F(1, 44) = 6.48, p = .014, η_p^2 = .13, which suggests that males (M_{adj} = 0.030, SEM = 0.004) exhibited greater SCRs than females (M_{adj} = 0.016, SEM = 0.004) in response to the CS+, Mdiff = 0.39, 95% CI [0.18, 0.60], p < .001, and males (M_{adj} = 0.029, SEM = 0.004) also exhibited greater SCRs than females (M_{adj} = 0.017, SEM = 0.004) in response to the CS-, Mdiff = 0.34, 95% CI [0.14, 0.55], p = .002. Please see Supplementary Data 5.3 for any additional interactions which were observed as part of this ANCOVA analysis.

Table 5.2 – *Skin conductance responses to the CS+ and CS- during each run of acquisition and extinction.*

	Adolescent				**Adult**			
	Male		*Female*		*Male*		*Female*	
	M_{adj} (SEM)		M_{adj} (SEM)		M_{adj} (SEM)		M_{adj} (SEM)	
	CS+	**CS-**	**CS+**	**CS-**	**CS+**	**CS-**	**CS+**	**CS-**
ACQ 1	0.058	0.060	0.043	0.052	0.038	0.032	0.020	0.026
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
ACQ 2	0.037	0.038	0.019	0.018	0.015	0.013	0.008	0.007
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
ACQ 3	0.040	0.034	0.020	0.019	0.013	0.012	0.005	0.005
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
ACQ 4	0.037	0.032	0.021	0.018	0.015	0.012	0.004	0.006
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
EXT 1	0.033	0.036	0.016	0.019	0.027	0.018	0.006	0.006
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
EXT 2	0.035	0.033	0.023	0.024	0.025	0.021	0.006	0.006
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
EXT 3	0.042	0.040	0.026	0.025	0.018	0.018	0.004	0.004
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
EXT 4	0.038	0.041	0.032	0.035	0.016	0.020	0.006	0.009
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Note: All means are adjusted for state anxiety.

5.3.2.2. Adolescents exhibited greater SCRs to the aversive US, compared with adults

Regarding participants' autonomic responses to the aversive US, adolescents demonstrated greater SCRs to the US compared with adults, and males demonstrated greater SCRs compared to females (Table 5.3). In order to assess age- and gender-dependent differences in autonomic responses to the aversive US, a mixed ANCOVA was conducted, with *Age* (Adolescent and Adult) as the between-subjects variable, *Acquisition Run* (1-4) as the within-subjects variable, and *State Anxiety* as the covariate. These results suggest a significant main effect of Age, $F(1, 46) = 8.41$, $p = .006$, $\eta_p^2 = .16$, with greater SCRs to the US observed in adolescents ($M_{adj} = 0.078$, SEM = 0.007) compared with adults ($M_{adj} = 0.046$, SEM = 0.008).

5.3.2.2.1 Additional exploratory gender effects

When gender was included as an additional between-subjects factor in the ANCOVA model, a significant main effect of *Gender* was observed, F(1, 44) = 5.63, p = .022, η_p^2 = .11, with greater SCRs to the US observed in males (M_{adj} = 0.072, SEM = 0.007) compared with females (M_{adj} = 0.052, SEM = 0.008). Additionally, a significant interaction between *Gender* and *Run* was observed, F(2.21, 97.38) = 8.34, p < .001, η_p^2 = 0.16. In males, pairwise comparisons revealed significantly greater SCRs to the US during acquisition *run 1* (M_{adj} = 0.104, SEM = 0.011) compared to *run 2* (M_{adj} = 0.074, SEM = 0.008), Mdiff = 0.16, 95% CI [0.08, 0.24], p < .001, *run 3* (M_{adj} = 0.055, SEM = 0.007), Mdiff = 0.34, 95% CI [0.21, 0.47], p < .001, and *run 4* (M_{adj} = 0.054, SEM = 0.006), Mdiff = 0.34, 95% CI [0.19, 0.49], p < .001, as well as between *runs 2* and *3*, Mdiff = 0.18, 95% CI [0.07, 0.28], p = .001. In females, pairwise comparisons revealed females had significantly greater SCRs to the US during acquisition *run 1* (M_{adj} = 0.096, SEM = 0.012) compared to *run 2* (M_{adj} = 0.051, SEM = 0.009), Mdiff = 0.34, 95% CI [0.36, 0.42], p < .001, *run 3* (M_{adj} = 0.036, SEM = 0.007), Mdiff = 0.56, 95% CI [0.42, 0.70], p < .001, and *run 4* (M_{adj} = 0.25, SEM = 0.006), Mdiff = 0.78, 95% CI [0.61, 0.94], p < .001, and also between *runs 2* and *3*, Mdiff = 0.22, 95% CI [0.11, 0.34], p < .001, *runs 2* and *4*, Mdiff = 0.43, 95% CI [0.30, 0.56], p < .001, and finally between *runs 3* and *4*, Mdiff = 0.21, 95% CI [0.08, 0.34], p = .002. In addition, pairwise comparisons revealed males had significantly greater SCRs in response to the US than females during *run 4* of acquisition, Mdiff = 0.44, 95% CI [0.17, 0.71], p = .002.

Table 5.3 – *Skin conductance responses to the US during each run of acquisition.*

	Adolescent		**Adult**	
	Male	*Female*	*Male*	*Female*
	M_{adj} (SEM)	M_{adj} (SEM)	M_{adj} (SEM)	M_{adj} (SEM)
ACQ 1	0.124 (0.02)	0.115 (0.02)	0.085 (0.02)	0.077 (0.02)
ACQ 2	0.09 (0.01)	0.066 (0.01)	0.059 (0.01)	0.037 (0.02)
ACQ 3	0.074 (0.01)	0.047 (0.01)	0.036 (0.01)	0.024 (0.01)
ACQ 4	0.073 (0.01)	0.034 (0.01)	0.035 (0.01)	0.016 (0.01)

Note: All means are adjusted for state anxiety.

5.3.3. ERP results

5.3.3.1. The visual P1 component

Visual P1 responses revealed both age- and gender-dependent differences during both acquisition and extinction phases (Figure *5-4*). To examine potential age- and gender-dependent differences on the effect of acquisition and extinction in early visual responses to CS+ and CS- cues, a mixed ANCOVA was conducted, with *Stimulus* (CS+ and CS-), *Task Phase* (Acquisition and Extinction) and *Hemisphere* (Left and Right) as the within-subjects variables, and *Age* (Adolescent and Adult) as the between-subjects variable, whilst controlling for *State Anxiety* as the covariate (see Supplementary Table 5.5 for the mean visual P1 amplitudes for each condition, split by age group and gender).

A significant interaction between *Phase* and *Age* was observed, $F(1, 50) = 6.96$, $p = .011$, $\eta_p^2 = .12$. Pairwise comparisons suggest greater P1 area amplitudes in adolescents compared with adults during acquisition, Mdiff = 0.22, 95% CI (0.12, 0.31) $p < .001$, and during extinction, Mdiff = 0.27, 95% CI (0.17, 0.37) $p < .001$. In addition, adolescent P1 responses were greater during extinction compared to acquisition, Mdiff

= 0.06, 95% CI (0.03, 0.08) $p < .001$. However, all other main effects and interaction were non-significant (all p's > .05).

5.3.3.1.1 Additional exploratory gender effects

Most importantly, when gender (Male and Female) was included as an exploratory factor in the ANCOVA model, results revealed a significant interaction between *Stimulus, Phase, Age, and Gender*, F(1, 48) = 10.01, $p = .003$, $\eta_p^2 = .17$. Pairwise comparisons suggest that adolescent males exhibited greater P1 area amplitudes in response to the CS+ compared with the CS- during the acquisition phase, Mdiff = 0.11, 95% CI [0.06, 0.15], $p < .001$. Given that this CS+/CS- differentiation was no longer observed in adolescent males during extinction, Mdiff = 0.01, 95% CI [-0.03, 0.06], $p = .583$, this suggests that their differential P1 area amplitudes to the CS+ versus CS- were extinguished. In contrast, adolescent females did not exhibit greater P1 area amplitudes in response to the CS+ compared with the CS- during acquisition, Mdiff = 0.02, 95% CI = [0.03, 0.06], $p = .525$. However, they did exhibit greater P1 area amplitudes in response to CS+ during extinction (M_{adj} = 0.49, SEM = 0.05) compared with acquisition (M_{adj} = 0.38, SEM = 0.05), Mdiff = 0.11, 95% CI = [0.07, 0.15], $p < .001$. In the adults, there were no significant differences between in P1 response to the CS+ and CS- during both acquisition, and extinction phases of the experiment (all p's < .05). Finally, there was also significant main effect of Age, F(1, 48) = 24.22, $p < .001$, $\eta_p^2 = .34$, with overall greater P1 area amplitudes observed in adolescents (M_{adj} = 0.41, SEM = 0.03) compared with adults (M_{adj} = 0.17, SEM = 0.04). Together, these results suggest that male adolescents showed reinforcement dependent potentiation of the P1 to the CS+ during fear acquisition, which was abolished during extinction; a phenomenon not observed in either female adolescents or male and female adults. Please see Supplementary Data 5.4 for any additional interactions which were observed as part of this ANCOVA analysis.

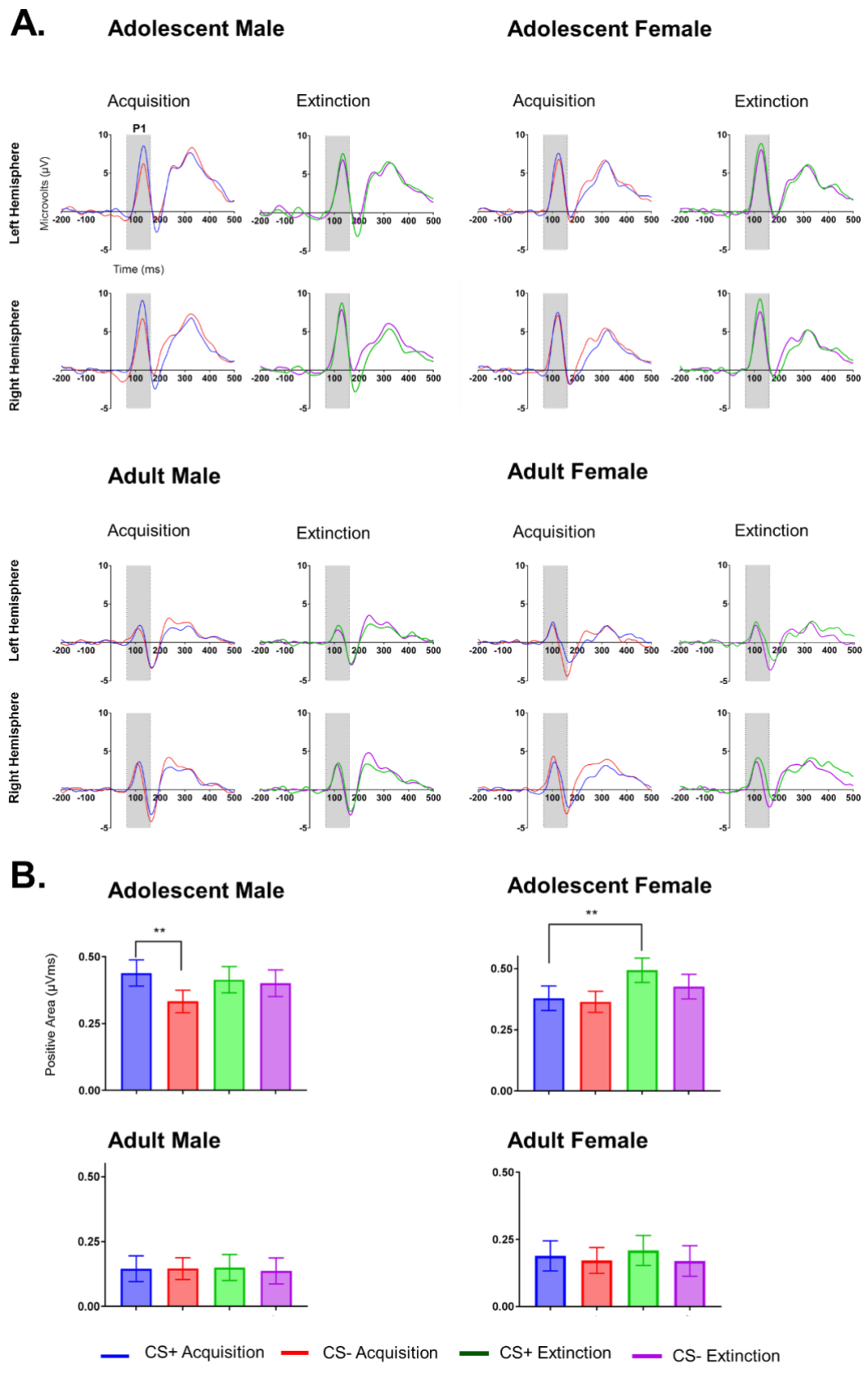

Figure 5-4: The visual P1 component. Pairing the CS+ with an aversive US led to P1 reinforcement-dependent potentiation to CS+ vs CS- in adolescent males only, which was abolished during extinction. In contrast, adolescent females showed greater potentiation of P1 responses to the CS+ (acquisition to extinction). Both adult males and females demonstrated no evidence of reinforcement-dependent potentiation of the CS+

compared to the CS-. **A:** Grand averaged ERP waveforms in response to CS+ and CS-, as well as in response to stimuli presented during acquisition and extinction, for male and female adolescents and adults. **B:** Bar graphs included to highlight the significant differences in positive P1 area observed between CS+ and CS- stimuli in adolescent males, and in response to the CS+ from acquisition to extinction in adolescent females only. $^{**}p < .001$. (Means adjusted for state anxiety, bars represent SEM).

5.3.3.2. The visual N1 component

Visual N1 responses revealed age-dependent differences during acquisition and extinction (Figure *5-5*). To examine potential age- and gender-dependent differences on the effect of conditioning and extinction in early visual responses to CS+ and CS- cues, a mixed ANCOVA was conducted, with *Stimulus* (CS+ and CS-), *Task Phase* (Acquisition and Extinction) and *Hemisphere* (Left and Right) as the within-subjects variables, and *Age* (Adolescent and Adult) as the between-subjects variables, whilst controlling for *State Anxiety* as the covariate (see Supplementary Table 5.5 for the mean visual N1 amplitudes for each condition, split by age group and gender).

The results revealed a significant interaction between *Stimulus* and *Age*, $F(1, 50) = 10.70$, $p = .002$, $\eta_p^2 = .18$. Pairwise comparisons suggest that the adolescent group, and not the adults, are exhibiting greater N1 responses to the CS+ compared to the CS-, Mdiff = 0.07, 95% CI [0.03, 0.10], $p < .001$. Because of the non-significant interaction between *Stimulus* and *Phase*, $F(1, 50) = 0.41$, $p = .501$, the interaction between *Stimulus* and *Age* indicates that the potentiation of the N1 to the CS+ cues that is being driven by adolescents was unaffected by *Task Phase*. This suggests that this CS+/CS- differentiation in adolescent N1 responses was not extinguished (Figure *5-5*). Unlike the P1, this N1 effect was not affected by gender.

There was also a significant interaction between *Task Phase* and *Age*, $F(1, 48) = 6.57$, $p = .013$, $\eta_p^2 = .12$. Pairwise comparisons revealed that adult N1 responses to both CS cues decreased during extinction ($M_{adj} = 0.19$, SEM = 0.03) compared with acquisition ($M_{adj} = 0.23$, SEM = 0.03), Mdiff = 0.04, 95% CI [0.01, 0.07], $p = .012$. There was also a significant interaction between Hemisphere and Age, $F(1, 50) = 4.68$, $p = .035$, $\eta_p^2 = .09$, however, pairwise comparisons were all found to be non-significant (all p's > .05). Any remaining main effects and interactions were non-significant (all *p*'s > .05).

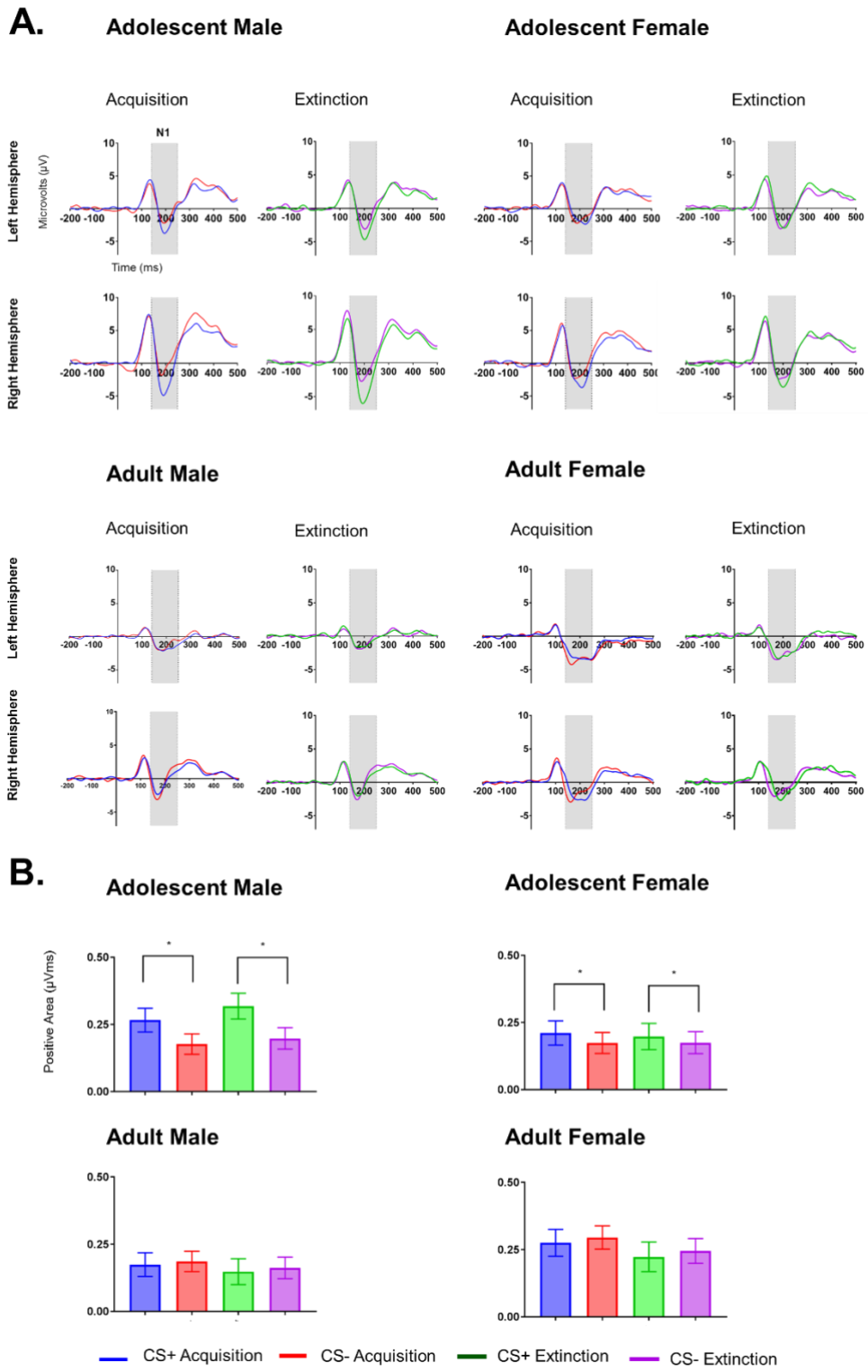

Figure 5-5: The visual N1 component. Pairing the CS+ with an aversive outcome resulted in potentiation of the N1 component in adolescents, but not adults. **A:** Grand averaged ERP waveforms in response to CS+ and CS-, for adolescents and adults across both the left and right hemispheres, **B:** Bar graphs included to highlight the differences in negative N1 area observed between CS+ and CS- stimuli in all participants. ***p* < .001. (Means adjusted for state anxiety, bars represent SEM).

5.3.3.3. The auditory N1 component

It is possible that the pattern of results observed in the visual P1 and N1 components were influenced by age and/or gender-dependent differences in participants' reactivity to the aversive US. This can be examined by comparing participants' auditory N1 responses to the US, because greater aversion towards the US could result in increased attentional facilitation from the auditory cortex, given that the auditory cortex shares connections with limbic structures such as the amygdala (Herry & Johansen, 2014; Romanski & LeDoux, 1992), which may result in enhanced N1 responses to the aversive auditory stimulus. Therefore, a mixed ANCOVA was conducted on auditory N1 area amplitudes, with *Electrode* (FCz and Cz) as the within subjects variable and *Age* (Adolescent and Adult) as the between-subjects variable, whilst controlling for *State Anxiety* as the covariate (see Supplementary Table 5.5 for the mean auditory amplitudes in response to the US, split by age group and gender). These results demonstrated non-significant main effects of *Age*, $F(1, 50) = 1.47$, $p = .231$, $\eta_p^2 = .03$, and *Electrode*, $F(1, 48) = 2.31$, $p = .134$, $\eta_p^2 = .04$, suggesting that participants' early visual processing of the fear conditioned cues were not directly influenced by developmental differences in their early auditory processing of the aversive US.

5.3.3.3.1 Additional exploratory gender effects

When gender was included as an additional between-subjects factor in the ANCOVA model, a marginally significant main effect of *Gender* was observed, $F(1, 48) = 3.71$, $p = .060$, $\eta_p^2 = .07$, which suggests that females ($M_{adj} = 0.48$, SEM = 0.05) exhibited greater auditory N1 responses than males ($M_{adj} = 0.35$, SEM = 0.05) in response to the aversive US. In addition, a significant interaction between *Electrode* and *Gender* was observed, $F(1, 48) = 8.31$, $p = .006$, $\eta_p^2 = .15$, with males showing greater auditory N1 responses at electrode Cz (M= 0.40, SEM = 0.05) compared with electrode FCz, Mdiff = 0.09, 95% CI [0.05, 0.14], $p < .001$. Any remaining main effects and interactions were non-significant (all p's > .05).

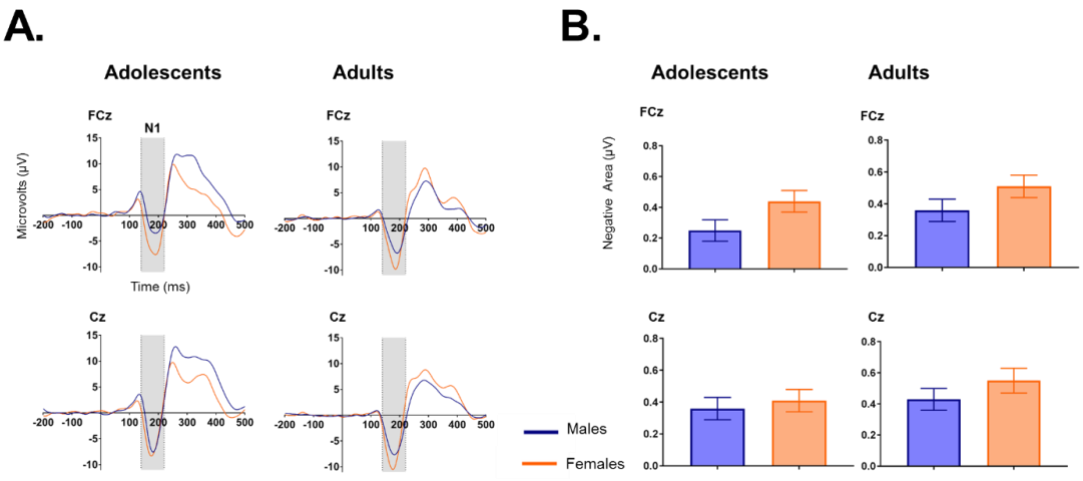

Figure 5-6: The auditory N1 component.Auditory N1 responses to the aversive US were not significantly different between adolescents and adults. **A:** Grand averaged ERP waveforms in response to the US, split by age and gender. **B:** Bar graphs to show the marginally significant difference between auditory N1 responses in females compared to males (p = .060).

5.4. Discussion

The current study presents, to my knowledge, the first examination of age and gender-dependent differences in the reinforcement-dependent potentiation of early visual processing during Pavlovian fear conditioning. To that end, a 50% partial reinforcement differential fear conditioning and extinction paradigm was designed, to be used with EEG.

First, regarding the implicit measures of fear conditioning, only adolescent males showed significant reinforcement-dependent potentiation of the P1 component in response to CS+ compared to CS- cues. This CS+/CS- discrimination was abolished during the extinction phase. In contrast, adolescent females did not discriminate between the CS+ and CS- at the level of the P1 component, but their responses to the CS+ increased significantly during the extinction phase of the experiment. Notably, however, during extinction their P1 responses to the CS+ were not significantly different from their responses to the CS-. Both male and female adults showed no evidence of CS+/CS- discrimination in visual P1 responses. Regarding the visual N1, adolescents, but not adults, showed greater visual N1 responses to CS+ versus CS- cues during acquisition, a phenomenon which was not abolished during extinction. Regarding the physiological measure of fear conditioning (SCR), the present study did not observe the expected dissociation between the CS+ and CS- (i.e., greater SCRs to

the CS+) in adolescents or adults, as reported in other studies (e.g., Johnson & Casey, 2015; Lau et al., 2011; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). Despite this, larger overall SCR responses were observed to both CS cues in adolescents compared to adults, consistent with previous work (Lau et al., 2011; Levita et al., 2015).

Second, regarding the explicit measures of fear conditioning, in line with the predictions, the behavioural results suggest the current fear conditioning protocol produced reliable fear conditioning in both adolescents and adults, as indexed by contingency awareness and evaluative ratings of the CSs. However, adolescents were slower to discriminate between CS+/CS- cues as measured by their awareness of the CS contingencies.

5.4.1. Implicit measures of conditioning and extinction

5.4.1.1. The P1 component

Gender differences in the degree of P1 potentiation between adolescent males and females could reflect differences in brain maturation rates during this developmental period. Specifically, the P1 results suggest a gender-dependent dissociation at one of the earliest measurable stages of visual sensory processing in response to Pavlovian fear conditioned cues during adolescence, whereby adolescent males show CS+/CS- discrimination, followed by immediate extinction, whilst adolescent females do not show this discrimination, and instead seem to engage greater attentional resources to the processing of the CS+ cue during extinction compared to acquisition. Past sMRI work suggests that adolescent male brain development is slower than females, with females exhibiting evidence of earlier maturation of cortical and thalamic grey matter volume during adolescence (Lenroot & Giedd, 2010), with some female brain changes occurring around 1-2 years earlier than males (Lenroot et al., 2007). These changes occur in line with earlier pubertal development in females (e.g., Carskadon & Acebo, 1993; Howsley & Levita, 2018; J. M. Tanner, 1971), which was also observed in this study. This could explain the differential P1 results between male and female adolescents observed in the current study, as adolescent females, who undergo significant brain maturational changes before male adolescents, exhibited P1 responses which were adult-like (i.e., no CS+/CS- differentiation during fear acquisition).

Notably, overall P1 area amplitudes were significantly smaller in adults compared with adolescents. This is highly consistent with other developmental studies on the P1, which have shown linear, age-related decreases in P1 amplitudes, among other ERPs (e.g., N1, LPP, N170), from childhood to adulthood (Brecelj, Štrucl, Zidar, & Tekavčič-Pompe, 2002; Crognale, 2002; Hirai, Watanabe, Honda, & Kakigi, 2009; Roxane J Itier & Margot J Taylor, 2004; Kuefner et al., 2010; MacNamara et al., 2016). Some work has argued that age-related reductions in P1 amplitudes may index greater efficiency of holistic visual processing (Itier & Taylor, 2004). However, this developmental difference could be unrelated to brain activity, instead reflecting changes in the conductive properties of the skull that occur throughout development, due to head growth and increases in skull thickness/density, leading to a reduction in ERP amplitudes (Segalowitz, Santesso, & Jetha, 2010).

5.4.1.2. The N1 Component

In contrast to the P1, the present study observed N1 potentiation in response to threat-predicting CS+ compared to CS- in both male and female adolescents, but not adults, which is consistent to some extent with previous EEG work examining reinforcement-dependent potentiation of visual responses to learned danger signals (Howsley & Levita, 2017; Levita et al., 2015). Firstly, Levita et al. (2015) observed greater reinforcement-dependent potentiation of the N170 component in response to threat-predicting cues in adolescents (12-15 years) compared to adults (18-32 years), as part of an instrumental conditioning procedure. In their second study, Howsley and Levita (2017) conducted a similar instrumental task, but this task not only examined responses to cues that predict a negative outcome, but also reward, indexed by the N170 and LPP components. They found significant developmental differences in LPP amplitudes, which were potentiated to the avoidance cues in adolescents (9-23 years), with only younger adolescents (9-12 years) demonstrating greater potentiation to the cues signalling reward. Importantly, participants learned in these studies that aversive outcomes could be avoided by making or withholding a motor action, so their results suggest that response-outcome contingencies may be important for the modulation of early visual responses in conditioning paradigms. However, the results of the present study suggest that adolescents' early visual responses can also be modulated by learned Pavlovian associations between a neutral stimulus and an aversive event, even in the absence of any response-outcome contingencies. Interestingly, Howsley and Levita

(2017) did not replicate the age-dependent potentiation of the N170 to threatening outcomes observed in their initial study (Levita et al., 2015). The authors suggest this null finding could be task-related, as their first study utilised the immediate threat of a primary reinforcer (an aversive tone), whereas their second study utilised a secondary reinforcer (loss of points in game). Therefore, the lack of N170 potentiation in Howsley and Levita (2017) could have resulted from the insufficient threat posed by a loss of points. The current study supports this suggestion, as enhanced N1 amplitudes were found in response to threat-predicting cues in adolescents, following the use of a primary reinforcer.

The CS+/CS- discrimination observed in adolescent visual N1 responses is consistent with previous adult EEG/MEG work, which demonstrated potentiation of early visual responses to fear conditioned CS+ cues (Baas, Kenemans, Böcker, & Verbaten, 2002; Dolan et al., 2006; Keil, Stolarova, Moratti, & Ray, 2007; Panitz, Hermann, & Mueller, 2015; Pizzagalli et al., 2003; P. S. Wong et al., 1997) as well as early auditory responses (N1, Montoya, Larbig, Pulvermuller, Flor, & Birbaumer, 1996). For example, Pizzagalli et al. (2003) found that the presentation of CS+ cues (two fearful faces signalling the potential delivery of an aversive noise) was associated with greater activation in ventral visual pathways (120 and 176 ms post-stimulus) than CS- cues (two control fearful faces). Critically, in the present study, greater adolescent N1 responses to CS+ cues were not abolished during extinction, consistent with previous work demonstrating blunted extinction processes in adolescents compared with adults, when implicit autonomic responses were examined (Johnson & Casey, 2015; Pattwell et al., 2012). Similar to the task protocol in the present study, both of these studies also conducted a differential fear conditioning task with a 50% partial reinforcement strategy, reporting attenuated fear extinction of autonomic (SCR) responses in adolescents (12-17 years) compared with adults (18-32 years in Johnson & Casey, 2015; 18-28 years in Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012) and also with children (5-11 years in Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), following a delayed (24 hr) extinction procedure. Taken together, the present study demonstrates modulation of visual N1 responses by threat-predicting CS+ cues in adolescents, and supports the idea that adolescents exhibit blunted fear extinction at the implicit perceptual level, whilst providing additional evidence that this effect can be indexed by early visual percepts following an immediate extinction procedure.

5.4.2. Mechanisms that might underlie potentiation of visual P1 and N1 components

The observed potentiation of early visual responses to threat-predicting CS+ cues in this study could be modulated by amygdala activation, as suggested by neuroimaging studies which have directly examined interactions between the amygdala and visual sensory cortices. Specifically, studies have shown that the amygdala interprets the emotional significance of a stimulus, and feeds this information back to the rostral inferotemporal and caudal occipital areas, leading to enhanced perceptual processing, by directing participants attention to threat-related stimuli (Amaral, Behniea, & Kelly, 2003; Sabatinelli et al., 2009). This may have been achieved via a dedicated subcortical pathway (superior-colliculus – posterior thalamus – amygdala; Linke, De Lima, Schwegler, & Pape, 1999), which contains a rapid route for transferring biologically salient visual sensory information to the amygdala. This has been further illustrated in a discriminative fear conditioning paradigm in adults (Tabbert et al., 2005), using two geometrical shapes as CS cues and electrical stimulation as the US. Their results demonstrated differential activation of both the amygdala and occipital visual regions in response to CS+/CS- cues. Overall, this work suggests that initially neutral visual cues that acquire a learned threat value can rapidly modulate neural processing in visual sensory cortices, via a dedicated subcortical pathway involving the amygdala, which was observed in adolescents in the present study.

Surprisingly, however, this CS+/CS- discrimination was not found in either the P1 or N1 visual ERPs in the adult participants. This is inconsistent with a number of studies which have found CS+/CS- discrimination in occipital visual regions in adults (e.g., Dolan et al., 2006; Pizzagalli et al., 2003; P. S. Wong et al., 1997). The lack of effect in this study cannot be explained by a lack of fear conditioning, as both age groups demonstrated successful fear conditioning and immediate extinction according to their explicit behavioural responses. Furthermore, adolescents and adults rated the US as being equally as unpleasant, and allocated a similar degree of early attentional auditory resources to processing the US, so the lack of effect cannot be explained by a lack of fear. Instead, the lack of discrimination in adult P1 and N1 responses to the CS+ versus CS- may be because adolescents and adults engage different fear networks when processing learned danger cues, as at the implicit neural level (visual N1) only

adolescents' demonstrated potentiation to the CS+ compared to the CS-, which did not reduce following an immediate extinction protocol. This is supported by work by Stolarova et al. (2006) who did not find CS+/CS- differentiation in adult P1 and N1 responses either. In that study, participants (M age = 25.6 years) were presented with grating patterns as CS cues and unpleasant affective pictures as the US cues. Whilst these authors did find greater visual C1 responses to CS+ compared with CS- in adults during conditioning, adults did not demonstrate CS+/CS- discrimination in visual P1 and N1 responses. The authors argued that studies which report modulations of visual P1 and N1 components often utilise salient affective pictures, such as emotional faces (e.g., Dolan et al., 2006; Pizzagalli et al., 2003; P. S. Wong et al., 1997). This contrasts with the small black and white gratings employed in their study, and with the small geometrical shapes employed in the present study. As such, it possible that the inherent salience of the emotional faces interacted with the fear conditioning process in adults in these previous studies (Dolan et al., 2006; Pizzagalli et al., 2003; P. S. Wong et al., 1997). However, this would need to be examined in future work, by replicating the current study with more salient CS cues.

Additionally, inconsistencies between the present study and others (Dolan et al., 2006; Pizzagalli et al., 2003; P. S. Wong et al., 1997) concerning adult P1/N1 modulation during fear conditioning, may have resulted from age-related differences in the adult populations examined by each study. For example, studies which report no adult P1/N1 modulations in response to CS+/CS- cues utilised adult age groups which better reflect adulthood (25-26 years in the present study, 25.6 years in Stolarova et al., 2006). In contrast, studies which do report adult P1/N1 modulations utilise adult age groups which also consist of late adolescents (mean age = 23.9 years in Baas et al., 2002; mean age = 19.1 years in Pizzagalli et al., 2003). The inclusion of late adolescents as part of adult groups was highlighted as a significant issue in the systematic review of the adolescent fear conditioning literature in Chapter 4, and suggests instead that the CS+/CS- differentiation observed in adults' early visual responses in these previous studies (Dolan et al., 2006; Pizzagalli et al., 2003; P. S. Wong et al., 1997) may better reflect late adolescent neural activity.

Alternatively, instead of the ERP results reflecting immaturity of visual areas, it is possible that the modulation of ERPs observed in the adolescent group may reflect differences in emotion processing between adolescents and adults. Specifically, that adolescents did show greater implicit fear during the conditioning experiment, which

resulted in greater N1 responses to the CS+ versus the CS-. As discussed in Chapter 1, neurobiological models of adolescence have suggested that adolescence is characterised by early maturation of the emotional subcortical system and late maturation of the cognitive cortical system. Because of this developmental mismatch, adolescents are theorised to be more emotionally responsive and less able to exercise cognitive control over these emotional reactions (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010). For example, previous work has shown exaggerated activation of the amygdala in adolescents who encountered or anticipated aversive stimuli, relative to adults and children (Galvan & McGlennen, 2013; Hare et al., 2008). In the context of the current Chapter, it could be argued that adolescents expressed a greater degree of fear towards the conditioned stimuli that was sufficient enough to elicit enhanced activation of the amygdala, which led to subsequent modulation of adolescents' early visual responses to the stimuli. In contrast, although the adults may have found the US fearful enough to learn the CS-US association, as demonstrated by their behavioural responses, their level of fear may not have been sufficient enough to elicit activation of the amygdala and subsequently modulate adults' visual responses to the stimuli. Together, these differences in emotional responding may explain why the adult group did not show evidence of amygdala-mediated implicit conditioning when their ERPs were examined.

This theory would have been strengthened by examining differences in the degree of autonomic fear conditioning using SCR, as these responses also reflect implicit fear conditioning associated with amygdala activation (Cheng, Richards, Helmstetter, 2007). However, because I did not observe significant fear conditioning in either age group using SCR, the degree of the CS-US associations cannot be compared using this measure. Similarly, without fMRI data, it is not possible to confirm whether or not there were differences in amygdala activation between the adolescent and adult age groups.

5.4.3. Explicit measures of conditioning and extinction

During fear acquisition, the present study found no age differences in the degree of conditioning when examining evaluative CS valence ratings, consistent with other studies (Den et al., 2015; Experiment 1, Lau et al., 2011; Waters et al., 2017). However, adolescents were slower to discriminate between CS+ and CS- cues compared to adults, as indexed by their CS-US contingency awareness ratings, which is consistent with

previous work utilising US expectancy (Den et al., 2015). In that study, adolescents incorrectly rated the CS- as being significantly more likely to be followed by the US, relative to adults (Den et al., 2015). During fear extinction, no age differences in the degree of immediate extinction were found, as indexed by participants' explicit knowledge of the CS+/CS- contingencies and post-extinction evaluative CS ratings, consistent with previous studies which also measured immediate extinction using self-reported fear ratings and/or US expectancy (Den et al., 2015; Waters et al., 2017).

Despite this, Waters et al. (2017) did observe age differences in extinction learning when examining trial-by-trial evaluative CS ratings. Specifically, the authors reported blunted extinction both adolescents (15-18 years) and adults (25+ years), with adolescents also maintaining less pleasant ratings of both the CS+ and CS- relative to the adults. This suggests that adolescents were slower to re-evaluate their negative perceptions of the CS cues during extinction, a phenomenon that could only be observed when extinction was tracked more frequently. Therefore, more frequent assessment of behavioural ratings (i.e., trial-by-trial) may provide a more sensitive index of extinction learning. As such, the lack of age differences in the evaluative CS ratings of the current study, relative to the age differences observed in contingency awareness ratings, could be because the evaluative ratings were only examined at the end of each task phase (consisting of 4 runs), instead of being measured after each run. This suggestion is strengthened when examining the implicit ERP results, which established clear age differences.

As discussed in Chapter 4 (section 4.4.2.2) differences between implicit and explicit outcome measures may reflect the engagement of different fear networks, which may mature at different rates in the transition from adolescence to adulthood. For example, adolescents were slower to explicitly discriminate between CS contingencies, whilst adults were explicitly aware of the contingencies after each run of acquisition and extinction. Conversely, adolescents demonstrated successful modulation of early visual N1 responses to fear learned CS+ versus CS- cues, whilst adults did not show such modulation. Together, these results suggest that adolescents and adults may engage different fear networks during Pavlovian fear conditioning, with evidence that explicit learning may continue to mature throughout adolescence.

These developmental differences across implicit and explicit measures share some consistencies with a dual process account of fear conditioning, which also proposes a dissociation between explicit and implicit measures of conditioning.

Specifically, dual process accounts suggest that conditioning can occur implicitly without explicit awareness (Bechara et al., 1995; Schultz & Helmstetter, 2010). These differences were highlighted in an fMRI study by (Knight et al., 2009). In that study, participants took part in a Pavlovian fear conditioning task, in which tone CS cues were presented either just above (perceived trials) or just below (unperceived trials) their perceptual detection threshold, to examine whether explicit (US expectancy) and implicit (SCRs) measures are mediated by separate fear learning processes. Their results demonstrated that participants were unable to explicitly learn the CS+/CS- contingencies when the CS cues were presented below their perceptual threshold. However, when CS cues were presented above their perceptual threshold, participants did learn the contingencies, with greater contingency awareness supported by greater hippocampal and parahippocampal activity in response to CS+ versus CS-, suggesting a role for these brain regions during explicit fear learning. In contrast, participants demonstrated implicit autonomic conditioned responses regardless of whether or not participants perceived the CS cues, which were accompanied by greater amygdala activity in response to CS+ versus CS-. These results suggest that the amygdala can modulate conditioned SCRs even when participants are not explicitly aware of the contingencies. Together, these findings demonstrate that both implicit and explicit fear learning processes exist, but function according to different neural networks, with explicit awareness associated with hippocampal activity and implicit autonomic awareness associated with amygdala activity. The results of the present study add to this body of work, and also provide evidence to suggest that adolescents and adults may engage different fear networks during conditioning, with adolescents (not adults) demonstrating reinforcement-dependent potentiation of early visual responses to threat, with this study additionally demonstrating developmental differences in the maturation rates of participants' explicit contingency awareness knowledge – with adolescents taking longer to learn the CS contingencies relative to adults.

5.4.4. Implications for neurobiological models of adolescence

Our results do not support the view that adolescents are hypo-responsive to aversive stimuli (Spear, 2011, 2013). According to Spear's neurobiological model of adolescence (2011; Chapter 1, section 1.7.3), adolescents should show weaker acquisition of conditioned fear responses, because adolescents should be less sensitive to the aversive properties of the US (Doremus-Fitzwater & Spear, 2016). However, in

the current study, adolescents and adults demonstrated comparable levels of fear conditioning and extinction according to their evaluative CS ratings and awareness of the CS contingencies, albeit with adolescents taking longer to learn the CS contingencies compared with adults. Moreover, only adolescents demonstrated greater reinforcement-dependent potentiation of implicit visual N1 responses to the CS+ versus CS-, which did not extinguish. Therefore, whilst evidence from nonhuman animal studies characterises adolescents as being hypo-responsive to aversive stimuli (Doremus-Fitzwater & Spear, 2016; Doremus-Fitzwater et al., 2010; Schramm-Sapyta et al., 2006; Torres et al., 2008), the present study with human participants, and others (Barkley-Levenson et al., 2013; Howsley & Levita, 2017; Levita et al., 2015), do not support this view.

Instead, this work provides tentative evidence that adolescents may be hyper-responsive to threatening stimuli, as demonstrated by enhanced early visual N1 responses to threat-predicting CS+ versus CS- cues, compared to adults. Hyper-responsivity of visual cortical brain regions in adolescents cannot currently be explained by any of the neurobiological models of adolescence (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008), as these models are focused on adolescent behaviours in the context of early-maturing subcortical and late-maturing prefrontal cortical brain regions. As a result, current models would need to be extended to incorporate developmental changes to early visual neural networks.

5.4.5. Study limitations and future directions

The present findings should be considered in light of the study limitations. First, I employed an immediate extinction procedure, in which participants underwent extinction straight after the acquisition phase of the experiment. However, past research that has reported attenuated extinction learning in adolescents' autonomic SCRs compared with adults has assessed delayed (24 hrs) extinction (e.g., Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). Therefore, future studies should seek to replicate the current paradigm and assess extinction 24 hours after conditioning. This will enable a fuller understanding of potential developmental and gender-dependent differences in the extinction of early visual responses to aversive CS+ cues.

Second, the SCR results, showing a lack of dissociation between CS+ and CS-, was not expected, and is inconsistent with other fear conditioning studies which utilized 50% reinforcement and did reveal greater SCRs to CS+ compared to CS- cues (Johnson & Casey, 2015; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). This lack of effect could be due to the greater number of conditioning trials used in this paradigm, which were needed to accurately capture the ERP components. Moreover, the SCR analysis included all CS+/CS- trials that were presented throughout the acquisition and extinction phases, which contrasts with studies which often only report data for selected segments of their conditioning and extinction procedures. For example, in (Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012), acquisition was assessed by comparing SCRs to the CS+ versus the CS- in last of three runs of acquisition, and extinction was quantified by subtracting the mean SCR of the last two CS+ trials from the first two CS+ trials. In (Johnson & Casey, 2015), extinction was quantified by comparing the first five and the last five CS+/CS- trials. Furthermore, in order to present a sufficient number of ERP trials for each condition, trial times were reduced (4500-5000 ms), which constrained the time window that could be used to examine autonomic responses to the CS cues (1000-4000 ms post-stimulus). However, to overcome this issue, I conducted a continuous decomposition analysis to separate skin conductance data into tonic and phasic activity, to assess underlying sudomotor activity in a temporally precise way, whilst avoiding confounds relating to overlapping SCRs. Given the lack of fear acquisition observed in the autonomic responses of this study, future work could examine this effect further by implementing longer trial windows in their EEG fear learning paradigms.

Third, based on earlier pilot work, it was determined that 80 trials per condition would be optimal for the present study, to ensure there were enough trials to reliably measure the P1 and N1 visual components. Reducing the trial numbers in the current study would have significantly increased the signal-to-noise ratio, resulting in potentially spurious results (false positives or false negatives). However, the majority of classic fear conditioning studies use 5-20 trials to observe conditioned fear responses (Lonsdorf et al., 2017). Therefore, there is a discrepancy between the number of trials needed to observe fear conditioning and the number of trials needed to accurately measure early visual components. This suggests that reinforcement-dependent potentiation may have been present in the adult group early on during the experiment, but their visual responses habituated as the experiment went on. This seems unlikely,

given early EEG work that used a similar number of trials per condition (n = 56) and observed reinforcement-dependent modulation of the visual P1 and N1 components (Pizzagalli et al., 2003), and with MEG work that observed P1 modulation in adults following a fear conditioning study with 400 CS+ trials and 400 CS- trials (Dolan et al., 2006). Future work could utilise larger trial numbers and examine differences between early and late acquisition/extinction trials, to determine whether adults do show earlier habituation of visual P1/N1 responses.

5.4.6. Suitability of a Pavlovian fear conditioning paradigm to examine emotion processing in both adolescents and adults

Due to the small condition effects, task difficulty, and issues regarding the use of emotional face processing models to examine emotion processing in adolescents and adults in Chapters' 2 and 3, I decided not to extend these chapters for use with a younger adolescent population. Instead I opted to examine emotion processing in adolescents and adults using Pavlovian fear conditioning, a highly-controlled and well-established model of threat processing. However, the relative weaknesses of this paradigm should also be considered, as well as their impact on the study of potential age group differences in emotion processing from adolescence to adulthood.

First and foremost, because effect sizes were not estimated in advance of conducting the current study (as in Chapters 2 and 3), this study could have again resulted in small condition effects between the CS+ and CS-, which would have made it difficult to detect age group differences. Secondly, adults did not demonstrate fear conditioned responses in their P1 and N1 ERP responses, which makes it difficult to make direct comparisons between the adolescent and adult visual responses to the CS+/CS- cues. In an optimal scenario, both groups would have shown some CS+/CS- differentiation at the level of the P1 and N1, so that differences in the strength of acquisition and extinction could be examined. Thirdly, the absence of fear conditioning effects reported in adults visual P1 and N1 responses may have resulted from age differences in the rate of acquisition. For example, perhaps adults' ERP fear responses to the CS+ habituated faster that adolescents', which resulted in a lack of CS+/CS- differentiation when early and late ERP trials were averaged together. If this were the case, age differences in the rate of acquisition could be being masked by the use of

many ERP trials in the current chapter (80 per condition). Therefore, whilst the current fear conditioning paradigm took onboard the pitfalls associated with high task difficulty and of isolating emotion-specific processes from facial expressions (as observed in previous paradigms in Chapters' 2 and 3 of this doctoral work), this approach did result in additional difficulties when attempting to examine threat processing from adolescence to adulthood.

5.5. Conclusions

Adolescence has been characterised as a sensitive period for heightened levels of anxiety and the development of anxiety disorders (Beesdo et al., 2009; Kessler et al., 2007; Kessler et al., 2009; Lijster et al., 2017). Some theorize that this increased anxiety results from a reduction in fear extinction during adolescence (Johnson & Casey, 2015; Morriss et al., 2018; Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). However, our current understanding of adolescent fear learning is limited. The present study took a multi-modal approach in the study of adolescent fear learning, by examining behavioural, physiological, and neural correlates in response to visually presented CS+ and CS- cues during a differential fear conditioning and extinction task. The results of this study provide support for a developmental dissociation between implicit and explicit measures of fear learning. Specifically, regarding their implicit visual responses, only adolescent males demonstrated significant potentiation of P1 responses to the CS+ versus CS-, which were immediately extinguished. Furthermore, adolescent males and females demonstrated greater N1 responses to CS+ versus CS- cues, which were not extinguished. There was no evidence of P1/N1 modulation in adult male or female responses during conditioning or extinction. In contrast, all participants demonstrated successful conditioning and extinction according to their explicit knowledge of the CS contingencies and their evaluative CS ratings, although adolescents were slower to learn the CS contingencies relative to adults. Together, the results of this study provide support for a dual process account of fear conditioning, in which implicit (ERPs) and explicit (contingency awareness, CS evaluative ratings) learning are mediated by different fear networks. Moreover, this study provides evidence to suggest these different fear networks mature at different rates, resulting in a developmental dissociation between adolescents' and adults' implicit and explicit measures of fear conditioning and extinction.

Chapter 6. General Discussion

6.1. Introduction

Adolescence is a transitional phase of development, characterised by a cascade of developmental changes which can impact behaviour, and which have been associated with increased risk-taking (S. Burnett et al., 2010; L. Steinberg, 2008), and with increased emotionality (Casey, Jones, et al., 2010). These behavioural changes occur in tandem with maturational changes to key cortical (i.e., cognitive) and subcortical (i.e., affective) brain regions throughout adolescence (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010; Shulman et al., 2016). A number of neurobiological models of adolescence have been proposed to account for these changes (Chapter 1, Section 0), with a view to explaining why adolescence is associated with increased risk-taking behaviours (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008). However, these models, and the research that supports them, have not yet fully assessed the mechanisms of increased emotionality reported during adolescence (Casey, Jones, et al., 2010), as compared with adulthood. Understanding this increase in emotionality during adolescence is important, to help explain why adolescents are at an increased risk of developing symptoms of anxiety and anxiety disorders (Beesdo et al., 2010; Kessler et al., 2007; Kessler et al., 2005; Lijster et al., 2017; McGorry et al., 2011; Pine et al., 1998).

Consequently, throughout this thesis, my main aim was to provide a better understanding of adolescent emotion processing, and how this develops in the transition to adulthood. To that end, this doctoral work presents an examination of the suitability of both a temporal recalibration paradigm (Chapter 2) and an emotional voice-face integration task (Chapter 3) for use with a younger developmental population, by first examining the ERP and/or behavioural responses of two late adolescent populations. Following this, evidence was presented in favour of a Pavlovian fear conditioning approach in providing a highly-controlled measure of adolescent threat processing. Subsequently, a systematic review of the fear conditioning and extinction literature was conducted, with all available adolescent data (Chapter 4), to guide the design and implementation of a Pavlovian fear conditioning task with both adolescents (13-14 years) and adults (25-26 years) (Chapter 5). The present chapter will begin by outlining the key findings of this doctoral work (section 6.2) and the main conclusions which can be drawn from these findings (section 6.3), followed by a detailed discussion of the strengths (section 6.4) and limitations (section 6.5) of this research. Importantly, this

Chapter will discuss the implications for future research based on my findings (section 6.6), before drawing a number of final conclusions (section 6.7).

6.2. Summary of key findings

Chapter 2 investigated how late adolescents bound motor-sensory events together in time, as part of a temporal recalibration paradigm, and whether this process could be influenced by threat-related faces. Previous temporal recalibration studies have focused on the precise temporal processing of simple artificial stimuli, such as beeping or flashing cues (e.g., Fujisaki et al., 2004; Stetson et al., 2006; Timm et al., 2014; Vroomen et al., 2004). However, Chapter 2 provides an extension of this work, by presenting the first known examination of how biologically-significant facial stimuli interact with the process of temporal recalibration, in a late adolescent sample. The results of this chapter demonstrated an enhanced temporal recalibration transference effect for fearful faces, compared with neutral faces, which suggests that temporal recalibration processes can be influenced by stimulus-driven attention for the visual processing of faces which signal threat. However, for reasons relating to high task difficulty and small condition effects, it was concluded that this paradigm would not be optimal for use with younger developmental populations.

Chapter 3 took on board the difficulties associated with conducting a temporal recalibration task with younger adolescents, and instead implemented a simple emotion categorisation task, which could be used with EEG. Specifically, participants were presented with emotional vocalisations (laughter and crying) followed by the presentation of congruent or incongruent emotional faces (happy and sad). Unexpectedly, an enhancing effect of emotional congruency on response times was not observed during the emotional categorisation task. However, the results of this study demonstrated enhanced visual P1 responses to happy congruent relative to happy incongruent voice-face pairs. Given that P1 modulation was not reported for sad congruent relative to sad incongruent voice-face pairs, these results provide support for a valence-dependent enhancing effect of congruency on early visual responses to emotional face stimuli. Modulation of the N170 component by emotional voice-face congruency was not observed. However greater N170 amplitudes were associated with faster reaction times in each of the conditions, which provided evidence instead for a functional dissociation between the visual P1 and N170 components. Following a

discussion regarding the difficulties associated with isolating the emotion-specific effects of face processing, it was determined that a return to first principles (i.e., Pavlovian associative learning) would be optimal for assessing emotion processing in the transition from adolescence to adulthood.

Chapter 4 consisted of a systematic review which synthesised the existing literature on adolescent Pavlovian fear conditioning and extinction. The results of this review provided evidence of successful fear acquisition in both adolescents and adults when implicit autonomic responses are measured, with some evidence to suggest adolescents exhibit poorer CS+/CS- discrimination when explicit self-report measures are examined. Regarding extinction, the review suggests both adolescents and adults demonstrate successful immediate extinction of implicit autonomic responses and explicit self-report measures. However, adolescent-specific impairments in delayed extinction, as well as extinction recall, were observed in autonomic responses of adolescents relative to adults. The fMRI work outlined in this review suggested that impairments in extinction processes may result from immaturity of PFC regions that are integral to successful fear extinction (e.g., vmPFC and dlPFC). Regarding the quality of the studies appraised, this review highlighted that current knowledge of fear conditioning and extinction processes in human adolescents is based on a very small number of developmental studies. More specifically, the majority of work that has assessed fear learning processes in adolescence has neglected to include adult or child comparison groups. In addition, a lack of consistency regarding experimental task protocols appear to be contributing to the contradictory findings that have been reported so far regarding adolescent fear acquisition and extinction learning. For example, different types of CS-US cues (e.g., lights, faces, shapes), and different outcome measures (e.g., self-report, skin conductance, EEG), have created challenges in understanding the precise differences in fear conditioning and extinction processes in adolescent populations. Notably however, the evidence outlined in this review does provide support for a dual process account of fear learning, in which explicit (self-report) and implicit (autonomic, brain activation) processes of fear conditioning appeared to mature at difference rates in adolescent relative to adult populations. This chapter concludes by making recommendations for future studies in this area, specifically, to make comparisons between age-appropriate adolescent and adult populations, utilise emotionally-neutral CS-US cues, and take a multi-modal approach to fear learning.

Chapter 5 took on board the recommendations made following the systematic review presented in Chapter 4, by examining Pavlovian fear conditioning and extinction in two developmentally distinct samples of adolescents (13-14 years) and adults (25-26 years). In order to gain a more precise understanding of fear learning processes, multiple outcome measures of conditioning and extinction were obtained, including explicit (self-report), physiological (SCR) and electrophysiological (ERP) measures. The behavioural measures indicated successful conditioning and extinction in both adolescents and adults, with some age-differences observed in the rate of acquisition, indexed by participants' awareness of the CS+/CS- contingencies. However, despite observing similarities in adolescents' and adults' explicit behavioural responses, the ERP results demonstrated both age and gender differences in the early visual processing of fear conditioned cues. Firstly, at the level of the visual P1, only male adolescents showed enhanced P1 amplitudes in response to threat-predicting CS+ versus CS- cues, a difference that was abolished during extinction. Secondly, at the level of the visual N1, adolescents, but not adults, showed enhanced N1 amplitudes in response to CS+ versus CS- cues during acquisition, a dissociation which remained during the extinction phase. As adults did not demonstrate CS+/CS- differentiation in their early visual responses, these results suggest different neural networks may be engaged when adolescents and adults process learned danger cues.

6.3. Conclusions from key findings

The work presented in this thesis has assessed the suitability of three separate experimental approaches for studying emotion processing in adolescence (Chapters 2, 3, and 5). Overall, the findings of this body of work propose that the study of emotional face processing may not be an optimal model for adolescent emotion processing, due to issues when attempting to isolate emotion-specific processes, over-and-above the effects of social information processing and structural face processing. Conversely, this doctoral work suggests that a Pavlovian fear conditioning approach may be a more useful model for the study of emotion (e.g., threat) processing in adolescence. This approach was favoured as it provides a well-established model of threat processing and emotional learning, which has been used previously to assess transitions into and out of adolescence, whilst also demonstrating strong translational value across species (e.g., Pattwell, Duhoux, Hartley, Johnson, Jing, Elliott, et al., 2012). As such, Chapter 5 provides a validated paradigm to assess fear conditioning and extinction in adolescents,

which is also suitable for use with EEG. Importantly, the data from this thesis supports the implementation of a multi-modal approach to studying emotion processing, through the use of behavioural, physiological, and EEG measures. Next, the following sections will outline how the results from this doctoral work can inform current neurobiological models of adolescence.

6.3.1. Implications for current neurobiological models of adolescence

Instead of emotional face processing, Chapters 4 and 5 present evidence to suggest that a Pavlovian fear conditioning approach may provide a more fruitful model in which to study adolescent behaviour and brain development, which could improve current neurobiological models of adolescence. As discussed in Chapter 1, a number of limitations restrict the explanatory power of these models. First, studies of emotion processing during adolescence have largely focused on data from fMRI studies and nonhuman animals, which have neglected to study how emotional stimuli are processed by the visual system. Second, whilst all six models (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008), attempt to explain how adolescents process rewarding stimuli, just three of these models (Casey, Getz, et al., 2008; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006) attempt to explain how adolescents process threatening stimuli. Third, of the three models that assess adolescent threat processing, each provides a contradictory explanation regarding precisely how threatening stimuli are processed. For example, whilst the Triadic Model (Ernst, 2014; Ernst et al., 2006), and Spear's Reward-Centricity Model (Doremus-Fitzwater & Spear, 2016), suggest that adolescents are hypo-responsive to threatening or aversive stimuli, the imbalance model (Casey, Getz, et al., 2008; Casey, Jones, et al., 2010) proposes that adolescents may be hyper-responsive to threatening stimuli.

Consequently, the work presented in this thesis addresses these limitations in a number of ways. First and foremost, this doctoral work uses EEG to study the precise temporal processing of emotional stimuli in early perceptual areas, adding to the weight of literature that has focused on fMRI and nonhuman animals thus far. Furthermore, the use of EEG enabled a direct examination of the posterior visual regions that are discussed as part of the Social Information Processing Network (Nelson et al., 2005), but have so far been assumed to be largely mature by adolescence. Second, Chapter 4

presents a systematic review of the fear conditioning literature in the transition from adolescence to adulthood, to overcome a lack of consideration for adolescent threat processing in current neurobiological models of adolescence. Third, Chapter 5 directly assesses adolescents (13-14 years) and adults (25-26 years) behavioural, physiological, and early visual responses to CS+ versus CS- cues, to elucidate the debate regarding whether adolescents are hypo- or hyper-responsive to threatening stimuli. In addition, given a lack of consideration for potential gender differences in current neurobiological models, the findings of this work also indicate how gender may be interacting with the processes of fear learning, and how this might contribute to our understanding of fear mechanisms. Together, this doctoral work overcomes a number of limitations regarding current neurobiological accounts of adolescent behaviour and development.

Overall, the results presented in this thesis provide tentative evidence that adolescence may be characterised as a period of hyper-responsivity to threatening cues. The EEG findings of Chapter 5 are consistent with this argument, as they suggest developmental differences in the engagement of early perceptual processes during fear conditioning and extinction, in adolescents relative to adults. Specifically, adolescents exhibited enhanced visual N1 responses to threat-predicting CS+ cues compared with CS- cues, which did not reduce following an immediate extinction procedure. In contrast, adults did not show conditioning of early visual responses to CS+ versus CS- cues. Moreover, in that study adolescents demonstrated significantly greater autonomic responses to the aversive US compared with adults. These findings are in line with work showing greater activation of key brain regions in response to aversive stimuli in adolescents compared with adults (e.g., the amydala and ventral striatum; Britton et al., 2013; Galvan & McGlennen, 2013), and add weight to the argument that adolescents may exhibit hyper-responsivity to threatening stimuli.

However, if adolescents do show hyper-responsivity to threatening or aversive stimuli, why is adolescence associated with increased risk-taking? Increased risk-taking during adolescence (e.g., S. Burnett et al., 2010; S. H. Mitchell et al., 2008; L. Steinberg, 2010), has been explained by developmental changes to adolescent reward systems, with extensive behavioural and fMRI literature suggesting that adolescents exhibit hyper-responsivity to rewarding stimuli (Ernst et al., 2005; Galvan et al., 2007; Galvan et al., 2006). Therefore, it is possible that adolescent hyper-responsivity to threatening cues may be overridden when adolescents find themselves in risky situations in which there is a potential for reward. This could be due to hyper-activity of

the reward neurocircuitry (e.g., Doremus-Fitzwater & Spear, 2016), in combination with adolescents' weaker ability to engage top-down cognitive control processes (Casey et al., 2000; Giedd et al., 1999; Sowell et al., 2001). However, this theory is currently only speculation and, as a result, the interaction between threat and reward systems and its relation to adolescent risk-taking would need to be explored further in future studies.

The results of this doctoral work have provided no support for Spear and colleagues view that adolescents' exhibit hypo-responsivity to threatening or aversive stimuli (Doremus-Fitzwater & Spear, 2016; Doremus-Fitzwater et al., 2010). By their notion, adolescents should have exhibited weaker acquisition of fear conditioned responses in Chapter 5, because adolescents are expected to be more resistant to the effects of aversive stimuli. In addition, adolescents should have exhibited weaker reactivity to the aversive US in Chapter 5, but instead the results suggested equivalent reactivity when assessing unpleasantness ratings and early auditory processing of the US, and even demonstrated significantly greater physiological reactivity (SCR) to the US in adolescents compared to adults. Spear's view is based on findings from nonhuman animal studies, which have suggested adolescents may be hyper-responsive to rewarding stimuli but hypo-responsive to aversive stimuli. For example, this evidence suggests that adolescent rats are more sensitive to the rewarding effects of potential drugs of abuse (e.g., Brenhouse & Andersen, 2008; Torres et al., 2008; Vastola et al., 2002; Zakharova et al., 2009) and are less sensitive to their aversive effects (Cobuzzi et al., 2014; Ramirez & Spear, 2010; Schramm-Sapyta et al., 2006), when compared to adult rats. However, whilst human work also suggests that adolescents are hyper-responsive to rewards (Ernst et al., 2005; Galvan et al., 2007; Galvan et al., 2006), human work suggesting adolescents are hypo-responsive to threats is currently lacking (Moutsiana et al., 2013). Thus, whilst human and nonhuman animal literature consistently support the theory that adolescents are hyper-responsive to rewarding stimuli, the human and nonhuman animal literature on adolescent hypo-responsivity to threatening stimuli is less conclusive.

The question remains why there are contradictions between Spear's work with nonhuman animals, suggesting adolescents are hypo-responsive to threatening stimuli (Doremus-Fitzwater & Spear, 2016; Doremus-Fitzwater et al., 2010) and with human work presented in this thesis and elsewhere in the literature, suggesting adolescents are hyper-responsive to threatening stimuli (Howsley & Levita, 2017; Levita et al., 2015). As discussed in Chapter 1 (section 1.7.3), it is possible that methodological differences

across research groups could explain these inconsistencies. For example, Spear and colleagues examine adolescent responses to the aversive effects of drugs of abuse with both positive and negative reinforcing properties, such as repeated doses of cocaine, MDMA, or methamphetamine. However, when stimuli with solely aversive properties are used (e.g., loud tones, aversive liquids), adolescents demonstrate enhanced visual responses to avoidance-related cues (Howsley & Levita, 2017; Levita et al., 2015), and enhanced activation of subcortical affective brain regions in response to aversive cues (e.g., the amygdala and ventral striatum; Britton et al., 2013; Galvan & McGlennen, 2013). This work suggests that adolescents may respond differently to threats depending on the properties of the aversive stimuli employed.

These differences in responses depending on the nature of the aversive stimulus are further illustrated by the impact of primary and secondary reinforcers. For example, a primary reinforcer can elicit an innate response from an organism (e.g., shocks, aversive liquids, loud sounds), whereas a secondary reinforcer acquires its properties through association with a primary reinforcer (e.g., money Delgado et al., 2011). This was demonstrated in Chapter 5 and in other work from my laboratory (Levita et al., 2015), which reported enhanced early visual responses (e.g., N1 and N170) to threat-predicting cues when using a primary reinforcer (e.g., loud tone), whilst other recent work failed to replicate this enhancing effect on the N170 when using a secondary reinforcer (e.g., loss of points in a game, Howsley & Levita, 2017). This suggests that developmental differences in early visual processes during conditioning tasks may be partially explained by differences in the relative salience of the aversive cues employed across different task paradigms. Taken together, this work suggests that the properties of an aversive stimulus must be appropriately considered when examining adolescent threat responses, as differences in primary and secondary reinforcement could lead to significantly different interpretations of adolescent fear mechanisms.

Importantly, the work in this thesis, which demonstrates evidence of enhanced perceptual processing of learned threat cues in adolescents relative to adults, cannot be clearly mapped onto any of the current models of adolescence. This highlights a lack of completeness of each of these models, as most have focused on mechanisms of reward, and how these mechanisms develop in the transition from adolescence to adulthood (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006; Luna & Wright, 2016; Nelson et al., 2005; L. Steinberg, 2008). In addition, each of these models have focused on a subset of cortical (i.e., cognitive) and subcortical (i.e.,

affective) brain regions, which neglects potential developmental differences in other neural networks, such as in posterior visual regions. Although three of these models provide some possible explanations of adolescent threat processing (Casey, Jones, et al., 2010; Doremus-Fitzwater & Spear, 2016; Ernst et al., 2006) research on this topic is still in its infancy, and future replications of the conditioning model used in this doctoral work are necessary to further explore the possibility of adolescent hyper-responsivity to threat. Overall though, based on my findings, I suggest that current neurobiological models of adolescence should be extended to incorporate developmental changes in visual cortical regions during both reward and threat processing.

6.3.2. Gender differences in fear learning

The work presented in this thesis highlights the importance of considering gender differences as part of adolescent emotion processing research. For example, Chapter 5 reports a gender-dependent difference in adolescent visual responses to threat-predicting CS+ versus CS- cues, at one of the earliest measurable visual ERP responses (P1 component). Specifically, adolescent males demonstrated greater P1 responses to CS+ versus CS- cues during fear acquisition, which significantly reduced during fear extinction. In contrast, adolescent females did not show CS+/CS discrimination in P1 responses during fear acquisition, and P1 responses to the CS+ increased from acquisition to extinction. This is early evidence to suggest that adolescent male and females respond to fear learned cues differently, when examining implicit perceptual responses. This finding could be explained by differences in brain development, based on work which as demonstrated the existence of gender differences in cortical and thalamic grey matter volume during adolescence (see Lenroot & Giedd, 2010 for a review), and based on work which demonstrated a peak in grey matter volume that occurs 1-2 years earlier in females compared with males (Lenroot et al., 2007). Furthermore, the observed gender differences in the processing of learned fear cues may also be related to other gender differences that have been reported in the literature, such as the increased risk of anxiety experienced by females compared to males (Abe & Suzuki, 1986; Lewinsohn et al., 1998), as well as the increased risk-taking behaviours observed in males compared to females (Byrnes et al., 1999). However, despite this evidence, an understanding of gender differences in adolescent behaviour and brain development is still in its infancy, and would need to be explored in more detail in future work.

6.4. Strengths of this work

One of the main strengths of this doctoral work regards its successful validation of a Pavlovian fear conditioning study, that implements EEG methodology, and which can be used to study developmental differences in threat processing. The use of a Pavlovian conditioning model is particularly useful, given its ability to examine transitions into and out of adolescence, as well as its ability to examine how behaviours are translated across different species (Casey, Duhoux, & Cohen, 2010). The strength of Pavlovian conditioning as both a transitional and translational model was well-demonstrated in a study by Pattwell et al., (2012), which assessed fear conditioning and extinction in a sample of human children (5-11 years), adolescents (12-17 years) and adults (18-32 years), whilst running parallel study with mice that had reached postnatal day 23 (P23), early adolescence (P29), or early adulthood (P70). The authors reported evidence that both adolescent humans and adolescent mice demonstrated attenuated fear extinction processes, relative to the children and adults. These results are consistent with other human and non-human animal work showing attenuated fear extinction processes in adolescents (e.g., Johnson & Casey, 2015; McCallum et al., 2010), and with the results of Chapter 5, which showed evidence of attenuated fear extinction in adolescents early visual N1 responses to CS+ versus CS- cues, compared with adults. Together, these studies continue to strengthen the argument that Pavlovian fear conditioning is an appropriate tool for studying transitional and translational changes in adolescent brain and behaviour, and that adolescents may be experiencing impairments in extinction learning relative to adults.

EEG has proved to be a particularly useful methodology in the current doctoral thesis. As discussed in Chapter 1 (section 1.7.1.2), the majority of work that has assessed emotion processing in adolescence has used fMRI technology (e.g., Britton et al., 2013; Ernst et al., 2005; Galvan et al., 2007; Galvan et al., 2006; Galvan & McGlennen, 2013; Hare et al., 2008; Lau et al., 2011; Monk et al., 2003). This fMRI work has been integral to our current understanding of adolescent brain development, and how subcortical and cortical brain regions function during both threat and reward processing. However, whilst fMRI work has proved useful in delineating the specific brain regions involved in adolescent emotion processing, its temporal resolution is poor. As a result, fMRI results cannot tell the whole story, which is why an examination into the precise neural time course of emotion-specific processes using EEG has played a central role in this doctoral thesis. EEG is a relatively inexpensive tool, and can be

easily implemented in developmental studies to examine neural activation in response to emotional stimuli, causing participants little-to-no discomfort. Whilst its spatial resolution is poor, EEG provides excellent temporal resolution, providing additional information alongside fMRI measures. For example, the EEG results from Chapter's 3 and 5 provide evidence that emotional information can modulate ERP components as early as 100 ms post-stimulus, a finding that could not have been observed using fMRI. Together, the use of both fMRI and EEG technology can provide a more nuanced understanding of emotion processing in adolescence.

In addition, one of the key strengths of this work was the use of a multi-modal approach in the study of adolescent emotion processing. As such, this doctoral work utilised various behavioural, physiological and EEG measures to examine emotion processing during the adolescent period. The use of multiple outcome measures was proven to be of particular importance to this field of research, in lieu of the findings of Chapter 4, which suggested that the degree of fear conditioning and extinction observed in adolescents was highly dependent on the outcome measures used in each study. This issue was further highlighted by the findings of Chapter 5, whereby immediate fear extinction was demonstrated in adolescents and adults when examining explicit measures of conditioning (e.g., contingency awareness, evaluative ratings), but attenuated extinction was demonstrated in adolescents when examining implicit measures of conditioning (e.g., N1 responses). It has been suggested that these different measures of conditioning most likely represent different aspects of fear learning (as discussed in chapter 4 section 4.4.2.2). This provides support for a dual process account of fear learning, which suggests that implicit fear conditioning can occur even when participants are not explicitly aware of the CS+/CS- contingencies (Bechara et al., 1995; Schultz & Helmstetter, 2010). Arguably, this dissociation between implicit and explicit measures of conditioning is believed to occur because these measures reflect the activation of different neural fear networks (Hamm & Vaitl, 1996; Hamm & Weike, 2005; Knight et al., 2009; Sevenster et al., 2012). Together, the differences reported in Chapters 4 and 5 demonstrate the importance of examining multiple outcome measures when assessing developmental changes in acquisition and extinction, as the results of individual measures may contradict one another, depending on the fear networks that they represent.

6.5. Limitations of this work

The findings of this doctoral work must also be considered in light of a number of limitations, which are discussed in the following sections.

First and foremost, the work of Chapters 2 and 3 were limited to an examination of emotion processing in late adolescent groups only, without making comparisons with an adult group. Although this clearly limits what these chapters can conclude regarding adolescent emotion processing, this was an intentional decision, as the first main aim of this thesis was to identify an appropriate emotion processing paradigm for use with a younger adolescent population. To achieve this aim, I wanted to explore the utility of a range of potential tasks, which meant first examining these tasks in an older population. Once a meaningful interpretation of the results of these paradigms were obtained using a late adolescent sample, it would then be appropriate to recruit from a developmental population. Despite the lack of younger adolescent groups in these studies, this work has made a significant contribution to knowledge, by providing evidence to suggest that the process of temporal recalibration, which is important for determining the causality between our own actions and externally-generated events, can be modulated by threat-related facial expressions, and by providing evidence to suggest that the neural correlates of early face processing can be influenced by the prior presentation of emotionally-charged auditory information.

Secondly, whilst the focus of this work was on adolescent visual responses to affective stimuli (e.g., emotional faces, fear-conditioned cues), indexed by early visual ERP components (P1, N1, N170), this thesis did not examine the impact of emotional cues on frontally-meditated scalp activity, which would have been indexed by frontal ERP components. Frontal ERP components have been theorised to represent activity from prefrontal cortex regions, such as the medial PFC (van Noordt & Segalowitz, 2012). Differences in frontal ERPs between adolescents and adults would have been expected, based on research that has demonstrated protracted maturation of the PFC, which continues to develop well into young adulthood (Casey et al., 2000; Giedd et al., 1999). This is particularly relevant for Chapters 4 and 5, as evidence suggests that the prelimbic prefrontal cortex is involved in the production of conditioned fear responses (Sotres-Bayon & Quirk, 2010), whilst the mPFC is needed for fear extinction (Quirk, Garcia, & González-Lima, 2006; Sotres-Bayon, Cain, & LeDoux, 2006). Therefore, examining frontal activity would have provided a measure of the top-down modulation of emotion processing during adolescence. However, a recommended strategy for

interpreting ERP components involves focusing on one or two specific components . This is because it becomes increasingly difficult to interpret variations across multiple components across multiple scalp locations, which are generated by different neural networks. Therefore, whilst this doctoral work provides a precise examination of the time course of early visual responses to emotional stimuli, future work would also need to consider the impact of emotional stimuli on frontally-mediated scalp activity for a more complete understanding of emotion processing in the transition from adolescence to adulthood.

Thirdly, this doctoral work did not directly study the potential impact of pubertal development on emotion processing in adolescence. Puberty refers to a series biological changes that are necessary to attain sexual maturation (Spear, 2000b). Work suggests that pubertal development occurs between 10-15 years of age, with females' pubertal development beginning around 1-2 years earlier than males (Carskadon & Acebo, 1993; J. M. Tanner, 1971). Pubertal development can include physical growth spurts, attentional and motivational changes, alterations to the voice and body, and changes to emotional and social processes (Blakemore et al., 2010), all of which can impact their behaviour. For example, in a study assessing adolescents' (10-17 years) reaction times when categorising facial emotions, the authors observed a 10-20% increase in reaction times when adolescents at the average age of puberty onset for boys (12-13 years) and girls (10-11 years) responded to emotional stimuli, an effect which decreased gradually between then and age 17 (McGivern, Andersen, Byrd, Mutter, & Reilly, 2002). Additional fMRI evidence has shown puberty to influence adolescent brain activation in response to emotional faces (Forbes, Phillips, Silk, Ryan, & Dahl, 2011; W. E. Moore et al., 2012), rewards (de Macks et al., 2011; Forbes et al., 2010), and social scenarios (Goddings, Burnett Heyes, Bird, Viner, & Blakemore, 2012; Klapwijk et al., 2013), independently of chronological age. Taken together, these results suggest that adolescents processing of emotional stimuli are intrinsically linked to pubertal development. As a result, the differences observed in early visual P1 responses reported in adolescent males and females in Chapter 5 could have also been influenced by differences in pubertal development between adolescent girls and boys. This is supported by the PDS scores collected in that study (Table 5.1), which suggested that adolescent females had reached a significantly later stage of their pubertal development compared to adolescent males. Importantly, however, puberty and chronological age are difficult to dissociate. This is because puberty and chronological age are often

correlated, with age measured easily and precisely, whilst puberty is only roughly estimated using measures which are difficult to validate (Blakemore et al., 2010). Because of these difficulties, this doctoral work focused instead on age and gender-dependent differences in threat processing, with a view to extending this research to study differences in pubertal development following the validation of a Pavlovian fear conditioning paradigm.

6.6. Recommendations for future research

The following sections will discuss recommendations for future work in this area.

6.6.1. Replication and extension

Due to the paucity of work which has examined adolescent threat processing, and the relatively small sample sizes utilised in this doctoral thesis, the main focus of future work in this area should be on the replication and extension of the present findings. Given earlier discussions which suggested that differences in fear conditioning and extinction were dependent on the outcome measures used (Chapter 4, section 4.4.2.2), extensions of this work should also continue to utilise multiple outcome measures, including EEG. In addition to studying early visual responses to learned fear cues, future work should also consider the impact of such cues on frontally-mediated scalp activity, as this may help to understand how top-down attentional processes impact early visual activity in adolescence and adulthood. The following sections will discuss a number of possible extensions to the Pavlovian fear conditioning paradigm that was validated for use with EEG in this doctoral thesis.

6.6.2. Immediate extinction versus delayed extinction

Chapter 5 presents an examination of how fear conditioned responses are impacted by an immediate extinction procedure. Examining immediate, as opposed to delayed, extinction was a logical first step, because most human work employs this procedure (see Lonsdorf et al., 2017). This extinction procedure is also useful for practical reasons, as delayed extinction can result in increased attrition rates, with attrition being more common in participants who find the conditioning task particularly aversive. This was demonstrated by Lau et al. (2008), who found that adolescents who

reported the greatest fear of the CS+ were less likely to return for the extinction phase of the study. This could bias the extinction results of the remaining sample, as the most fearful individuals would no longer be represented in the study's findings. As Chapter 5 aimed to validate an EEG fear conditioning task for use with mid-adolescents (13-14 years) and adults (25-26 years), I aimed to limit the attrition rate as much as possible, so an immediate extinction procedure was preferable under these circumstances.

However, now that a potential developmental difference in the extinction of early visual responses has been established using a Pavlovian fear conditioning task, it is important to extend the current paradigm to investigate how this process might be impacted by a delayed (24 hr) fear extinction procedure. This is because the time interval between acquisition and extinction (i.e., immediate or delayed) can have differential effects on the degree of fear reduction observed. For example, a lack of long-term fear suppression has been observed in some studies that assessed extinction immediately after fear acquisition (Archbold, Bouton, & Nader, 2010; Chang & Maren, 2009; Maren, 2014; Merz, Hamacher-Dang, & Wolf, 2016). This is particularly relevant for therapeutic exposure treatments for anxiety, which rely on mechanisms of extinction. For example, a service-user may be exposed to the features of a previously traumatic event in a safe environment, with repeated exposure expected to reduce anxiety. However, it is possible that this type of exposure treatment may result in more successful long-term fear suppression when more time has passed between the treatment and the traumatic event. Despite this possibility, some work is inconsistent with this view, showing instead that immediate extinction did result in successful long-term fear suppression, whilst delayed extinction did not (Myers, Ressler, & Davis, 2006). As such, the precise mechanisms of immediate and delayed fear extinction have yet to be fully outlined. Therefore, an extension of Chapter 5 to incorporate a delayed extinction procedure could help to delineate the mechanisms of fear extinction and its relationship to pathological fear and anxiety in adolescents and adults.

6.6.3. Anxious populations

Adolescents face a greater risk of developing an anxiety disorder (Beesdo et al., 2010; Kessler et al., 2007; Kessler et al., 2005; Lijster et al., 2017; McGorry et al., 2011; Pine et al., 1998). The Pavlovian fear conditioning approach has been proposed to be a useful model for the study of anxiety (Duits et al., 2015; Lissek et al., 2005),

because anxiety maintained by excessive fear (Shin & Liberzon, 2010). As such, it would be advantageous to examine fear learning processes in clinically anxious adolescents and adults, as this could enable a clearer understanding of why adolescence is such a sensitive period for the development of anxiety. In chapter 5, the effect of state anxiety was controlled for when examining any age- or gender-dependent differences in behavioural, physiological, or ERP responses. This was carried out because adolescents had reported significantly greater state anxiety compared to adults. However, few results appeared to be influenced by state anxiety scores in adolescents or adults. This may have been because neither group reported feeling particularly anxious (Table 5.1). Conversely, previous fear conditioning work with clinically anxious adolescents suggests that they may exhibit exaggerated overall fear to CS cues (Haddad et al., 2015; Lau et al., 2008), compared with non-anxious adolescents. This effect of greater fear could result from anxious adolescents over-generalising their fear from the CS+ to the CS-, or could result from difficulty in inhibiting their fear in response to the CS-. However, to the author's knowledge, an examination of developmental differences between anxious adolescents and adults is largely missing. Therefore, future work should also focus on developmental differences in fear learning among anxious individuals, to provide additional insight into mechanisms of pathological fear and anxiety.

6.6.4. Socially-relevant conditioned stimuli

It has been suggested that the strength of the conditioned fear response is also impacted by the inherent salience of the CS cues. For example, emotional CSs such as angry or fearful faces may result in stronger fear conditioning and a resistance to extinction, when compared to emotionally-neutral CSs (Mineka & Öhman, 2002). However, emotionally intense CS cues may lead to increased amygdala activation on their own, which may overshadow the CS+/CS- discrimination effects induced during fear conditioning (Lonsdorf et al., 2017). This is particularly problematic when interpreting the results of adolescent fear conditioning work (Chapter 4), as there is currently a lack of basic research examining how adolescents respond to emotionally-neutral CS cues. This makes it difficult to interpret the results of work which has used emotional faces as CS cues, as there is no baseline measurement of adolescent fear acquisition and extinction responses. To overcome such issues, the fear conditioning paradigm employed in Chapter 5 successfully utilised 'neutral' non-face cues (a

hexagon and a circle) as the conditioned stimuli. Following this success, which has provided a baseline measurement of adolescent fear conditioning and extinction, relative to adults, the results of this study could now be extended to examine the impact of emotional CS cues such as facial expressions. This would be an important extension of the paradigm, based on evidence from the social information processing network (outlined in Chapter 1, section 1.7.4) which suggests adolescents undergo intense changes in the processing of socially-relevant stimuli, and based on work which suggests changes in gonadal hormones can influence adolescent emotional face processing (e.g., Scherf et al., 2012).

6.6.5. Peer group influence

This doctoral work has focused on adolescence as characterised by a developmental cascade of maturational changes to the brain's structure and function, and how these changes influence how adolescents process threatening stimuli. However, adolescent peer groups could also strongly influence how adolescents respond in the face of danger. For example, research has shown that adolescents were more likely to participate in risk-taking behaviours when members of their peer group were present during the experiment (Gardner & Steinberg, 2005). This was demonstrated as part of a simulated driving task, whereby adolescents (14-19 years), young adults (19-22 years), and adults (24-29 years) took part in the driving task both alone and when their peers were present (Chein, Albert, O'Brien, Uckert, & Steinberg, 2011). Their fMRI results suggested greater activation of brain regions important for reward processing (e.g., ventral striatum and orbitofrontal cortex) when adolescents' task performance was observed by peers, and that the degree of activation predicted subsequent risk-taking. Recent work suggests that this peer group effect is present even in the absence of risk-taking, when playing a card game that could lead to rewards (Smith, Steinberg, Strang, & Chein, 2015). In that study, adolescents (14-19 years) demonstrated greater ventral striatal activation during the card game when they were being observed by peers, compared with adults (25-35 years). Given this evidence that suggests the presence of peer groups could lead to enhanced activation of reward-related brain regions, it would be interesting to examine whether peer groups can also influence how adolescents respond to threatening cues. An examination of threat processing in the presence of peers could help to explain why adolescents take risks even in the presence of potential danger (L. Steinberg, 2008).

6.6.6. Longitudinal studies

Finally, it would be beneficial to conduct longitudinal studies of threat processing in the transition from adolescence to adulthood, as this would overcome a number of current methodological weaknesses in the research area. For example, as highlighted in Chapter 4, adolescent threat processing is often studied using wide age ranges (e.g., 12-17 years) in which individuals in different stages of adolescence are collapsed into one category, with adult comparison groups (when included) often consisting of late adolescent populations (e.g., 18-24 years). These methodological choices could be masking potential differences in fear learning or, similarly, highlighting differences in age groups which do not exist. In order to reduce the issues related to age categorisation, Chapter 5 conducted a cross-sectional study of two developmentally distinct groups of adolescents (13-14 years) and adults (25-26 years). However, cross-sectional studies do not completely alleviate the issue, as there may also be differences in responses to threat throughout the different stages of adolescence. This was highlighted by (Howsley & Levita, 2017), who observed differences in the reinforcement-dependent potentiation of visual ERP responses to threatening cues in pre-adolescents (9-12 years), adolescents (13-17 years), and late adolescents (18-23 years). In addition, recent work has suggested that some behaviours may exhibit a non-linear developmental trajectory from childhood to adolescence to adulthood (Chapter 4, Figure 17; Casey, 2015), which cross-sectional studies may struggle to capture. In contrast, longitudinal studies involve repeated observations of the same cohort of individuals at multiple time points, which can significantly reduce the error variance associated with comparing different individuals from different age groups. There are some weaknesses associated with a longitudinal approach, in relation to the high cost and large amount of time needed to conduct research over multiple years. Despite these imperfections, a longitudinal study would enable an examination of how each individuals' learned fear responses develop as they move through different stages of adolescence and into adulthood.

6.7. Final conclusions

This doctoral work aimed to advance our current understanding of adolescent emotion processing, and contribute to current neurobiological models of adolescence.

To achieve this aim, I took a multi-modal approach to understanding emotion processing in this age group, by assessing the suitability of a variety of emotion processing paradigms, and by utilising various behavioural, physiological and EEG measures. The results of this body of work argues for the strength of the Pavlovian fear conditioning approach in the study of emotion processing in the transition from adolescence to adulthood. However, this work is still in its infancy. Future work should seek to replicate and extend the results of this EEG Pavlovian conditioning work, through close examination of the mechanisms of extinction and its relationship to anxiety, including an examination of the impact of learned threat cues on frontally-mediated EEG activity. Given that adolescence represents a phase of significant social development (Blakemore, 2008), threat processing in this age group should also be assessed through the use of socially-relevant stimuli, and within the context of peer group influences. Lastly, the implementation of longitudinal work would alleviate many of the issues encountered so far regarding the age categorisation of adolescent populations and the selection of age-appropriate adult comparison groups. Together, this doctoral work provides a basis for which the developmental trajectory of adolescent threat processing can be further investigated.

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Appendices

Appendix 1 – Chapter 3 Supplementary Tables

Supplementary Table 3.1 – *Pearson's correlation coefficients between P1 mean amplitudes and reaction times for each of the corresponding conditions.*

	Happy Congruent		**Happy Incongruent**		**Sad Congruent**		**Sad Incongruent**	
Hemisphere	Left	Right	Left	Right	Left	Right	Left	Right
	-.19	-.13	-.24	-.24	-.25	-.15	-.21	-.24

* $p < .05$, ** $p < .001$.

Supplementary Table 3.2 – *Pearson's correlation coefficients between N170 mean amplitudes and reaction times for each of the corresponding conditions.*

	Happy Congruent		**Happy Incongruent**		**Sad Congruent**		**Sad Incongruent**	
Hemisphere	Left	Right	Left	Right	Left	Right	Left	Right
	.24	.76**	.19	.65*	.28	.61*	.26	.61*

* $p < .05$, ** $p < .001$.

Appendix 2 – Chapter 4 Supplementary Tables

Supplementary Table 4.1 – *Summary of the key search terms used to systematically search for articles within the three electronic databases: Web of Science, PsycINFO, and PubMed.*

Key Terms					
"Conditioning"	AND	Fear			
"Extinction"	AND	Fear			
"Conditioning"	AND	Fear	AND	Adolescents*	
"Extinction"	AND	Fear	AND	Adolescents*	
"Conditioning"	AND	Fear	AND	Children*	
"Extinction"	AND	Fear	AND	Children*	
"Fear"	AND	Aversive	AND	Pavlovian*	AND Children*
"Fear"	AND	Aversive	AND	Pavlovian*	AND Children*

Supplementary Table 4.2 - *Details of the eight studies that were identified in the literature search.*

Authors (Year)	N	ACQ measured?	EXT measured?	% female	Age M (SD)	Type of task	CS	US	DV	# of Trials		Evidence of ACQ?	Evidence of EXT?
										ACQ	EXT		
1. Cohen Kadosh et al. (2015) HA = High anxiety LA = Low Anxiety	Total: 33 HA:19 LA:24	Yes	No	HA: 47.37 LA: 29.17	HA: 14.6 (1.6) LA: 14.0 (1.2)	Cue and Context	Face Context: one of three rooms	Scream	Outcome expectancy FPS	24 predictable 24 unpredictable 24 no-scream	N/A N/A	Yes	N/A
2. Den et al. (2015)	Adol: 59 Adult: 46	Yes	Yes Immediate	Adol: 61.01 Adult: 80.43	range = 12-17 range = 38-57	Differential	Faces	Face and scream	Outcome expectancy Eye-tracking	7 CS+ 7 CS-	5 CS+ 5 CS-	Yes	Yes
3. Dvorak-Bertsch et al., (2007)	39	Yes	No	43.59	range = 17-21	Differential	Coloured Letters	Shock	FPS	Unclear	N/A	Yes	N/A
4. Ganella et al., (2017)	31	Yes	Yes Immediate	Adol: 58.82 Adult: 42.85	Adol: 14-16 Adult: 25-35	Differential	Faces	Face and Scream	SCR fMRI	15 CS+ 15 CS-	15 CS+ 15 CS-	Results not reported	Yes but impairment in extinction recall
5. Ganella et al., (2018)	31	Yes	Yes Immediate	Same sample as above	Same sample as above	Differential	Faces	Face and Scream	SCR fMRI	15 CS+ 15 CS-	15 CS+ 15 CS-	Yes	Yes but impairment in extinction recall
6. Haddad et al., (2015)	Anx: 15 HC: 11	Yes	No	Anx: 86.67 HC: 54.54	Anx: 15.2 (1.5) HC: 15.6 (1.3)	Differential	Face	Face and Scream	Evaluative CS rating fMRI	15 CS+ 15 CS- 15 Control	N/A	Yes	N/A

Authors (Year)	N	ACQ measured?	EXT measured?	% female	Age M (SD)	Type of task	CS	US	DV	# of Trials		Evidence of ACQ?	Evidence of EXT?
										ACQ	EXT		
					range = 11-17								
7. Johnson and Casey (2015)	Adol: 36 Adult: 36	Yes	No	Adol: 52.63 Adult: 50	range = 12-17 range = 18 to 32	Cue and Context	Coloured Windows Context: Bedroom or Kitchen	Picture and Sound	SCR	16 CS+ 16 CS-	16 CS+ 16 CS-	Yes	Not in the adolescents
8. Lau et al., (2011)	Exp 1 Adol: 21 Adult: 21	Yes	No	Adol: 28.57 Adult: 47.62	Adol: 13.09 (2.95) Adult: 27.10 (6.25)	Differential	Face	Face and Scream	Evaluative CS ratings SCR fMRI	10 CS+ 10 CS-	N/A	Yes	N/A
	Exp 2 Adol: 15 Adult: 20	Yes	No	Adol: 33.33 Adult: 35	Adol: 13.33 (2.35) Adult: 28.90 (8.77) range = 10-17 range = 18-50					30 CS+ 30 CS-	N/A	Yes	N/A
9. Lau et al., (2008)	Anxious: 15 HC: 39	Yes	Yes Delayed (M interval = 16 days)	Anx: 60 HC: 53.84	Anx: 13.64 (2.37) HC: 12.84 (2.47)	Differential	Face	Face and Scream	Evaluative CS ratings	16 CS+ 16 CS-	15 CS+ 15 CS-	Yes	No

Authors (Year)	N	ACQ measured?	EXT measured?	% female	Age M (SD)	Type of task	CS	US	DV	# of Trials		Evidence of ACQ?	Evidence of EXT?
										ACQ	EXT		
10. Morrow et al., (1969)	42	Yes	No	19.05	11.2 20.5 68.25 range = 10-12 range = 19-21 range = 62-75	Differential	Lights	Shock	SCR	20 CS+ 20 CS-	N/A	Yes	N/A
11. Pattwell et al., (2012)	Total: 83 Children: 30 Adol: 28 Adult: 25	Yes	Yes Delayed (24 hr)	Children: 53.33 Adol: 46.42 Adult: 52	Children: 8.8 Adol: 13.9 Adult: 22.8 range = 5-11 12-17 18-28	Differential	Shape	Sound	SCR	24 CS+ 24 CS-	24 CS+ 24 CS-	Yes	Not in adolescents
12. Raes et al., (2009)	38	Yes	Yes Immediate	89.5	18.47 (0.69)	Differential	Face	White Noise	Outcome expectancy SCR	5 CS+ 5 CS- (high load) 5 CS+ 5 CS- (low load)	9 CS+ 9 CS-	Yes	Partial (Dependent on condition)
13. Waters et al., (2014)	Fear disorder: 20 Distress disorder: 9 HC: 29	Yes	No	Fear disorder: 65 Distress disorder: 66.67 HC: 65.52	17 (0.35) range = 16-18	Cue and Context	Sentence	Muscle Contraction	Anxiety rating FPS	8 Safe 8 Danger	N/A	Yes	N/A
14. Waters et al., (2017)	123	Yes	Yes Immediate	Children: 45.65 Adol: 48.57 Adult: 76.10	8.8 (0.9) 16.1 (0.9) 32.3 (8.3) range = 7-10 15-18 25+	Differential	Shapes	Sound	Outcome expectancy Evaluative CS ratings	12 CS+ 12 CS-	12 CS+ 12 CS+ Then reinstatement 3 US Then re-test 3 CS+ 3 CS-	Yes	Yes (US expectancy) No (Evaluative ratings)

Appendix 3 – Chapter 5 Supplementary Data and Tables

Note: The following results reported here are part of the main ANCOVA models for each outcome measure in Chapter 5, and were removed from the main text as they were less theoretically informative in answering the primary research predictions (outlined in Section 5.1.3).

Supplementary Table 5.1 – *Mean contingency awareness and evaluative ratings for each condition, split by age group and gender.*

		Adolescent				**Adult**			
		Male		*Female*		*Male*		*Female*	
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
CS-US Contingency Awareness (M, SEM)	*ACQ 1*	1.92 (0.06)	2.08 (0.15)	1.92 (0.01)	2.16 (0.15)	2.01 (0.06)	2.49 (0.15)	1.92 (0.07)	2.53 (0.17)
	ACQ 2	1.62 (0.13)	3.01 (0.09)	1.54 (0.13)	3.01 (0.09)	1.67 (0.13)	2.71 (0.09)	1.85 (0.15)	2.90 (0.11)
	ACQ 3	1.72 (0.12)	2.94 (0.10)	1.58 (0.12)	3.01 (0.10)	1.99 (0.12)	2.71 (0.10)	1.90 (0.13)	2.90 (0.11)
	ACQ 4	1.78 (0.11)	2.86 (0.07)	1.64 (0.11)	3.00 (0.07)	1.93 (0.11)	2.93 (0.07)	1.92 (0.12)	2.91 (0.08)
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
CS Evaluative Ratings (M, SEM)	*Post_ACQ*	-1.18 (0.40)	0.56 (0.37)	-1.30 (0.41)	0.41 (0.37)	-1.54 (0.40)	1.23 (0.37)	-1.34 (0.46)	0.93 (0.42)
	Post_EXT	1.55 (0.39)	-0.21 (0.35)	1.03 (0.39)	-0.14 (0.35)	0.36 (0.39)	-0.36 (0.35)	0.16 (0.44)	-0.27 (0.40)

Supplementary Data 5.2 – *Evaluative ratings of the CS cues.*

There was a significant interaction between *Stimulus* and *Age*, F(1, 51) = 14.40, $p < .001$, $\eta_p^2 = .23$. Follow-up pairwise comparisons showed that the significantly greater overall negative ratings of the CS+ (M_{adj} = -0.59, SEM = 0.12) compared to the CS- (M_{adj} = 0.38, SEM = 0.13), was being driven by the adult group, Mdiff = 0.97, 95% CI [0.65, 1.29], $p < .001$. This result would suggest that although presentations of the CS+ resulted in ratings which were significantly more negative compared to the CS-, this effect was primarily driven by our adult group and not our adolescents. This is supported by an additional pairwise comparison showing greater negative ratings of the CS+ in adults (M_{adj} = -0.59, SEM = 0.12) compared to adolescents (M_{adj} = 0.03, SEM = 0.13), Mdiff = 0.62, 95% CI [0.27, 0.96], $p = .001$.

Supplementary Data 5.3 – *Physiological Responses to CS cues.*

There was a significant interaction between *Run* and *Age*, F(1, 46) = 3.48, p = .018, η_p^2 = .07. Pairwise comparisons revealed that adolescents had greater SCRs than adults during *run 2*, Mdiff = 0.37, 95% CI [0.12, 0.61], p = .004, r*un 3*, Mdiff = 0.45, 95% CI [0.21, 0.68], p < .001, and *run 4*, Mdiff = 0.42, 95% CI [0.15, 0.68], p = .003. In addition, adolescent SCRs were greatest during *run 1* (M_{adj} = 0.039, SEM = 0.005) compared to *run 2* (M_{adj} = 0.028, SEM = 0.004), Mdiff = 0.12, 95% CI [0.05, 0.19], p = .001, whereas adult SCRS were greatest during *run 1* (M_{adj} = 0.023, SEM = 0.005) compared to *run 2* (M_{adj} = 0.014, SEM = 0.004), Mdiff = 0.20, 95% CI [0.12, 0.28], p < .001, *run 3* (M_{adj} = 0.011, SEM = 0.005), Mdiff = 0.27, 95% CI [0.18, 0.37], p < .001, and *run 4* (M_{adj} = 0.012, SEM = 0.005), Mdiff = 0.24, 95% CI [0.15, 0.34], p < .001.

There was also a significant interaction between *Phase, Run*, and *Gender*, F(3, 129) = 3.62, p = .015, η_p^2 = .08. Pairwise comparisons revealed males (M_{adj} = 0.024, SEM = 0.004) had greater SCRs than females (M_{adj} = 0.012, SEM = 0.004) during acquisition *run 4*, Mdiff = 0.42, 95% CI [0.18, 0.67], p = .001, and also had greater SCRs than females during extinction *run 2*, Mdiff = 0.51, 95% CI [0.24, 0.78], p < .001, and *run 3*, Mdiff = 0.44, 95% CI [0.20, 0.69], p = .001. Males had greater SCRs during acquisition *run 1* (M_{adj} = 0.047, SEM = 0.006) compared to extinction *run 1* (M_{adj} = 0.028, SEM = 0.004), Mdiff = 0.31, 95% CI [0.15, 0.47], p < .001. Similarly, females had greater SCRs during acquisition *Run 1* (M_{adj} = 0.034, SEM = 0.007) compared to extinction *run 1* (M_{adj} = 0.011, SEM = 0.005), Mdiff = 0.63, 95% CI [0.46, 0.80], p < .001. Males had significantly greater SCRs during acquisition *run 1* compared to *run 2* (M_{adj} = 0.026, SEM = 0.004), Mdiff = 0.33, 95% CI [0.24, 0.42], p < .001, *run 3* (M_{adj} = 0.025, SEM = 0.004, Mdiff = 0.34, 95% CI [0.22, 0.47], p < .001, and *run 4* (M_{adj} = 0.024, SEM = 0.004), Mdiff = 0.32, 95% CI [0.19, 0.46], p < .001. Similarly, females had significantly greater SCRs during acquisition *Run 1* compared to *run 2* (M_{adj} = 0.013, SEM = 0.004), Mdiff = 0.43, 95% CI [0.33, 0.52], p < .001, *run 3* (M_{adj} = 0.012, SEM = 0.004), Mdiff = 0.57, 95% CI [0.43, 0.70], p < .001, and *run 4* (M_{adj} = 0.012, SEM = 0.004), Mdiff = 0.61, 95% CI [0.46, 0.76], p < .001.

There was a significant interaction between *Age, Task phase*, and *Gender*, F(1, 44) = 7.81, p = .008, η_p^2 = .15. Pairwise comparisons revealed that adult females had greater SCRs during the acquisition phase (M_{adj} = 0.010, SEM = 0.007) compared to the extinction phase (M_{adj} = 0.006, SEM = 0.007), Mdiff = 0.36, 95% CI [0.17, 0.55], p < .001. During extinction, adult males (M_{adj} = 0.020, SEM = 0.006) had greater SCRs

than adult females, Mdiff = 0.69, 95% CI [0.31, 1.07], $p = .001$, and adolescent females ($M_{adj} = 0.025$, SEM = 0.005) had greater SCRs than adult females, Mdiff = 0.74, 95% CI [0.37, 1.11], $p < .001$.

Supplementary Data 5.4 – *Visual P1 Responses.*

Each of these interactions preceded the final interaction term (Stimulus*Phase*Age*Gender) reported in the main text (Section 5.3.3.1.1)

There was a significant interaction between *Phase* and *Gender*, F(1, 48) = 4.76, p =.034, η_p^2 = .05. Pairwise comparisons revealed that females exhibited greater P1 amplitudes during extinction compared to acquisition, Mdiff = 0.05, 95% CI (0.02, 0.08) p < .001.

Finally, there was a significant interaction between *Stimulus*, *Phase*, and *Gender*, F(1, 48) = 12.98, p = .001, η_p^2 = .21. Pairwise comparisons revealed males showed greater P1 responses to the CS+ compared with the CS- during acquisition, Mdiff = 0.05, 95% CI (0.02, 0.09) p = .002. In addition, greater P1 responses to the CS+ during extinction compared to acquisition was being driven by females, Mdiff = 0.05, 95% CI (0.02, 0.09) p = .003. Finally, females showed significantly greater P1 responses to the CS+ during extinction, compared with acquisition, Mdiff = 0.07, 95% CI (0.024, 0.10) p < .001.

Supplementary Table 5.5 – *Mean ERP area amplitudes for the auditory N1, visual P1, and visual N1 for each condition, split by age group and gender.*

		Adolescent				**Adult**			
		Male		*Female*		*Male*		*Female*	
Auditory N1		0.30		0.43		0.39		0.53	
(M, SEM)		(0.07)		(0.07)		(0.07)		(0.07)	
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Visual P1	*ACQ*	0.44 (0.05)	0.33 (0.05)	0.38 (0.05)	0.36 (0.04)	0.15 (0.05)	0.15 (0.04)	0.19 (0.06)	0.17 (0.05)
(M, SEM)	*EXT*	0.41 (0.05)	0.40 (0.05)	0.49 (0.05)	0.43 (0.05)	0.15 (0.05)	0.14 (0.05)	0.21 (0.06)	0.17 (0.06)
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Visual N1	*ACQ*	0.27 (0.04)	0.18 (0.04)	0.21 (0.05)	0.17 (0.04)	0.17 (0.04)	0.19 (0.04)	0.28 (0.05)	0.30 (0.04)
(M, SEM)	*EXT*	0.32 (0.05)	0.20 (0.04)	0.20 (0.05)	0.18 (0.04)	0.15 (0.05)	0.16 (0.04)	0.22 (0.06)	0.25 (0.05)