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Emotion and motor function: a clinical and developmental perspective

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Abstract

The idea that emotions and physical actions are strongly intertwined has been widely accepted for quite some time. Yet surprisingly, in both the affective neuroscience and movement neuroscience literature, relatively little empirical attention has been paid to the (psycho)neurophysiological processes underpinning emotion-motor interactions. Therefore, the primary aim of this doctoral work was to further our understanding of emotion-motor interactions by investigating this relationship at different stages of brain development and in a clinical population characterized by episodic manifestations of altered motor and sensory function often associated with emotion regulation deficits, namely patients experiencing psychogenic non-epileptic seizures (PNES). To do this, I adopted a multidisciplinary approach encompassing both electroencephalography (EEG) and structural magnetic resonance imaging (sMRI).

The first Study (Chapter 2) investigated the relationship between emotion and motor function by measuring an electrophysiological index of anticipation, the contingent negative variation (CNV), to examine how anticipation of angry, happy and neutral faces influence motor preparation and subsequent action in early adolescents, late adolescents and young adults. In addition to which I also investigated whether automated attentional capture, as indexed by the visual P1 and N170 ERP components, differed at different stages of brain maturation. The results of this EEG study found no significant effects of emotion on the CNV during the anticipatory period, either within or between age groups. Both the visual P1 and N170 ERP components were found to be larger in response to angry faces relative to neutral faces but not happy faces, suggesting that early non-conscious automated attentional capture was facilitated by negative facial expressions.

Study 2, presented in Chapter 3, used a systematic meta-analytical approach to critically appraise the evidence for, and examine the convergence of, the neurobiological correlates of abnormal neurological functioning in PNES. Findings based on this systematic meta-review suggest that PNES may be associated with changes in brain morphology and/or persistent or recurrent functional changes in the brain. These neurobiological correlates may act as predisposing, precipitating and/or perpetuating factors in PNES. Study 3, presented in Chapter 4, used sMRI to measure cortical thickness and gyrification patterns in individuals experiencing PNES and age- and gender matched healthy controls. This was done to investigate whether cortical thickness differences between groups would be found in motor, frontal and occipital regions in addition to brain regions involved in emotion processing. In addition to cortical thickness measures, additional analyses were conducted to investigate whether patients with PNES would show evidence of abnormalities on a measure thought to reflect prenatal and early childhood cortical development and organization, namely local Gyrification Index. In this study I observed differences in cortical thickness between patients with PNES and healthy controls in (pre)frontal, temporal and occipital cortical regions which may suggest that atypical neuroplasticity may be implicated in precipitating and/or perpetuating PNES symptomatology.

This body of work provides new insights into emotion-motor interactions by furthering our understanding of the temporal relationship between emotion and motor preparation and motor output during different stages of brain maturation as well as the neurobiological correlates of abnormal motor output in the form of non-epileptic seizures.

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Publications arising from Thesis

Mcsweeney, M., Reuber, M., & Levita, L. (2017). Neuroimaging studies in patients with psychogenic non-epileptic seizures: a systematic meta-review. *NeuroImage: Clinical, 16*, 210-221.

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Authors' contributions

For Chapter 1, Marco Mcsweeney (M.M) took part in drafting/revising this chapter with the final version approved by Dr Liat Levita (L.L). For Chapter 2, M.M took part in the study concept and design, data collection, statistical analyses and interpretation, drafts and revisions under the supervision of L.L with additional support by Dr Myles Jones (M.J). For Chapter 3, M.M took part in the study concept and design, data collection, statistical analyses and interpretation, drafts and revisions under the supervision of L.L with additional support by Professor Markus Reuber (M.R). All three authors (M.M, L.L, M.R) gave final approval of the version to be published. For Chapter 4, M.M took part in the study design, data collection, statistical analyses and interpretation, drafts and revisions under the supervision of L. L and M.R with additional support by Professor Nigel Hoggard (N.H). L.L, M.R and N.H contributed to the initial study concept and design. M.R and N.H contributed to the screening of patients and N.H contributed to the retrieval of MRI scans. All four authors (M.M, L.L, M.R, N.H) were involved in the final draft and gave final approval of the version to be published. For Chapter 5, M.M took part in drafting/revising this chapter with the final version approved by L.L.

Chapter 1. General introduction

1.1. Introduction

The idea that emotions and actions are strongly intertwined has been widely accepted for quite some time (Sander, 2013). Yet, to date, the majority of empirical investigations of affect-behaviour interactions in humans have primarily focused on how emotions influence attention, working memory, learning, cognitive control and/or emotion regulation strategies (Okon-Singer, Hendler, Pessoa, & Shackman, 2015). In the realm of movement neuroscience, researchers have largely overlooked emotion and have primarily focused on motor learning and motor control and the cortical and subcortical generators of movement and inhibition of movement (Alexander, DeLong, & Strick, 1986; Alexander & Crutcher, 1990; Frith, Blakemore, & Wolpert, 2000; Schmidt, Lee, Winstein, Wulf, & Zelaznik, 2018; Wolpert & Flanagan, 2001). As a result, in both the affective neuroscience and movement neuroscience literature, relatively little empirical attention has been paid to the neural correlates of emotion-motor interactions. This is in spite of the fact that many studies involving humans in the cognitive and affective neurosciences have employed experimental paradigms using affective stimuli while measuring reaction times (RTs), yet have overlooked the neural correlates of emotion-motor interactions (Blakemore & Vuilleumier, 2017).

The relative lack of empirical work into how human emotion influences preparatory motor processes and overt physical action (or vice versa) has hindered progress in understanding neurotypical and non-neurotypical brain development, psychiatric disorders and disorders involving aberrant motor function resulting from organic or non-organic causation. This oversight may be an important one, given that the degree to which emotion and motor processes are successfully integrated may well depend on the functional integrity of neural circuitry involving numerous cortical and subcortical regions of the brain – the prefrontal and frontal cortex (including inferior

frontal gyri and supplementary motor area), amygdala, basal-ganglia, periaqueductal gray, cerebellum and anterior cingulate being some of the prominent examples (Blakemore & Vuilleumier, 2017).

However, the convergence of affective and movement neuroscience over the last decade or so, has led to empirical research in some areas, afforded valuable insights and pointed to promising new avenues of investigation. For example, in the main stream affective sciences a great deal of attention has been paid to approach-avoidance behaviours (Coombes, Cauraugh, & Janelle, 2006; Lang & Bradley, 2010; LeDoux, 2000; Sege, Bradley, Weymar, & Lang, 2017). In movement neuroscience the role of emotion in movement disorders such as Parkinson's disease has received some attention (Metz, 2007). In the realm of psychiatry, aberrant emotion-motor interactions have been associated with functional neurological disorders (Aybek et al., 2014; Aybek et al., 2015; Blakemore, Sinanaj, Galli, Aybek, & Vuilleumier., 2016; Pick, Goldstein, Perez, & Nicholson, 2019; Voon, Brezing, Gallea, & Hallett., 2011; Voon et al., 2016; Vuilleumier, 2014) and depression (Buyukdura, McClintock, & Croarkin, 2011) while abnormalities in the perception of action have been associated with schizophrenia (Frith, Blakemore, & Wolpert, 2000).

Yet, questions still remain as to how human emotions influence action at the neural level in real time, and how interactions between emotion and action develop at different stages of brain development. This is particularly the case in adolescence, a time in which emotional experiences may be more potent and/or consequential due to the maturational mismatch between relatively immature (pre)frontal structure/circuitry and relatively more mature limbic regions of the brain (Casey & Jones, 2010; Giedd, 2004; Giedd & Rapoport, 2010; Gogtay et al., 2004; Gogtay & Thompson, 2010; Hare et al., 2008; Shaw et al., 2008; Somerville, Jones, & Casey, 2010). Indeed,

phylogenetically older lower-order limbic regions of the brain have been shown to mature earlier than phylogenetically newer higher-order regions of the brain (parietal, frontal and association areas, Gogtay et al., 2004) and this is likely to have a significant bearing on how emotion-motor interactions may change at different stages of brain development. Therefore, the period of adolescence may be an ideal time to pose such a question, to better elucidate how different affect states influence motor function, which I examined in this thesis in Chapter 2.

The regulation of emotional experiences is critical to maintaining coherent and successful cognitive and behavioural patterns in response to internal and/or external experiences. Psychopathology during adulthood may often appear earlier with the manifestation of symptoms occurring during adolescence or even childhood (Kozlowska et al., 2018; Kessler et al., 2005; Reuber, 2008). Gaining an insight into how anticipatory and motor related activity in the brain may be attenuated or enhanced under different emotional conditions is of importance to getting a better understanding of how emotion and its regulation may be involved in conditions in which altered sensory and/or motor function is believed to result from psychological and/or emotional distress (psychogenic non-epileptic seizures; PNES). Moreover, the manifestation of symptoms associated with functional neurological disorders like PNES during adulthood often appear earlier in adolescence or even childhood (Kozlowska et al., 2018; Reuber, 2008). Study 1 presented in Chapter 2 may represent a first step in the development of future experimental paradigms which could involve clinical populations during different stages of brain development or possibly different stages of their condition, including patients experiencing PNES.

PNES is a highly heterogeneous condition, clearly disabling and often misunderstood by both patients and non-expert healthcare professionals (Green, Payne,

& Barnitt, 2004). In the absence of easily identifiable physiological change, PNES patients may be accused of "faking" their seizures (Benbadis, 2005), which increases patients' sense of helplessness, confusion and isolation (Thompson, Isaac, Rowse, Tooth, & Reuber, 2009). Therefore, in PNES research, there is a need to bring the psychosocial and biological together if we are to gain a better understanding of predisposition to, and consequence of this condition. At the very least, this may help to address the issue of stigmatization which often surrounds this condition.

Multiple predisposing, precipitating and perpetuating factors have been implicated in PNES, including childhood sexual abuse, other trauma or neglect in early life, subsequent adverse life events, a dysfunctional social environment, learning disabilities and other psychiatric comorbidities or comorbid brain disorders (Reuber, Howlett, Khan, & Grünewald , 2007). While biopsychosocial models conceptualize PNES as resulting from of a complex interplay of these risk factors (Reuber, Howlett, Khan, & Grünewald , 2007; Reuber, 2009), to date no single necessary and sufficient aetiological cause of the disorder has been identified (Reuber, 2009). However, it has been suggested that non-epileptic seizure-like episodes may be the result of emotional regulation deficits (Bakvis et al., 2009; Reuber, 2009). For example, evidence suggests that patients with non-epileptic seizure-like episodes may have reduced accuracy in recognising emotional facial expressions (Pick, Mellers, & Goldstein, 2016) and show attentional bias to threatening or negative information (e.g., Bakvis et al., 2009; Bakvis et al., 2010; Bakvis, Spinhoven, Zitman, & Roelofs, 2011). If this is indeed the case, then one might see structural and/or functional correlates of such deficits in patients with PNES. In fact, some studies have shown increased amygdala activity in patients with conversion disorder during the processing of emotional facial expression (Aybeck et al., 2015; Hassa et al., 2017; Voon et al., 2010a). Therefore, I further investigated the

evidence for the possible neurobiological underpinnings of PNES in Chapter 3. Further, I did this in an attempt to assess whether the available empirical evidence was convergent on brain regions involved in emotion processing and motor function. Additionally, very little is known about the structure of the cerebral cortex in PNES and I examined this further in Chapter 4. The intention was to investigate whether cortical thickness in regions associated with emotion processing, sensorimotor functions and motor control were affected in PNES in addition to characteristics of early cortical development and organisation, namely gyrification patterns.

In summary, the purpose of this doctoral thesis was to first, investigate whether emotion-motor interactions were valence dependent and whether they differed significantly between adolescents and adults, and to second, examine the available neuroimaging evidence for the existence of neurobiological substrates of aberrant emotion-motor interactions in patients with PNES. Lastly, the purpose of this doctoral thesis was to investigate whether group level analyses of structural magnetic resonance imaging (sMRI) scans would be able to differentiate patients with PNES and age- and gender-matched healthy controls and whether this differentiation would be localised to brain regions associated with emotion processing, sensorimotor functions and motor control.

The following literature review primarily focuses on research investigating emotion and motor function, their physical substrates in the brain and how these may change in concert with brain maturational processes/mechanisms. Instances in which aberrant motor function occurs free of neurological causation will also be covered. This review will begin by giving an overview of the structural brain changes that occur primarily during adolescence into adulthood and why they are important. This will be followed by an overview of emotion as a concept and how emotional experiences and

the regulation of emotion may be influenced by brain maturation. I will then give an overview of emotion-motor interactions and how these interactions precipitate and/or regulate internal states and adaptation to external cues/stimuli. This will be followed by an overview of anticipatory behaviour and the event-related potential (ERP) known as the contingent negative variation (CNV), an index of anticipation and preparatory brain activity. I will then describe some the studies that have investigated modulation of the CNV with the use emotional stimuli and studies that have investigated the developmental trajectory of this ERP component. The available neuro-scientific evidence describing the relationship between emotion and motor function, and the neurobiological correlates underpinning emotion-motor interactions will also be provided.

The final part of this review will cover studies examining how structural and/or functional changes in the brain may facilitate or indeed hinder the regulation of motor activity and emotional experiences and why this might be of importance in functional neurological disorders (FND) more generally. Additional literature specifically related to psychogenic non-epileptic seizures (PNES) will be covered in Chapter 3 and in Chapter 4. For purposes of clarity, the use of the term *action tendency* or *action readiness* is intended to describe preparation or readiness to enact movement while the term *action* describes the overt movement itself.

1.2. Brain development

Differences in cognitive ability, behaviour, emotion expression and emotion regulation between children, adolescents and adults is fairly evident. While there is still no clear and definitive explanatory model linking behaviour to neurocognitive development during this transition period, neuro- and developmental psychologists have

known for some time that the ability to regulate emotion and behaviour is relatively late to mature and that these regulatory functions are likely linked to the maturation of prefrontal regions of the brain (Steinberg, 2005). Indeed, while early studies in human brain development were greatly limited by the availability and number of post-mortem human brains at different ages (particularly at younger ages), these studies suggested that parts of the human brain, particularly the prefrontal cortex, continue to develop beyond early childhood (Huttenlocher, 1979; Huttenlocher, de Courten, Garey, & Van der Loos, 1982). More recently, the advent of nuclear magnetic resonance imaging (NMR), and advances in neuroimaging techniques over the last 20 years, have made it possible to investigate human brain development with large samples in a non-invasive way (*in vivo*). These technological advances have greatly added to a better understanding of human brain development.

It is now generally accepted that brain maturation continues from early childhood through adolescence into adulthood. Although the overall size of the human brain does not appear to change dramatically from the age of 6 (95% of the adult size brain) to age 20 (Giedd., 2004; Giedd & Rapoport, 2010), subdivisions of the brain undergo subtle yet substantial dynamic structural change during childhood and from adolescence into young adulthood (Blakemore, 2012; Blakemore & Choudhury, 2006; Casey, Giedd, & Thomas, 2000; Cao et al., 2017; Dosenbach et al., 2010; Goddings et al., 2014; Mills et al., 2016; Paus, 2005; Segalowitz, Santesso, & Jetha, 2010). Evidence for this will be examined next.

1.2.1. Developmental trajectories in white matter

White matter is primarily made up of myelinated axons or fibres and supporting glia. Myelin are lipid-rich (fatty) sheaths produced by oligodendrocytes that wrap around the axons of neurons dramatically increasing the speed of neural transmission

(Morell, Quarles, & Norton, 1999). The organisation of densely packed myelinated axons, fibre bundles or white matter tracts, beneath the surface of the cerebral cortex enables communication between the hemispheres (*commissural*), different cortical regions (*associational*) and between the cerebral cortex and other regions of the central nervous system (*projectional)*. Findings from cross-sectional and longitudinal sMRI studies examining the developmental trajectories in the structure of the brain consistently point to linear increases in white matter volume throughout the entire brain during childhood and early to late adolescence (Giedd, 2004; Giedd et al., 1999; Lenroot et al., 2007; Paus, 2005; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999).

Developmental change in a number of important white matter tracts, particularly the corpus callosum and left arcuate fasciculus have been reported (Giedd et al., 1996; Giedd, 2004; Lenroot et al., 2007; Paus et al., 1999). Connections in the corpus callosum generally take the shortest route so anterior sections connect frontal regions of the brain, middle sections connect middle cortical areas, and posterior sections connect posterior regions. The corpus callosum integrates information coming from both cerebral hemispheres. This integration is important for motor output, integration of sensory input, attention and language. Anterior sections of the corpus callosum appear to mature relatively early compared to mid regions and posterior sections which show greater age-related change during adolescence (Giedd et al., 1996; Giedd, 2004). It is plausible that functions relating to the structural integrity of the corpus callosum continue to improve during childhood and adolescence (Geidd, 2004). However, it is still unclear as to why anterior portions of the corpus callosum appear to mature before middle and posterior regions, especially given that prefrontal regions of the brain show comparatively protracted development (Giedd, 2004). Increases in white matter density during childhood and adolescence have also been reported in the internal capsule and

the left arcuate fasciculus, a white matter tract connecting anterior (Broca) and posterior (Wernicke) regions of the brain associated with language comprehension and speech (Paus et al., 1999). This may be one plausible explanation for improvements in language comprehension during childhood and adolescence.

1.2.2. Developmental trajectories in grey matter

Grey matter primarily consists of neuronal cell bodies and dendrites, as well as myelinated and unmyelinated axons, glial cells and capillaries. The vast majority of neuronal cell bodies in the central nervous system are located in the cerebellum (approximately 101 billion) and the cerebral cortex (approximately 21-26 billion), the convoluted sheath of grey matter (approximately 1.6mm to 4mm in thickness) located on the outer surface of the brain. Different regions of the cerebral cortex are subservient to a variety of functions underpinning behaviour, from primary functions (sensory experiences and innervation of movement) to higher-order functions (the organization of behaviour and intellect). Pre-pubescent increases followed by regionally dependent post-pubescent decreases in cortical grey matter volume, cortical thickness (surface of the brain) and some subcortical grey matter nuclei (structures inside the cortex) occur at different times during the transition from childhood to adulthood (Giedd et al., 1996; Giedd et al., 1999; Geidd & Rapoport, 2010; Mills, Goddings, Clasen, Giedd, & Blakemore, 2014; Mills et al., 2016; Shaw et al., 2008). Total cerebral grey matter volume appears to decline from its maximal level roughly between 7 -12 years of age (Giedd et al., 1999; Mills et al., 2016; Sowell, Thompson, Tessner, & Toga, 2001) and continues to decrease post-puberty into late adolescence and young adulthood, most notably in the prefrontal and parietal lobes relative to the temporal (middle and inferior) and occipital lobes (Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Lenroot et al., 2007; Paus, 2005). Further regional differences have been found in terms of grey matter

density, with sensorimotor areas of the brain maturing first (in terms of grey matter loss), followed by higher-order association areas such as the prefrontal, inferior parietal and superior temporal regions of the brain (Giedd, 2004; Giedd & Rapoport, 2010; Gogtay et al., 2004; Shaw et al., 2008). This suggests that phylogenetically older regions of the brain associated with the more basic functions appear to mature first, followed by brain regions involved in the organisation, integration and execution of these basic functions (Gogtay et al., 2004). Indeed, the most consistent findings from developmental structural imaging studies in humans' point to protracted development of the dorsolateral prefrontal cortex relative to other cortical and subcortical regions of the brain (Mills, Goddings, Clasen, Giedd, & Blakemore, 2014). These findings are in accordance with post-mortem histological studies of the human brain at different ages (Huttenlocher, 1979; Huttenlocher, de Courten, Garey, & Van der Loos, 1982; Webb, Monk, & Nelson, 2001) suggesting that the human brain undergoes protracted and heterogeneous development with prefrontal regions of the brain being amongst the last regions to fully mature.

1.2.3. Developmental trajectories in subcortical grey matter

The developmental trajectories of subcortical grey matter nuclei are less understood. However, recent imaging studies again point to regional differences in the developmental trajectories of subcortical grey matter structures (Dennison et al., 2013; Giedd et al., 1996; Goddings et al., 2014; Østby et al., 2009). Increases or decreases in subcortical grey matter volume appear to be less dramatic than those observed in terms of white matter volume increases with age (proportional to total brain volume) (Sowell, Trauner, Gamst, & Jernigan, 2002). Between 6 and 30 years of age the majority of subcortical grey matter structures appear to show age-related decreases in volume, i.e., the caudate, thalamus, accumbens, putamen, and pallidum (Goddings et al., 2014; Mills,

Goddings, Clasen, Giedd, & Blakemore, 2014; Østby et al., 2009; Sowell, Trauner, Gamst, & Jernigan, 2002) while other regions of subcortical grey matter structures in medial temporal regions of the brain such as the amygdala and hippocampus show age-related increases in volume (Giedd et al., 1996; Goddings et al., 2014; Mills, Goddings, Clasen, Giedd, & Blakemore, 2014; Østby et al., 2009). Interestingly, a sMRI study examining the timing of maturation of the amygdala (associated with the processing emotional stimuli, especially threat related), nucleus accumbens (associated with reward processing), and prefrontal cortex (associated with cognitive control) found that in a longitudinal sample of thirty-three neurotypical participants, aged 7–30 years, maturation of the amygdala and nucleus accumbens occurred earlier than that of the prefrontal cortex. The authors suggest that this maturational mismatch may be one possible explanation for why children, and in particular adolescents, may find it more difficult than adults to regulate their emotions and behaviours (Mills, Goddings, Clasen, Giedd, & Blakemore, 2014).

In summary, the neuroimaging evidence outlined above makes it clear that the brain undergoes substantive change from early childhood to adulthood. Both the cerebral cortex and subcortical grey matter is subject to temporally distinct maturational change. This is coupled with increases in white matter volume which facilitates communication between brain regions. It is therefore likely that if emotion has an influential effect on motor preparation and action, that this might be most evident in neurotypical individuals during the adolescent period, which again I examine in Chapter 2.

1.3. What is an emotion?

I am interested in how emotion can affect motor function at different stages of brain development and how emotion can affect motor function in PNES. To be able to

do this it is first important to understand what an emotion is. The question, what is an emotion, is a contentious one not only historically but also in contemporary theories of emotion. This is because emotions are difficult to pin down conceptually and as such are not amenable to definition or quantification. As pointed out by Frijda (2016), Kleinginna and Kleinginna (1981), Izard (2010), Scherer (2005), Sander (2013) and Oatley and Laird (2014), the major obstacle to the scientific investigation of emotion is the numerous definitions that have been proposed. For example, based on a list of almost 100 definitions derived from psychology dictionaries and texts on emotion, motivation, physiological psychology, and introductory psychology, Kleinginna and Kleinginna (1981) describe 11 categorical definitions of emotion: (1) *affective* definitions (emphasising excitement/depression or pleasure/displeasure); (2) *cognitive* definitions (emphasising appraisals and/or labelling processes); (3) *external emotional stimuli* definitions (emphasising external emotion-eliciting stimuli); (4) *physiological* definitions (emphasising internal physical mechanisms of emotion); (5) *emotional/expressive* definitions (emphasising observable emotional responses); (6) *disruptive* definitions (emphasising the disruptive/maladaptive nature of emotion); (7) *adaptive* definitions (emphasising goal-directed behaviours/the function of emotion in meeting an organism's needs); (8) *multiaspect* definitions (emphasising the different facets of emotion); (9) *restrictive* definitions (emphasising the differentiation of emotion from other aspects of cognition); (10) *motivational* definition (emphasising overlap of emotion and motivation). The eleventh category (*skeptical* statements) has been left out as this is not a category of emotion per se.

A second and related difficulty may in part be due to the fact that historically, many theories of emotion view emotion and action as two separate phenomena and disagreements still exist as to how these concepts interact and the direction of causality.

That is, do emotions cause physiological changes or changes in action tendencies and overt behaviour (Frijda, 2016; Scherer, 2009), or do actions cause emotions, or at the very least form part of the cause of emotions – physiological arousal leads to the post-hoc labelling of said arousal as an emotion based on the situational context (Hommell, Moors, Sander, & Deonna, 2017; Russell, 2003, 2009; Schachter & Singer, 1962). These two views of emotion-action interactions, which are largely based on older assumptions (James/Cannon debate), appear to be diametrically opposed – one viewing emotion as instigator of action with foci in the central nervous system (primarily influenced by Cannon, 1927) and the other viewing action as instigator of emotion with foci in the peripheral nervous system (primarily influenced by James, 1884; Lange, 1885). The common sense notion that emotion often results in action supports the former while the observation that emotion does not necessarily result in action supports the latter (Scarantino, 2017). However, this contentious debate over causality may be misleading and may have led to the confusion surrounding the concept of what an emotion actually is (Scherer, 2005).

Notwithstanding this variety in the definition of emotion and the seemingly diametrically opposed views on emotion-action interactions, in the current cognitive and affective neuroscience literature there does appear to be some consensus among the dominant theoretical frameworks of emotion on at least four fronts. Namely: (1) emotions are multicomponent phenomena – an emotion involves a *subjective* component (feelings or affect), a *cognitive* component (information processing), a *motivational* component often described as changes in action tendencies, a *somatic* component (changes in physiological responses), and a *motor* component (overt behaviour); (2) emotions are two-step processes involving *emotion elicitation* (internal elicitation – appraisals, memory associations, reflexes, and affect, and external

elicitation – direct sensory triggering) that provoke *emotional responses* (overt behaviours, action tendencies, feelings, autonomic responses, and changes to perception, attention, memory, decision making, and moral judgements); (3) emotions have relevant objects (only objects/events that are perceived as relevant elicit emotions, i.e. increased probability of satisfaction or dissatisfaction for an organism); (4) emotions are brief occurrences (quick onset and limited duration as opposed to affect states, moods or dispositions) (Sander, 2013).

Additionally, most emotion theories agree that emotions are not functionally irrational, i.e., emotions have relevant objects. Emotions serve the function of providing salient information about an event or object of import to the perceiver's past, present or futures goals (Hommell, Moors, Sander & Deonna, 2017). For this reason, many cognitive and affective neuroscience theories of emotion emphasise the significance of emotion as a tool of adaptation and change. In other words, an emotional episode is likely to include appraisals and concerns (Frijda, 2016; Izard, 2010; Scherer, 2005) that occur in an environmental or cultural context (Izard, 2010; Lang & Bradley, 2010; Russell, 2003) that are driven by motivational factors (pleasure/displeasure, approach/avoidance, appetitive/defensive) (Frijda, 2016; Izard, 2010; Lang & Bradley, 2010; Russell, 2003; Scherer, 2005). Emotion affords relevance or significance to objects or events that promote readiness for action or action itself in an attempt to change the relationship between an organism and its internal or external environment. Indeed, appetitive and aversive conditions are particularly likely to elicit emotions (Bradley, Cuthbert, & Lang, 1993; Bradley, 2009; Frijda, 2016; Lazarus, 1991; Sander, 2013; Scherer, 2009).

According to Jaak Panksepp, emotions originate in subcortical neural circuitry, interpreted later as emotions (subjective feelings). The subcortical emotional network is

highly conserved across mammals, including humans (Panksepp, 2004, 2016). This emotional network is built around the amygdala, which when activated, for example by threat, generates the subjective feeling of fear and also instigates defensive behaviours supported by physiological arousal to help the organism defend against attack (tenseness of muscles, solid stance and clenched fist – all preparatory activity for a swift defence). Moreover, the ability of an observer to recognize and classify "expressive movements" and to infer the corresponding emotional state is well documented (Witkower & Tracy, 2019). For example, feelings of joy/happiness are expressed through body posture as upward body movements, upward head tilt, and illustrative gestures, while feelings of fear are expressed in the body as backward movement or backward leaning, collapsed body, arms in front of body and shielding of the face (Witkower & Tracy, 2019). Just based on these examples, one can see that emotions can have a direct impact on physical behaviours/overt action, whether the action is in response to perceived threat or sense of elation.

In summary, given the diversity of views of how emotion-action interactions occur, a helpful approach to better understand how emotion-action interactions occur is to take action to in part cause emotion and emotion to in part cause action. This is because an emotion is not a single phenomenon but rather an emotion reflects the interaction between the subsystems, functions and components of emotion which include, as mentioned above, cognitive components (appraisals), neurophysiological components (bodily sensations), motivational components (action tendencies), motor components (overt behaviour), and subjective components (emotional experience). For example, emotionally relevant stimuli (appetitive or aversive) may influence action on the one hand (to approach or to avoid), while facial and/or bodily movement may influence emotional experiences on the other (Hommell, Moors, Sander & Deonna,

2017). To understand how emotion and action may be related during adolescence, again it is first important to understand how brain maturation may affect how emotions are experienced and expressed during adolescence and how this differs compared to adults or even children. This will be examined next.

1.3.1. Emotion in development

Adolescence is a critical transition period from childhood to adulthood involving multiple changes in almost all domains of an individual's life. Indeed, adolescence is often described as a period of heightened stress (Spear, 2000). In fact, adolescence is the most common developmental period for psychiatric illness onset with half of all lifetime cases starting at fourteen years of age and three quarters by twenty-four years of age (Kessler et al., 2005). Within this context, it is also a time in which cognitive and emotional change occurs in concert with brain maturational processes (Paus, Keshavan, & Giedd, 2008). For a detailed description of brain development see Section 1.2.

Significant changes in brain morphology and function during adolescence have been interpreted as having a number of consequential outcomes on adolescent behaviour, such as increased risk-taking and reward seeking (Casey & Jones, 2010; Doremus-Fitzwater & Spear, 2016; Ernst, Pine, & Hardin, 2006; Luna & Wright, 2016; Nelson, Leibenluft, McClure, & Pine, 2005; Steinberg, 2008, 2010) as well as increased emotionality (Casey & Jones, 2010). According to Casey's "imbalance" model (Casey & Jones, 2010; Somerville, Jones, & Casey, 2010), relative to adults and children, adolescents often display enhanced responsivity to salient environmental cues while at the same time lacking the appropriate behavioural inhibitory skills needed for making optimal decisions. This partly occurs due to the mismatch between relatively mature subcortical limbic regions (amygdala and nucleus accumbens) associated with affect and motive states (approach/avoidance) coupled with relatively immature prefrontal

cortical regions associated with emotion regulation, impulse control and decision making (ventral, medial, orbital, dorsal prefrontal cortex). This hypothesis is supported by a number of neuroimaging studies which have found greater activation of the nucleus accumbens in response to rewards in adolescents compared to children and adults (Galvan, Hare, Voss, Glover, & Casey, 2007) as well as greater activation of the amygdala coupled with under-recruitment of the ventral prefrontal cortex during anticipation of fearful, happy and calm facial expressions in adolescents compared to children and adults (Hare et al., 2008). Additionally, white matter tract integrity (size, density and organization) appears to increase throughout adolescence well into adulthood (Schmithorst, Wilke, Dardzinski, & Holland, 2002; Snook, Paulson, Roy, Phillips, & Beaulieu, 2005). White matter tracts facilitate more efficient and effective cross-communication between different regions of the brain. Presumably immature white matter tract integrity between prefrontal regions involved in regulatory functions, and subcortical regions involved in emotion- and motive-driven behaviours, results in increased emotionality and less top-down or regulatory control (Somerville, Jones, & Casey, 2010). Lastly, this increased emotional reactivity or emotionality seen during adolescence is not so evident in younger children and adults because in children both the limbic system and prefrontal regions are both relatively immature while in adults both have reached maturity.

As has been suggested in the introduction (Section 1.1), investigating preparatory neural activity during the anticipation of emotional stimuli when maturation of the prefrontal cortex is not yet complete may be an ideal time (developmentally) to investigate how brain maturation may modulate motor function and also how (pre)frontal-limbic interactions modulate action. In instances where emotionally-laden stimuli act as instigators of action and are perceived as such, it follows that motor

preparation may be closely aligned with the concurrent motive state (Lang & Bradley, 2010). However, little is known about how emotional contexts interact with the process of motor preparation and action, particularly during developmental stages when maturation of the prefrontal cortex is not yet complete. This is particularly the case during the anticipatory state when preparation or readiness for action is involved, and when future events are known to be pleasant or unpleasant. This is directly relevant to Chapter 2. Indeed, the primary aim of Study 1 presented in Chapter 2 was to examine this process using EEG during the anticipation of angry, happy, and neutral facial expressions during three different developmental periods, namely early adolescence (13-14-year olds), late adolescence (18-20 year olds) and young adults (18-20). I did this to investigate how brain maturation may modulate motor function and also how (pre)frontal-limbic interactions modulate action. To the best of my knowledge, study 1 presented in Chapter 2 is the first developmental study of emotion-motor interactions. Therefore, it is not possible to present the findings of previous developmental studies using similar paradigms in this chapter. However, empirical evidence does exist for emotion-motor interactions and this evidence will be examined next.

1.3.2. Emotion in action

Emotions are powerful determinants of subjective experiences, physiological arousal, motivation and behaviour. Indeed, the presence of emotional stimuli has been shown to both disrupt and enhance the orienting and capture of attention during experimental tasks (Dolcos & McCarthy, 2006; Hart, Lucena, Cleary, Belger, & Donkers, 2012; Schupp et al., 2007). In terms of peripheral physiology, at a basic level, the influence of emotion is probably most evident during fear conditioning paradigms, in which the presentation of aversive stimuli typically elicits defensive behaviours (flight, fight or freezing response), autonomic arousal (increases in blood pressure, heart

rate, sweating etc.) and endocrine responses (hormone release) (LeDoux, 2000). Presumably, such robust changes to central and peripheral neurophysiology reflect the engagement of motivational systems. Indeed, a number of studies point to the modulation of voluntary movement execution by motive states triggered by affective stimuli.

Coombes, Cauraugh & Janelle (2006) found that in a sample of forty-five healthy undergraduate students' (age range 18-29), exposure to unpleasant images (attack scene, disfigured child) resulted in greater mean force production, as measured by sustained voluntary muscle contractions of the wrist and finger extensors, relative to pleasant (erotic couples), neutral (face, wicker basket) and blank images. However, variability of mean force production did not vary as a function of affect. They conclude that when presented with threatening situations organisms have evolved to execute sustained voluntary movements with greater force without the loss of movement stability. They posit the possible involvement of two motor control loops (open and closed loops) to account for these findings, one involving the amygdala and cortico-basal ganglia connections which was subject to modulation by affect (open loop) and the other involving the basal ganglia only (closed loop).

A number of transcranial magnetic stimulation (TMS) studies have also reported modulation of movement generation and production by emotion. Schutter, Hofman, & Van Honk (2008) found that, in a sample of twelve healthy right-handed volunteers (age range 19-29), applying focal TMS over the left primary motor cortex 300ms after viewing fearful, happy and neutral facial expressions led to changes in corticospinal motor tract (CST) excitability with selective increases in motor evoked potentials (MEPs) recorded with the electromyogram (EMG) to fearful facial expressions compared to happy and neutral facial expressions. Using a similar approach Coombes et

al. (2009) applied TMS delivered to the motor cortex of twenty-three healthy male volunteers (age range 18-36) while they viewed pleasant, unpleasant and neutral images. They found reduced reaction times (RTs) during exposure to unpleasant images relative to pleasant and neutral images, increased force amplitude in the unpleasant condition relative to the pleasant and neutral condition, and larger MEPs in the unpleasant condition relative to the neutral condition. A non-significant marginal difference was found between MEPs elicited by pleasant and unpleasant images with a trend toward larger MEPs in the pleasant condition. As the authors point out, the modulation of the motor system by emotion may vary on differing levels and thus measurements may vary across behavioural and neurophysiological measures. A more recent study by Nogueira-Campos et al. (2014) also observed a valence effect on CST excitability in fourteen healthy undergraduate and postgraduate male participants (age range 21-36), where CST excitability was higher during preparation to grasp transparent cylinders containing emotionally-laden unpleasant objects compared to transparent cylinders containing emotionally-laden pleasant objects. However, no significant effect of valence on reaction times was found.

The evidence outlined above suggests that action is dependent upon the context in which the action occurs (Coombes et al., 2009; Schutter, Hofman, & Van Honk, 2008) and implies that the selection of motor programs during motor preparation may depend on the goal of an action (Nogueira-Campos et al., 2014; Phaf, Mohr, Rotteveel, & Wicherts, 2014). This is consistent with the view of emotion as instigator of a survival network, which facilitates adaptation to environmental concerns and has evolved to avoid threat and to enhance survival probabilities (Lang & Bradley, 2010), and that human emotions prime the human body for action (Frijda, 1986, 2009; Frijda, Kuipers, & Ter Schure, 1989; Izard, 1994). If human emotions prime the body for

action, then one might expect that anticipation of a positive outcome might be different than anticipation of a negative outcome, which again I investigate in Chapter 2. To understand better how this might occur, it is first important to understand what anticipation is and how it can be measured in the lab using electroencephalography (EEG).

1.4. The anticipatory brain – the CNV

Anticipation is integral to efficient and effective preparation, navigation, and interaction with an environment (Leuthold, Sommer, & Ulrich, 2004; van Boxtel & Böcker, 2004). If an individual perceives that at some point in the future, they will be presented with a certain stimulus, event, or likely to experience a particular state (affective or situational), they can adaptively prepare for that experience. Movement execution on the other hand is a more readily observable physical event resulting from the innervation of efferent signals emanating from the motor cortex to the skeletal muscle fibres and reafferent signals (sensory feedback) to the central nervous system (Frith, Blakemore, & Wolpert, 2000; Miall & Wolpert, 1996). Although movement execution is in part more readily observable than anticipatory processes linked to motor preparation and instigation of action, in many instances the execution of movement is reliant on conscious intent (volition) and unconscious preparatory neural activity which together, within a given context, facilitate execution or inhibition of motor related activity (Frith, Blakemore, & Wolpert, 2000).

Anticipatory states are physiologically active states which involve the pre-activation of sensory brain regions during the anticipation of forthcoming stimuli or events, and in most instances the pre-activation of motor regions of the brain when the execution of an action is required (Walter, Cooper, Aldridge, McCallum, & Winter, 1964; McCallum, 1988; Birbaumer, Elbert, Canavan, & Rockstroh, 1990). Given that

the sequential change in state from perception to action and thus termination of the anticipatory state is in essence a temporal process, it is therefore logical to assert that anticipation and action sit on a perception-action continuum with perception (information processing of a cue or trigger) at one end and action at the other (van Boxtel & Böcker, 2004). Motor preparation occurs between perception and action, and involves response selection and motor response initiation beginning after the perceptual processing stage, prior to movement execution (Blakemore & Vuilleumier, 2017; Schmidt & Lee, 2014).

Given that anticipation, motor preparation and action appear to unfold in a sequential manner on a perception-action continuum, EEG is ideally suited to capture the temporal characteristics of emotion-motor interactions. This is why I adopted this approach to investigate how emotion-motor interactions may or may not be age-dependent (Chapter 2). Anticipatory behaviour and motor preparation are most often studied in ERP research using slow cortical potentials like the contingent negative variation (CNV; Walter, Cooper, Aldridge, McCallum, & Winter, 1964), stimulus preceding negativity (SPN; Brunia, 1988) and *Bereitschaftspotential* (BP; Kornhuber & Deecke, 1965). The CNV is typically elicited during a reaction time task involving a cue/warning stimulus (S_1) and a target/imperative stimulus (S_2). If the inter-stimulus interval between S_1 and S_2 is long enough, two subcomponents can be identified, an early/initial CNV (iCNV) associated with an orienting response to S_1 (peaking about 0.7 to 1 s after S_1 onset with a fronto-central scalp topography) and a late/terminal CNV (tCNV) associated with anticipation of and response preparation to S_2 (peaking 500ms to 200ms prior to S_2 onset with a central scalp topography) (Jonkman, Lansbergen, & Strauder, 2003; Loveless & Sanford, 1974; McCallum, 1988; Rohrbaugh & Gaillard, 1983). The SPN is similar to the CNV but can be elicited when no motor response is

required (van Boxtel & Brunia, 1994; Böcker & Van Boxtel, 1997). The BP is most often elicited prior to movement execution during a self-paced task (Kornhuber & Deecke, 1965).

Centrally distributed CNV amplitudes have been found to increase depending on the motor parameters as set out by the experimental paradigm, for example, forceful responses, responses requiring rapid increases in force or fast compared to slow responses (Low & McSherry, 1968; Rohrbaugh, Syndulko, & Lindsley, 1976; Van Boxtel, Van den Boogaart, & Brunia, 1993). CNV amplitudes are reduced or not present during NoGo trials in which no motor response is required versus Go trials in which a motor response is required (Funderud et al., 2012; Segalowitz & Davies, 2004; Taylor, Gavin, & Davies, 2016). In paradigms that require a motor response, the tCNV component is comprised of motor preparedness to, and pre-evaluation of S_2, particularly in instances where the S_1 indicates the nature of the forthcoming S_2. In paradigms that are more perceptual then motor, it is likely that the tCNV represents a continuation of the iCNV component as amplitudes are reduced and scalp distributions are shifted to more posterior topographies for perceptual stimuli (Gaillard & Perdock, 1980) or more frontal topographies when mental effort is required (Van Boxtel & Brunia, 1994). Therefore, it is likely that the CNV, to a large extent is subject to manipulation depending on the type of experimental paradigm used and that both the iCNV and the tCNV components reflect aspects of cognition and action which include perception, expectancy, motor preparation, decision making, and when mental effort is required as well as somatosensory feedback (Donchin, Gerbrandt, Leifer, & Tucker, 1972; Hamano et al., 1997).

In general, the CNV, as described in the majority of the literature, appears to represent the expectancy of forthcoming stimuli/events when shorter ISI's are used, and

two distinct associative processes when ISI's longer than 3 seconds are used, namely attentive orientation to the cue/warning stimulus and anticipatory attention and motor preparation leading up to the presentation of the imperative stimulus (see Figure 1.1). In essence, the CNV encompasses perceptual and motor preparation (paradigm dependent) and reflects the summation of excitatory postsynaptic potentials (EPSPs) in the cerebral cortex evoked during the anticipatory state. The use of the CNV in the measurement of voluntary movement is well established.

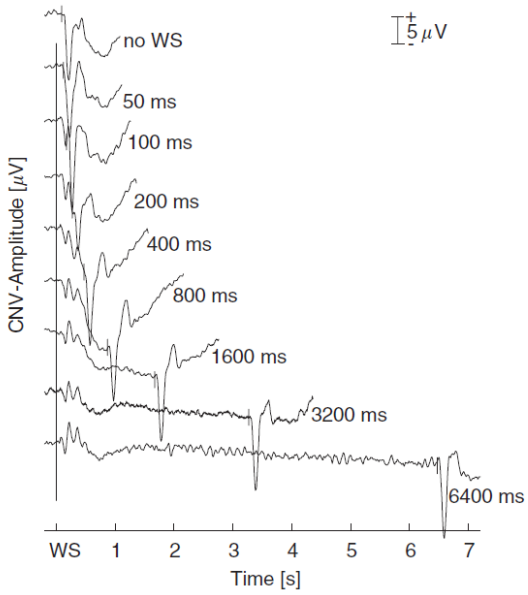


Figure 1.1. The CNV recorded at Cz with different ISIs. The CNV was recorded during a choice reaction time task involving visual cues as the warning stimulus and tones as the imperative stimulus. The long vertical line that spans each of the CNVs depicted represents the onset of the warning stimulus while the short vertical lines marked on each of the CNVs individually represent the onset of the imperative stimulus. Figure reprinted with the permission from MÜller-Gethmann, Ulrich, & Rinkenauer, 2003.

Studies investigating the cortical generators of the CNV using intracranial recordings in animals and intracranial and scalp recordings in humans have localized the CNV to prefrontal, premotor and motor cortices (Hablitz, 1973; Rebert, 1972; Walter, 1967), while a study using subdural electrode implants in patients with

intractable epilepsy, noted a patchy distribution in prefrontal and supplementary sensorimotor areas for the iCNV component and again in the prefrontal and supplementary sensorimotor areas as well as the primary motor, primary sensory, temporal and occipital cortices for the tCNV component (Hamano et al., 1997). Studies using magnetic field encephalography (MEG) during Go/NoGo tasks point to multi-focal generators of the contingent magnetic variation (CMV), the CNVs magnetic counterpart, including frontal, temporal, parietal and occipital areas as well as motor, sensory and association cortices (Elbert, Rockstroh, Hampson, Pantev, & Hoke, 1994; Fenwick et al., 1993). Studies using low-resolution electromagnetic tomography (LORETA) have observed cortical activation in prefrontal, primary and supplementary motor area, anterior cingulate cortex and superior and middle frontal areas for the CNV (Gómez, Marco, & Grau, 2003; Gómez, Flores, & Ledesma, 2007). In addition, recent functional magnetic resonance imaging (fMRI) studies investigating the neurobiological substrates of the CNV have noted the involvement of basal ganglia-thalamo-cortical circuitry in the generation of CNV amplitudes (Nagai et al., 2004) and it has been suggested that thalamic activation during CNV generation might reflect GABAergic regulation by the thalamic reticular nucleus during anticipatory attention resulting in an increase in CNV amplitudes (Brunia, 1999; Brunia & Van Boxtel, 2001).

1.4.1. Anticipation and emotion

A limited number of EEG studies have been conducted to examine how anticipation of emotionally-laden stimuli modulate anticipation, motor preparation and action. Casement and colleagues (2008) reported that in a group of fifteen healthy controls (age range 18-65), anticipation of positive, negative, and neutral adjectives resulted in larger CNV amplitudes prior to positive compared to neutral or negative adjectives. In contrast, in twelve dysthymia patients (age range 18-65) they observed a

non-significant trend for larger CNV amplitudes prior to negative compared to neutral adjectives. Using an emotional variant of the S_1-S_2 CNV paradigm in thirty participants (age range 20-32), grouped according to state and trait anxiety scores, Carretié, Mercado, Hinojosa, Martın-Loeches, & Sotillo. (2004) observed greater initial CNV (iCNV) amplitude in the high anxiety group (those that showed high levels of state anxiety and combined state and trait scores) during anticipation of negative images (insect) relative to positive images (opposite sex nude) and neutral images (telephone). This negativity bias was not present in the non-anxious group. The authors suggest that this implies a greater use of attentional resources towards negative/threatening stimuli compared to positive/pleasant and neutral stimuli in highly anxious individuals and points to a valence effect rather than general emotionality/arousal.

Moser, Krompinger, Dietz, & Simons. (2009) examined ERP modulation during anticipation of unpleasant (human and animal mutilation and threat) and neutral images (household items and neutral faces) under different emotion regulation instructions in sixteen undergraduate students (age range not reported). They noted that preparation to increase or decrease emotional intensity to unpleasant and neutral pictures resulted in modulation of the SPN, a slow cortical potential associated with anticipation of emotional stimuli when no motor response is required (Hajcak, Weinberg, MacNamara, & Foti, 2012; van Boxtel & Böcker, 2004). Anticipation of down regulation but not up regulation to unpleasant images resulted in an increase in both early SPN (associated with orienting and processing of the cue stimulus) and late SPN (associated with anticipation of stimuli) amplitude relative to passively viewing the unpleasant images. These findings point to enhanced preparatory activity during instructions to down regulate rather than up regulate emotions and suggest that increased mental preparation

for forthcoming affective stimuli facilitates emotion regulation through increased prefrontal cortical activity.

Using aversive and neutral picture presentations during a delayed Stroop task, Hart, Lucena, Cleary, Belger, & Donkers (2012) observed a relative decrease in CNV amplitude for both initial (iCNV) and terminal (tCNV) phases during aversive trials compared to neutral trials in twelve healthy participants (age range 19-34). However, iCNV amplitude attenuation in the aversive condition only occurred during incongruent trials in which increased cognitive control was needed, for example, the word RED printed in green ink. In contrast, reductions in tCNV amplitude during aversive trials relative to neutral trials were evident regardless of the difficulty of the task. Additionally, longer RTs were reported for incongruent trials compared to congruent trials and for trials involving aversive pictures compared to neutral pictures. The authors suggest that greater executive control during early stages of processing may exacerbate the effect of emotion while the later phase of motor preparation may be affected by emotional interference regardless.

Finally, using a novel self-paced task in which fifteen healthy participants (mean age = 23.6, *SD* = 4) viewed positive, negative, neutral and neutral scrambled pictures initiated with a key press, Perri et al. (2014) reported bilateral positive activity over prefrontal and occipital electrode sites during expectancy of highly arousing pictures relative to neutral and neutral scrambled pictures. However, a direct link to more established movement related potentials as recorded by scalp electrodes was somewhat inconclusive given that BP amplitudes did not differ significantly between conditions. However, this may have resulted from insufficient power given the small sample size and/or some overlap of ERP components with the positive activity over prefrontal and occipital electrodes obscuring the BP. Also, because participants instigated picture onset

by pressing a key, motor preparation and anticipatory processes may have overlapped due to the methodological design employed (self-paced rather than cued design).

In summary, and again, while some of the results outlined above paint a somewhat inconsistent picture and questions remain in relation to whether positive and negative stimuli differentially modulate motor related activity beyond the level of arousal (Coombes et al., 2009), the evidence does again suggest that neural activity associated with anticipation, motor preparation and action is dependent to some degree upon the context within which it occurs. However, something that has not been examined by any of the studies described above is how does anticipation of positive, negative or neutral stimuli differentially modulate motor preparation and action during different stages of brain development. This is important, especially given that different stages of brain development often coincide with periods of heightened emotional experience and expression. In fact, to date, no studies have investigated emotion-motor interactions at different stages of brain development but there are a handful of studies that have used the CNV to investigate maturation of the (pre)frontal cortex. These will be examined next.

1.4.2. Anticipation in development – the CNV

Preparatory neural activity as indexed by the CNV reflects different cognitive and neurophysiological processes that occur in parallel and are facilitated by functionally related yet segregated cortical and subcortical pathways. Indeed, the CNV is believed to represent the integration of sensory input and the preparation for action, driven by the (pre)frontal cortex and connected structures (Segalowitz, Santesso, & Jetha, 2010). While there is a plethora of studies examining CNV amplitudes in animals and adult humans, less is known about anticipatory behaviour as indexed by the CNV during different stages of human brain development. Indeed, only a handful of

comparative neurodevelopmental studies currently exist which have sought to measure the CNV at different stages of brain maturation in neurotypical humans (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Flores, Digiacomo, Meneres, Trigo, & Gómez, 2009; Killikelly & Szűcs, 2013; Klein & Feige, 2005; Jonkman, 2006; Klorman, 1975; Perchet & Garcia-Larrea, 2005; Segalowitz, Unsal, & Dywan, 1992a; Segalowitz & Davies, 2004; Taylor, Gavin, & Davies, 2016; Timsit-Berthier & Hausman, 1972). While small in number, most studies point to linear increases in iCNV and tCNV amplitude with increasing age. This is true for most developmental CNV studies with the exception of the Timsit-Berthier & Hausman (1972) study which found adult-like CNV levels by eleven years of age in healthy children.

Nonetheless, consensus points to a linear relationship between increases in CNV amplitude with increasing age. This has been interpreted as reflecting the protracted development of the prefrontal cortex. In fact, the CNV has been found to be either completely absent during the first decade of life (Perchet & Garcia-Larrea, 2005; Timsit-Berthier & Hausman, 1972) or significantly reduced in children compared to young adults (Jonkman, 2006; Segalowitz, Unsal, & Dywan, 1992a). Furthermore, patients with severe head injury show no CNV negativity or CNV negativity occurring on both Go and Nogo trials (Segalowitz & Davies, 2004). Moreover, greater CNV amplitude has been associated with greater attentional capacity (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Segalowitz & Davies, 2004) and higher IQ (Segalowitz, Unsal, & Dywan, 1992a; Segalowitz & Davies, 2004) suggesting that individual differences in CNV may be due to other factors apart from chronological age. However, to the best of my knowledge, no study has explored how emotional factors differentially modulate motor related activity during neurotypical adolescence.

Many of the neurodevelopmental studies outlined above have a number of limitations. First, from a developmental perspective, age categorization can be a significant limitation. For example, when comparing group means Segalowitz & Davies (2004) included seven to seventeen-year olds in the child group versus nineteen to twenty-five-year olds in the young adult group. Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax. (2005) included six to eleven-year olds in the child group versus twelve to eighteen-year olds in the adolescent group. Flores, Digiacomo, Meneres, Trigo, & Gómez. (2009) included eight to thirteen-year olds in the child group versus eighteen to twenty-three year olds in the late adolescent group, and Taylor, Gavin, & Davies (2016) included seven to thirteen year olds in the child group versus nineteen to twenty-eight year olds in the late adolescent/young adult group. Given the numerous changes that occur in brain morphology and circuitry during the transition from middle/late childhood to young adulthood, it would seem wise to restrict age ranges to a minimum when comparing EEG measures between age groups, and not include different developmental stages (e.g. child and adolescent) in the same age group. The large degrees of variance in age between participants within each age group may obscure any significant differences that would be found if the age ranges were more tightly controlled.

Second, with respect to measuring motor preparation, and this may be true for other measures as well, given that children, adolescents and adults appear to show different brain activation patterns due to differential recruitment of brain regions, it is likely that when completing the same task, possibly involving taxing cognitive demands, children, adolescents and adults may employ different strategies and engage different cortical and subcortical regions of the brain in order to accomplish the same goal (Flores, Digiacomo, Meneres, Trigo, & Gómez, 2009; Killikelly & Szűcs, 2013;

Segalowitz & Davies, 2004). If this is true, then it would be important to ensure, as much as possible, that the experimental paradigm employed would tap into the same strategies if we want to compare and contrast any real differences between the age groups. One way to do this is to ensure that the experimental task is as simple as possible with as few cognitive demands as is reasonable (Segalowitz & Davies, 2004) and I attempted to do this in Study 1 presented in chapter 2.

In this doctoral work I am interested in how emotion is expressed through the preparatory or anticipatory state and through physical action or movement. Further, I am interested in how emotion-motor interactions change over time and how brain regions associated with emotion-motor interactions may be implicated in PNES. To be able to study this it is first important to have a general understanding of how altered sensory and/or motor function may result from psychological and/or emotional distress.

1.5. Functional neurological disorders – FND

This section is primarily included in this thesis to give the reader an introduction to functional neurological disorders, of which PNES is only one of many differing presentations. Again, additional literature specifically related to PNES will be covered in Chapter 3 and in Chapter 4.

FND is a common condition found in neurology clinics (Stone et al., 2010) and sits at the intersection between neurology and psychiatry (Carson et al., 2012). FND symptoms show varying presentations which manifest as alterations in motor, sensory and/or cognitive function which occur beyond conscious control, are not easily explained by a general medical condition and/or brain disease, and are not accounted for by the direct effects of substance use. The most common symptoms include paroxysmal seizure-like episodes or non-epileptic seizures, functional sensory and movement disorders (hemisensory, visual, tremors), muscle weakness, and gait abnormalities

(Stone et al., 2010). Functional symptoms can occur in isolation but can also occur alongside other neurological or psychiatric conditions (Stone et al., 2010). Functional symptoms are now believed to be multifactorial. That is, many different risk factors may be involved in the development of this disorder.

While the exact prevalence of FND is unknown, as stated above, FNDs are common, presenting in approximately 16% of patients in neurology out-patient clinics, beating epilepsy (14%) and second only to headache (Stone et al., 2010). Functional symptoms are more often diagnosed in women than in men (3:1) (Matin et al., 2017), although this may be an overrepresentation given that women tend to be 1.5 times more likely to seek healthcare assistance compared to men (Carson & Lehn, 2016). Importantly, functional symptoms are real and patients with functional symptoms report similar levels of disability to both epilepsy and multiple sclerosis patients (Stone, Warlow, & Sharpe, 2010). However, despite the prevalence of FND in neurology clinics, the underlying mechanisms that cause FND remain poorly understood.

While aetiology remains elusive, the most prominent psychosocial etiological factors often include (but not always) a history of sexual or non-sexual trauma, subsequent adverse life events, familial discord/conflict, and bereavement of a significant other (Reuber, Howlett, Khan, & Grünewald, 2007). Additional factors often include psychiatric comorbidity or comorbid brain disorders, health anxiety or ongoing health issues (Reuber, Howlett, Khan, & Grünewald, 2007) as well as physical precipitators such as physical illness or injury (Pareés et al., 2014; Stone et al., 2009).

Nomenclature is both controversial and problematic in clinical and research settings. Historically, labels such as “hysteria”, “psychosomatic” or “conversion” have been used, implying that symptom formation and expression is solely due to the conversion of emotional distress/conflict to “somatic” or bodily sensations and actions

(Perez et al., 2015). Other labels such as “psychogenic” or “pseudoseizures” are common, again implying that symptoms are to some extent “in the mind” rather than being “organic” i.e., in the body. In clinical reality, the presence of a triggering factor such as a traumatic event, is not a necessary precondition to the diagnosis of a functional disorder nor should the presence of co-existing psychopathology be used as the sole precursor to an FND diagnosis, as psychopathology occurs without functional symptoms and co-existing psychopathology can often occur in other neurological or movement related disorders (Edwards & Bhatia, 2012). Therefore, a diagnosis that is solely based on psychological causation due to the exclusion of alternative “physical” explanations for the existence of functional symptoms is problematic. Instead, where possible, a diagnosis of FND or a functional movement disorder should be a transparent process that is in part based on identifying positive signs which may be indicative of causation. For example, in addition to a detailed patient history, Hoover’s sign can be used to help identify functional weakness (Factor, Podskalny, & Molho, 1995) while the use of distractibility and entrainment can be used to distinguish functional tremors form organic tremor (McAuley & Rothwell, 2004). In addition to a detailed patient history and video-EEG, the presence of pre-movement-related potentials and spectral power changes in EEG may in the future aid in the differential diagnosis of non-epileptic seizures (Edwards & Bhatia, 2012; Blakemore, Hyland, Hammond-Tooke, & Anson, 2015; Meppelink et al., 2017), although this last point represents a recent development, one which requires further replication and validation.

More recently, the use of the term “functional” has emerged as a possible compromise that is arguably more accurate in its descriptive sense on the one hand and more acceptable to patients on the other because it is perceived as less offensive by patients than a psychiatric or negative diagnosis (Edwards & Bhatia, 2012; Stone et al.,

2003). At a basic level, the term functional is used in this context to describe the hypothesised alterations in the functioning of the nervous system rather than the underlying structures per se. While it is clearly reasonable to suggest that brain structure and function is correlated (brain disease or injury studies, lesion studies, developmental studies etc.), the use of the term functional in FND is analogous to a "software" versus "hardware" problem (Bègue, Adams, Stone, & Perez, 2019). That is, while visual inspection of brain MRI (hardware) may result in clinically non-significant findings beyond clinically irrelevant incidental observations, the way in which different regions of the brain communicate (software) may be the key to symptom formation and expression. That is, functional symptoms may result from problems in how the brain and body send and receive signals.

However, the use of functional as a prefix to this condition also has its limitations, especially given the recent emergence of comparative imaging studies showing structural differences between patients with PNES and matched controls (Bolen, Koontz, & Pritchard, 2016; Devinsky, Mesad, & Alper, 2001; Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer, 2015; Labate et al., 2012; Lee et al., 2015; Reuber, Fernandez, Helmstaedter, Qurishi, & Elger, 2002; Ristić et al., 2015), in addition to known morphological changes resulting from trauma exposure for example (Kelly et al., 2013). What is more, we also know that the structure and functioning of the brain is dynamic rather than static. That is to say, the structure and functioning of the brain is not set in stone after a certain period of development after which time the brain goes into monotonic decline as we progress into old age. In fact, we know that learning new skills can lead to experience-dependent subtle yet demonstrable changes to grey and white matter in the brain (Zatorre, Fields, & Johansen-Berg, 2012). Therefore, it may be more advantageous and clinically relevant to consider a more nuanced

approach (Bègue, Adams, Stone, & Perez., 2019), one which considers the interplay between psychosocial stressors and neurobiological substrates (structural and functional brain changes) as predisposing factors and/or precipitators and perpetuators of FND. An understanding of such an interplay is key to getting a better understanding of the development of functional symptoms and their treatment.

In addition to models of FND that emphasise the role of dissociation (Nijenhuis, Spinhoven, Van Dyck, van der Hart, & Vanderlinden, 1998; van der Kruijs et al., 2014), attentional mechanisms and expectancy, illness beliefs and sense of agency (Edwards, Fotopoulou, & Pareés, 2013), given that functional symptoms are associated with significant disability and often occur alongside other neurological or psychiatric disorders, it is not surprising to find that previous studies have shown impairments in emotion processing in patients with non-epileptic seizures (Novakova, Howlett, Baker, & Reuber, 2015; Williams, Levita, & Reuber, 2018). For the purposes of this literature review, I will focus on the role of emotion processing and its interaction with motor function, as this is the central area of interest of this thesis. This will be examined next.

1.5.1. Neurobiological correlates of emotion-motor interactions in FND

As discussed above, for some patients' early life stress and trauma may play a key role in the development of FND and psychogenic non-epileptic seizures in particular (Holman, Kirkby, Duncan, & Brown, 2008). Notably early life stress has persistent negative effects on cortical and subcortical brain regions involved in emotional regulation (Cohen et al., 2013). It has been proposed that aberrant prefrontal and amygdalar neuroplastic changes resulting from chronic exposure to stressors may facilitate the development and subsequent expression of functional symptoms (Perez et al., 2015). Further, relative to controls, several recent neuroimaging studies have observed increased connectivity between the amygdala and the supplementary motor

area (SMA) in FND in response to emotional stimuli (Aybeck et al., 2014, 2015; Hassa et al., 2017; Voon et al., 2010a, 2010b). Compared to epilepsy patients, in conversion disorder patients Szaflarski et al. (2018) observed abnormal motor (putamen) and limbic (parahippocampal) activations during a facial emotion processing task. Blakemore et al. (2016) found that compared to controls, patients with FND were able to maintain a higher force hand grip in response to negative (but not positive) emotional stimuli and this was associated with activation in the cerebellar vermis, hippocampus, and posterior cingulate. Conversely, during the task, the healthy controls engaged medial prefrontal cortices and regions of the inferior frontal gyri (IFG), two areas of the brain associated with motor control and motor inhibition. Similarly, in a MEG study Fiess, Rockstroh, Schmidt and Steffen. (2015) observed that during an emotion regulation task, FND patients activated the sensorimotor cortex during emotion regulation but unlike the healthy controls they were lacking in frontal cortical activity.

In agreement with event related studies (task based studies), van der Kruijs et al. (2012) noted increased resting state functional connectivity between brain regions associated with emotion regulation and self-awareness (insula) and brain regions involved in motor preparation (precentral sulcus) in PNES relative to controls and that these functional connectivity values correlated with self-reported dissociation scores. During an action selection task, Voon, Brezing, Gallea and Hallet (2011) observed that patients with conversion disorder showed lower left SMA activity but higher right SMA, right amygdala, left anterior insula, and bilateral posterior cingulate activity relative to healthy controls. In addition, during internally versus externally generated action, they also observed that the left SMA showed lower connectivity values with bilateral prefrontal cortices compared to controls.

All of the above suggests that functional symptoms may result from aberrant neural circuitry, whereby stronger functional connectivity between brain regions associated with emotion processing and brain regions associated with motor function are not subject to, or at the very least are less hindered by, regulatory functions emanating from prefrontal cortical regions associated with response inhibition, down regulation of emotional responses and other executive functions. However, the question still remains, how and why are functional movements experienced as involuntary when in effect, the same voluntary pathways may be employed.

A fMRI study conducted by Voon et al. (2010b) involving eight patients with conversion disorder noted that, when comparing functional tremors to voluntary mimicked tremors, patients showed right temporoparietal junction (TPJ) hypoactivity with lower right TPJ connectivity to sensorimotor regions (sensorimotor cortices and cerebellar vermis), and limbic regions (ventral anterior cingulate and right ventral striatum) of the brain. Conversely, another study conducted by Aybeck et al. (2014) found that relative to healthy controls, patients with conversion disorder showed increased activity in right TPJ and right amygdala during the recall of relevant life events. While these findings appear contradictory, the inconsistencies may result from the fact the Voon et al. study did not involve controls and used a very different experimental paradigm. However, notwithstanding these differences, while activity in this region of the brain (TPJ) may not be sufficient to explain the existence of functional symptoms in these patients, it may represent an important node in the process and may partly help to explain why patients with FND experience their symptoms as involuntary, i.e., beyond their conscious control. The right TPJ has been implicated as a controller involved in the integration of sensory feedback and motor commands, i.e., prediction and outcome (Voon et al., 2016). This suggests that while the same voluntary pathways

may be employed, functional movements are experienced as involuntary (Voon et al., 2016). Justification for this underlying assumption is the phenomenon of entrainment. For patients with functional tremors, the "entrainment test" often provides a clear positive indication that the tremor is indeed functional, whereby the affected limb becomes entrained to the voluntary paced movement of the unaffected limb (tremor frequency changes or tremor frequency changes to match frequency of tapping). This again points to a "fault" in the circuitry (software) rather than an issue relating to the structures of the brain (hardware).

In summary, notable brain regions implicated in the alterations of brain circuitry in FND and in non-epileptic seizures in particular include the anterior cingulate, the amygdala, the dorsolateral and ventromedial prefrontal cortices as well as the insula, the pre- and post-central gyri and central sulcus in addition to the inferior frontal gyri, SMA, the cerebellum and TPJ. Differences found in the structure and function of these regions have been used to explain why impairments in emotion processing and motor control are evident in patients with FND compared to controls. This is not an exhaustive list but has been included in this chapter to introduce the reader to the neurobiology underpinnings of FND. However, given the high levels of psychiatric comorbidity in these patients and the small sample sizes involved (a characteristic of the majority of imaging studies in this patient population), the results presented here need to be considered with these caveats in mind. These issues will be addressed in more detail in Chapter 3.

1.6. Thesis outline

The primary aim of this PhD thesis was to examine this relationship between emotion and motor function. This was done by adopting a multidisciplinary approach encompassing both electroencephalography (EEG) and structural magnetic resonance

imaging (sMRI). Study 1, presented in Chapter 2, investigated the relationship between emotion and motor function by recording electrophysiological changes in the brain during the anticipation of emotion eliciting stimuli in early adolescents, late adolescents and young adults. More specifically, this study used an electrophysiological index of anticipation, the contingent negative variation (CNV), to investigate how anticipation of angry, happy and neutral faces influence orienting responses, motor preparation and subsequent action. Well established CNV paradigms are available and have demonstrated robust and replicable findings (Brunia, van Boxtel, & Böcker, 2012). However, many of these have failed to acknowledge the role that emotion plays in motivational behaviours (Mercado, Hinojosa, Peñacoba, & Carretié, 2008).

Study 2, presented in Chapter 3, used a systematic meta-analytical approach to critically appraise the evidence for, and examine the convergence of, the neurobiological correlates of abnormal neurological functioning in PNES. This was important due to the limited number of reviews available, most of which were not systematic. Therefore, these reviews may have missed important studies in this area. Additionally, no previous review sought to uncover convergent neuroimaging findings in patients with PNES to better determine the neurobiological correlates of this condition.

Study 3, presented in Chapter 4, used sMRI to measure cortical thickness and gyrification patterns in individuals experiencing PNES and age- and gender-matched healthy controls. This was done to investigate whether cortical thickness differences between groups would be found in motor, frontal and occipital regions in addition to brain regions involved in emotion processing. In addition to cortical thickness measures, additional analyses were conducted to investigate whether patients with PNES would show evidence of abnormalities on a measure thought to reflect prenatal

and early childhood cortical development and organization, namely local Gyrification Index (Schaer et al., 2012).

Chapter 5 summarises and discusses the findings from this doctoral thesis, highlights the strengths and weaknesses, and considers future directions.

Chapter 2. Neural correlates of emotion-motor interactions and automated attentional capture from early adolescence to young adulthood

Abstract

Anticipation is integral to efficient and effective preparation, navigation, and interaction with an environment while emotions are powerful determinants of subjective experiences, physiological arousal, motivation and behaviour. The successful integration of emotional experiences and motor function may be critical to an individual's functional well-being. Yet little is known about how emotions modulate preparation for action, particularly at different stages of brain development. Therefore, in this study I examined the relationship between emotion and motor function by recording electrophysiological changes in the brain during the anticipation of angry, happy and neutral faces in 18 early adolescents, 18 late adolescents and 18 young adults. I also investigated whether viewing angry, happy and neutral facial expressions would differentially modulate visual P1 and N170 amplitudes. However, there were no significant effects of emotion on the CNV during the anticipatory period, either within or between age groups. Both the visual P1 and N170 ERP components were found to be larger in response to angry faces relative to neutral faces but not happy faces, suggesting that early non-conscious automated attentional capture was facilitated by negative facial expressions over and above neutral facial expressions. For the early adolescent group only, mean RTs were found to be significantly faster to happy faces relative to neutral faces, suggesting that for early adolescents a relatively faster and more accurate identification of happy faces may have occurred resulting in faster reaction times in happy trials compared to neutral trials. This may suggest that, for the early adolescent group only, we observed heightened emotional responses to happy faces which may reflect increased reward seeking behaviours in this age group compared to the two older age groups. However, further work is needed to better delineate valence dependent changes in motor preparation and action.

2.1. Introduction

EEG is well suited to chart both the structural and functional characteristics of the brain as it goes through different stages of brain development. Indeed, continuous EEG scalp recordings of the electrical activity of the brain are often associated with structural maturation, while ERPs (averaging EEG recordings time-locked to stimulus onset) are often associated with functional properties such as attention, working memory, visual processing of stimuli, motor preparation etc. (Segalowitz & Davies, 2004; Segalowitz, Santesso, & Jetha, 2010). Given that the generators of CNV amplitudes are largely associated with the frontal lobes, examining anticipatory behaviour during different stages of brain development may be key to gaining a better understanding of not only how brain maturation may modulate motor function but also how (pre)frontal-limbic interactions modulate action.

The CNV was selected for the current study over other measures of motor preparation such as the BP, partly due to the way in which the CNV is elicited in EEG experiments (S_1- S_2 CNV paradigm). For example, in the S_1- S_2 CNV paradigm there is the need to use a pre-cue stimulus/warning stimulus (S_1) which is open to manipulation and an imperative/target stimulus (S_2) which requires a motor response. This allows for the manipulation of the type of information processed by the brain during the anticipatory state (between S_1 and S_2). This allows for the testing of the main hypothesis of this study, that is, does the emotional context as defined by the information provided in S_1 modulate motor preparation and action. It would be difficult to address this hypothesises by using a self-paced finger tapping task such as that used by Kornhuber & Deecke. (1965) or even the Libet, Gleason, Wright, & Pearl. (1993) clock task, both experimental tasks associated with self-initiated movement and often used to elicit the BP. Furthermore, while the CNV is unlikely to reflect all aspects involved in motor

function (conscious versus unconscious/automated reflex versus cognitively controlled), as described in Section 1.4. it has been taken to be a robust and replicable measure of anticipatory attention/expectancy and to incorporate aspects of motor preparation. In contrast, doubt has been cast on the validity of the BP as a measure of motor preparation (Schurger, Sitt, & Dehaene, 2012).

Therefore, I employed high density EEG recordings during a simple emotional variant of the S_1- S_2 CNV paradigm in which the type of cue stimulus (S_1 shape) was always predictive of the forthcoming imperative stimulus (S_2 facial expression), at which point participants were required to press a button as fast as they could as soon as the face appeared on screen. Again, because the cue (S_1) in effect signalled the need to prepare for the upcoming response, it was possible to examine how discrete affect states elicited during anticipation of angry, happy and neutral faces influenced not only motor preparation as indexed by the CNV, but also how these anticipatory processes influence motor output, as assessed by the speed of participant's responses. In addition, I also investigated how discrete affect states elicited by S_1 and S_2 influenced attentional processes as indexed by early visually evoked potentials, namely the visual P1 and N170. In addition, I took measures of state and trait anxiety (as measured by the STAI), depression, anxiety and stress (as measured by the DASS-21), autistic traits (as measured by the AQ-10), as well as cognitive reappraisal and emotion suppression strategies (as measured by the ERQ). To address some of the concerns raised about previous ERP developmental studies (see Section 1.4.2), the experimental paradigm was kept as simple as possible with as few cognitive demands as was reasonable and restricted the age range in each age group to two years.

Previous ERP studies have shown modulation of anticipatory behaviour by emotional stimuli (Carretié, Mercado, Hinojosa, Martın-Loeches, & Sotillo, 2004;

Casement et al., 2008; Hart, Lucena, Cleary, Belger, & Donkers, 2012; Moser, Krompinger, Dietz, & Simons, 2009; Perri et al., 2014) and given that adolescents often show increased levels of emotionality, I hypothesised the following: First, reaction times to angry, happy and neutral faces would differ significantly within and between age groups; second, that iCNV, tCNV and total CNV amplitudes during the anticipatory period between S_1 and S_2 would differ significantly between conditions (angry, happy, neutral) and between age groups (early adolescents, late adolescents and young adults), that is, CNV amplitudes would be condition dependent and a greater degree of modulation of the iCNV, tCNV and total CNV by emotional stimuli would occur in early adolescents compared to late adolescents and young adults. However, given that this is essentially an exploratory study and to the best of my knowledge the first of its kind, I did not hypothesize the direction of the results; third, because previous studies have shown a weak yet consistent relationship between larger CNV amplitudes and faster reaction times, suggesting that roughly 13% of the variability in reaction time data could be accounted for by tCNV amplitude (Rebert & Tecce, 1973; Smith, Johnstone, & Barry, 2006), I carried out additional post-hoc analyses in which I hypothesised that iCNV and tCNV as well as state anxiety scores would be predictive of mean reaction times in each of the three conditions. However, it should be noted again that to the best of my knowledge no previous study has examined this relationship in response to emotional stimuli in the transition from adolescence to adulthood. As these analyses were not part of my main hypotheses, they can be found in the Appendices.

Additionally, given that the cue (S_1) was always predictive of the forthcoming imperative stimulus (S_2), I hypothesised that visual P1 peak amplitude in response to S_1 would be condition dependent and that I would again see differences between age groups. I also hypothesised that visual P1 peak amplitudes and N170 amplitudes in

response to S_2 would differ significantly between conditions and between age groups. Both the P1 and N170 are early visually evoked potentials associated with automatic attentional mechanisms and the neural processing of face stimuli, respectively (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010; Itier & Taylor, 2004b; Luck, 2005). The visual P1 (or P100) is an early positive bilateral occipital component that peaks around 80-130ms following stimulus onset (Mangun, 1995) and decreases in amplitude and latency with increasing age (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010). The P1 is believed to originate from striate and extrastriate visual areas in the occipital lobe (Clark, Fan, & Hillyard, 1994; Di Russo, Martínez, Sereno, Pitzalis, & Hillyard, 2002). It has been proposed that decreases in P1 latency between early childhood and adulthood may be a result of increases in speed and efficiency in visual cortical areas due to increases in myelination with increasing age (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010). Decreases in P1 amplitude may result from structural brain changes during adolescence associated with grey matter volume and synaptic density and/or alternatively changes in underlying tissue conductivity (bone, skin etc.) and cortical folding patterns (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010).

The functional relevance of the visual P1 in the current study is that potentiation of visual P1 peak amplitudes have been shown to occur in response to negative stimuli compared to positive and neutral stimuli (Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia, 2004). This has been interpreted as the prioritizing of biologically salient danger signals over and above other percepts (Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia, 2004). More specifically, enhancement or attenuation of visual P1 peak amplitudes in response to cues (S_1) predicting emotional stimuli may prove informative with regards to approach/avoidant behaviours which may be reflected by

anticipatory processes and motor preparedness indexed by the CNV. In terms of visual P1 responses to the faces themselves, based on the findings of Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia. (2004), I hypothesised that I would see larger visual P1 peak amplitudes in response to angry faces compared to happy and neutral faces.

The N170 is a face sensitive negative component that peaks roughly around 170ms and is maximal over occipital-temporal electrodes with a right hemisphere advantage (Duchaine & Yovel, 2015; Rossion, 2014). The N170 also appears to be sensitive to emotional facial expression with studies showing greater N170 amplitudes evoked by happy and sad faces compared to neutral faces (Batty & Taylor, 2003; Blau, Maurer, Tottenham, & McCandliss, 2007). Generators of the N170 have been localised to the fusiform and inferior and superior temporal gyri (Gauthier et al., 2000; Itier & Taylor, 2004b). N170 latency appears to reach adult-like levels by around nine to eleven years of age (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010) while N170 amplitude appears to be large and stable up to nine years of age and then decreases steadily until adulthood (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010). Changes in N170 latency and amplitude may be the result of the same brain maturational processes and/or tissue conductivity considerations as the visual P1. Again, similar to my hypotheses regarding the visual P1 in response to S_2 (faces), if the prioritizing of biologically salient danger signals does indeed occur at such a relatively early stage in emotional face processing, I hypothesised that I would see larger N170 amplitudes in response to angry faces compared to happy and neutral faces.

2.2. Method

2.2.1. Participants

In total, sixty participants completed the study. However, one participant was excluded due to being left-handed and five participants were excluded due to excessive artefacts, which for some participants resulted in an excessive number of trials being rejected (> 30%). The final sample included in the analyses comprised of eighteen young adolescents aged 13-15 (9 male), eighteen late adolescents aged 18-20 (9 male), and eighteen young adults aged 25-27 (9 male) (see Table 2.1). All participants reported a medical history free of epilepsy, fits, blackouts, fainting turns or unexplained loss of consciousness, recurrent headaches or migraines. All participants were free of any developmental or psychiatric conditions (ADHD, Autism spectrum disorder, depression or anxiety) and were not taking any form of medication which may have affected the CNV results (benzodiazepines, anticonvulsants; Brunia, van Boxtel & Böcker, 2012). No participant reported having any problems with their sight (e.g. scotoma, colour blindness, blindness in one eye, night blindness, reduced visual field, blurred vision, or detached retina) and had normal or corrected-to-normal vision.

Table 2.1. *Group characteristics (N = 54).*

			Age		
Age group	**Gender**	***n***	***Mean (SD)***	**Range**	**Handedness (%)***
Early Adolescents	Male	9	13.44 (0.72)	13 – 15	Right-handed (100%)
	Female	9	13.88 (0.92)	13 – 15	Right-handed (100%)
	Total	18	13.67 (0.84)		
Late Adolescents	Male	9	18.88 (0.78)	18 – 20	Right-handed (100%)
	Female	9	19.11 (0.78)	18 – 20	Right-handed (100%)
	Total	18	19.00 (0.76)		
Young Adults	Male	9	26.33 (0.70)	25 – 27	Right-handed (100%)
	Female	9	26.11 (0.92)	25 – 27	Right-handed (100%)
	Total	18	26.11 (0.92)		

Note. Handedness was reported by the participant on the screening form.

Participants were recruited via adverts on online social media platforms, flyers distributed to local schools, cafes, and businesses, and via email invitation to online

staff and student volunteer lists maintained by the UoS Psychology Department. To determine inclusion/exclusion of potential participants, individuals who indicated their willingness to take part were sent a participant screening form, along with a participant information sheet as well as a leaflet about EEG and how to prepare for an EEG experiment. Prior to participation, written consent was obtained from each participant, or where applicable from their parent/guardian (participants below 18 years of age). All participants received £10 compensation for taking part. This study was approved by the Department of Psychology Ethics Committee, University of Sheffield (see Appendices for copy of ethical approval).

2.2.2. Self-report measures

Each participant completed a set of self-report questionnaires prior to taking part in the EEG experiment. These questionnaires were used to assess their emotional and functional well-being (copies of the measures used are provided in the Appendices). The following questionnaires were used.

Adolescent questionnaires (13 – 15 years of age)

The Autism Spectrum Quotient (AQ -10, Adolescent Version; Allison, Auyeung, & Baron-Cohen, 2012) is a short version of the Autism Quotient and consists of 10 descriptive items designed to measure 'autistic traits' in children and adolescents aged 12 – 15 years. With the assistance of their parent/guardian participants indicated whether they definitely agree, slightly agree, slightly disagree or definitely disagree to each item. Item 1 for example asks whether 'S/he notices patterns in things all the time', item 2 asks whether 'S/he usually concentrates more on the whole picture, rather than the small details'. Items 1, 5, 8 and 10 are scored 1 point each if participants respond with definitely or slightly agree and items 2, 3, 4, 6, 7 and 9 are scored 1 point each if participants respond with definitely or slightly disagree. In a sample of 162 adolescents

aged 12 – 15 with an autism spectrum condition (ASC) using a cut-off point of 6, Allison, Auyeung, & Baron-Cohen (2012) reported a sensitivity of 0.93, specificity of 0.95, and positive predictive value (PPV) of 0.86.

The Self-Administered Rating Scale for Pubertal Development (Carskadon & Acebo, 1993) was used to assess pubertal development. Items ask about the adolescent's stage of development. For example, "Have you noticed any skin changes, especially pimples?", "Have you begun to menstruate?" for girls, "Have you begun to grow hair on your face?" for boys etc. Items 1 to 5 for boys are scored 1 – 4 points or are marked as missing if the response is unknown. Items 1 to 4 for girls are scored 1 – 4 points or are marked as missing if the response is unknown and item 5a is scored 4 points if menstruation has occurred or 1 point if menstruation has not yet occurred. These point values are averaged to give a pubertal development scale.

The State-Trait Anxiety Inventory for Children (STAI – C; Spielberger, 1973) was used to assess anxiety levels in the early adolescent group. The STAI – C is a 40-item measure of anxiety and consists of two scales, a state scale which measures current levels of anxiety (20-items) and a trait scale which measures a general disposition to anxiety traits (20-items). Participants are asked to read a number of statements and indicate how they feel to each one. For example, on the state scale participants are asked to indicate whether they feel 'very calm', 'calm' or 'not calm'. On the trait scale participants are asked to indicate whether anxious feelings occur 'hardly ever', 'sometimes' or 'often'. Each item is scored on a 3-point Likert scale (1 – 3-point values) with some items on the state scale reversed scored. The minimum possible score for each scale is 20 and the maximum possible score is 60.

Emotion Regulation Questionnaire for Children & Adolescents (ERQ – CA; Gullone & Taffe, 2012) is a revised version of the Emotion Regulation Questionnaire

(ERQ; Gross & John, 2003) and consists of 10 items used to asses two emotion regulation strategies, namely cognitive reappraisal (CR) and emotion suppression (ES). Revisions include simplification of item wording (e.g., “I control my emotions by not expressing them” reworded to “I control my feelings by not showing them”). In addition, the original 7-point Likert scale is reduced to 5 responses ranging from 1 (strongly disagree) to 5 (strongly agree). Items 1, 3, 5, 7, 8, and 10 assess cognitive reappraisal and items 2, 4, 6 and 9 assess emotion suppression. Higher scores indicate the greater use of the respective emotion regulation (ER) strategy. For the 6-item CR scale, the alpha reliability coefficients were reported as 0.83 and for the 4-item ES scale the alpha coefficient was reported 0.75 (Gullone & Taffe, 2012).

The Depression Anxiety Stress Scale (DASS-21; Lovibond & Lovibond, 1995) is a shortened version of the DASS which consists of 21 items designed to measure three facets of psychopathology; depressive symptoms (7 items, e.g. “I felt that life was meaningless”), anxious symptoms (7 items, e.g. “I felt scared without any good reason”), and stress (7 items, “I found it difficult to relax”). The items are scored on a 4-point Likert scale ranging from 0 (‘Did not apply to me at all’) to 4 (‘Applied to me very much or most of the time’). The DASS-21 demonstrates acceptable psychometric properties with alpha coefficients ranging from 0.88 for the depression subscale, 0.82 for the anxiety subscale, and 0.90 for the stress subscale (Henry & Crawford, 2005).

Late adolescent and young adults’ questionnaires (18 – 20 & 25 – 27 years of age)

The Autism Spectrum Quotient (AQ -10, Adult Version; Allison, Auyeung, & Baron-Cohen, 2012) is again a short version of the Autism Quotient and consists of 10 descriptive items designed to measure ‘autistic traits’ in adults. As with the adolescent version participants indicated whether they definitely agree, slightly agree, slightly disagree or definitely disagree to each item. Item 1 for example asks whether ‘I often

notice small sounds when others do not', item 2 asks whether 'I usually concentrate more on the whole picture, rather than the small details'. Items 1, 7, 8 and 10 are scored 1 point each if participants respond with definitely or slightly agree and items 2, 3, 4, 5, 6 and 9 are scored 1 point each if participants respond with definitely or slightly disagree. In a sample of 449 adults with ASC using a cut-off point of 6, Allison, Auyeung & Baron-Cohen (2012) reported a sensitivity of 0.88, specificity of 0.91, and positive predictive value (PPV) of 0.85.

The State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) was used to assess anxiety levels in the late adolescent and young adult group. Similar to the STAI-C described above, the STAI consists of 40 items with a state scale which measures current levels of anxiety (STAI-S, 20-items) and a trait scale which measures a general disposition to anxiety traits (STAI-T, 20-items). Again, similar to STAI-C, participants are asked to read a series of statements and respond. Unlike the STAI-C, for both state and trait scales, participants respond using a 4-point Likert scale and both scales have reverse scoring to some of the items. For example, on the STAI-S participants are asked to rate statements such as 'I feel calm', 'I feel secure', 'I feel tense' by responding 'not at all', 'somewhat', 'moderately so' or 'very much so'. Examples on the STAI-T include 'I feel pleasant', 'I feel nervous and restless', 'I feel rested'. Participants indicate how they generally feel by selecting either 'almost never', 'sometimes', 'often', or 'almost always'. The minimum possible score for each scale is 20 and the maximum possible score is 80.

The Emotion Regulation Questionnaire (ERQ, Gross & John, 2003). Like the ERQ-CA (described above), the ERQ comprises 10 items assessing cognitive reappraisal (CR, 6 items) and emotion suppression (ES, 4 items). Unlike the ERQ-CA, ERQ items are rated on a 7-point Likert scale. Again, higher scores indicate the greater

use of the respective ER strategy. The ERQ has been reported to have good internal consistency (0.73 for CR, 0.69 for ES).

The Depression, Anxiety and Stress Scale – 21 (DASS-21; Lovibond & Lovibond, 1995). Exactly the same scale as used in the early adolescent group. See above description.

2.2.3. Comparing child/adolescent questionnaire responses to adult questionnaire responses.

Given that the STAI child and STAI adult versions as well as the ERQ child/adolescent version and ERQ adult version use different rating scales (3-point vs 4-point and 5-point vs 7-point respectively), it was necessary to compute a percentage score in order to compare early adolescent group responses to late adolescent and young adult group responses. For each scale, each participants score was divided by the maximum possible score of that scale and multiplied by 100. For example, if a young adult participant scored 25 for the STAI state scale, the percentage score was calculated by dividing 25 by 80 (max possible score) multiplied by 100. If an early adolescent scored 30 on the STAI-C state scale, the percentage score was calculated by dividing 30 by 60 (max possible score) multiplied by 100. This was repeated for the ERQ and the ERQ-CA with the appropriate maximum score used in the calculation. The same DASS-21 questionnaire was used in all groups and the AQ-10 used the same scoring system so there was no need to compute a new score. Results are shown in Supplementary Table 2.1. Cronbach's alpha for each self-report measure are shown in Supplementary Table 2.2. For group differences on self-report measures see Supplementary Table 2.3.

2.2.4. EEG Procedure

Following electrode-cap placement, participants were seated approximately 1 meter distance from the monitor. All stimuli were presented on a grey background using Psychtoolbox 3 (Brainard, 1997; Pelli & Vision, 1997; Kleiner, Brainard, & Pelli, 2007). Before beginning the behavioural experiment, participants were informed that they would see three different shapes (cue stimuli) followed shortly after by a facial expression (imperative stimuli) at which point they needed to press the space bar with their dominant hand as quickly as possible. For all participants, this was their right hand. Participants were informed that they would see three different facial expressions and that their reaction times would be recorded and displayed on the screen at the end of each block. Participants were informed that the experiment initially involved a practice session consisting of 30 trials and that at the end of the practice session they would be asked to answer 3 questions. Participants were informed about the number of blocks/sessions involved and how long it would take to complete the experiment.

Participants were informed about the causes of EEG artefacts that can occur during EEG recordings and were asked to avoid excessive movements of the body and/or face during the trials and to avoid touching the electrode-cap during the experiment as a whole. Participants were also asked to turn off their mobile phones for the duration of the experiment. Participants were asked to keep looking at the fixation cross in the centre of the screen for the duration of each block/session. Participants were informed that they would have breaks between blocks/sessions and that they could take as long as they needed before starting the next block/session. Informed verbal consent was again sought and participants were again informed that they were free to stop at any time if they felt at all uncomfortable. Experimental instructions were repeated again on instructional slides at the beginning of the experiment and additional instructions were

presented throughout. During the experiment ceiling lights were turned off and the door to the room was kept closed. A baby cam was used to allow the experimenter to monitor the participant and to facilitate 2-way communication during the EEG recordings. To avoid possible eye irritation due to glare from the monitor, a rechargeable LED table lamp was used to provide some background lighting.

2.2.5. Stimuli and experimental paradigm

The use of faces in attentional studies has been well documented (Mack, Pappas, Silverman, & Gay, 2002; Vuilleumier, 2000), suggesting that facial stimuli are advantageous over other stimuli categories in early visual sensory perception (Calvo & Beltrán, 2013; Bindemann, Burton, Hooge, Jenkins, & De Haan, 2005; Hare et al., 2008). This may result from there relevance due to their salient nature as they encompass both biological and socially important information (Bindemann, Burton, Hooge, Jenkins, & De Haan, 2005). Given that attention is often oriented toward salient stimuli regardless of an individual's behavioural goals and that the presence of such stimuli may impair performance when used as distractors (Sato & Kawahara, 2015), face stimuli appear to be uniquely placed as stimulus-response activators provoking both affect and instigating motivationally driven behaviours. Moreover, enhanced capture of attention by threat-related stimuli such as human faces depicting anger, has been associated with increased anxiety levels in adults (Fox, Russo, Bowles, & Dutton, 2001) and children (Roy et al., 2008; Waters, Henry, Mogg, Bradley, & Pine, 2010).

In total, 192 naturalistic colour photographs of models depicting angry mouth closed, happy mouth open and neutral mouth closed facial expressions were used in the experiment. These images were obtained from the NimStim set of Facial Expressions (30 images; Tottenham et al., 2009, http://www.macbrain.org/resources.htm) and the 'FACES' database (162 images; Ebner, Riediger, & Lindenberger, 2010). Given the age

range in the present study, only images of the youngest faces were used (FACES database, age range 19-31). In an attempt to avoid replication during the experiment as a whole, images obtained from the NimStim set were used during practice trials only (N = 30; 5 male X 3 facial expressions, 5 female X 3 facial expressions). Images form the FACES database were used during the experimental trials (N = 162; 27 male models X 3 facial expressions, 27 female models X 3 facial expressions, age range 19-31). Due to the insufficient number of different model images available, it was necessary to use the same model in the 3 different conditions, i.e. depicting either an angry, happy or neutral facial expression. However, the same model was never shown twice during the same block.

The practice session consisted of 30 presentations of a warning stimulus (S_1; 500ms duration) followed 250ms later by the imperative stimulus (S_2; 500ms duration). Geometric shapes (triangle, circle, square) were used for S_1 while facial expressions (angry, happy, neutral) were used for S_2. On presentation of S_2, participants had to press the spacebar with their dominant hand as quickly as possible. The inter-trial interval was 2s. A fixation cross remained on screen throughout except during the presentation of the S_2. S_1 was always indicative of whether the facial expression that followed was either angry, happy or neutral. Three versions of the experiment were used. In version 1 of the experimental paradigm a triangle always predicted the onset of an angry face, a circle always predicted the onset of a happy face, while a square always predicted the onset of a neutral face. In version 2 a circle predicted the onset of an angry face, a square predicted on the onset of a happy face and a triangle predicted the onset of a neutral face. For version 3 a square was used in the angry condition, a triangle was used in the happy condition and a circle was used in the neutral condition. At the end of the practice session participants were presented with three slides asking which geometric

shape predicted which type of facial expression. Participants responded by pressing 1, 2 or 3 on the number pad. If a participant responded correctly to all three slides, they were free to start the experiment proper by pressing the space bar at a time of their choosing. If a participant gave one incorrect response, they were required to repeat the practice session until they gave three correct responses.

During the experiment proper, the presentation of images was pseudorandomized so that an angry, happy or neutral facial expression was never followed by another angry, happy or neutral facial expression. In addition, in each block for each participant the same ratio of male to female angry, happy and neutral facial expressions were presented (9 different male models X 3 conditions, 9 different female models X 3 conditions). Each image was 500 X 584 pixels in size and presented centrally on a grey background on a 24-inch Iiyama 1080p LED monitor with a refresh rate of 144 Hz.

In total, 162 experimental trials divided into 9 blocks (18 trials per block) were recorded for each participant. Each participant completed 54 trials for the angry condition, 54 trials for the happy condition, and 54 trials for the neutral condition. Each block lasted approximately 3mins and 30s. Each block began with the presentation of a black fixation cross in the centre of the screen. At the start of each trial the warning stimulus S_1 (triangle, circle or square) was presented for 500ms duration followed later by the imperative stimulus S_2 (angry, happy or neutral facial expression) for 500ms duration. The inter-stimulus interval (ISI) between S_1 offset and S_2 onset was 3 s and the inter-trial interval (ITI) was pseudorandomized to vary between 6, 7, and 8 s. Each ITI occurred an equal number of times in each block and an equal number of times before each condition (male face or female face). The fixation cross remained on screen throughout the trial except during the S_2 presentation. As in the practice sessions, three

versions of the paradigm were used. Version 2 and version 3 of the paradigm were counterbalanced with respect to version 1 (as noted above in the description of the practice sessions). (Figure 2.1).

For each age group, six participants took part in version 1 (3 male), six participants took part in version 2 (3 male), and six participants took part in version 3 (3 male). Again, participants were always required to press the space with their dominant hand as quickly as possible when the imperative S_2 facial expression appeared on the screen. Reaction times were recorded only if the button press occurred within a 1 s time window following S_2 onset. In an attempt to increase engagement and to motivate participants, mean reaction times were presented at the end of each block. If participants recorded a faster time than in the previous block, they were shown a congratulations slide. If participants recorded the fastest mean time in block 3 – 9 they were shown a congratulations best score slide. At the end of each block participants were also informed as to how many blocks they had completed/how many blocks left to go. At the end of block 3, block 6 and block 9 participants were again presented with three slides asking them which shape predicted which facial expression. Again, participants responded by pressing 1, 2, or 3 on the number pad. If they responded incorrectly the slides were shown again until correct responses to all 3 slides were given.

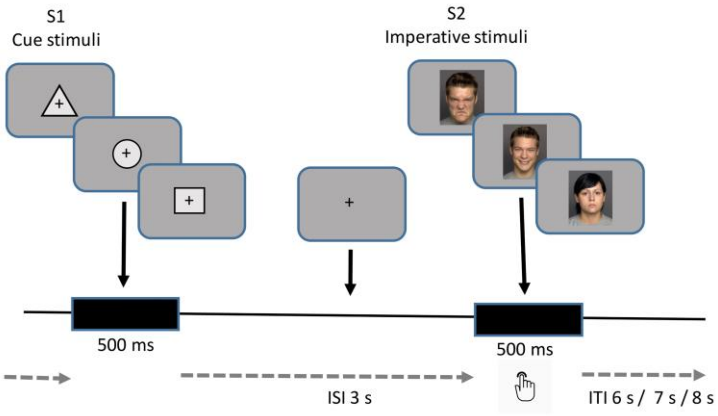


Figure 2.1. Experimental design of the emotional variant of the S_1-S_2 CNV paradigm.

2.2.6. EEG recording

The EEG signal was recorded using a Biosemi ActiveTwo system (Amsterdam, the Netherlands) from 64 'Pin-Type' Ag-AgCl active electrodes placed on the scalp and held in place by an appropriately sized cap according to the 10–20 system (Figure 2.2). Two additional sensors, a common mode sense (CMS) and driven right leg (DRL), were used as reference and ground respectively. After fitting the cap, measurements for head circumference, nasion to inion and from left pre-auricular to right pre-auricular were used to locate the position of the vertex (Cz). Before fitting the electrodes to the cap, a highly conductive saline based electrode gel (Signa Gel, produced by Parker Laboratories Inc; http://www.parkerlabs.com/signagel.asp) was applied to the scalp after slight abrasion of the skin. Electrode impedance levels were within the ± 25μV range for all participants. EEG signals were recorded continuously at a sampling rate of 2048 Hz using Biosemi ActiView software, and later down sampled to 512 Hz offline using Biosemi Decimator software. EEG signal preprocessing, averaging and measurement were conducted offline using EEGLAB 13.5.b (Delorme & Makeig, 2004) and ERPLAB 7.0.0 Matlab toolboxes (Lopez-Calderon & Luck, 2014). An important point to note here is that prior to artefact rejection and correction, each participant was randomly assigned a new participant ID. In so doing each of the preprocessing steps outlined in the following sections were performed blind without knowledge of group membership. Once the ERPs had been computed the original participants' IDs were then re-assigned.

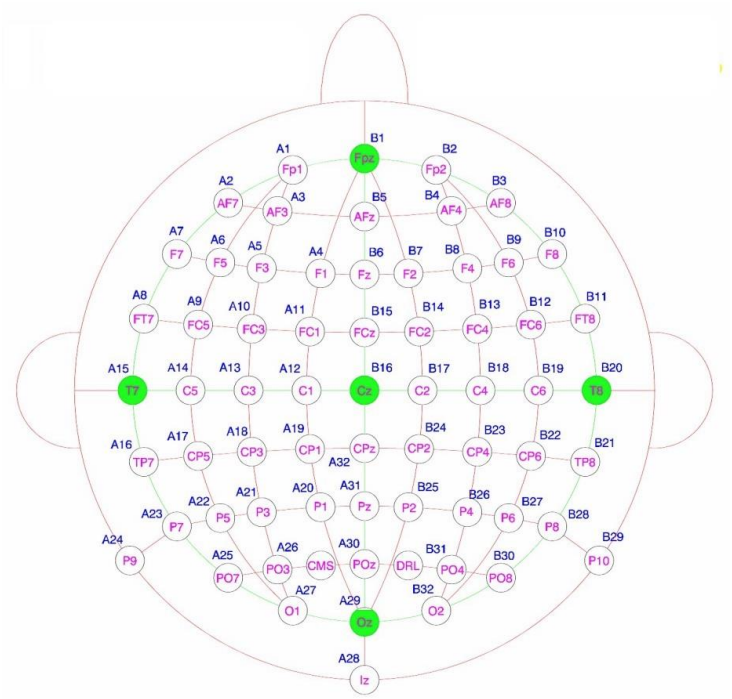


Figure 2.2. Illustration of channel locations according to the 10-20 system. Image shown from https://www.biosemi.com/headcap.htm

2.2.7. Importing data, DC offset and high-pass filter

The EEG BioSemi.bdf data files were imported and referenced to Cz. This initial referencing step was necessary as BioSemi ActiveTwo amplifiers allow for reference free recordings by way of the CMS-DRL loop (www.biosemi.com/faq/cms&drl.htm). When no electrode is chosen post hoc as reference during import, the signals are displayed with respect to CMS which results in a lower common-mode rejection ratio (CMMR) leading to 40db of unnecessary noise in the signal (www.biosemi/faq/cms&drl.htm). To deal with DC trends in the data, a subtraction of the average voltage of the whole EEG data waveform from each point in the waveform was applied before applying a high-pass filter (https://github.com/lucklab/erplab/wiki/Filtering). Continuous EEG data was filtered offline in ERPLAB with an IIR Butterworth high-pass noncausal filter of 0.05 Hz (order 2, 12dB/octave -6 d/B half-amplitude roll-off). In ERPLAB IIR filters are applied in

both directions to produce a zero phase-shift (https://github.com/lucklab/erplab/wiki/Filtering).

2.2.8. Artefact rejection and correction

Although EEG is designed to record neural signals generated in the cerebral cortex, it also records electrical activity arising from other physiological and non-physiological sources. Typical artefacts in the EEG signal include eye blinks, lateral eye movements, muscle contractions such as clenching of the jaw and frowning or swallowing and chewing, as well as cardiac/pulse artefacts and sweating across the scalp. Non-physiological artefacts can result from poor electrode impedances or changes in electrode impedances during recordings in addition to sources of environmental electrical noise. For example, 50 Hz line noise oscillations in the EEG signal results from AC power line fluctuations, electrical equipment and fluorescent lights etc. In the present study, 50 Hz line noise was removed using the Cleanline toolbox (EEGLAB plugin). Cleanline is advantageous over the use of notch filters because notch filters often employ a notch width of 10 Hz or larger which can lead to band-holes in the signal and distortions, in this case in the 40Hz – 60Hz range (Bigdely-Shamlo, Mullen, Kothe, Su, & Robbins, 2015). Cleanline uses an alternative approach to reduce line noise by applying a sliding window to the EEG data which adaptively estimates and removes the 50 Hz line noise sinusoidal artefact without creating distortions in the signal. However, the default settings did not work well for all participants. In these cases, a sliding window of 2s rather than the 1s default sliding window worked well in most instances. However, this step was not essential as a low-pass filter of 30 Hz was applied at a later stage of preprocessing but this step left open the possibility of analysing the data in different ways.

After applying Cleanline to remove 50 Hz line noise, the continuous EEG data was visually inspected for bad sections of data. That is, sections of continuous data that included ECG artefacts, EMG artefacts, slow drifts due to sweating on the scalp and/or non-physiological sources of noise. These bad sections of data were removed. In addition, bad channels were identified by way of visual inspection of the continuous data in conjunction with the plotting of channel spectra and maps. Channels that showed clear deviations or noise throughout the recording were removed before applying the average reference. This was done to avoid including bad channels and therefore bad data in the average reference. The data was then re-referenced to the average prior to the use of independent component analysis (ICA).

ICA is a data driven blind source separation technique which has been shown to effectively identify artefacts in a signal – eye blinks, eye movements, muscle contractions (EMG), and line noise (Jung et al., 1998a, 1998b; Jung et al., 2000). While ICA can and has been used for other purposes such as source localisation (Makeig, Debener, Onton, & Delorme, 2004), in the present study, an extended ICA decomposition algorithm (Delorme & Makeig, 2004) was used to facilitate the removal of eye blinks and lateral eye movement contaminants from the EEG signal. In essence, ICA reduces the overall signal data into independent and distinct components which make up that signal. This allows for separating out and the removal of ocular artefacts. The use of artefact correction – in conjunction with rather than the exclusive use of artefact rejection – allows for the inclusion of additional data which would have otherwise been removed if only artefact rejection had been used. This increases the signal-to-noise ratio of the data because the removal of artefactual data inevitably removes underlying neural activity as well. The exclusive use of artefact rejection is

particularly problematic in clinical or developmental studies in which patients or young participants often have much noisier data.

Following decomposition, the ADJUST toolbox (Mognon, Jovicich, Bruzzone, & Buiatti, 2011) was used to help identify and correct ocular artefacts in the signal. Additionally, the independent components were plotted in the continuous data through the Plot > Components activation scroll function in the EEGLAB menu. Following ICA and ocular artefact correction, previously removed channels were interpolated using the spherical spline interpolation option (Perrin, Pernier, Bertrand, & Echallier, 1989). Following interpolation, a 30 Hz low-pass IIR Butterworth filter (order 2, 12dB/octave - 6 d/B half-amplitude roll-off) was applied to the data. For CNV analyses, data was then epoched -500ms to 5000ms locked to S_1 onset with a -500ms to 0ms baseline correction. For P1 analyses to S_1, data was epoched -200ms to 1000ms, locked to S_1 onset, with a -200ms to 0ms baseline correction. For P1/N170 analyses to S_2, data was epoched -200ms to 1000ms, locked to S_2 onset, with a -200ms to 0ms baseline correction. Epochs with amplitude fluctuations of ± 150 μV were detected and excluded from the grand averaged ERPs by using the ERPLAB moving window peak-to-peak threshold function (channels 1:64, Moving Window Full Width 200ms, Window Step 100ms). For each epoch this function uses a moving window length in ms to calculate the peak-to-peak amplitude (difference between the most positive and negative peak) and compares these to the threshold set by the user. When the threshold is met or exceeded the epoch is marked for rejection (https://github.com/lucklab/erplab/wiki/Artifact-Detection-in-Epoched-Data). In addition, epochs which contained incorrect button presses were removed (button press to S_1, button press during ISI, no button press at all). Finally, ERPs for each condition were computed in ERPLAB by averaging across the remaining epochs. For the number

of channels removed, head size and room temperatures at the time of the recordings and button press errors see Supplementary Table 2.4. For the number of trials included in the analyses see Supplementary Table 2.5. A flow chart depicting each step in the preprocessing pipeline is presented in Appendix 35 (Figure. 35.1).

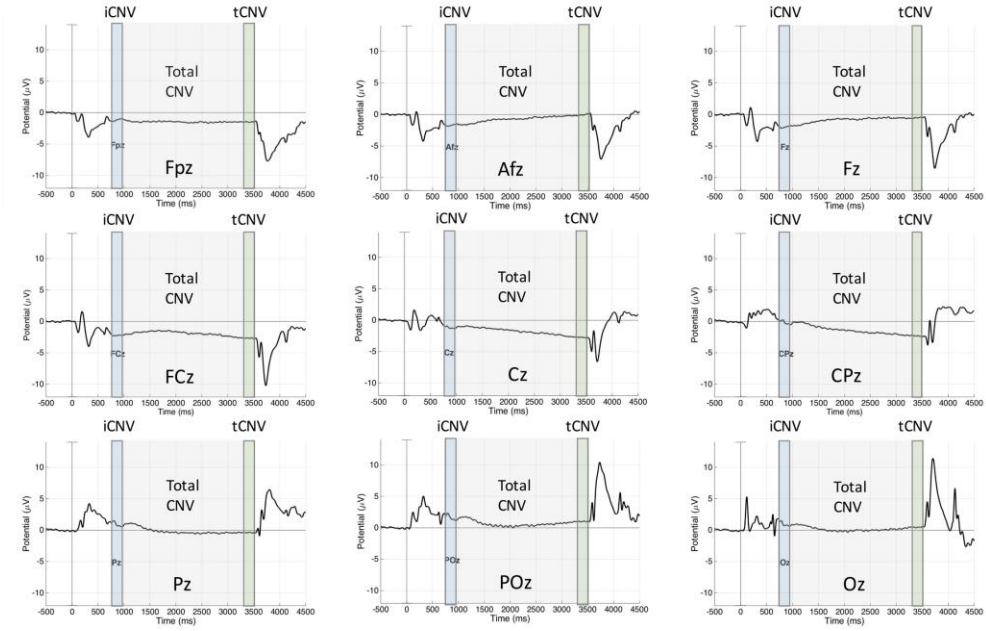
2.2.9. Selecting electrodes for analyses

To determine the location of maximal activity, grand-grand averaged ERP waveforms (waveforms containing all participants and all conditions) were created for the CNV and P1 stimulus-locked to S_1 onset, and the P1 and N170 stimulus-locked to S_2 onset. Descriptive statistics and visual inspection of the grand-grand averaged ERP waveforms were used to make a final determination of which electrodes to use for the ERP analyses. This was done in an attempt to avoid bias in the selection of the electrodes of interest thereby protecting against only choosing electrodes that showed the maximum difference between age groups. The use of collapsed localizers to determine electrodes of interest has become increasingly common in ERP research (Luck & Gaspelin, 2017).

The iCNV was measured as the mean amplitude in a time window of 750ms-950ms following S_1 onset at midline electrode sites FPz, Afz, FZ, FCz, Cz, CPz, PZ, Poz, Oz and lz. Mean amplitudes and visual inspection of CNV topographical maps indicated a frontal distribution with the maximum negativity observed at FCz (*M*=-2.21 μV) followed by Fz (*M*=-2.00 μV) and Afz (*M*=-1.71 μV). The tCNV was measured as the mean amplitude in the last 200ms prior to S_2 onset. Mean amplitudes at midline electrode sites FPz, Afz, FZ, FCz, Cz, CPz, PZ, Poz, Oz and lz and visual inspection of CNV topographical maps indicated a central distribution with the maximum negativity observed at the vertex Cz (*M*=-2.71 μV) followed by FCz (*M*=-2.66 μV) and CPz (*M*=-2.30 μV). Mean amplitudes for the total CNV (750ms – 3500ms) were maximal at

electrode sites FCz (*M*=-1.99 μV), followed by Cz (*M*=-1.73 μV) and FPz (*M*=-1.43). Based on the grand-grand averaged waveforms, a single electrode site was chosen for the iCNV (FCz), for the tCNV (Cz), and for the total CNV (FCz) (see Figure 2.3).

A



B

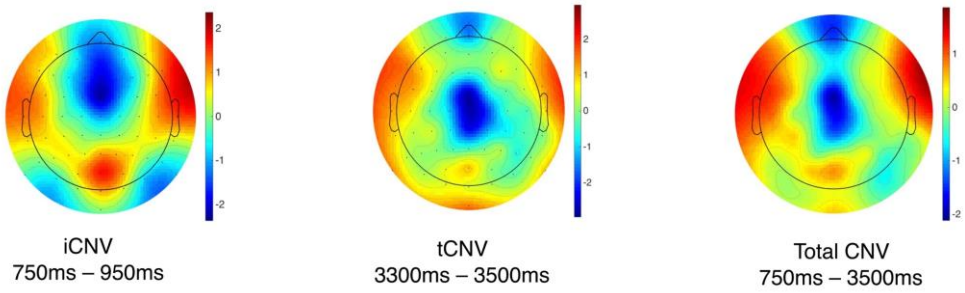


Figure 2.3. **(A).** Grand-grand averaged ERP waveforms at midline electrodes for the iCNV, tCNV and total CNV. **(B).** ERP scalp topographies showing mean amplitude for the iCNV between 750-950ms, tCNV between 3300-3500ms and total CNV between 750-3500ms after S_1 onset (N=54).

Based on the grand-grand averaged P1 and N170 ERP waveforms, P1 local peak amplitudes were defined as the maximum positive voltage measured between 80-120ms after stimulus onset (S_1 and S_2) and N170 local peak amplitudes were defined as the

maximum negative going voltage measured between 120-180ms after stimulus onset (S_2). In respect to P1, in response to S_1 I observed the largest P1 local peak amplitudes at occipital electrodes O1 (*M*=4.78 μV) and O2 (*M*=5.02 μV) followed by PO7 (*M*=3.51 μV) and PO8 (*M*=4.57 μV) at approximately 110ms following S_1 onset with a right hemisphere advantage (see Figure 2.4). In response to S_2 I again observed the largest P1 local peak amplitudes at O1 (*M*=5.72 μV) and O2 (*M*=5.98 μV) and PO7 (*M*=4.55 μV) and PO8 (*M*=8.19 μV) at approximately 95ms following S_2 onset and again showing a clear right hemisphere advantage (see Figure 2.5). Due to the larger P1 peak amplitude response to faces in the right hemisphere at PO8 compared to O2 (with the reverse occurring in the left hemisphere), a new waveform was created in ERPLAB collapsing across electrodes O1/PO7 for the left hemisphere and O2/PO8 for the right. Based on the grand-grand averaged waveforms, two electrode sites were chosen for the P1 in response to S_1 (O1/O2) and two electrode clusters were chosen for the P1 in response to S_2 (O1/PO7 and O2/PO8).

Given that N170 amplitudes in early adolescents often occur in the positive range, it was necessary to use a subtraction method (P1 local peak amplitude – N170 local peak amplitude) to better isolate the N170. This was done to allow for more exact comparisons between age groups (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010). The N170 was identified as the first negative going peak immediately following the P1 and the maximum amplitude of N170 ERP waveforms were arrived at by using the subtraction method outlined above at each of the following electrode sties, P5/P6, P7/P8, P9/P10, PO3/PO4, PO7/PO8, and O1/O2. I observed N170 maximum peak to peak amplitude in response to face stimuli at bilateral electrodes P9 (*M*=6.49 μV) and P10 (*M*=8.58 μV) at approximately 145ms following S_2 onset (see Figure 2.5).

Based on these findings, these two electrode sites were chosen for the N170 in response to S_2 (P9/P10).

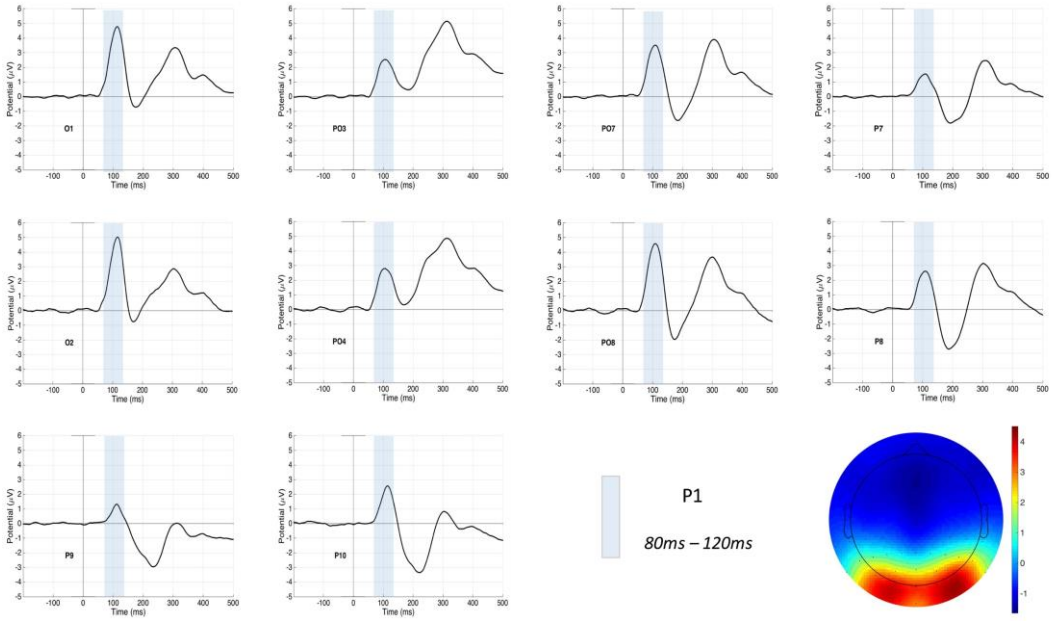
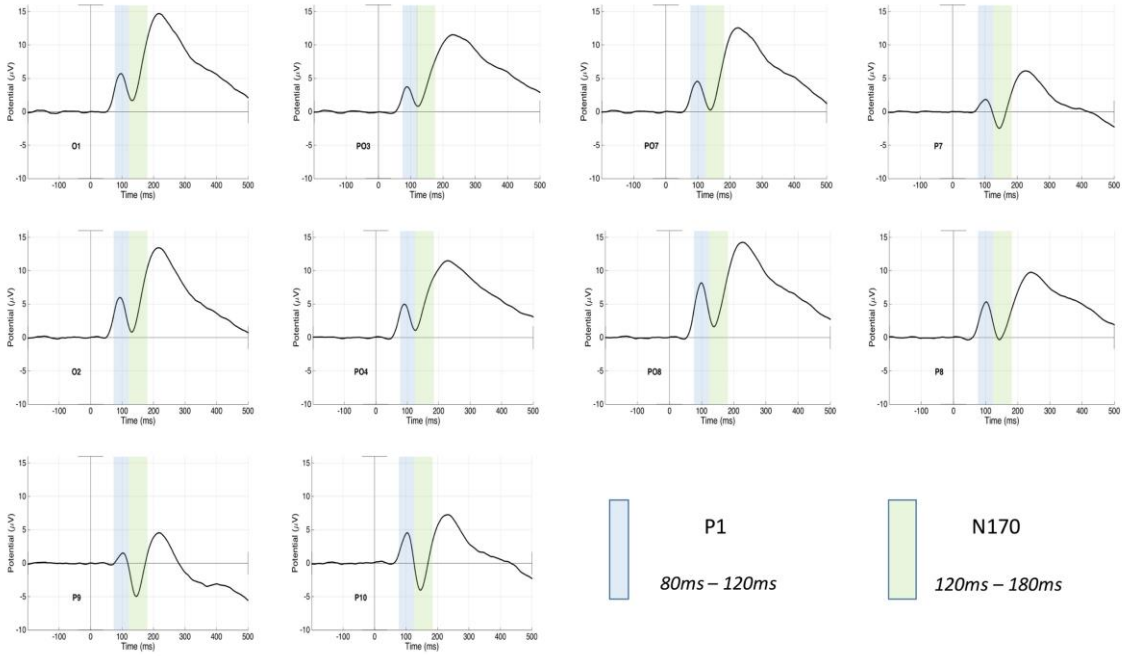


Figure 2.4. Grand-grand averaged ERP waveforms at posterior electrodes for the P1 in response to S_1 (shape) with accompanying ERP scalp topography showing mean amplitude between 80-120ms after S_1 onset (N=54).

A



B

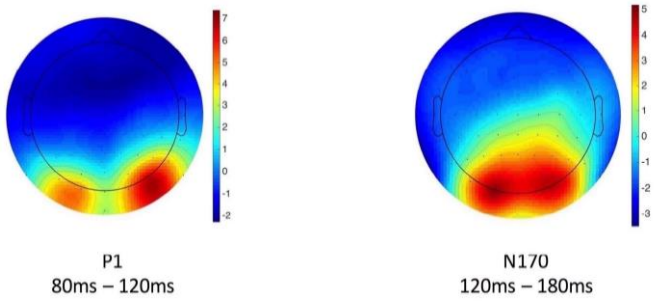


Figure 2.5. **(A).** Grand-grand averaged ERP waveforms at posterior electrodes for the P1 and N170 in response to S_2 (face). **(B).** P1 scalp topography showing mean amplitude between 80-120ms after S_2 onset and the N170 scalp topography showing mean amplitude between 120-180ms after S_2 onset (N=54).

2.2.10. Selecting time windows for analyses of individual ERP waveforms

Upon closer inspection of the data, it became clear that at an individual level, the time windows chosen for the visually evoked grand-grand averaged ERP waveforms (P1, N170) were inappropriate given the variance in component latency across subjects, in particular between the early adolescent group and the two older groups. Therefore, the P1 local peak amplitude in response to S_1 and S_2 was measured separately for each

participant and each condition in a 70ms-150ms time window for S_1 at electrodes O1/O2 and a 50ms-135ms time window for S_2 at electrode clusters O1/PO7 and O2/PO8. To determine the N170 local peak amplitude at electrodes P9/P10 for each participant in each condition, a time window of 50-135ms for P1 and a time window of 120ms-190ms for N170 was sufficient to accurately capture both. It was not necessary to tweak the time windows already used to measure iCNV, tCNV and total CNV of the grand-rand averages and therefore these time windows were retained for the analyses of CNV mean amplitude. An example of how measurements were taken using the ERPLAB measurement tool is presented in Appendix 36.

2.2.11. Statistical analyses

Reaction time data was analysed using a two-way mixed design analysis of variance (ANOVA) with trial type (condition) as the within-subjects factor and age group as the between-subjects factor. For iCNV, tCNV and total CNV analyses, three separate two-way mixed design ANOVAs were conducted, with trial type (condition) as the within-subjects factor and age group as the between-subjects factor. For the analyses of early visually evoked potentials (P1, N170) a series of three-way mixed design ANOVAs were conducted, with trial type (condition) and electrode location (left and right hemisphere) as the within-subjects factors and age group as the between-subjects factor. For the ERP and behavioural data analyses, if extreme outliers were found in the data, as assessed by studentized residual values being ± 3 standard deviations away from the mean, these extreme outliers were removed prior to running the ANOVA. If any cells of the design were found to be non-normally distributed after the removal of said outliers, as assessed by Shapiro-Wilk's test of normality ($p < .05$), but the skew was moderate and in the same direction it was decided to continue with this analysis. This is because ANOVAs are fairly "robust" to deviations from normality and there is

no non-parametric equivalent of a two-way or three-way mixed ANOVA. In instances were homogeneity of variance was violated, as assessed by Levene's test of homogeneity of variance ($p < .05$), but sample sizes in each group where the same, it was decided to continue with this analysis. This is because ANOVAs are considered to be somewhat robust to violations of homogeneity of variance if group samples are of equal size (Field, 2018; Tabachnick, Fidell, & Ullman, 2007). Greenhouse Geisser correction was used in situations where Sphericity was violated (Mauchley's test, $p < .05$). In addition to ANOVAs, standard multiple regression was used to investigate the relationship between early/initial and late/terminal CNV mean amplitudes (iCNV and tCNV respectively), state anxiety and mean reaction times (see Appendix 32). All statistical analyses were performed using an alpha level of $p < 0.05$ in the IBM SPSS Statistics for Mac software package, Version 24.0. Assumption testing for each ANOVA can be found in Appendix 31.

2.3. Results

2.3.1. Task performance summary

In this task, participants were asked to respond as quickly as possible when they saw a face (S_2) on the screen by pressing the space bar with their dominant hand. Participant reaction times were recorded in each condition (angry, happy, neutral) to determine whether the presentation of angry, happy, and neutral faces differentially modulated the speed of participants' responses. The analyses of reaction times resulted in two significant findings. First, and consistent with previous comparative developmental studies of reaction times in early adolescents (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Klein & Feige, 2005; Perchet & Garcia-Larrea, 2005), regardless of trial type, early adolescents had slower responses to the face stimuli compared to both the late adolescents and young adults. Second, for this younger age

group only, responses were found to be modulated by facial expression with faster reaction times to happy faces compared to faces showing neutral expressions . The statistical analyses used to make this determination is described below.

2.3.2. Mean reaction time ANOVA analyses

The results of the two-way mixed design ANOVA indicated that there was a statistically significant interaction effect of condition and age group on mean reaction time $F(2, 102) = 4.122$, $p = .004$, partial $\eta^2 = .139$. To investigate where these group difference where with respect to each measure of reaction times (angry, happy, neutral), a total of three between-subjects ANOVAs using the univariate function in SPSS were conducted. When examining the simple main effects of group on mean reaction times in response to the face stimuli, the results indicated that there was a statistically significant difference in mean reaction times between age groups in the angry condition $F(2, 51) = 10.079$, $p < .001$, partial $\eta^2 = .283$, happy condition $F(2, 51) = 8.997$, $p < .001$, partial $\eta^2 = .261$, and in the neutral condition $F(2, 51) = 13.040$, $p < .001$, partial $\eta^2 = .338$. In each case, Tukey HSD adjusted post-hoc tests showed that the early adolescent group had significantly slower RTs compared to both the late adolescent and young adult group (all p values < .01) (Table 2.2). There were no significant differences between the late adolescent group and the young adult group (all p values > .05).

Table 2.2. *Mean reaction times in each condition and standard deviations (SD).*

	Early adolescent (n = 18)	**Late adolescent** (n = 18)	**Young adult** (n = 18)	**1***	**2***
Condition		*Mean RT (SD)*		*p*	*p*
Angry	352.500 (41.812)	286.967 (44.658)	297.819 (53.546)	< .001	.003
Happy	345.823 (37.138)	293.209 (50.122)	290.559 (44.041)	.002	.001
Neutral	358.126 (44.402)	289.285 (43.015)	292.125 (49.609)	< .001	< .001

Note. 1 = early adolescents > late adolescents, 2 = early adolescents > young adults

To examine the simple main effects of condition on mean reaction time at each level of the group factor, the data was split by age group. For the early adolescent group there was a statistically significant effect of condition on mean reaction time, $F(2, 34) = 3.947$, $p = .029$, partial η2 = .188. This was not the case for late adolescents $F(2, 34) = 2.803$, $p = .075$, partial $\eta^2 = .142$ or young adults $F(2, 34) = 2.325$, $p = .113$, partial $\eta^2 = .120$. In addition to *MD* (mean difference) and CI (confidence interval), the data presented are marginal mean and ± standard error. Within the early adolescent group, Bonferroni corrected pairwise comparisons revealed that mean reaction times in response to the happy face stimuli were significantly faster (*M*=345.823, ± 8.754) compared to the neutral face stimuli (*M*=358.126, ± 10.466, *MD* = -12.304, *SE* = 4.300, $p = .032$, 95% CI = -23.72 to -.88). There was no statistically significant difference between mean reaction times in response to angry faces (*M*=352.500, ± 9.855) compared to the happy faces (*MD* = 6.677, *SE* = 5.058, $p = .613$, 95% CI = -6.75 to 20.10) or happy faces compared to neutral faces (*MD* = -5.627, *SE* = 3.687, $p = .436$, 95% CI = -15.41 to 4.16) (see Figure 2.6).

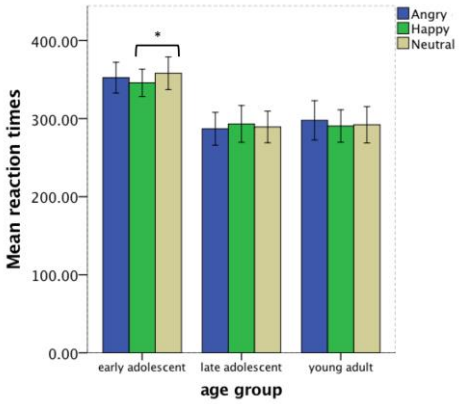


Figure 2.6. Mean reaction times in response to angry, happy and neutral faces for each age group. Error bars represent the standard error of the mean (SEM). Mean reaction times are in milliseconds (ms). *p < .05 (N=54).

2.3.3. No modulation of the CNV during anticipation of emotional and neutral faces

summary

The main aim of this study was to investigate whether anticipation of different facial expressions would differentially modulate early/initial (iCNV) and late/terminal (tCNV) phases of the CNV as well as total CNV in the transition from adolescence to adulthood. To determine whether this was or was not the case, I conducted three separate two-way mixed design ANOVAs, one for iCNV mean amplitude at electrode FCz, one for tCNV mean amplitude at electrode Cz and one for total CNV mean amplitude at electrode FCz as the dependent variables. Condition (angry, happy, neutral) was entered as the within-subjects factor and age group as the between-subjects factor. These analyses were all non-significant suggesting that the anticipation of faces showing emotional or neutral expressions did not differentially modulate neural activity previously associated with preparation for action. There was however a trend in the data suggesting that early adolescents had attenuated tCNV amplitudes compared to late adolescents and young adults. Mean amplitudes for each CNV measure are provided in Table 2.3.

Table 2.3. *Mean potential for iCNV, tCNV and total CNV in each condition for each age group (N = 54).*

	Early adolescent	**Late adolescent**	**Young adult**
Condition	*iCNV FCz (μV)*	*iCNV FCz (μV)*	*iCNV FCz (μV)*
Angry	-1.852	-2.300	-1.721
Happy	-2.405	-2.800	-1.607
Neutral	-2.478	-2.713	-2.080
	Early adolescent	**Late adolescent**	**Young adult**
Condition	*tCNV Cz (μV)*	*tCNV Cz (μV)*	*tCNV Cz (μV)*
Angry	-.904	-3.409	-3.015
Happy	-1.771	-4.172	-3.563
Neutral	-.991	-3.810	-2.654
	Early adolescent	**Late adolescent**	**Young adult**
Condition	*Total CNV FCz (μV)*	*Total CNV FCz (μV)*	*Total CNV FCz (μV)*
Angry	-1.630	-1.909	-2.070
Happy	-1.111	-2.769	-2.321
Neutral	-1.552	-2.300	-2.298

Note. iCNV = initial contingent negative variation; tCNV = terminal contingent negative variation

2.3.4. iCNV ANOVA analysis

First, I investigated the effect of the predictive cue stimuli on iCNV mean amplitude. That is, how was the information provided by the cue processed differently depending on the condition and between age groups. The results indicated that there was no statistically significant interaction effect of condition and age group on iCNV mean amplitude $F(4, 100) = .514$, $p = .726$, partial $\eta^2 = .020$. The main effect of condition was statistically non-significant $F(2, 100) = 1.459$, $p = .237$, partial $\eta^2 = .028$, as too was the main effect of group $F(2, 50) = .421$, $p = .659$, partial $\eta^2 = .017$. These results show that in our sample iCNV mean amplitude did not differ significantly between conditions depending on age group, nor was there significant differences in iCNV mean amplitude between conditions when ignoring group membership, nor was there significant differences in iCNV mean amplitude as a whole regardless of condition between early adolescents, late adolescents and young adults (see Figure 2.7).

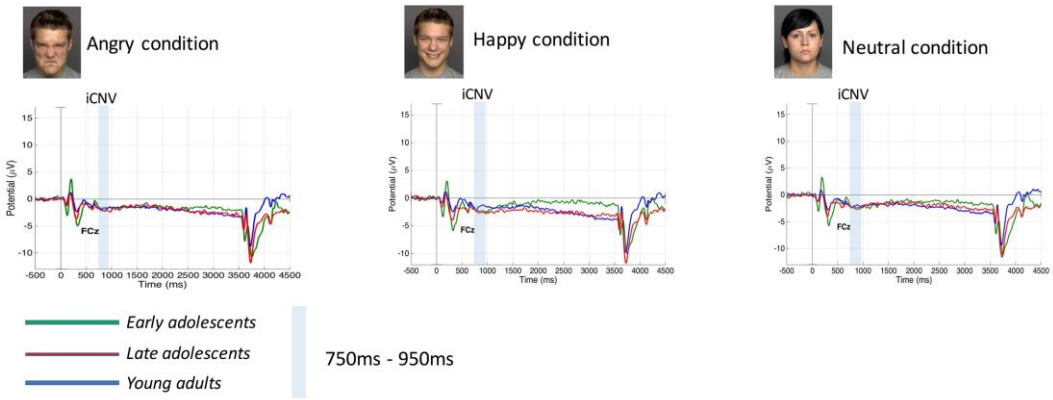


Figure 2.7. Grand averaged iCNV ERPs for each age group for each condition (N=53).

2.3.5. tCNV ANOVA analysis

Second, I investigated whether tCNV mean amplitude differed depending on the type of facial expression the participants were expecting to see and whether tCNV mean

amplitude differed between age groups. The results of the ANOVA showed that there was no statistically significant interaction effect of condition and age group on tCNV mean amplitude $F(3.566, 89.1154) = .171$, $p = .939$, partial $\eta^2 = .007$. The main effect of condition was statistically non-significant $F(1.783, 89.154) = 2.173$, $p = .125$, partial $\eta^2 = .042$, as too was the main effect of group $F(2, 50) = 3.100$, $p = .054$, partial $\eta^2 = .110$. These results show that in this sample tCNV mean amplitude did not differ significantly between conditions depending on age group, and that tCNV mean amplitude did not differ significantly between conditions when ignoring group membership. However, while there was no statistically significant difference in tCNV mean amplitude as a whole regardless of condition between early adolescents, late adolescents and young adults, there was a trend in the data suggesting that early adolescents did have attenuated tCNV amplitudes compared to late adolescents and young adults (see Figure 2.8).

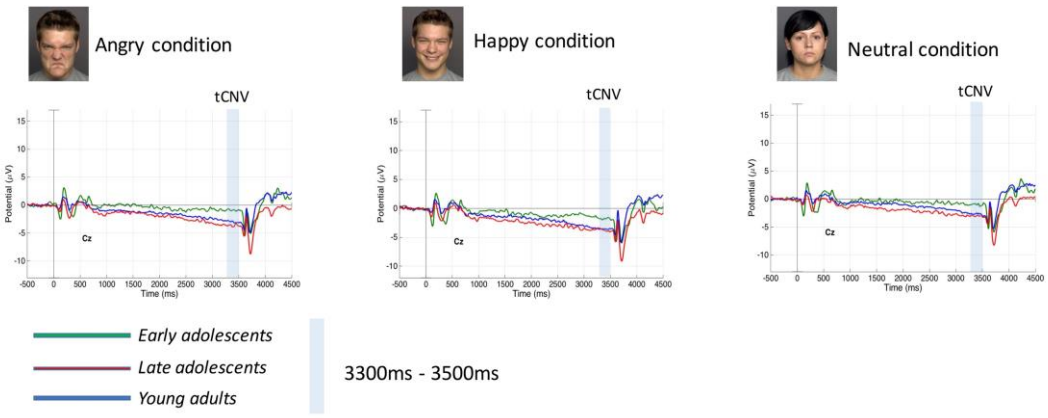


Figure 2.8. Grand averaged tCNV ERPs for each age group for each condition (N=53).

2.3.6. Total CNV ANOVA analysis

Lastly, I investigated whether anticipation of angry, happy or neutral faces differentially modulated the anticipatory period as a whole in an attempt to assess

whether a more general measure of anticipation/expectancy as indexed by the total CNV differed between conditions and age groups. The results of the ANOVA showed that there was no statistically significant interaction effect of condition and age group on total CNV mean amplitude at electrode site FCz $F(4, 102) = 1.080$, $p = .370$, partial $\eta^2 = .041$. Additionally, the main effect of condition was statistically non-significant $F(2, 102) = .315$, $p = .731$, partial $\eta^2 = .006$, as too was the main effect of age group $F(2, 51) = 0.945$, $p = 0.395$, partial $\eta^2 = .036$. As was the case for iCNV and tCNV, these results again show that in this sample total CNV mean amplitude did not differ significantly between conditions depending on age group. Total CNV mean amplitude did not differ significantly between conditions when ignoring group membership and total CNV mean amplitude across all condition did not differ significantly between early adolescents, late adolescents and young adults (see Figure 2.9).

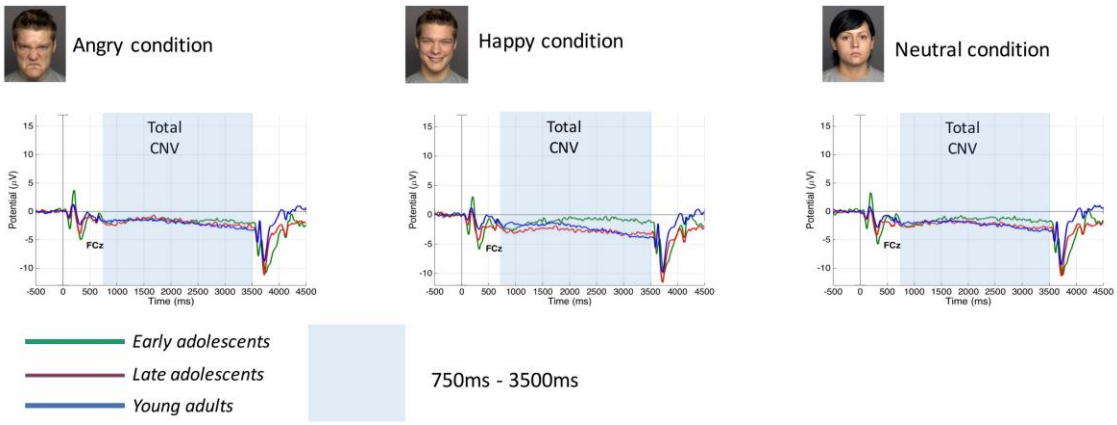


Figure 2.9. Grand averaged total CNV ERPs for each age group for each condition (N=54).

2.3.7. Modulation of early visually evoked potentials to S_1 and S_2 summary

A further aim of this study was to investigate whether the different cues (S_1) predicting angry, happy and neutral facial expressions (S_2) would result in differential modulation of the visual P1 in response to these cues, and whether the degree of modulation would differ significantly between early adolescents, late adolescents and young adults. I also investigated whether viewing angry, happy and neutral facial expressions (S_2) would result in differential modulation of the visual P1 and N170 to these faces. In other words, I was interested to find out whether the information portrayed by the type of predictive cue (S_1) and the type of facial expression (S_2) was processed differently depending on the condition and depending on age group. Again, as stated in the introduction (Section 2.1), these measures were taken in an attempt to make interpretations of the CNV findings more intelligible. More specifically, age-dependent modulation of the visual P1 and N170 by affect laden stimuli may prove to be informative with regard to how these stimuli are processed differently in the brain. Furthermore, because I didn't find any significant differences between conditions and between age groups in CNV mean amplitudes (CNV amplitudes were not condition dependent), if significant differences existed in these early visually evoked potentials if not in the CNV, this would at least suggest a degree of segregation or partitioning of the functions in the brain associated with the processing of emotional information.

To determine whether this was or was not the case, I conducted three separate three-way mixed design ANOVAs, one for the visual P1 to S_1, one for the visual P1 to S_2, and one for the N170 to S_2. The results of these analyses showed that while early adolescents had significantly larger visual P1 peak amplitudes in each condition for both the left and right hemisphere compared to late adolescents and young adults, across all age groups, visual P1 peak amplitudes were not modulated by the type of predictive

cue the participants saw. However, for the early adolescent group only, visual P1 peak amplitudes were found to be larger over the right hemisphere compared to the left hemisphere of the brain. For the visual P1 to the faces (S_2), across age groups, significant differences were found between viewing angry faces compared to neutral faces with greater peak amplitudes in the angry condition compared to the neutral condition. Additionally, greater visual P1 peak amplitudes were found over the right compared to the left hemisphere, and across all conditions, visual P1 peak amplitudes were greater in the early adolescent group compared to the late adolescent and the young adult group. For the N170 the results were slightly different. Across all age groups, while the N170 amplitudes were found to be significantly greater to angry faces compared to neutral faces and while N170 amplitudes were found to be significantly greater over the right compared to the left hemisphere of the brain, unlike the visual P1 to S_1 and S_2, age group differences in N170 amplitude were non-significant. The statistical analyses used to investigate the modulation these early visually evoked potentials are described below.

2.3.8. Visual P1 to S_1 ANOVA analyses

To determine whether there was an interaction effect of age group, condition, and electrode location (left vs right hemisphere) on visual P1 peak amplitude to the predictive cue stimuli (S_1), a three-way mixed design ANOVA was conducted with group as the between-subjects factor with three levels (early adolescent, late adolescent, young adult) and condition (angry, happy, neutral) and electrode location (O1/O2) as the two within-subjects factors. The three-way interaction between age group, condition, and electrode location was not statistically significant $F(4, 96) = 1.121$, $p = 0.500$, partial $\eta^2 = .034$, suggesting that visual P1 peak amplitudes in response to the

predictive cue stimuli (S_1) did not differ significantly between conditions and electrode location as a function of group membership. The ANOVA also did not find a statistically significant two-way interaction between condition and age group on visual P1 peak amplitudes in response to the predictive cue stimuli $F(4, 96) = .756$, $p = 0.556$, partial $\eta^2 = .031$ showing that when electrode location is ignored visual P1 peak amplitudes did not differ significantly between conditions depending on the age group. Nor was there a significant two-way interaction between condition and electrode location on visual P1 peak amplitudes in response to the predictive cue stimuli $F(2, 96) = .252$, $p = 0.778$, partial $\eta^2 = .005$ showing that when group membership is ignored visual P1 peak amplitudes did not significantly differ between conditions depending on the electrode location i.e. left hemisphere (O1) vs right hemisphere (O2).

There was however a statistically significant two-way interaction between electrode location and age group on visual P1 peak amplitudes in response to the predictive cue stimuli $F(2, 48) = 3.438$, $p = 0.040$, partial $\eta^2 = .125$. To investigate where these group difference where with respect to each measure of the visual P1 peak amplitude (angry, happy, neutral) at electrodes O1 and O2, a total of six between-subjects ANOVAs using the univariate function in SPSS were conducted. When examining the simple main effects of age group on visual P1 peak amplitude in response to the cue stimuli, the results indicated that there was a statistically significant difference in visual P1 peak amplitude between age groups at electrode O1 in the angry condition $F(2, 48) = 18.266$, $p < .001$, partial $\eta^2 = .432$, happy condition $F(2, 48) = 15.085$, $p < .001$, partial $\eta^2 = .386$, and in the neutral condition $F(2, 48) = 12.139$, $p < .001$, partial $\eta^2 = .336$. This was also the case for electrode site O2 which showed statistically significant differences in visual P1 peak amplitude between age groups in the angry condition $F(2, 48) = 26.026$, $p < .001$, partial $\eta^2 = .520$, happy condition $F(2,$

48) = 22.684, $p < .001$, partial $\eta^2 = .486$, and in the neutral condition $F(2, 48) = 13.390$, $p < .001$, partial $\eta^2 = .358$. In each case, Games-Howell adjusted post-hoc tests showed that the early adolescent group had significantly greater visual P1 peak amplitude compared to both the late adolescent and young adult group (all p values < .01) but there were no significant differences between the late adolescent group and the young adult group (all p values > .05) (see Figure 2.10, Supplementary Table 2.6).

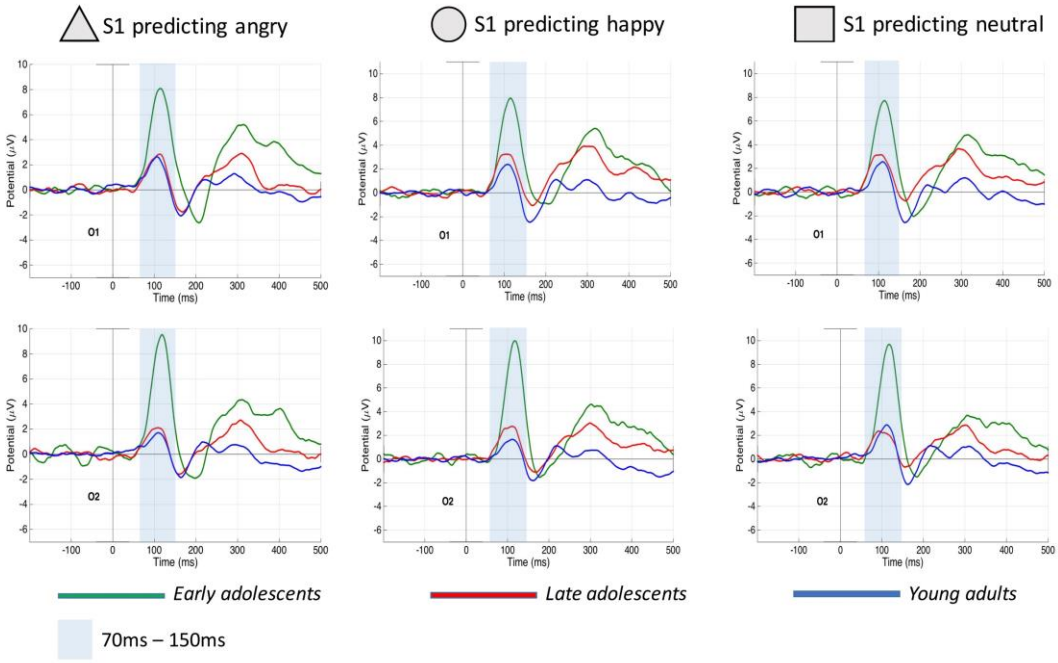


Figure 2.10. Visual P1 ERPs in response to cue stimuli in each age group for the left hemisphere (O1) and the right hemisphere (O2) (N=51). Note. S_1 shape was counterbalanced across participants. Shapes shown in figure are for illustrative purposes only.

To examine the simple main effects of electrode location on visual P1 peak amplitude, the data was split on the age group factor. For the early adolescent group there was a statistically significant difference in visual P1 peak amplitude between O1 and O2 $F(1, 16) = 14.161$, $p = .002$, partial $\eta^2 = .470$. This was not the case for late adolescents $F(1, 16) = .034$, $p = .857$, partial $\eta^2 = .002$, nor young adults $F(1, 16) = 1.568$, $p = .229$, partial $\eta^2 = .089$. In addition to *MD* (mean difference) and CI

(confidence interval), the data presented are marginal mean and ± standard error. Within the early adolescent group, Bonferroni corrected pairwise comparisons revealed that visual P1 peak amplitudes in response to the predictive cue stimuli were significantly greater in the right hemisphere (O2) with a mean of 10.320 ± 1.123 compared to the left hemisphere (O1) with a mean of 9.179 ± 1.049 (*MD* = 1.208, *SE* = .321, *p* = .002, 95% CI = .52 to 1.88) (see Figure 2.11).

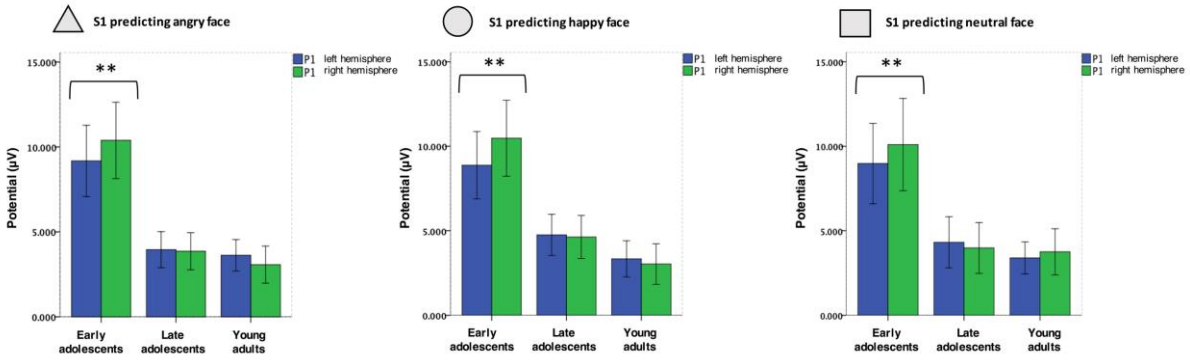


Figure 2.11. Visual P1 peak amplitudes in response to cue stimuli in each age group for the left hemisphere (O1) and the right hemisphere (O2). Greater P1 peak amplitudes were observed in the right hemisphere compared to the left hemisphere in early adolescents to each of the three cues. Error bars represent the standard error of the mean (SEM). **p < .001.

2.3.9. Visual P1 to S_2 ANOVA analyses

I also investigated whether viewing positive, negative and neutral emotional facial expressions (S_2) would result in differential modulation of the visual P1 and N170. For the visual P1, a three-way mixed design ANOVA with age group (early adolescent, late adolescent, young adult) as the between-subjects factor and condition (angry, happy, neutral) and electrode location (O1/PO7 and O2/PO8) as the two within-subjects factors. The results of the ANOVA indicated that the three-way interaction between age group, condition, and electrode location was not statistically significant $F(3.487, 88.916) = 1.710$, $p = 0.163$, partial $\eta^2 = .063$. The ANOVA also did not find a statistically significant two-way interaction between condition and age group on visual

P1 peak amplitudes in response to the face stimuli $F(3.832, 97.72) = .470$, $p = 0.750$, partial $\eta^2 = .018$, nor was there a significant two-way interaction between electrode location and age group on visual P1 peak amplitudes in response to the face stimuli $F(2, 51) = .976$, $p = 0.384$, partial $\eta^2 = .037$. Nor was there a significant two-way interaction between condition and electrode location on visual P1 peak amplitudes in response to the face stimuli $F(1.743, 88.917) = 1.701$, $p = 0.192$, partial $\eta^2 = .032$ showing that when group membership is ignored visual P1 peak amplitudes did not significantly differ between conditions depending on the electrode location i.e. left hemisphere (O1/PO7) vs right hemisphere (O2/PO8).

However, the results of the ANOVA did indicate that there was a significant main effect of condition $F(2, 102) = 5.689$, $p = 0.005$, partial $\eta^2 = .100$, a significant main effect of electrode location $F(1, 51) = 26.609$, $p < .001$, partial $\eta^2 = .343$ and a significant main effect of age group $F(2, 51) = 19.941$, $p < .001$, partial $\eta^2 = .439$. In addition to *MD* (mean difference) and CI (confidence interval), the data presented are marginal mean and ± standard error. Bonferroni corrected pairwise comparisons revealed that visual P1 peak amplitudes in response to the angry face stimuli (*M*=9.353, ± .730) were significantly greater (*MD* = .724, *SE* = .213, *p* = .004, 95% CI = .20 to 1.25) than those found in response to the neutral face stimuli (*M*=8.630, ± .687) but not the happy face stimuli (*MD* = .471, *SE* = .238, *p* = .161, 95% CI = -.20 to 1.06) which had a mean of 8.882 ± .657. In addition, Bonferroni corrected pairwise comparisons revealed that visual P1 peak amplitudes were significantly greater in the right hemisphere (*M*=10.126, ± .789) compared to the left hemisphere (*M*=7.784, ± .637) (*MD* = 2.342, *SE* = .454, *p* < .001, 95% CI = 1.43 to 3.25). Finally, Bonferroni corrected pairwise comparisons revealed that across conditions and electrode locations visual P1 peak amplitudes were significantly greater in the early adolescent group (*M*=15.000, ±

1.178) compared to the late adolescent group (*M*=6.468, ± 1.178) (*MD* = 8.532, *SE* = 1.667, $p < .001$, 95% CI = 4.40 to 12.65) and young adult group (*M*=5.398, ± 1.178) (*MD* = 9.602, *SE* = 1.667, $p < .001$, 95% CI = 5.47 to 13.72). There was no significant difference between late adolescents and young adults ($p > .05$) (see Figure 2.12).

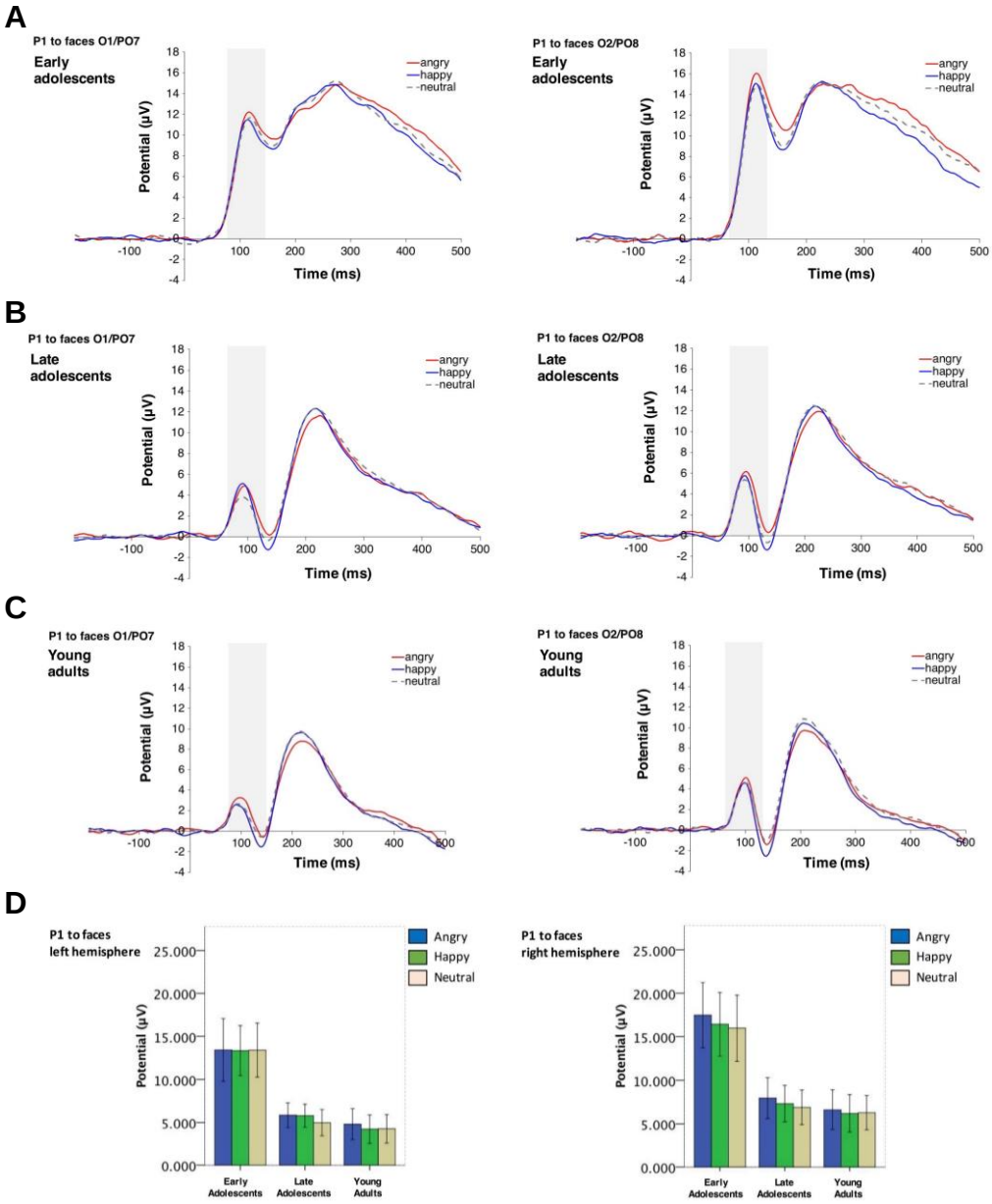


Figure 2.12. **(A).** Visual P1 ERPs in response to face stimuli in the early adolescent group for the left hemisphere (P1/PO7) and the right hemisphere (O2/PO8). **(B).** Visual P1 ERPs in response to face stimuli in the late adolescent group for the left hemisphere (P1/PO7) and the right hemisphere (O2/PO8). **(C).** Visual P1 ERPs in response to face stimuli in the young adult group for the left hemisphere (P1/PO7) and the right hemisphere (O2/PO8). **(D).** Visual P1 peak amplitudes in response to the face stimuli in each age group for the left hemisphere (O1/PO7) and the right hemisphere (O2/PO8). Error bars represent the standard error of the mean (SEM). (N=54).

2.3.10. N170 to S_2 ANOVA analyses

For the N170, a three-way mixed design ANOVA was conducted with age group (early adolescent, late adolescent, young adult) as the between-subjects factor and condition (angry, happy, neutral) and electrode location (P9/P10) as the two within-subjects factors. The results of the ANOVA indicated that the three-way interaction between age group, condition, and electrode location on N170 amplitude was not statistically significant $F(4, 96) = .229$, $p = .922$, partial $\eta^2 = .009$. The results of the ANOVA also indicated that there were no statistically significant two-way interactions between condition and age group $F(4, 96) = .592$, $p = .669$, partial $\eta^2 = .024$, electrode location and age group $F(2, 48) = 1.485$, $p = .237$, partial $\eta^2 = .058$, and between condition and electrode location $F(2, 96) = .469$, $p = .627$, partial $\eta^2 = .010$. There was however a significant main effect of condition $F(1.761, 84.510) = 5.246$, $p = .007$, partial $\eta^2 = .099$ and a significant main effect of electrode location $F(1, 48) = 4.874$, $p = .032$, partial $\eta^2 = .092$, but unlike visual P1 responses to the face stimuli, there was no significant main effect of age group $F(2, 48) = .970$, $p = .386$, partial $\eta^2 = .039$. In addition to *MD* (mean difference) and CI (confidence interval), the data presented are marginal mean and ± standard error. Bonferroni corrected pairwise comparisons revealed that N170 amplitudes in response to the angry face stimuli (M=9.881, ± .523) were significantly greater (MD = .697, SE = .258, p = .028, 95% CI = .05 to 1.33) than those found in response to the neutral face stimuli (M=9.184, ± .497) but not the happy face stimuli (MD = .219, SE = .200, p = .839, 95% CI = -.27 to .716) which had a mean of 9.662 ± .531. There was no significant difference in N170 amplitude between the happy and neutral conditions ($p > .05$). Additionally, Bonferroni corrected pairwise comparisons revealed that N170 amplitudes were greater in the right hemisphere (M=10.254, ± .631) compared to the left hemisphere (M=8.897, ± .541) (MD = 1.357,

SE = .614, *p* = .032, 95% CI = .12 to 2.60) (Figure 2.13).

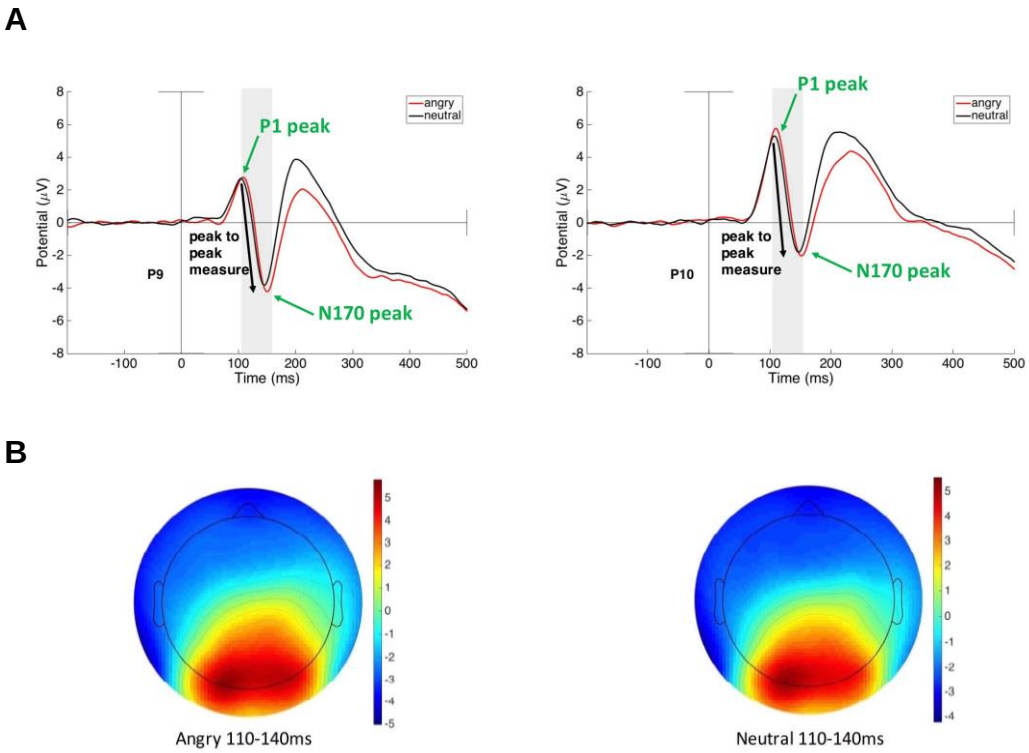


Figure 2.13. **(A).** N170 ERPs in response to cue stimuli collapsed across age groups for the left hemisphere (P9) and the right hemisphere (P10). **(B).** N170 scalp topographies collapsed across age groups depicting mean amplitudes between 110-140ms for the angry and neutral condition following S_2 onset (N=51).

2.4. Discussion

2.4.1. Summary of main findings

The primary aim of this study was to investigate whether anticipation of different emotional facial expressions differentially modulated motor preparation and action and whether this modulation differed significantly at different stages of brain development. The rationale behind this approach was based upon two main assumptions derived from the literature. First, previous research in this area has shown that preparation to act and action itself is often modulated by the emotional context in which it occurs (Carretié, Mercado, Hinojosa, Martın-Loeches, & Sotillo, 2004; Casement et al., 2008; Coombes, Cauraugh, & Janelle., 2006; Coombes et al., 2009; Hart, Lucena,

Cleary, Belger, & Donkers, 2012; Nogueira-Campos et al., 2014; Perri et al., 2014; Schutter, Hofman, & Honk., 2008). Second, as discussed above, relative to childhood and adulthood evidence suggests that adolescence is a period of heightened emotional reactivity (Casey & Jones, 2010; Doremus-Fitzwater & Spear, 2016; Ernst, Pine, & Hardin, 2006; Kessler et al., 2005; Luna & Wright, 2016; Nelson, Leibenluft, McClure, & Pine, 2005; Somerville, Jones, & Casey, 2010; Spear, 2000; Steinberg, 2008, 2010). Therefore, I hypothesised that I would see a greater degree of affect-related modulation of anticipatory behaviour as indexed by the CNV during early adolescence relative to late adolescence and young adulthood. However, contrary to my hypotheses that CNV amplitudes would be differentially modulated by emotionally salient versus non-emotionally salient stimuli (angry, happy and neutral faces) and that this modulation would be more prominent during early adolescence due to the hypothesised maturational mismatch between (pre)frontal and limbic regions of the brain, I found no statistically significant interaction effect of emotion on CNV amplitudes at the within subjects' or between subjects' level.

However, the results of the tCNV analyses were consistent with previous developmental studies investigating anticipatory behaviour in humans (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Flores, Digiacomo, Meneres, Trigo, & Gómez, 2009; Killikelly & Szűcs, 2013; Klein & Feige, 2005; Jonkman, 2006; Klorman, 1975; Perchet & Garcia-Larrea, 2005; Segalowitz, Unsal, & Dywan, 1992a Segalowitz & Davies, 2004; Taylor, Gavin, & Davies, 2016), showing that although non-significant, there was a trend in the data toward attenuation of motor preparedness (tCNV) in early adolescents compared to late adolescents and young adults ($p = .054$). This again suggests the possibility of poorer regulation of motor control or possibly attentional resources during early adolescence, which is in line with previous research

showing increases in CNV negativity with increasing age (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Segalowitz & Davies, 2004). However, this was clearly not the case for iCNV (p = .65) suggesting that at the group level early adolescents, late adolescents and young adults showed a similar orienting response to the cues predicting S_2, and therefore presumably similar levels of prefrontal cortical activity and therefore prefrontal attentional capacity during this earlier part of the CNV wave. However, it must be noted that these effects in tCNV are minimal (p = .054), and may therefore represent a serendipitous trend and so my interpretations relating to age-related changes in tCNV should be viewed with some degree of caution.

In addition, I also investigated how anticipation of, and response to, emotional facial expressions influenced motor output as assessed by the speed of participants' responses to S_2. Based on the initial mean reaction time ANOVA analyses (Section 2.3.2) and consistent with previous developmental studies (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Klein & Feige, 2005; Perchet & Garcia-Larrea, 2005), RTs in early adolescents, regardless of trial type, were significantly slower in comparison to late adolescents and young adults. However, for the early adolescent group responses were significantly faster to happy faces compared to neutral faces. Interestingly, this was not the case for late adolescents and young adults. While slower responses in the early adolescent group relative to the two older age groups across all conditions may suggest that the trend observed in attenuated tCNV amplitudes in the early adolescent group was related to the RT data in our study, supplementary post-hoc analyses showed only tCNV amplitude in the neutral condition proved to be a significant predictor of mean RT (p = .01) (see Appendix 32). In fact, based on this supplementary post-hoc analyses, semipartial correlations indicated that tCNV in the neutral condition uniquely accounting for just 8.2% of the variance in mean RT. This is

consistent with previous studies showing a weak relationship between tCNV amplitudes and RTs (Rebert & Tecce, 1973; Smith, Johnstone, & Barry, 2006). Taken together, these results suggest that factors other than CNV amplitude were responsible for the significant age group differences found in RTs and further suggests that motor preparation as indexed by the CNV is not a good predictor of the speed of participants motor responses.

I also investigated whether viewing different facial expressions led to differential modulation of the visual P1 and N170, and whether this differed between age groups. First, I found that visual P1 peak amplitudes were larger in responses to both S_1 and S_2 in the early adolescent group compared to late adolescents and young adults. These findings are in agreement with previous studies showing decreases in P1 amplitude with increasing age (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010; Peters & Kemner, 2017). Second and contrary to my hypothesized association between the information provided by the cues (S_1) and visual P1 peak amplitudes, I found that visual P1 peak amplitudes were not modulated by the type of predictive cue the participants saw (S_1). However, my hypothesis regarding visual P1 responses to faces (S_2) was confirmed , showing that the visual P1 was modulated by the type of facial expression the participants saw. I found a significant main effect of condition, with larger visual P1 peak amplitudes to angry faces compared to neutral faces but not happy faces. These findings are again in agreement with previous research showing potentiation of early visually evoked potentials to negative stimuli (Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia, 2004). I also found a significant main effect of electrode location, with larger visual P1 peak amplitudes to faces over the right hemisphere compared to the left hemisphere of the brain. However, it is again important to note that I did not find any significant condition by age group interaction for the

visual P1 in response to the emotional face stimuli showing that, at the level of ERP measurement, there was no significant difference between age groups in the way they processed the emotional face stimuli.

For the N170, the results were mostly the same as the results for the visual P1 to faces. Again, consistent with my hypothesized association between N170 amplitudes and the type of faces the participants saw, I found a main effect of condition, with N170 amplitudes found to be significantly larger to angry faces compared to neutral faces. However, like the visual P1 to faces, no significant condition by age group interaction for the N170 was found. Again, like the visual P1 to faces, N170 amplitudes were found to be significantly larger over the right compared to the left hemisphere of the brain. However, unlike the visual P1 to S_1 (cues) and S_2 (faces), age group differences in N170 amplitude were non-significant. This is again in agreement with a recent study showing that age-related changes in N170 amplitudes are minimal, even between four and twenty-nine years of age (Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010).

2.4.2. Contingent negative variation

The fact that I did not observe any significant condition dependent changes in the early or late phases of the CNV in addition to any meaningful changes in CNV during the anticipatory period as a whole was at first, somewhat surprising. This suggests that in our sample of neurotypical participants, at the level of ERP measurement from scalp electrodes, there was little or no significant cross-over between limbic and motor circuitry, at least at the level of motor preparation if not motor output. This suggests that a degree of segregation was maintained between parallel circuits in the brain associated with the processing of emotional information on the one hand (possibly via the anterior cingulate $\rightarrow$ medial orbitofrontal pathway), and circuits

responsible for motor preparation on the other (via supplementary motor area (SMA), premotor cortex, motor cortex and somatosensory cortex → subcortical pathway) (Tekin & Cummings, 2002). Or alternatively, there was no emotion-motor interaction via amygdala-SMA (Voon et al., 2016). This may have been the case, especially given that all other electrophysiological and behavioural measures showed the expected developmental trajectories.

Furthermore, visual inspection of CNV topographical maps indicated a clear fronto-central maximum negativity for iCNV and a clear maximum negativity for tCNV at the vertex, and with group level CNV amplitudes that are within normal limits. This suggests that the experimental paradigm was effectual in capturing both early/initial and late/terminal phases of the CNV. Moreover, the supplementary post-hoc analyses found that tCNV amplitude at electrode Cz in the neutral condition correlated more strongly with mean RT in response to neutral faces than in any other condition ($r = .439$, $p < .001$). This is comparable to a previous study using non-emotional stimuli that reported correlations between tCNV and RTs at electrode Cz of $r = .367$ (Smith, Johnstone, & Barry, 2006). Consistencies between the present study and previous studies investigating visually evoked ERPs and the CNV lends support to the interpretation that the processing of emotional information did not, in any significant way, modulate brain processes responsible for motor preparation as indexed by the CNV in the current study.

Given the available evidence in the literature showing the existence of emotion-motor interactions and that goal-directed behaviour is often aligned to the motive state (see Section 1.3.2), a number of important considerations should be taken into account when interpreting the CNV results. First, motor preparation is a multi-factorial process on many levels, not a unitary preparatory state, in which different components may have only a weak to moderate relationship with one another (Jennings, van der Molen, &

Steinhauer, 1998; MÜller-Gethmann, Ulrich, & Rinkenauer, 2003). This may help to explain why I did not observe any significant effects of emotion on the CNV, that is to say, the preparatory state observed in the present study was possibly only a reflection of a readiness to act (gradual increase or ramping up of neuronal activity preceding imperative stimulus onset) devoid of any significant input from other brain processes related to emotion processing or action selection. As suggested by MÜller-Gethmann, Ulrich, & Rinkenauer. (2003), while ERPs like the CNV may reflect neuronal activity involved in the readiness of the motor system, it remains debatable whether this neural correlate of preparation is predictive of the actual motor output (accuracy or speed of motor responses). For example, Jennings, van der Molen, & Steinhauer. (1998) found that in a simple RT task, correlations between psychophysiological variables such as heart rate, pupillary diameter and brain potentials and RTs was in fact low.

Therefore, it may be that the effects of emotion on motor function actually occurred later during the movement instigation stage, between the onset of the face and the button press response. This would concur with why I observed a significant main effect of condition for early visually evoked potentials but not motor preparation as indexed by the CNV. Further, if the effects of emotion on motor function actually occurred after the onset of the emotional facial expressions, this may in part explain why for early adolescents, RTs in the happy condition were significantly faster than those found in the neutral condition, which was not reflected in CNV amplitudes. In hindsight, an alternative or rather a complimentary approach to investigating the effects of emotion on the temporal characteristics of motor function (motor preparation, movement instigation and motor output) would be to use multiple indices spanning the time period between perception and action by using measures of motor preparedness stimulus locked to S_1 as well as S_2 and indices of movement instigation stimulus locked

to the participants response (button press) alongside measures of the accuracy (button press errors) and the speed of motor responses (RTs).

2.4.3. Early visually evoked potentials

This study found, that when group membership was ignored, visual P1 peak amplitudes were not modulated by the type of predictive cue (S_1) the participants saw but they were modulated by the type of facial expression the participants saw (S_2), with larger visual P1 peak amplitudes found in response to angry faces compared to neutral faces. This pattern was repeated for the N170, with N170 potentiation occurring in response to angry faces compared to neutral faces. These findings are in agreement with previous studies showing that negative stimuli representing threat or danger mobilize attentional resources more than positive, neutral or non-emotional stimuli (Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia, 2004). Further, the fact that visual P1 peak amplitudes (across conditions) were larger in response to faces (S_2) compared to the predictive cues (S_1) and that visual P1 peaks were larger to angry faces compared to neutral faces further suggests that the visual processing of salient stimuli can be differentiated from that of non-salient stimuli as early as 95 ms following stimulus onset. This finding is consistent with a previous ERP study showing a global effect of emotional facial expressions on visual processing occurring as early as 90 ms post stimulus onset (P1) in addition to larger N170 amplitudes evoked by angry facial expressions compared to neutral facial expressions (Batty & Taylor, 2003). From a biological point of view, this aspect of the attentional capture by environmental salient stimuli supports evolutionary theories which emphasize the prioritization of threat-related stimuli for protection and survival.

Both the visual P1 and the N170 occur very early on in the processing of visual information in the brain. Indeed, across all participants on average, visual P1 peak

latency in response to the faces (S_2) occurred as early as 95 ms and the N170 peak latency in response to the faces (S_2) occurred at approximately 145 msec. It is unlikely that conscious recognition of facial expressions occurs so early, particularly for the P1 ERP component (Calvo & Beltrán, 2013; Carretié, Hinojosa, Martín-Loeches, Mercado, & Tapia, 2004; Vuilleumier, 2005) if not so much for the N170 ERP component (Blau, Maurer, Tottenham, & McCandliss., 2007). Indeed, it has been suggested that the effect of emotional stimuli on perception may occur at different stages of visual processing (Bocanegra & Zeelenberg, 2009; Vuilleumier, 2005), so attentional capture and conscious awareness of the meaning of emotional stimuli may have distinct temporal qualities with the former occurring much more quickly than the latter.

It has been proposed that the amygdala may facilitate or boost sensory processing of emotional and configural aspects of faces via connections to the visual cortex (Vuilleumier, 2005). These configural or coarser grained aspects of emotional stimuli (for example position of facial features and coarse emotional cues such as eyes wide open in fearful faces) are relayed mostly through low spatial frequencies (LSF) via the magnocellular pathway, which facilitates the rapid transfer of information to the visual cortex, amygdala, and prefrontal cortex, thereby speeding up threat detection and initiating rapid threat-responses (Peters & Kemner, 2017). More detailed or fine grained information (for example identity, facial age) is reliant on high spatial frequencies (HSF) being relayed via the parvocellular pathway, which projects solely to the visual cortex and takes more processing time than does coarse grained information (Goffaux et al., 2010; Merigan & Maunsell, 1993; Peters & Kemner, 2017). Studies investigating the recruitment of these two distinct visual pathways often use filtered images that have either a high spatial frequency (HSF) or low spatial frequency (LSF) (Vuilleumier, Armony, Driver, & Dolan, 2003). With regard to the rapid transfer of information via

the magnocellular pathway, neuroimaging studies have shown that there is a relationship between amygdala activity and enhanced responses to emotional stimuli in visual cortex, particularly threat-related stimuli (Vuilleumier, 2005), with some fMRI studies showing increased coupling of the fusiform gyrus (Morris et al., 1998) and primary visual cortex (Pessoa, McKenna, Gutierrez, & Ungerleider, 2002) when viewing fearful faces versus neutral faces. The fusiform gyrus is important for facial recognition. Of particular interest here, is that neuroimaging studies have shown that these structures in the brain are not fully matured till adulthood with children showing more HSF-driven processing of facial expressions while adults show more advanced configural processing driven by LSF content (Peters & Kemner, 2017).

Yet, contrary to my hypotheses that I would see condition dependent age group differences in the visual P1 and N170, I failed to find any significant condition by age group effects for either. Moreover, previous fMRI studies have observed greater amygdala activity in adolescents compared to children and adults when viewing emotional compared to neutral facial expressions (Hare et al., 2008; Monk et al., 2003). With this in mind, the lack of any significant condition by age group effects for the visual P1 and N170 in the current study may be unexpected, although there was a clear change in the morphology of visual P1 across age groups, which would be expected (P1 peak amplitudes were significantly larger in the early adolescent group compared to the two older age groups). Therefore, future developmental studies using EEG and simultaneous fMRI could better examine the developmental trajectory of threat-detection and related visual pathways in response to different emotional facial expressions by using filtered images that contain either LSF or HSF information. This could be further investigated in relation to prefrontal cortical activity. This may have implications for our understanding of why we see increases in sensation seeking or risk-

taking behaviours during early adolescence (Arnett, 1992; Steinberg & Morris, 2001). That is, if early adolescents have levels of sensitivity to threat approaching those of late adolescents and young adults (Peters & Kemner, 2017), increases in risk-taking behaviours may be due more to functions associated with the prefrontal cortex (due in part to protracted development of prefrontal cortical areas) rather than any ability to detect threat in the environment. This may mean that in addition to peer pressure (Gardner & Steinberg, 2005), early adolescents may be more prone to less effective regulation resulting in an increases in risk-taking or sensation seeking behaviours when compared to adults due to the relative immaturity of the prefrontal lobe (Casey & Jones, 2010; Hare et al., 2008; Somerville, Jones, & Casey, 2010). Future studies using the approach suggested may help to uncover the psychophysiological substrates of threat-detection and top-down regulation of adolescent behaviour. Another possible reason for non-significant age group effects could be that in this study these facial expressions were predicted by S_1, which is effectively a conditioned stimulus. Subjects would have expected the facial expression they saw, which could have attenuated/modulated their P1/N170 responses.

2.4.4. Reaction times to emotional facial expressions

Faster RTs to happy faces relative to neutral faces in the early adolescent group is consistent with previous studies showing faster RTs to happy faces relative to fearful, calm, and neutral faces (Calvo & Beltrán, 2013; Hare et al., 2008). This was not the case for late adolescents or young adults. One possible explanation for this finding in early adolescents comes from previous research highlighting a happy face recognition advantage over and above other facial expressions (neutral, angry, sad, disgusted, surprised and fearful), suggesting that happy facial expressions are identified faster and more accurately than others (Calvo & Lundqvist, 2008; Calvo & Beltrán, 2013). For the

early adolescent group only, a relatively faster and more accurate identification of happy faces may have occurred resulting in faster reaction times in happy trials compared to neutral trials or alternatively for early adolescents' happy faces may have preferentially instigated action/approach more than angry and/or neutral faces.

At first glance, this may seem somewhat at odds with the notion that we prioritize threat-related stimuli over and above other types of environmental cues as suggested above. However, while attentional capture by threat-related stimuli may occur more quickly than the attentional capture by positive or neutral stimuli (happy faces or neutral faces), this does not mean that we might predict faster RTs to angry faces. This is because it is likely that angry faces may in fact result in slower RTs due to a desire to avoid rather than approach such threatening stimuli. Also, it is likely that more elaborate and discriminative top-down conscious mechanisms are engaged at later stages of visual processing (P2, N2) and that it is at these later stages of visual processing that response selection and decision making is more likely to occur (Calvo & Beltrán, 2013). This is consistent with research showing that signals of potential reward are only attended to after the degree of threat has been evaluated and safety has been assured (Calvo & Beltrán, 2013). In other words, approach behaviours are initiated when it is safe to do so, and in our experiment, for the early adolescent group only, this may have occurred after the conscious recognition and categorization of the facial expressions had happened. One possible explanation for why this was significant in the early adolescent group but not the two older age groups may be partly explained by changes in the structure and function of the adolescent brain that have been linked to increased reward seeking behaviours as well as increased emotionality (see Section 1.3.1). It is possible that early adolescents were more responsive to happy faces relative to neutral faces compared to the two older age groups due to greater input from

subcortical regions of the brain associated with increased reward seeking behaviours and this occurred at later stages of visual processing (Ernst et al., 2005; Galvan, Hare, Voss, Glover, & Casey, 2007).

A related point of interest is that early adolescents made significantly more errors during trials than both late adolescents and young adults. The most common errors were the inability to suppress responses to S_1 (cues) and/or responses made immediately prior to S_2 onset (faces). This was the case for all of the participants, just significantly more so for early adolescents. It is possible that incorrect responses during trials may have reflected relatively immature prefrontal connectivity and therefore poorer regulation of motor control during early adolescence, as reflected also by the trend in attenuated tCNV amplitudes. So, while early adolescents found it more difficult to inhibit or suppress incorrect responses during trials, they also responded relatively more quickly to happy faces relative to angry and neutral faces compared to late adolescents and young adults. This tentatively lends support to the interpretation that in this study, early adolescents showed increased reward seeking behaviours as indexed by faster RTs to happy faces, possibly reflecting greater input form the ventral striatum, coupled with significantly more incorrect responses during trials. Thereby showing relative immaturity in prefrontal cortical regions associated with response inhibition and impulse control (Casey & Jones, 2010; Hare et al., 2008; Somerville, Jones, & Casey, 2010) and/or relative immaturity in the motor circuit. So again, relative to late adolescents and young adults, the early adolescents may have displayed enhanced responsivity to positive salient environmental cues while at the same time lacking the appropriate behavioural inhibitory skills needed for making optimal decisions.

2.4.5. Study limitations and future directions

Several limitations should be considered when interpreting the study findings presented here. First, this study lacked sufficient power to allow for the examination of sex differences in CNV amplitudes and early visually evoked potentials in the transition from early adolescence to young adulthood. Age-related brain changes in structure and organization are likely influenced by puberty and sex-specific hormone levels, with some brain changes occurring around 1-2 years earlier in females compared to males (Lenroot et al., 2007). It is possible that the inclusion of both male and female adolescent participants in the same age group (13-15 years of age) may have obscured brain developmental differences which may have been present if I had examined gender differences, but again sample sizes were relatively small in this study. Although I restricted age ranges in each group to two years, this limitation holds true even though I recruited the same number male and female participants in each age group. This is because the developmental gap between early adolescents and late adolescents may have narrowed due to earlier brain maturation in females compared to males in the early adolescent group. Replication with a larger sample size would allow for an estimation of the influence of pubertal development and gender on motor preparation and action in addition to pubertal stages and gender on early visually evoked potentials.

A further limitation relates to the arousal and/or valence effects of the images and the chosen presentation timings of the faces during the experimental paradigm. First, due to the large number of images used during the EEG experiment in addition to task duration, it was not feasible to ask participants to rate the images for arousal and/or valence. Furthermore, because this study involved adolescents aged between thirteen and fifteen, I intentionally used relatively mild images. It is of course possible that using stronger imagery (thereby eliciting stronger emotional responses) in an adult only group

would have resulted in significant condition dependent changes in CNV. Second, the presentation of emotional cues during the interval between the predictive cue (S_1) and the target stimulus (S_2) may have been a more effectual approach when investigating the effects of emotional stimuli on motor preparation and action. This would avoid the necessity and reliance on the strength of association between S_1 and S_2. Although it was originally my intention to conduct such a follow up study, time limitations did not allow for this. Additionally, in this study I did not include a no-go condition. Inclusion of a no-go condition in future developmental EEG studies using a similar paradigm would allow for a better understanding of the developmental trajectories of response inhibition to emotionally salient stimuli, particularly during early adolescence.

In addition, the inclusion of an additional and complimentary alternative analytic approach to the analyses of the EEG data may have proved more informative (see Section 2.4.2). Time frequency analyses would have allowed for a more in-depth investigation of motor preparation, more closely aligned to the underlying neurophysiology. For example, a recent study by Li et al. (2018) found that in a pre-cue reaction time task, the time coarse of EEG changes in the time domain (CNV) and time-frequency domain (mu event-related-desynchronization/ERD) were closely aligned but showed different topographical features, with the CNV again showing the largest voltage change at Cz while the mu ERD presenting mostly in the contralateral sensory motor cortex area with respect to left versus right wrist movements during motor preparation (C3 and C4 respectively). The authors suggest that these findings show that brain activity during motor preparation (mu ERD) has a contralateral feature which may be more sensitive indicator of movement intentions. Again, this highlights the fact that motor preparation may be multifaceted and suggests that future studies investigating

motor preparation and action would benefit by incorporating a more comprehensive approach.

Finally, an important consideration relates to the sample itself. All participants were neurotypical and free of any neurodevelopmental or psychological disorder. Therefore, it is of course not possible to generalize these findings to groups of individuals who may be or have experienced trauma/emotional distress, anhedonia or forms of psychopathology or indeed functional movement disorders for example. It may be the case that the use of similar paradigms in the future investigating movement related potentials in the brain in response to emotional stimuli in such groups of individuals may prove to be of interest. For example, Blakemore, Hyland, Hammond-Tooke, & Anson. (2015) found that in patients with conversion paresis, CNV amplitudes were significantly attenuated compared to controls but only when the pre-cue stimulus indicated the need to use the symptomatic limb. This suggests that abnormalities of motor preparation may be involved in conversion disorders. The use of EEG paradigms with emotionally salient stimuli to investigate pre-movement-related potentials in the brain could be applied to PNES and other functional movement disorders before and after psychotherapy or physiotherapy to investigate whether the alleviation of motor-related symptoms correlate in any way with preparatory motor related activity in the brain as indexed by the CNV or other movement related potentials.

2.4.6. Conclusion

In conclusion, while the results of the current study are largely consistent with previous studies showing developmental differences in task performance (RTs) and electrophysiology (ERPs), there were no significant effects of emotion on CNV amplitudes during the anticipatory period. In addition, a weak relationship was found

between tCNV amplitudes and mean RTs, but only in the neutral condition, suggesting that the CNV may not be the best predictor of RT. These findings suggest that while the experimental paradigm successfully captured both early and late phase of the CNV, it is likely that segregation was maintained between emotional circuits and circuits involved in motor preparation and that electrophysiological changes due to emotional influences may have occurred during movement instigation after S_2 onset not before. Both the visual P1 and N170 ERP components were found to be larger in response to angry faces relative to neutral faces, suggesting that early non-conscious automated attentional capture was facilitated by angry facial expressions over and above neutral facial expressions. However, I did not observe any significant age group differences in the early visual processing of different emotional facial expression. For the early adolescent group only, mean RTs were found to be significantly faster to happy faces relative to neutral faces, suggesting that for early adolescents a relatively faster and more accurate identification of happy faces may have occurred resulting in faster reaction times in happy trials compared to neutral trials. This may have reflected greater input from subcortical regions of the brain associated with increased reward-seeking behaviours in the early adolescent group. In addition, given that early adolescents made significantly more errors during trials than both late adolescents and young adults, it is possible that this reflects relatively immature (pre)frontal connectivity and therefore poorer regulation of motor control and decision making during early adolescence.

Chapter 3. Neuroimaging studies in patients with psychogenic non-epileptic seizures: A systematic meta-review

Abstract

Psychogenic Non-epileptic Seizures (PNES) are ‘medically unexplained’ seizure-like episodes which superficially resemble epileptic seizures but which are not caused by epileptiform discharges in the brain. While many experts see PNES disorder as a multifactorial biopsychosocial condition, little is known about the neurobiological processes which may predispose, precipitate and/or perpetuate PNES symptomology. This systematic meta-review advances our knowledge and understanding of the neurobiological correlates of PNES by providing an up-to-date assessment of neuroimaging studies performed on individuals with PNES. Although the results presented appear inconclusive, they are consistent with an association between structural and functional brain abnormalities and PNES. These findings have implications for the way in which we think about this “medically unexplained” disorder and how we communicate the diagnosis to patients. However, it is also evident that neuroimaging studies in this area suffer from a number of significant limitations and future larger studies will need to better address these if we are to improve our understanding of the neurobiological correlates of predisposition to and/or manifestation of PNES.

3.1. Introduction

Psychogenic Non-epileptic Seizures (PNES)[1] are episodic functional neurological symptoms which superficially resemble epileptic seizures but which are not caused by epileptic discharges in the brain (LaFrance, Reuber, & Goldstein, 2013). Current medical nosologies class most PNES episodes as a manifestation of conversion/somatoform (DSM 5) or dissociative disorder (ICD-10) without providing any additional insights into the likely neurobiological underpinnings of the disorder (American Psychiatric Association, 2013; World Health Organization, 1992). In fact, the traditional dualistic approach to the understanding of functional disorders such as PNES has only provided psychoanalytic/psychodynamic perspectives, characterizing these disorders as "medically unexplained", and while a host of studies have provided insights into the psychosocial characteristics of PNES (Brown & Reuber, 2016a; Reuber, Howlett, Khan, & Grünewald, 2007; Wiseman & Reuber, 2015), the biological underpinnings of this disorder have received much less attention.

This is in spite of the fact that many experts see PNES as a biopsychosocial condition (Reuber, Howlett, Khan, & Grünewald, 2007; Reuber, 2009) and that patients find it difficult to understand how a physical problem such as a seizure could be caused by "purely" psychological processes or emotional problems. As a result, patients often feel misunderstood, dismissed and stigmatized when they are presented with a psychological model of their disorder (Thompson, Isaac, Rowse, Tooth, & Reuber, 2009). In fact, patients may reject their PNES diagnosis altogether due to their subjectively physical seizure experiences on the one hand and their dualistic concept of their condition on the other (Rawlings & Reuber, 2016). One could argue that the relative lack of understanding of PNES from a biological perspective does not only hinder our understanding but also has significant implications for the way in which

diagnosis is communicated to patients (Green, Payne, & Barnitt, 2004). However, over the last two decades, researchers have begun to employ novel neuroimaging techniques to investigate the neurobiological correlates of PNES. Like other mental health conditions which are not categorised as “medically unexplained”, we may now be getting closer to providing a neurobiological perspective which may help to improve our understanding of how neurobiological changes could play a part in the aetiology and maintenance of this disorder.

Although neuroimaging studies focusing on PNES have been summarised previously (Allendorfer & Szaflarski, 2014; Asadi-pooya, 2015; Baslet, 2011; Perez et al., 2015; Sundararajan, Tesar, & Jimenez, 2016), most previous reviews were not systematic and may have missed important studies in this area. In addition, no previous review has sought to uncover convergent neuroimaging findings in patients with PNES to better determine the neurobiological correlates of this condition. To that end, this systematic meta-review provides an up-to-date synthesis and quantification of both structural and functional neuroimaging studies performed on individuals with PNES. Having summarised the research in this area, we provide a critical appraisal of each methodological approach from which the conclusions where derived. This may better inform future research and current theoretical models.

3.2. Method

3.2.1. Literature search

The literature search for this review was closed on the 2nd of May 2017. The search terms used to identify relevant publications were 'dissociative seizure*' OR 'non-epileptic attack disorder' OR 'non-epileptic seizure*' OR 'psychogenic non-epileptic seizure*' OR 'conversion seizure*' OR 'pseudoseizure*', AND ‘MRI’ OR ‘fMRI’ OR

'imaging' OR 'neuroimaging' in the Web of Science core collection (1960 – May 2017; 189), ovid Medline (1960 to May 2017; 209), and Psychinfo (1960 to May Week 1 2017; 392). Our initial literature search identified a total of 790 publications. After a multistage selection process 17 empirical publications were retained and form the basis of this review (see Figure 3.1).

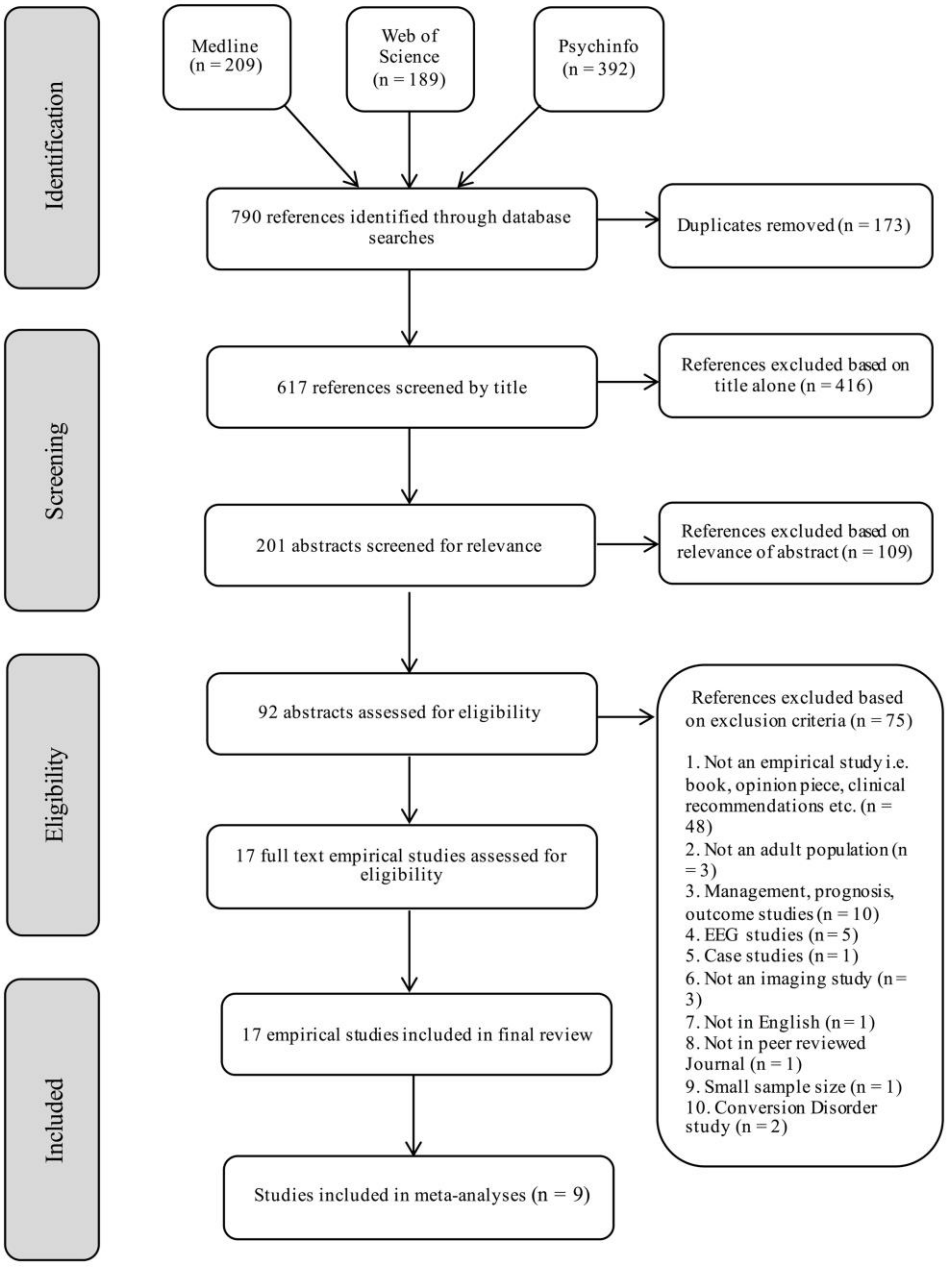


Figure 3.1. PRISMA flow diagram showing results of the multistage search process

3.2.2. Quality assessment of studies

Due to the absence of a suitable rating system specific to studies in this area, a bespoke rating system was employed. This rating system is similar to one used recently by Brown and Reuber (2016a) and was adapted with neuroimaging of patients with PNES specifically in mind. The ratings are based on the proportion of "yes" responses to the following criteria; 1) video-EEG confirmed PNES diagnosis; 2) comparison groups matched for age and gender; 3) patients with mixed diagnosis (PNES plus epilepsy) excluded from the PNES group. If not, was this group compared to a PNES group free of a mixed diagnosis (PNES with no epilepsy); 4) co-existing psychiatric conditions excluded from the PNES group; 5) other central nervous system pathologies excluded from the PNES group; 6) other functional neurological disorders excluded from the PNES group; 7) effects of medication controlled for; 8) image acquisition and analysis discussed in sufficient detail to allow for study replication. The final item relates to sample size. Studies with group sizes ≥ 50 were rated as good, studies with group sizes between16 and 49 were rated as moderate, and studies with group sizes ≤ 15 received a poor rating.

The overall rating was based on the summation of "yes" responses to items 1 – 8 in addition to weighted scores for sample size. Each item was assigned a score of 0.1 for yes and 0.0 for no, with the exception of sample size (item 9) which was given the score of 0.0 for poor, 0.1 for moderate and 0.2 for good. Therefore, the highest possible rating was 1.0. In addition, studies that reported on the prevalence of brain abnormalities in PNES groups relating to lesions, tumours, evidence of stroke, cysts etcetera were given a score of 0.1 for item 5 (other central nervous system pathologies excluded from the PNES group). It was not deemed appropriate to mark these down when the presence of neurological/CNS pathologies was the primary focus of these studies. In cases in which

it was unclear whether or not a study met any of the items described above or where only some of the participants but not all met these criteria, a score of 0.0 was allocated. These ratings were then used to assess the overall quality of the respective research methodology from which the conclusions were derived. Studies with ratings ≥ 0.8 (based on yes item response, score of 0.8 out of 1.0) were rated as high quality. Studies with ratings between 0.5 – 0.7 were rated as moderate and those with ratings between 0.2 – 0.4 were rated as poor.

3.2.3. Meta-analyses

Nine of the seventeen studies included in this review were eligible for inclusion in our meta-analysis (Table 3.1). Given that a number of different neuroimaging approaches were used and in order to identify which brain regions were most consistently implicated in PNES across these studies, we conducted a coordinate-based Activation Likelihood Estimation (ALE) meta-analysis using GingerAle 2.3.6 (Eickhoff et al., 2009; Eickhoff, Bzdok, Laird, Kurth, & Fox, 2012; Turkeltaub et al., 2012).

This method is capable of integrating findings from multiple imaging modalities and to identify converging brain areas across different experiments/different contrasts and statistically determines whether the convergent brain areas or clusters reported are greater than expected by chance. It is important to note here that this method does not ask any questions about effect sizes but rather is primarily concerned with the spatial location of results. This procedure uses the follwing steps. First, a text file is created in which groups of coordinates or foci are listed under the heading of the respective study which also needs to include the number of subjects in the study. Based on this list of coordinates, GingerALE creates Modelled Activation (MA) maps by entering the reported cooridinates (x, y and z maxima) into a 3D image for each group of foci (coordinates), i.e., creates MA maps for each study individually. These foci are assigned

a value of 1 and everything else is assigned a value of 0. Because the maxima often reported in studies (coordinates with maximumn statistic value from voxel or vertex) have spatial uncertainty, in the ALE procedure, the reported coordinates (those assigned a value of 1) are modeled using a three-dimensional Gaussian probability distribution, the width of which is determined from the number of subjects in the respective study. Foci derived from studies with smaller samples are subjected to a greater degree of spatial blurring to lessen their impact on localisation in the meta-analysis (Eickhoff et al., 2009). Second, each study MA map is then combined (pooled) into a single common ALE-map by taking the union of probabilities (the ALE value for each voxel in the ALE-map is the result of taking the MA-values over the number of studies entered into the meta-analysis). The resulting ALE-map can then be thresholded under the null distribution, i.e. that foci are randomly spread throughout the brain. This is done by calculating all possible ALE values that can be obtained by making all possible combinations of voxels in the MA maps. The resulting ALE values can then be used to create histograms which can then be devided by the total number of voxels in a MA map to create a table of probablilities of finding each voxel in the MA map. Combining the probabilities results in a table of *p* values for ALE scores. The ALE image and the table of *p* values are then used to create a 3D *p* value image. In the final step of this procedure, the 3D *p* value image can then be subjected to multiple comparison correction by using either voxelwise (uncorrected values, False Discovery Rate FDR or Family Wise Error FWE), or alternatively by conducting multiple comparison correction on the cluster-level. The cluster-level inference corrected threshold sets the minimum cluster-level volume so that only a certain precentage (5% for example) of the simulated data's clusters exceed the specified size (brainmap.org). Anatomical labels of the resulting clusters are provided by Talairch Daemon (Talairach.org).

For the current meta-analyses, all available coordinates were transformed from MNI space to Talairach space using icbm2tal transform (Laird et al., 2010; Lancaster et al., 2007) provided by brainmap.org (Eickhoff, 2014). Given that this was an exploratory analysis, and as noted by Eickhoff, Bzdok, Laird, Kurth, & Fox (2012), both uncorrected *p* values and FDR corrected thresholds are not always optimal, we opted for a less conservative correction by implementing cluster-level inference. This threshold algorithm uses a "cluster-forming threshold" with an uncorrected *p* value of 0.001 as the cluster-forming threshold with a cluster-level inference of 0.05 with 1000 permutations, as recommended by brainmap.org. Mango (v 4.0) (rii.uthscsa.edu/mango) was used to view the threshold maps and the ALE results were superimposed on the high-resolution standard anatomical brain image provided by brainmap.org (Colin_tlrc.nii).

Given that all of the imaging studies entered into our meta-analysis involved group comparisons, we summed the number of PNES patients and the number of participants in the comparison groups to quantify the number of participants in each study. Where studies came from the same research group and used the same participants (Ding et al., 2014; Li et al., 2014, 2015) we subsumed these participants into a single group of coordinate results in order to avoid any overestimation of these participants in the results. Three different meta-analyses were conducted. The first analysis combined both structural and functional findings from all nine studies. The second analyses focused solely on studies reporting functional connectivity patterns in PNES patients compared to healthy controls. The third and final analysis focused solely on imaging studies reporting structural brain differences between PNES patients and controls. All reported foci (MNI or Talairach coordinates) from these publications entered the ALE analysis. In the results, brain areas within +/- $5mm^3$ of any significant cluster above the

corrected *p* value threshold are also reported. See Appendix 37 for a description of the procedure used for this ALE meta-analyses.

3.3. Results/Discussion

The results of this review have been divided into three sections. The first section describes the results of the quality assessment. The second section is sub-divided into the different neuroimaging modalities used in which limitations are discussed and future directions proposed. The third section outlines the results of the meta-analyses.

3.3.1. Quality assessment results and imaging methods

Of the seventeen studies assessed, none were rated as high quality, fourteen were of moderate quality, and three were rated as poor. Eleven (65%) were case control studies and six (35%) adopted a retrospective methodology. Sample sizes were considered good in three (17.6%), moderate in nine (53%) and poor in five studies (29.4%). All studies included both male and female participants, all over the age of 16. Across all seventeen studies the median total sample size was 38 (range 13 - 256, mean 66). The total number of participants was 1004. In total, the studies included 402 patients with PNES (range 8 - 79, mean 29, median 17). Sample sizes and groups characteristics for each of the seventeen studies are shown in Table 3.1. Results of the rating system are presented in Table 3.2. Table 3.3 summarizes findings and limitations separately for each imaging modality.

Table 3.1. *Sample size and group characteristics.*

Study	PNES	ES	Healthy controls	PNES + ES	ES + Psych	Non-diagnostic	IED	Total sample	Semiology features (PNES)
Arthuis et al. (2015)*	16	-	16	-	-	-	-	32	dystonic attacks with primary gestural activity, paucikinetic attacks with preserved responsiveness, pseudosyncope
Bolen et al. (2016)	68	111	-	19	-	32	26	256	major convulsions, tremors, unresponsiveness, subjective.
Devinsky et al. (2001)	79	51	-	-	71	-	-	201	motor events (seizures), weakness
Ding et al. (2013)	17	-	20	-	-	-	-	37	hypermotor movements of extremities, trembling, trembling of the extremities
Ding et al. (2014)*	18	-	20	-	-	-	-	38	hypermotor movements of extremities, trembling, trembling of the extremities
Ettinger et al. (1998)	11	11	-	-	-	-	-	22	Impaired consciousness
Hernando et al. 2015)	8	-	8	-	-	-	-	16	major motor events, minor motor events (waxy flexibility), electric feeling back of head followed by inability to talk or move (subjective event)
Labate et al. (2012)*	20	-	40	-	-	-	-	60	convulsive components (tonic-like, clonic-like or bizarre motor manifestations), no non-motor events such as paralysis, sensory feelings or unresponsiveness
Lee et al. (2015)*	16	-	16	-	-	-	-	32	major motor, minor motor, waxy flexibility, subjective events
Li, R et al. (2014)*	18	-	20	-	-	-	-	38	hypermotor movements of extremities, trembling, trembling of the extremities
Li, R et al. (2015)*	18	-	20	-	-	-	-	38	hypermotor movements of extremities, trembling, trembling of the extremities
Neiman et al. (2009)	13	-	-	-	-	-	-	13	major motor events, minor motor events, unresponsiveness, dystonic posturing, subjective experiences, pelvic trusting, back arching, weakness, head turning
Reuber et al. (2002)	74	-	-	95	-	-	-	169	convulsive components (tonic-clonic-like, tonic-like), flaccid, sensory
Ristić et al. (2015)*	37	-	37	-	-	-	-	74	dialeptic-like-loss of consciousness without motor symptoms, astatic-like-loss of consciousness and muscle tone with fall, motor-different phenomenon, and multiple
van der Kruijs et al. (2012)*	11	-	12	-	-	-	-	23	major motor events, unresponsiveness
van der Kruijs et al. (2014)*	21	-	27	-	-	-	-	48	major motor events, unresponsiveness without motor events
Varma et al. (1996)	10	10	-	-	-	-	-	20	alterations in consciousness, bilateral motor phenomena

PNES = psychogenic non-epileptic seizures; ES = epilepsy; Psych = psychiatric disorder; IED = interictal epileptiform discharges; * = indicates studies included in meta-analysis

Table 3.2. *Results of the rating system.*

Study	Video-EEG confirmed	Matched controls	Epilepsy excluded	Psych excluded	Other CNS excluded	Other FND excluded	Medication accounted for	Imaging acquisition & analysis	Sample size	Score out of 1	Overall rating
Arthuis et al. (2015)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Moderate	0.7	Moderate
Bolen et al. (2016)	Yes	No	Yes	No	N/A	No	No	Yes	Good	0.6	Moderate
Devinsky et al. (2001)	Yes	No	Yes	No	N/A	No	No	No	Good	0.5	Moderate
Ding et al. (2013)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Moderate	0.7	Moderate
Ding et al. (2014)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Moderate	0.7	Moderate
Ettinger et al. (1998)	Yes	No	Yes	No	No	No	Yes	Yes	Poor	0.4	Poor
Hernando et al. 2015)	Yes	Yes	Yes	No	No	No	No	Yes	Poor	0.4	Poor
Labate et al. (2012)	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Moderate	0.7	Moderate
Lee et al. (2015)	Yes	Yes	Yes	No	Yes	No	No	Yes	Moderate	0.6	Moderate
Li, R et al. (2014)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Moderate	0.7	Moderate
Li, R et al. (2015)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Moderate	0.7	Moderate
Neiman et al. (2009)	Yes	No	Yes	No	No	No	No	Yes	Poor	0.3	Poor
Reuber et al. (2002)	Yes	No	Yes	No	N/A	No	Yes	Yes	Good	0.7	Moderate
Ristić et al. (2015)	Yes	No	Yes	No	Yes	No	Yes	Yes	Moderate	0.6	Moderate
van der Kruijs et al. (2012)	Not all	No	Yes	Yes	Yes	No	Yes	Yes	Poor	0.5	Moderate
van der Kruijs et al. (2014)	Not all	No	Yes	Yes	Yes	No	Yes	Yes	Moderate	0.6	Moderate
Varma et al. (1996)	Yes	Yes	Yes	No	Yes	No	No	Yes	Poor	0.5	Moderate

Psych = psychiatric conditions; CNS = central nervous system; FND = functional neurological disorders

Table 3.3. *Neuroimaging studies of PNES and summary of results.*

Study	Design	Imaging	Results/brain regions	Limitations
Arthuis et al. (2015)	Retrospective	FDG-PET	PNES hypometabolism in RT IFG/central and bilateral ACC > HCs ($p < 0.001$)	Retrospective, small sample size, PTSD/anxiety not controlled for, did not measure dissociative traits, no psychiatric group
Bolen et al. (2016)	Retrospective	sMRI	PNES increased MF abnormalities > ES ($p < 0.018$;); PNES decreased TL abnormalities > ES ($p < 0.003$)	Retrospective, no HCs, no psychiatric controls
Devinsky et al. (2001)	Retrospective	sMRI, CT, EEG	PNES predominance of RT hemisphere abnormalities (85%) > combined epilepsy groups (78%; $p < 0.02$)	Retrospective, no HCs , no account for the effects of anticonvulsants and/or psychiatric medications
Ding et al. (2013)	Case control	rsfMRI, DTI	PNES decreased of coupling strength between FC – SC > HCs ($p < 0.0006$)	Small sample size, no psychiatric group
Ding et al. (2014)	Case control	fMRI	PNES increased SRFC in LT MFG, SFG, ACC, SMA, and bilateral MCC; SRFC decreased in RT MOG > HCs ($p < 0.05$); PNES increased LRFC in bilateral CF, LG, SMA, and RT STG, insula, pre- and post-CG, left PL; PNES decreased LRFC in RT MPFC, MFG, IFG , SFG, SMG, and IPG > HCs ($p < 0.05$)	Small sample size, weak correction for multiple comparisons (AlphaSim program), correlations between illness duration and altered FCD were not corrected for multiple comparisons, no psychiatric group
Ettinger et al. (1998)	Case control	SPECT, interictal, postictal	PNES abnormal SPECT interictal (27%), postictal (27%) all hypoperfusion > ES abnormal SPECT interictal (36%), postictal (64%), 6 hypoperfusion, 1 hyperperfusion; postictal vs interictal SPECT PNES vs ES ($p < 0.12$)	Small sample size, no HCs, psychiatric conditions not controlled for, abnormal sMRI in 2 PNES cases
Hernando et al. (2015)	Case control	DTI	PNES greater No. UF streamlines in RT hemisphere > HCs ($p < 0.021$); UF asymmetry significantly correlated with age at illness onset ($p < 0.0045$)	Small sample size, may have incorporated other fibers associated with other pathways other than UF, did not include all of the UF due to the technical limitations, mostly female sample (87%), 2 different scanners used
Labate et al. (2012)	Case control	sMRI, CTH, VBM	PNES VBM GM reductions in bilateral cerebellum, RT precentral gyrus, MFG, ACC and SMA > HCs ($p < 0.05$); PNES CTH reductions in RT precentral gyrus, SFG, precuneus, PL > HCs ($p < 0.05$)	Small sample size, no psychiatric group

Table 3.3. *(continued)*

Study	Design	Imaging	Results/brain regions	Limitations
Lee et al. (2015)	Retrospective	DTI	PNES increased connectivity LT hemisphere, internal and external capsules, carona radiata, UF and STG > HCs ($p < 0.05$)	Small sample size, retrospective, 2 different scanners used, no psychiatric group
Li et al. (2014)	Case control	rsfMRI	PNES increased FC between insular subregions and LT SPG, putamen, postcentral gyrus, RT LG and bilateral SMA > HCs ($p < 0.05$)	Small sample size, no psychiatric group
Li et al. (2015)	Case control	rsfMRI	PNES increased fALFF in LT SFG, precuneus, PL, SMA and RT postcentral gyrus and decreased fALFF in LT IFG > HCs ($p < 0.05$); PNES increased FC in precuneus, ACC, MCC, postcentral gyrus, frontal and parietal cortices, and decreased FC in MFG > HCs ($p < 0.05$)	Small sample size, weak correction for multiple comparisons (AlphaSIm program). No sig, correlations between fALFF and FC, no psychiatric group
Neiman et al. (2009)	Retrospective	SPECT, SISOM	PNES abnormal SISCOM (15%) in LT insula, RT insula, RT frontal regions, all hyperperfusion	Small sample size, no HCs, retrospective, high levels of psychiatric comorbidity in PNES sample, semiology consistent with partial seizures in 9/13 PNES patients, abnormal sMRI in 5/13 PNES patients, abnormal interictal EEG in 5/13 PNES patients
Reuber et al. (2002)	Retrospective	sMRI, EEG	PNES brain abnormalities (27%) > PNES plus ES (77.9%) ($p < 0.001$); PNES No sig. diff. in lateralization > HCs	Retrospective, no HCs, no psychiatric group
Ristić et al. (2015)	Case control	sMRI, CTH	PNES CTH increases in LT insula, MOF, LOF, and RT MOF > HCs ($p < 0.001$); PNES CTH decreased in RT precentral, enthorinal, LOC and LT precentral gyrus > HCs ($p < 0.001$)	No psychiatric group
van der Kruijs et al. (2012)	Case control	rsfMRI, event-related fMRI	PNES increased FC in insular subregions, CS, PCC and ACC, POF > HCs; No sig. diff. for activation patterns to fMRI tasks; PNES increased dissociation scores > HCs ($p < 0.05$)	Small sample size, no psychiatric group

Table 3.3. *(continued)*

Study	Design	Imaging	Results/brain regions	Limitations
van der Kruijs et al. (2014)	Case control	rsfMRI	PNES increased coactivation of OFC, insular and subcallosal cortex in F-P RSN; cingulate and insular cortex in EC RSN; cingulate gyrus, SPL, pre- and post CG and SMA in sensorimotor RSN; precuneus, (para-) cingulate gyri in DMN RSN ($p < 0.05$); PNES decreased coactivation OFC in EC RSN; precuneus in sensorimotor RSN > HCs ($p < 0.05$)	Small sample size, no psychiatric group
Varma et al. (1996)	Case control	SPECT, HMPAO	PNES abnormal SPECT (30%) hypoperfusion in bifrontal, LT F-P, RT middle temporal region > ES abnormal SPECT hypoperfusion (80%)	Small sample size, no HCs, high levels of psychiatric comorbidity in PNES sample

ACC = anterior cingulate cortex; CF = calcarine fissure; CG = central gyrus; CS = central sulcus; CT = computational tomography; CTH = cortical thickness; DMN = default mode network; DTI = diffusion tensor imaging; EC = executive control; EEG = electroencephalography; ES = epilepsy; fALFF = fractional amplitude low-frequency fluctuations; FC = functional connectivity; FDG-PET = fluorodeoxyglucose – positron emission tomography; fMRI = functional magnetic resonance imaging; F-P = fronto-parietal; GM = gray matter; HCs = healthy controls; HMPAO = hexamethyl propylene amine oxime; IFG = inferior frontal gyrus; IPG = inferior parietal gyrus; LG = lingual gyrus; LOC = lateral occipital cortex; LOF = lateral orbitofrontal; LRFC = long range functional connectivity; LT = left; MCC = middle cingulate cortex; MF = multifocal; MFG = middle frontal gyrus; MOF = medial orbitofrontal; MOG = middle occipital gyrus; MPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; PCC = posterior cingulate cortex; PL = paracentral lobule; PNES = psychogenic nonepileptic seizures; POF = parietal occipital fissure; PTSD = post-traumatic stress disorder; rsfMRI = resting state functional magnetic resonance imaging; RSN = resting state network; RT = right; SC = structural connectivity; SFG = superior frontal gyrus; SISCOM = subtraction ictal SPECT coregistered to MRI; SMA = supplementary motor area; SMG = superior marginal gyrus; sMRI = structural magnetic resonance imaging; SPECT = single photon emission computed tomography; SPL = superior parietal lobe; SRFC = short range functional connectivity; STG = superior temporal gyrus; SPG = superior parietal gyrus; TL = temporal lobe; UF = uncinate fasciculus; VBM = voxel based morphology

3.3.2. Structural magnetic resonance imaging

3.3.2.1 Pathological brain changes in patients with PNES

Initial information about possible brain changes associated with PNES can be extracted from studies in which visual inspection of structural MRI was used to look for potentially pathological brain abnormalities. Indeed, several researchers have identified brain abnormalities such as tumours, cysts, aneurysms, evidence of stroke, white matter lesions, hippocampal sclerosis, venous angioma, and general atrophy in PNES patients with or without epilepsy. The studies described below noted such findings in considerably more patients with PNES than expected in healthy volunteers in whom such findings are identified in 4.8% to 13.6% of cases (Katzman, Dagher, & Patronas, 1999; Vernooij et al., 2007).

Based on an initial sample of 311 patients with a diagnosis of PNES with or without epilepsy, Devinsky, Mesad, & Alper (2001) documented cerebral structural or electroencephalographic abnormalities in roughly 25.4% of these patients (n = 79). Of these, 76% demonstrated unilateral abnormalities (n = 60) of which 85% were structural (MRI, CT). When comparing this group of PNES patients to a comparison group with epilepsy without PNES (n = 102), Devinsky, Mesad, & Alper (2001) found significantly more right-sided abnormalities in the PNES group (71%) compared to the epilepsy group without PNES (46.5%). While Reuber, Fernandez, Helmstaedter, Qurishi, & Elger (2002) also found evidence of brain disease in PNES only patients (27%) compared to patients with PNES plus epilepsy (78%), in contrast to Devinsky, Mesad, & Alper (2001), Reuber, Fernandez, Helmstaedter, Qurishi, & Elger (2002) observed no significant difference in lateralization between PNES only patients and patients with PNES plus epilepsy and both groups showed abnormalities in frontal (PNES only = 5%; PNES plus epilepsy = 18.9%) as well as temporal brain regions (PNES only = 40%; PNES plus epilepsy = 54.1%). A more recent study by Bolen, Koontz, & Pritchard (2016) reported similar prevalence rates, with 33.8% of patients with PNES only compared to 57.7% of patients with epilepsy showing structural brain abnormalities. They also noted significantly more multifocal abnormalities in frontal, temporal, parietal, occipital, cerebellar and brainstem brain regions in the PNES only patients (47.8%) compared to the epilepsy group (21.9%), in which significantly more temporal abnormalities were detected for those with epilepsy only (57.8% vs 21.7%).

While all of the authors of these studies suggest that these findings point to a plausible mechanism through which non-epileptic seizures might occur due to pathological brain changes, all three studies have a number of significant limitations. Firstly, these studies were retrospective, and therefore it is unclear whether the observed

brain abnormalities occurred before or after PNES onset and therefore predisposition or consequence cannot be determined. Furthermore, because all three studies lacked healthy control subjects, the authors have to draw on other studies demonstrating that the prevalence of brain abnormalities in the general population is lower than that found in their respective PNES groups and therefore the frequency of markers of physical brain disease for these studies remains unclear. Additionally, all of these studies were undertaken in well characterised but also particularly disabled patient populations at specialist centres. This may have introduced a certain degree of selection bias which may have resulted in a higher prevalence rate of brain abnormalities as measured by MRI or CT than might be expected in the wider PNES patient population.

Secondly, given the high levels of psychiatric comorbidity in patients with PNES (Diprose, Sundram, & Menkes, 2016), it is impossible to infer that these brain abnormalities are specifically associated with this seizure disorder and not other co-existing psychopathologies. While Bolen, Koontz, & Pritchard (2016) suggest that the significant trend towards multifocal abnormalities in their PNES sample may be directly related to the underlying co-existing psychopathology, this is not clear because instances of concurrent psychopathology was not reported in their sample. In addition, there may also be other psychological reasons why an individual with structural brain abnormalities may develop PNES and this again is unclear. Thirdly, the hypothesis put forward by Devinsky, Mesad, & Alper (2001) that the prevalence of right-sided abnormalities might facilitate conversion due to non-dominant hemispheric injury or damage is not supported by either Reuber, Fernandez, Helmstaedter, Qurishi, & Elger (2002) nor Bolen, Koontz, & Pritchard (2016). In addition, it is unclear how the emotion dysregulation hypothesis that they put forward for PNES is directly associated with seizure like episodes because emotional processing was not directly measured in

their study. Rather, the lack of clear hemispheric dominance or lobar preponderance emanating from these studies supports the notion of a heterogeneous aetiology and phenomenology of PNES.

While the three studies described above observed pathological brain abnormalities in a proportion of patients with PNES, the majority of patients with PNES, on visual inspection, do not appear to show any evidence of brain disease or injury. One way to look for differences at the morphological level not apparent on visual inspection of individual scans, is to use computer-aided analysis of structural brain imaging using T1-weighted volumetric MRI scans of the brain. This method allows for the non-invasive quantification of different anatomical features of the brain in terms of shape, volume and density. In contrast to individual or even group level visual inspection of MRI scans or manual measurement of structures of interest, morphometric brain measurements are largely automated and allow for larger scale un-biased group comparisons.

Two common brain morphometry techniques are voxel based morphometry (VBM) and surface-based morphometry. VBM essentially performs statistical tests on all of the voxels in the T1-weighted MRI image and can be used to measures overall gray matter and white matter volume as well as increases/decreases in cerebral spinal fluid (Ashburner & Friston, 2000; Whitwell, 2009). Surface-based morphometry is a technique in which, once the brain is segmented, the boundary between different classes of tissue can be reconstructed as a surface on which morphometric analysis can proceed, for example cortical thickness, cortical surface area and cortical folding patterns (Fischl & Dale, 2000).

3.3.2.2 Morphological brain changes in patients with PNES

To date only two morphological studies have examined structural brain changes in individuals with PNES compared to healthy controls. Labate and colleagues (2012) combined two approaches, VBM and surface-based morphometry. VBM analysis revealed significant gray matter volume reductions in the cerebellum (bilateral), the right precentral gyrus, right middle frontal gyrus, right anterior cingulate cortex, and right supplementary motor area in PNES patients (n = 20) compared to age and gender matched healthy controls (n = 40). Cortical thickness analysis results revealed cortical thinning in the right precentral gyrus, right superior frontal gyrus, right precuneus and right paracentral gyrus in PNES patients compared to the matched healthy controls. Additional analyses revealed negative correlations between depression scores and atrophy involving the right dorsal premotor cortex, the right paracentral gyrus, the right superior frontal gyrus and right orbitofrontal sulcus thickness, as well as negative correlations between dissociation scores and atrophy in the left inferior frontal gyrus (pars opercularis) and the left central sulcus in patients with PNES.

The findings from a second surface-based morphometric study by Ristić et al (2015) differ somewhat from those reported by Labate et al (2012). In this study Ristić et al (2015) found that compared to healthy controls (n = 37), patients with PNES (n = 37) showed increases in cortical thickness in the left insula, left and right medial orbitofrontal and left orbitofrontal regions, as well as decreases in cortical thickness in the right precentral gyrus, right entorhinal, right lateral occipital and left precentral areas. In addition, they also noted increased sulcal depth in the left and right insular sulci, right rostral anterior cingulate, right posterior cingulate, and left cuneus, and reduced sulcal depth in the right and left medial orbito-frontal sulci in patients with PNES compared to controls. Correlational analysis between cortical thickness results

and clinical features revealed weak to moderate negative correlations between the left insula thickness and disease onset (r = -0.37), the left precentral thickness and illness duration (r = -0.34), and a weak to moderate positive correlation between the right enthorinal thickness and disease onset (r = 0.37). However, no other significant correlations were found for history of abuse, a stressful event identified as a trigger, seizure frequency, semiology, or number of antiepileptic drugs (AEDs) taken.

While both studies report cortical thickness decreases in patients with PNES, the results of these two studies also differ, with one reporting cortical thickness decreases only in the right hemisphere (Labate et al., 2012) and the other reporting bilateral cortical thickness decreases as well as cortical thickness increases in limbic and orbitofrontal regions (Ristić et al., 2015). While this may reflect differences in patient selection and sample size, differences between the two sets of results may be best interpreted as again reflecting the heterogeneous nature of this condition. Moreover, while both studies suggest that there is an association between emotion dysregulation linked to dissociative experiences (Ristić et al., 2015), or "psychogenic causation" due to trauma (Labate et al., 2012), this is not scientifically valid as neither study used other physiological or self-report measures to assess emotion regulatory abilities. Moreover, changes in brain morphometry may also occur for reasons other than pathology (Draganski et al., 2006; Zatorre, Fields, & Johansen-Berg, 2012).

3.3.2.3. Structural connectivity in patients with PNES – Diffusion tensor imaging

Another way to look at structural brain changes more closely associated with brain function is by looking at the strength and integrity of connections between different parts of the brain (how the brain is wired). One technique that has revolutionized our ability to examine structural connectivity between different brain regions is diffusion tensor imaging (DTI). DTI is an in vivo non-invasive technique

used to examine cerebral white matter fibre bundles or tracts that facilitate inter-regional neural communication.

Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer (2015) used DTI indices including fractional anisotropy and diffusion tensor tractography to examine the white matter structural connectivity of the uncinate fasciculus in PNES patients (n = 8) and age and gender matched healthy controls (n = 8). The uncinate fasciculus is a prominent tract for connecting medial prefrontal regions with limbic areas which include the amygdala and hippocampus (Ebeling & von Cramon, 1992; Seminowicz et al., 2004), which play key roles in emotion and memory processes (Schmahmann, Smith, Eichler, & Filley, 2008). They found a significantly greater number of uncinate fasciculus streamlines (visual and statistical representation of white matter tracts) in the right hemisphere when compared to the left hemisphere in patients with PNES and these differences were not evident in the healthy controls. This pattern of connectivity suggests that individuals with PNES may have a stronger connection between prefrontal regions and limbic regions in the right hemisphere compared to the left hemisphere, and like Devisky et al (2001), Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer (2015) suggest that this rightward asymmetry may have detrimental effects on emotion regulation. However, another DTI study by Lee et al (2015) using fractional anisotropy (FA) and mean diffusivity to measure differences in white matter tracts in the whole-brain between PNES patients (n = 16) and age and gender matched healthy controls (n = 16) found increased connectivity in the uncinate fasciculus and superior temporal gyrus in the left hemispheric areas, not the right, in addition to the corona radiata and internal and external capsule associated with motor function. Notably, the authors found no significant differences between average FA in regions with increased FA and clinical measures including event frequency and duration of illness.

Taken together, these findings are again somewhat contradictory with one study reporting right hemisphere differences (Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer, 2015) and the other left hemisphere differences between PNES patients and healthy controls (Lee et al., 2015). While both DTI studies propose that non-epileptic seizures may be associated with changes or abnormalities in white matter tracts such as the uncinate fasciculus, and that greater structural connectivity between prefrontal regions and limbic regions may predispose individuals to PNES through emotion dysregulation, this conclusion is highly overstated as neither study empirically tested this hypothesis. Additionally, it is not clear from either study how such a hypothesis easily translates to brain function in so far as it could be argued that greater connectivity of the uncinate fasciculus may in fact strengthen the ability to downregulate emotional responses rather than cause emotion dysregulation. Furthermore, given the complexity of structural connectivity of white matter and the vast number of subcortical brain connections, it is very possible that other fibre tracts involved in other pathways and therefore function were also included, an important limitation recognised by both study reports.

In summary, while all of the structural MRI studies reviewed in this section lend support for the view that structural brain changes may be present in patients with PNES, a number of limitations are also evident in addition to the heterogeneity of the results. Therefore, these results may be incidental and related to a third factor independently associated with PNES, such as a history of trauma, neglect in early life or concurrent psychopathology. Future studies should better attempt to account for these confounds by describing psychopathology in greater detail alongside personal history and personality characteristics so that the effects of different manifestations of psychopathology can be better aligned to the imaging results. It would also be helpful if

future studies recruited control groups with certain types of psychopathology or different levels of trauma exposure. This necessarily implies that future studies will need to be much larger so that clinically different subpopulations do not have to be analysed together which may cancel out significant findings.

3.3.3. Brain activation patterns and resting state networks in patients with PNES

In the previous section it was hypothesised that structural brain changes may have adverse effects on brain function, potentially contributing to phenomena such as seizure like episodes. One way to investigate links between brain function and PNES is to use imaging modalities which assess real time functional brain activity in individuals with non-epileptic seizures.

3.3.3.1. Positron emission tomography

Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj (2015) used interictal 18FDG - PET to examine resting state brain metabolic alterations in PNES patients. 18FDG or fludeoxyglucose F18 is a radiopharmaceutical used in PET to assess tissue uptake of glucose, and can provide an indirect measure of brain metabolic function/ activation. Compared to healthy controls (n = 16), PNES patients (n = 16) showed significant hypometabolism (lower glucose uptake) in two specific brain areas, namely the right inferior parietal/central brain region and bilateral anterior cingulate. No significant differences in hypermetabolism was observed in patients with PNES compared to healthy controls. The authors further examined how metabolic activity in these two clusters was associated with metabolic activity across the whole brain in both PNES patients and healthy controls. Compared to healthy controls, PNES patients showed significant correlations in metabolic activity between the right inferior parietal/central brain region and bilateral cerebellum and between bilateral anterior

cingulate and the left hippocampal gyrus. However, the authors did not find any significant correlations between the metabolic activity of the clusters reported and clinical features in the PNES group (age, age at onset, frequency, duration or semiology).

From their results Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj (2015) concluded that interictal resting state metabolic brain changes in PNES may reflect disturbances in brain function. The authors suggest that these disturbances may relate to two distinct pathophysiological mechanisms involved in PNES, namely emotion dysregulation (bilateral anterior cingulate hypometabolism) and dysfunctional processes associated with self-awareness/consciousness of one self and the environment (right inferior parietal hypometabolism). However, as the authors point out, these findings need to be interpreted with a certain degree of caution. This is because parameters relating to dissociative traits, emotion processing and certain psychiatric comorbidities such as anxiety, depression and PTSD were not formally assessed. Thus, co-existing psychopathology may have had a significant effect on the results, especially given that the anterior cingulate cortex has been implicated in both anxiety and PTSD (Bishop, Duncan, Brett, & Lawrence, 2004; Shin et al., 2001). Additionally, it is difficult to interpret the association between metabolic activity in the right inferior parietal/central brain region and bilateral cerebellum, and between bilateral anterior cingulate and the left hippocampal gyrus. The significance of these findings is unclear. Moreover, the lack of any significant findings relating to metabolic activity in the brain and clinical features of PNES could suggest that these brain changes observed are not related to PNES. However, there is also a strong possibility that the imaging method employed in this study, and this generalizes to other neuroimaging methodologies, may

not be sensitive enough to correctly identify associations between brain activity and symptomology.

3.3.3.2. Single photon emission computed tomography

Another unique and potentially informative approach to examining potential brain abnormalities in PNES is single photon emission computed tomography (SPECT). This imaging modality integrates two technologies, computed tomography (CT), and the use a radioactive tracer injected into the patient before the scan. SPECT differs from a PET scan in that the tracer stays in the blood stream rather than being absorbed by surrounding tissues, thereby limiting the images to areas where blood flows in the brain. During seizures, regional cerebral flow may increase at the brain site of epileptic origin (hyperperfusion) while interictally, the epileptic focus may demonstrate decreases in regional cerebral blood flow (hypoperfusion) (Devous Sr, Thisted, Morgan, Leroy, & Rowe, 1998). This procedure facilitates the localization of the epileptic focus of the seizures themselves when seizure brain activity is present but remains undetectable by scalp-recorded EEG.

To date SPECT has been solely used in difficult cases involving complex medical histories suggestive of both PNES and epilepsy in which differential diagnosis remains questionable. In such cases, SPECT has proven useful in differentiating epileptic from non-epileptic episodes by using computer-aided quantification of ictal, inter-ictal and postictal changes in regional cerebral blood flow (Ettinger et al., 1998; Neiman, Noe, Drazkowski, Sirven, & Roarke, 2009; Varma et al., 1996). The use of SPECT in PNES is important because it supports the proposition that PNES is indeed different from epilepsy in the majority of PNES cases. However, like structural MRI studies which have observed instances of brain disease or injury in a sub-population of

PNES patients ranging from 25% to 34% (Bolen, Koontz, & Pritchard, 2016; Devinsky, Mesad, & Alper, 2001; Reuber, Fernandez, Helmstaedter, Qurishi, & Elger, 2002), the SPECT studies outlined below have observed similar prevalence rates of abnormal regional cerebral blood flow in a subset of patients with confirmed PNES diagnosis (range 15% - 30%).

An early study to utilize SPECT in patients with PNES was conducted by Varma et al (1996). In this study they observed abnormal SPECT results in 30% of patients with PNES only (n = 3/10; bifrontal, left frontoparietal, right medial temporal hypoperfusion) compared to 80% of age and gender matched epilepsy patients, who demonstrated clear focal hypoperfusion suggestive of epileptogenic origin (n = 8/10). In line with these findings, Ettinger et al (1998) observed abnormal postictal SPECT scan results in 27% of patients who experienced non-epileptic seizures (n = 3/11; all hypoperfusion) compared to 64% of patients with epileptic episodes (n = 7/11; hypoperfusion in six, hyperperfusion in one). Consistent with Varma et al (1996) and Ettinger et al (1998), a more recent SPECT study by Neiman, Noe, Drazkowski, Sirven, & Roarke (2009), this time using subtraction ictal SPECT coregistered to MRI (SISCOM), observed abnormal SISCOM results in 15% of patients with non-epileptic seizures (n = 2/13; posterior lateral right frontal and right insular hyperperfusion).

In the majority of cases, SPECT studies support the differential diagnosis of PNES based on the absence of a clear epileptogenic origin in the brain. Nonetheless, observed brain abnormalities in regional cerebral blood flow appear to be present in a minority of PNES cases. This suggests that, to date, our understanding of this disorder as purely psychogenic may need to be reconsidered and the use of PNES as an umbrella term/diagnosis fails to appropriately classify PNES sub-populations. However, these findings are difficult to interpret given the small sample size, the use of a highly

selective PNES sub-population, and the absence of age and gender matched healthy controls. Additionally, abnormal SPECT scans reported for a certain percentage of PNES patients may also result from having other nonpsychogenic conditions such as brain disease or injury, cardiovascular disease and/or other psychiatric comorbid conditions (Camargo, 2001). All of the above again emphasises the importance of the clinical context in which diagnosis, treatment, and studies involving individuals with PNES are conducted.

3.3.3.3. Functional magnetic resonance imaging

Another way to look at brain activity is to use functional magnetic resonance imaging (fMRI). fMRI can be used to measure fluctuations in the blood oxygenation level-dependent signal or BOLD, which is an indirect correlate of neural activity. In addition, resting state functional magnetic resonance imaging (rsfMRI) can be used to measure the same BOLD signal during rest. During rest, co-activation patterns in different brain regions can be used to assess functional connectivity patterns in resting state networks (van den Heuvel & Pol, 2010). An important point to note here is that four of the six fMRI studies reviewed in this section come from the same research group and have used the same participants in their analysis (Ding et al., 2013; Ding et al., 2014; Li et al., 2014; Li et al., 2015).

To date, there is only one study that simultaneously investigated structural and functional connectivity in patients with PNES using rsfMRI and DTI. In this study Ding et al (2013) found that PNES patients (n = 17) compared to healthy controls (n = 20) demonstrated significant decreases in the strength of both structural connections and functional connectivity in brain regions associated with attention, sensorimotor, and the default mode network. Moreover, the coupling strength of structural-functional connectivity was decreased in patients with PNES and this showed high sensitivity

(75%) and specificity (77%) in differentiating PNES patients from healthy controls. Building on this work, Ding et al (2014) used functional connectivity density mapping based on the same rsfMRI data to assess whether a more detailed examination of both long-range and short-range functional connectivity would differentiate PNES patients (n = 18) from healthy controls (n = 20). Compared to healthy controls, Ding et al. (2014) found that PNES patients demonstrated bilateral differences in both long-range and short-range functional connectivity mainly in frontal, sensorimotor, cingulate, insular and occipital brain regions. Interestingly, three regions with increased long-range functional connectivity values correlated positively with illness duration, namely the right calcarine fissure (r = 0.64), the left lingual gyrus (r = 0.63) and the right lingual gyrus (r = 0.66).

Again, using the same rsfMRI data as Ding et al. (2013, 2014) but this time focusing on the distinct functional connectivity patterns of insular subregions (Cauda et al., 2011; Craig, 2009; Deen, Pitskel, & Pelphrey, 2011; Kurth, Zilles, Fox, Laird, & Eickhoff, 2010b), Li et al. (2014) found that functional connectivity maps based on the left ventral anterior insula (vAI), the right dorsal anterior insula (dAI) and the right posterior insula (PI) showed significant group differences in connectivity values between PNES patients and healthy controls. Both right dAI and PI showed stronger functional connectivity values with the left superior parietal gyrus and left putamen in patients with PNES compared to healthy controls. In addition, the left vAI showed stronger functional connectivity with the right lingual gyrus, left postcentral gyrus and bilateral supplementary motor area (SMA). Also, based on the left vAI seed, functional connectivity values of the left and right SMA were positively correlated with frequency of PNES (SMA_left, r = 0.59, SMA_right, r = 0.60).

A second follow up study by Li and colleagues (2015) using the same rsfMRI data, this time using a combination of fractional amplitude low-frequency fluctuations (fALFF; the measurement of spontaneous fluctuations in the BOLD-fMRI regional signal intensity) and functional connectivity values, found that PNES patients compared to healthy controls showed increased synchronous regional activity mainly in the dorsolateral prefrontal cortex (DLPFC), parietal, and motor regions, and decreased regional activity in the right triangular inferior frontal gyrus which is part of the ventrolateral prefrontal cortex linked, amongst other things, to response inhibition (Aron & Poldrack, 2006). Moreover, PNES patients also showed increased functional connectivity between the DLPFC, sensorimotor and limbic regions and decreased functional connectivity in the ventrolateral prefrontal cortex. Correlational analysis revealed that functional connectivity values between the SMA and the anterior cingulate cortex positively correlated with the frequency of PNES episodes ($r = 0.58$).

The findings from these four studies suggest that alterations in functional connectivity in brain regions associated with attention and regulatory processes, memory, emotion processing and sensory and motor function may be compromised in patients with PNES. These alterations imply less effective communication between different parts of the brain and therefore disruption in information processing, possibly resulting from life experiences, leading to aberrant sensori-motor interactions beyond the conscious control of the individual. Moreover, the inability to down regulate behavioural responses to emotional stimuli (Li et al., 2015) may result from hyper-connectivity between insular subregions and sensori-motor, parietal and occipital brain regions (Li et al., 2014), which may result in a form of maladaptive long-term hypervigilance to external stimuli (Ding et al., 2014). This suggests that alterations in cognitive-emotional-behavioural brain mechanisms may result from adverse life

experiences and/or experiential learning leading to PNES (Devinsky, Mesad, & Alper, 2001; Li et al., 2015; Brown & Reuber, 2016b).

Partially supporting the findings by Ding et al. (2013) and Li et al. (2015), a study by van der Kruijs et al. (2014) found that, compared to healthy controls (n = 27), PNES patients (n = 21) showed increased functional connectivity in resting state networks associated with fronto-parietal activation, executive control, sensorimotor functions, and the default mode. The default mode network is of particular interest as it has been associated with self-awareness/sense of agency and consciousness (Gusnard, Akbudak, Shulman, & Raichle, 2001; Schneider et al., 2008) which may be directly linked to the tendency to dissociate and the expression of motor symptoms occurring as involuntary movements observed in PNES. Moreover, the connectivity strength in resting state networks that showed differences in activation between PNES patients and healthy controls (fronto-parietal, default mode, executive control, and sensorimotor network) were positively correlated with dissociation scores, further implicating the role of these networks in PNES, and lending support to the view that PNES are a manifestation of dissociation (Nijenhuis & van der Hart, 2011). However, the extrapolation of resting state results to hypothesized activation patterns in response to external stimuli or events is questionable. That is, do individuals with PNES respond differently to external stimuli compared to healthy individuals and can this be measured inside the scanner. To date only one fMRI study has examined brain activation patterns to external stimuli in patients with PNES.

In this study, van der Kruijs et al (2012) conducted four fMRI scans during one scanning session (two resting state scans, one event-related picture-encoding task scan, and one event-related stroop paradigm scan) in patients with PNES (n = 11) and healthy controls (n = 12). While whole-brain analysis revealed no differences in either task-

related fMRI paradigm between PNES patients and healthy controls, functional connectivity maps based on the rsfMRI scans showed significantly stronger functional connectivity patterns in PNES patients compared to controls in areas involved in emotion (insula), executive control and sensory information processing (inferior frontal gyrus and parietal cortex) and movement (precentral sulcus). In addition, functional connectivity values based on the average of both rsfMRI scans showed a significant positive correlation between the precentral sulcus-posterior insula and reported dissociation scores (Spearman's $r_s = 0.56$). Moreover, linear regression analysis with functional connectivity values of the precentral sulcus-posterior insula connection as the dependent variable and dissociation scores as the independent variable, showed that dissociation scores was a significant predictor of the functional connectivity of these two brain regions ($\beta = 0.066$, $p = 0.04$). The authors suggest that a higher tendency to dissociate in PNES, may reflect a vulnerability or predisposition to PNES, whereby the hyper-connectivity between brain regions involved in emotion processing (insula) and motor function (precentral sulcus) goes unchecked by frontal brain regions involved in inhibitory control, resulting in non-epileptic seizure like episodes. These findings are important because they tentatively propose an underlying physical PNES substrate in the brain for dissociation, which has significant implications for how we view PNES (van der Kruijs et al., 2012).

All of the fMRI studies reviewed in this section provide plausible explanations for associations between fMRI findings and non-epileptic seizures, but there are a significant number of limitations. First and foremost, again these fMRI studies cannot infer any causal relationship between the brain imaging results and PNES. This again leaves open the possibility that these findings may also be incidental, or that they may be related to other factors not under investigation. Furthermore, given that a single brain

region may be involved in many different mental processes, it is not clear from the functional studies presented here, that activation patterns involving specific brain regions are solely associated with specific mental processes such as emotion regulation or dissociation, hypothesised to precipitate and perpetuate PNES symptomatology. Moreover, these behaviours have been characterized as paroxysmal rather than chronic and therefore, alterations in the interictal resting state networks may not necessarily be indicative of changes in brain activation patterns during an actual seizure like episode. Another possible confound of these studies relates to whether patients with PNES are engaging the same mental processes as healthy controls while in the scanner. This is arguably unlikely given that PNES patients often have other conditions such as PTSD, depression and anxiety.

Therefore, a certain degree of reverse inference (Aguirre, Feinberg, & Farah, 2003; Poldrack, 2006, 2008) may have led to premature conclusions. One could further speculate that because these studies are not longitudinal, a single scan at a single time point cannot tell us if the observed activation patterns reflect state or trait properties. A recent longitudinal study investigating brain function and a broad range of psychological and biological variables in a single human, has in fact demonstrated that brain function has temporal qualities related to both psychological and biological variability and that sensory, motor, and attentional networks actually showed the greatest variability across multiple fMRI sessions (Poldrack et al., 2015). Therefore, future studies in this area should attempt to control for this by scanning individuals with PNES at multiple stages of their disorder.

Moreover, if brain changes are responsible for the aetiology and maintenance of PNES, it is important to know how they relate to clinical features associated with the non-epileptic patients. Again, it is difficult to see clear agreement on the relationship

between the imaging results and clinical features. More importantly, self-report measures (including seizure frequency and symptoms) may not be that reliable, especially if they are applied cross-sectionally. It may be more meaningful if the patient reports on the frequency of events or the types of symptoms experienced at several different time points. Furthermore, the relationship between objective measures and self-report measures is poor in many areas of psychopathology. Therefore, it may be advisable to look for correlations between the imaging data and other objective measures such as neuroendocrine measures, heart rate variability changes, EEG, and/or epigenetic data. This may allow us to better understand the relationship between fMRI results and functional connectivity patterns in patients with PNES.

3.3.4. Meta-analysis

Given the heterogeneity of results summarised in the preceding sections, we were keen to explore whether any convergent findings could be extracted from the imaging studies carried out in patients with PNES. To that end, we carried out three different ALE meta-analyses using GingerAle (version 2.3.6).

The first meta-analysis included all nine functional and structural studies for which MNI or Talairach coordinates were available and included 307 subjects (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015; Ding et al., 2014; Labate et al., 2012; Lee et al., 2015; Li et al., 2014, 2015; Ristić et al., 2015; van der Kruijs et al., 2012, 2014). This analysis resulted in no significant clusters. The second meta-analysis which focused on all of the functional connectivity studies in PNES patients included six studies with a total of 141 subjects (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015; Ding et al., 2014; Li et al., 2014, 2015; van der Kruijs et al., 2012, 2014). Again, this analysis resulted in no significant clusters. However, the third and final meta-analysis which focused on three imaging studies

reporting structural brain differences between PNES patients and controls (Labate et al., 2012; Lee et al., 2015; Ristić et al., 2015) yielded significant findings. This meta-analysis included 166 subjects and resulted in 26 foci. This cluster-level analysis resulted in one significant cluster above the chosen minimum cluster size of 424 mm3 in the left temporal lobe region (Brodmann area 21). Table 3.4 outlines the results of this ALE meta-analysis showing brain areas within +/- 5mm3 of this significant cluster above the corrected p value threshold. Figure 3.2 depicts the results of the ALE meta-analysis conducted on the sMRI studies only, showing an overlay of this significant cluster, at the left temporal lobe, superimposed on the high-resolution standard anatomical brain image provided by brainmap.org (Colin_tlrc.nii).

Table 3.4. *ALE cluster-analysis results for structural studies (N = 3).*

Cluster	Size / Volume mm^3	Weighted Centre	Brain areas within +/- $5mm^3$	Max. ALE value	x, y, z of max. ALE	Contributors to cluster 1	Studies included
1	424	X = -35.3 Y = -4.6 Z = -9.3	BA 21: Left cerebrum / Temporal lobe	0.0143	-36, -4, -10	Lee et al (2015), Ristić et al., 2015).	Labate et al (2012), Lee et al (2015), Ristić et al (2015).
			Nearest gray within +/- $5mm^3$				
	200		Left cerebrum / sub-lobar / claustrum gray matter				
	168		Left cerebrum / temporal lobe / sub-gyral gray matter: BA21				
	48		Left cerebrum / limbic lobe / parahippocampal gyrus and amygdala gray matter				
	8		Left cerebrum / sub-lobar / insula gray matter: BA 13				

Brodmann Area (BA), X, Y, Z coordinates in Talairach space.

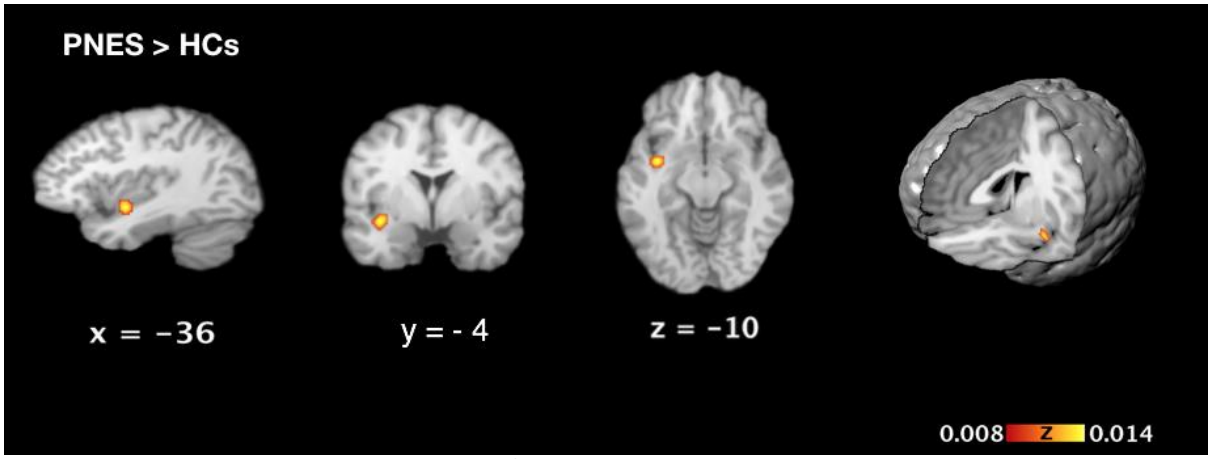


Figure 3.2. **Activation likelihood estimation (ALE) significance maps based on sMRI studies comparing PNES patients to healthy controls.** The only area showing a significant cluster to survive the cluster forming threshold with an uncorrected *p*-value of 0.001 was found in the left temporal lobe only. Numbers represent the sagittal (x), coronal (y), and axial (z) coordinates of each slice in Talairach space. Scale bar shows z-scores of ALE statistics with increasing significance from left to right. PNES = Psychogenic non-epileptic seizures; HCs = Healthy controls.

A link between a common abnormality in the temporal lobe and patients with PNES would be in keeping with the results of the studies by Bolen, Koontz, & Pritchard (2016) and Reuber, Fernandez, Helmstaedter, Qurishi, & Elger (2012) which identified higher prevalence rates of pathological brain abnormalities in this part of the brain (22% and 40% respectively). However, it is notable that this analysis was only based on three studies, and it is important to take account of the fact that no other convergent brain areas where found when we examined all of the nine studies together or when we examined six studies reporting functional connectivity patterns in PNES patients compared to controls. Considering the small sizes of the studies, the lack of high quality methodological approaches and the dearth of convergent findings, this may indicate that at least some of the studies report chance findings which may not be replicable in larger studies. However, the varied results may also reflect the true aetiological and phenomenological heterogeneity of patients with PNES, pointing to individualized phenotypes and patterns of abnormal brain activation, possibly resulting from individual differences and thus group differences in genetic makeup, anatomical variation, medical history, life experiences, semiology and state and trait characteristics.

3.3.5. Limitations

This review has a number of limitations. The first relates to the small number of neuroimaging studies in patients with PNES. Although an extensive literature search was conducted only seventeen empirical studies were included in this review. Secondly, it is difficult to draw direct comparisons between the results due to the different imaging methods used and differences in group characteristics which may have influenced the results. Thirdly, the lack of convergence across nine of the studies included in the combined meta-analysis may reflect the heterogeneity of this patient population compounded by the limitations highlighted above in relation to the lack of serial MRI scans taken at different stages of the disorder.

3.3.6. Conclusion

The purpose of this systematic meta-review was to provide an up-to-date synthesis and quantification of both structural and functional neuroimaging studies performed on individuals with PNES. The overarching aim was to present the available evidence in an attempt to assess the strength and limitations of these studies to improve our neurobiological understanding of this condition. Although the results presented here appear inconclusive, they nonetheless provide some evidence for an association between structural and functional brain abnormalities in patients with PNES, which may contribute toward a biopsychosocial account of a condition often described as "medically unexplained". The identification of such neurobiological correlates does not sit well with the understanding of PNES as a purely "psychological" or "psychogenic" disorder without any discernible "physical" correlates. In addition, given the heterogeneity of patients with this condition, characterising individuals in a narrowly defined manner based singularly on the expression of seizure like episodes does little to advance our knowledge base and fails to sufficiently account for sub-populations which

will need to be considered separately in future neuroimaging studies. Furthermore, clear international consensus about PNES diagnosis and semiology is required if we are to standardise measures that can be used in future neuroimaging studies of PNES. Given that psychiatric comorbidities appear overrepresented in PNES, future studies will need to better address this issue by the use of consecutive recruitment of patients with PNES with or without concurrent psychopathologies and comparing their neuroimaging data to patients with psychiatric conditions free of PNES and age and gender matched healthy controls from similar demographic backgrounds. Future studies will also need to address other limitations highlighted by this review by adopting multimodal approaches in conjunction with a detailed medical history when dealing with individuals who have PNES. Advances in these areas will allow for a better and more detailed understanding of the neurobiological correlates of this disorder, which may have implications for both diagnosis and better treatment options.

Footnote 1. While the authors have concerns with adopting the term psychogenic non-epileptic seizures (PNES), this was done because this was the most commonly used term in the scientific literature presented in this review. However, defining this condition as 'psychogenic' necessarily implies a purely psychological mechanism underlying non-epileptic seizures. While the psychological aspects of conversion are very helpful to our understanding and treatment of functional neurological disorders such as PNES, it is not clear if they are always necessary or indeed sufficient for the development or maintenance of this condition. Alternative biological explanations of PNES may provide important additional information, which when presented, should be given due consideration.

Chapter 4. Cortical thickness and gyrification patterns in patients with psychogenic non-epileptic seizures

Abstract

Psychogenic non-epileptic seizures (PNES) are often viewed as manifestations of altered motor and sensory function resulting from psychological responses to adverse experiences. Yet many patients and non-expert healthcare professionals find it difficult to understand how severe disturbances in normal neurological functioning can solely result from underlying psychological mechanisms to the exclusion of other physical causes. Perhaps importantly, recent advances using neuroimaging techniques point to possible structural and functional correlates in PNES. In an attempt to further our understanding of the neurobiological correlates of this condition, we compared the brain scans of 20 patients with PNES (14 females, mean age 41.05, range 19 – 62) and 20 age- and gender-matched healthy controls (14 females, mean age 40.65, range 21 - 61) to investigate group differences for cortical thickness and gyrification patterns using FreeSurfer. Compared to controls, patients with PNES showed cortical thickness increases in motor, sensory and occipital brain regions as well as cortical thickness decreases in temporal and frontal brain regions. In addition, we observed age-related changes in cortical thickness in the right lateral occipital area. However, contrary to our prediction that atypical gyrification may be present, we did not find any evidence of abnormalities on a measure thought to reflect prenatal and early childhood cortical development and organization. Nor did we find significant correlations between cortical thickness results and clinical features. These findings partly corroborate, but also differ from previous morphometry-based MRI findings in PNES. These inconsistencies likely reflect the aetiology and phenomenological heterogeneity of PNES.

4.1. Introduction

Psychogenic non-epileptic seizures (PNES) are characterized by seizures which superficially resemble epileptic seizures but in which seizure-like episodes are thought to result from underlying psychological mechanisms rather than being caused by epileptic discharges in the brain (LaFrance, Reuber, & Goldstein, 2013). In the absence of a clear and easily discernible "organic" cause, current medical nosologies class PNES as a conversion/somatoform (DSM 5, American Psychiatric Association, 2013) or dissociative disorder (ICD-10, World Health Organization, 1992). In light of this, explanations of this diagnosis have largely been rooted in psychoanalytic or psychological accounts (Monzoni, Duncan, Grünewald, & Reuber, 2011), often characterizing these disorders as medically unexplained (Brown, 2004). While the latter categorization is a diagnosis of convenience based on a highly reductionist view of what is considered medically explained, the former accounts reflect a contested, dualistic approach to the understanding of functional neurological disorders like PNES (Brown & Reuber, 2016b). However, there is now a growing body of evidence from structural and functional studies in PNES which suggests that PNES is best understood as a biopsychosocial disorder, a disorder in which structural and persistent or recurrent functional changes in the brain may act as predisposing or precipitating factors for PNES (Brown & Reuber, 2016b; Mcsweeney, Reuber, & Levita, 2017).

The present study was intended to add to this evidence by employing whole-brain cortical surface morphometric analyses of T1-weighted structural magnetic resonance imaging (sMRI) brain scans of individuals with PNES and age- and gender-matched healthy controls. First, we examined whether age-related changes in cortical thickness (controlling for gender) would differ between patients with PNES and controls. This is important because age-related changes in cortical thickness are well

documented (Salat et al., 2004), and disparity between groups in this regard would have significant implications for how subsequent group comparisons of cortical thickness are conducted. Secondly, the present study examined whether group differences in cortical thickness (controlling for age and gender) would differ between PNES patients and controls. Based on the two published morphometric studies in patients with PNES (Labate et al., 2012; Ristić et al., 2015), we hypothesised that we would see group differences in motor, frontal and occipital regions in addition to brain regions involved in emotion processing.

In addition to cortical thickness measures, we utilized a local Gyrification Index (*l*GI) measure based on that of Schaer et al. (2012). Because the degree of gyrification (gyral and sulcal formations) is largely determined early in life (primarily during the third trimester with additional changes during early childhood) and remains relatively stable from adolescence to adulthood (Armstrong, Schleicher, Omran, Curtis, & Zilles, 1995), this sensitive measure is thought to be particularly useful for investigating aberrant early neurodevelopmental changes, traces of which may be identifiable at any age (Schaer et al., 2012). While a later age at onset is more common, PNES manifestations have also been observed during early childhood (Reuber, 2008), and given the link between trauma and PNES (Brown & Reuber, 2016a) and atypical gyrification patterns previously described in children exposed to maltreatment (Kelly et al., 2013) and in individuals with panic disorder (Yoon et al., 2013), we hypothesised that individuals with PNES compared to controls would show atypical levels of gyrification. Finally, we conducted correlational analyses to explore the relationship between cortical thickness and PNES clinical features in cortical regions that showed increases or decreases in cortical thickness in PNES patients compared to controls.

4.2. Method

4.2.1. Participants

Fifty-three 3T T1-weighted MRI brain scans of patients with PNES acquired between 2009 and 2016 were retrieved retrospectively from the Radiology Department, Royal Hallamshire Hospital, United Kingdom. Inclusion of MRI brain scans was based upon (a) confirmed PNES clinical diagnosis by a Consultant Neurologist at the Royal Hallamshire Hospital (b) video-EEG recordings of typical attacks with semiological features of non-epileptic attacks and no associated electro-encephalographic (EEG) or electrocardiographic (ECG) changes suggestive of epilepsy (c) minimum age of 16 at the time of the scan. MRI brain scans were excluded if the patient was (a) likely to have had a mixed seizure disorder (epilepsy and PNES) or (b) had an MRI brain scan showing clinically significant abnormalities. From this initial PNES sample, twelve scans were excluded due to possible or definite co-existing epilepsy. Eight scans were excluded due to lack of video-EEG recordings showing habitual seizure-like episodes. Based upon visual inspection of the MRI scans, three scans were excluded due to MRI results showing clinically significant brain abnormalities (two for hippocampal reductions suggestive of mesial temporal sclerosis, and one 76-year-old with T2 hyperintensities which may have reflected a mini stroke), four scans were excluded due to blurring of the image, and six scans were excluded due to portions of the brain not being captured in the field of view. No cases were excluded due to age. In addition, fifty-six 3T T1-weighted MRI brain scans of age- and gender-matched healthy controls were retrieved retrospectively from an existing database of individuals who had previously volunteered for brain imaging studies (Radiology Department, Royal Hallamshire Hospital). In total, twenty patients with a “gold standard” PNES diagnosis and twenty age- and gender-matched healthy controls were included in the analyses. All

of the patients with PNES were right-handed. Age- and gender-matched healthy controls were free from neurological disease or psychiatric disorders. The retrospective retrieval of archival data was conducted in accordance with the guidelines set out by the NHS ethical approval granted by the South West – Exeter Research ethics committee and carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

4.2.2. PNES clinical features

Semiology features were categorized as 1) generalized motor seizures: seizures mainly characterized by tonic, clonic, or dystonic-like generalized movements, 2) akinetic seizures: seizures mainly characterized by unresponsiveness and the absence of movement with the exception of minor limb tremors, 3) seizures with subjective symptoms: seizures were mainly characterized by experiential phenomena reported by the patients during video-EEG recordings, and 4) focal motor seizures: seizures with focal motor movements (Magaudda et al., 2016). Symptom severity was assessed using a symptom severity scale, in which symptom severity was based upon the summation of previously described clinical features relevant to PNES: 1) ictal loss of consciousness 2) ictal incontinence 3) ictal tongue-biting 4) ictal injury 5) accident and emergency attendance for seizures 6) seizure episodes more than thirty minutes in duration 7) the continuation of symptoms without periods of remission up to the time of the MRI brain scan (Reuber, House, Pukrop, Bauer, & Elger, 2003). Age at onset, symptom duration, number of anti-epileptic and anti-depressant drugs taken at time of MRI, clinical psychiatric diagnosis and reports of trauma exposure were extracted retrospectively from patients' medical records. Types of trauma exposure were documented as 1) sexual abuse 2) physical abuse 3) psychological abuse 4) loss of child 5) other noted but unspecified traumatic experience 6) suicide attempt.

4.2.3 Image acquisition and FreeSurfer analyses

All brain MRI T1-weighted volumetric scans were acquired using the same Phillips 3 Tesla scanner at The Royal Hallamshire Hospital, Sheffield, U.K. For all participants, brain MRI was performed using matrix size 256 x 256, field of view 256, slice thickness 1mm, voxel size 1x1x1, flip angle 8º, coronal plane. Due to the retrospective nature of this study, it was not possible to limit the MRI pulse sequence parameters used to exactly the same timings across all of the subjects. Slight differences in echo time (TE) and repetition time (TR) were present in our PNES group (TE 3.75 – 4.86 ms, TR 8.16 – 10.42 ms) compared to controls (TE 4.80 ms and TR 10.50 ms). However, it is important to note that cortical thickness measurements remain relatively robust even when different MRI protocols or scanners are used (Fischl & Dale, 2000).

MRI-based measurements for each participant were obtained using FreeSurfer version 5.30 (http:// www.surfer.nmr.mgh.harvard.edu). In brief, FreeSurfer consists of two processing streams, a surface-based stream and a volume-based stream. The surface-based stream constructs models of the white matter/gray matter boundary and the boundary between the gray matter and cerebralspinal fluid (pial surface) from which cortical thickness measures are taken as the shortest distance between the two. The volume-based stream preprocesses MRI volumes and labels subcortical tissue classes allowing for the representation and measurement of subcortical structures (putamen, hippocampus, amygdala, ventricles etc.). Both cortical and subcortical labelling is based on a subject-independent atlas and the subject-specific values. These labels are then morphed onto a common space (average subject) to achieve a common point of reference for each subject. This coordinate system can be subsequently used to examine group differences by creating group maps. A more detailed description of FreeSurfer, the local Gyrification Index (*l*GI) and the procedure used to derive cortical thickness

values and *l*GI values is provided in Appendix 38. A full description of this procedure has been published elsewhere (Fischl & Dale, 2000; Fischl, 2012).

Following surface reconstruction and segmentation, the resulting output was visually inspected for quality and accuracy. If needed, edits were made to adjust for skull strip errors, intensity normalization failures (requiring addition of white matter control points), incorrect white matter segmentation, automated topological fixer errors, and pial surface inaccuracies. In the PNES group one skull strip error was adjusted. Edits to the pial surface were required in seven scans, topological defects (holes or handles) adjusted in eleven scans, and control points added in nine scans (263 in total). In the healthy control group, no skull strip errors occurred, edits to the pial surface were made in twelve scans, topological defects adjusted in thirteen scans, and control points added in eleven scans (277 in total). The recon-all processing stream was re-run from the appropriate stage to recreate the final surfaces in brains which required corrections to the initial segmentation. To achieve a common point of reference for each subject the recon-all –qcache flag was used to smooth and resample the data onto the FreeSurfer fsaverage (average subject made in MNI305 space). Prior to general linear model analyses (GLM), cortical thickness maps were smoothed with a 10-mm full-width at half-maximum (FWHM) Gaussian Kernel. No additional smoothing was applied in relation to *l*GI. This is because the final FWHM is a composite of the applied FWHM and the smoothness inherent in the data and *l*GI has a lot of inherent smoothness. In effect, this resulted in the degree of smoothness in our *l*GI data corresponding to a smoothing kernel of 10-mm. Analysis was run on the University of Sheffield high performance computing cluster (Iceberg; OS 64-bit Scientific Linux (Redhat); 2 X Intel Ivy bridge E5 2650V2 8-core processors based nodes).

GLM analyses to assess group differences in age-cortical thickness interactions and *l*GI-age interactions was run by implementing the mri_glmfit script, with DODS (different offset, different slope) as design matrix, diagnosis and gender as discrete factors, and age as covariate. DODS assumes a different offset but predicts a different effect of ageing in both groups (different slope). To examine group differences in cortical thickness and *l*GI (controlling for age), mri_glmfit was used with DOSS (different offset, same slope) as design matrix, diagnosis and gender as discrete factors, and age as nuisance factor. DOSS also allows for group differences to start at different points (different offset) but constrains the group data to change in a similar way, that is, a similar impact of ageing in both groups (same slope). DODS/DOSS are specific to FreeSurfer (for a detailed description see https://surfer.nmr.mgh.harvard.edu/fswiki/DodsDoss).

All of the vertex-wise group analyses were corrected for multiple comparisons using mri_glmfit-sim in FreeSurfer, with a cluster forming threshold of 3 ($p < 0.001$) and cluster-wise probability set to $p < 0.05$. P values were adjusted for both hemispheres using --s 2spaces flag in order to correct for the full search space. This was repeated for 10,000 iterations to derive the location of cluster sizes under the null hypothesis. Clusters surviving cluster-wise correction were then superimposed on fsaverage inflated surfaces using tksurfer, a GUI application available in FreeSurfer.

4.2.4 Correlation analyses with clinical features

We conducted correlational analyses to investigate the relationship between brain regions that showed increases or decreases in cortical thickness in patients with PNES compared to controls with clinical features in patients with PNES (age at onset; duration of symptoms; symptom severity; number of antiepileptic drugs taken). Regions of interest (ROIs) based on significant cluster-wise corrected cortical thickness results

were manually drawn on the fsaverage inflated surfaces in tksurfer and subsequently mapped back to each hemisphere for each subject using the mri_label2label command. The average cortical thickness for each cluster for each subject was then extracted using the mris_anatomical_stats command. Correlations were conducted using a bivariate nonparametric correlation procedure (Spearman's coefficient) with an alpha of 0.05 and subsequently corrected for multiple comparisons using Bonferroni correction. Kolmogorov-Smirnov and Shapiro-Wilk were used to test for normality. All statistical analyses were two-tailed and conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Macintosh, Version 24. Armonk, NY: IBM Corp.).

4.3. Results

4.3.1. Demographics

In total twenty patients with PNES (14 females, mean age at time of scan 41.05, standard deviation, SD 12.50, age range 19 - 62) and twenty age- and gender-matched healthy controls (14 females, mean age at time of scan 40.65, SD 12.40, age range 21 - 61) were included. The mean age at onset of PNES was 27.80 (SD 11.84, range 9 - 51) with a mean duration of symptoms in years prior to MRI of 10.18 (SD 13.73, range 0.25 – 50). Nine patients were taking anti-depressants (Table 4.1) and one patient was taking antipsychotic medication (Quetiapine). Telemetry data capturing typical attacks was available for all twenty PNES patients. The mean number of PNES habitual attacks recorded was 2.5 (range 1 – 8, Table 4.1). Based on the video-EEG recordings, 45% of patients (n = 9) were characterized as having predominantly generalized motor seizures/positive motor phenomena, 35% of patients as having predominantly akinetic seizures characterized mainly by blank spells with reduced responsiveness (n = 7), and 20% were characterized as having predominantly seizures with subjective symptoms

only but not loss of awareness (n = 4). None were characterized as having focal motor seizures. In all patients, brain MRI was visually inspected by an experienced neuroradiologist for signs of pathological brain abnormalities or brain injury. Three patients showed some abnormality. However, these abnormalities were not deemed clinically significant to the extent that these changes could explain their symptoms. Given that they affected the white matter and cerebellum they are unlikely to have affected our cortical thickness analyses. The same morphological analyses were run with these three missing in addition to their matched healthy controls. Exclusion or inclusion of these patients resulted in the same clusters and their order for both the left and right hemisphere. All other patients had unremarkable brain MRI results. PNES group characteristics are presented in Table 4.2. The results of PNES symptom severity scale are presented in Table 4.3.

Table 4.1. *Details of non-epileptic seizures captured by Video-EEG and medications taken at the time of MRI in patients with PNES.*

ID	Gender	Age	Video-EEG hours / attacks recorded	Semiology as captured during Video-EEG	Medications*	
					Anti-epileptics	Anti-depressants
1	Male	47	120hrs / 2 attacks	Fall, kicking of legs, whole-body rigidity, arching of back, dystonic generalized movements with some groaning, unresponsive	Lamotrigine	_
2	Female	24	72hrs / 1 attack	Eyes flickering, side to side head movements, violent shaking of whole body with pelvic trusts and clenching jaw, unresponsive.	Clonazepam Epilim chrono Levetiracetam	_
3	Male	25	48hrs / 4 attacks	Loss of consciousness, violent kicking of legs, jerks/jumps of whole-body, memory loss. unresponsive.	Epilim chrono	_
4	Female	37	48hrs / 1 attack	Unresponsive episode.	Lamotrigine	Sertraline
5	Male	49	24hrs / 2 attacks	Dizzy spells, blank spells, fully responsive and aware of surroundings.	Phenytoin Epilim chrono	_
6	Female	58	48hrs / 1 attack	Twitching of face, shaking of hands moved to shaking of four limbs.	Phenytoin Epilim chrono	Dosulepin
7	Female	36	24hrs / 1 attack	Generalised shaking, unresponsive.	_	Trazodone
8	Female	52	24hrs / 2 attacks	Electric shock sensation and twitching in both arms (not visible on camera), a sensation of dizziness in right-side of head.	_	Mirtazapine
9	Female	33	24hrs / 8 attacks	Unresponsive with left side of mouth dropping with left hand and arm jerking, eye blinks with right arm and hand jerking repetitively with legs jerking (fully aware throughout attack), bilateral asynchronous jerks both legs followed by contraction of right leg and foot (fully aware throughout attack).	Levetiracetam	_
10	Female	25	24hrs / 1 attack	Synchronous jerks of upper limbs, and jerking of legs (particularly right leg) and abdomen.	_	Fluoxetine
11	Female	34	72hrs / 1 attack	Unresponsive episode.	_	_
12	Female	19	48hrs / 1 attack	Twitching all over, eyes rolling into back of head (patient unaware of this), feeling faint at times.	Lamotrigine Pregablin	_
13	Male	62	72hrs / 3 attacks	Reported shouting but no evidence of this on camera, felt dizzy, headaches, intermittent jaw movements with mumbling and grimacing, shaking.	Gabapentin	_
14	Female	50	48hrs / 1 attack	Fuzzy headache followed by black out.	_	Fluoxetine
15	Male	35	48hrs / 3 attacks	Shaking of right arm, tenses up and head leads body to the right with arms shaking, rapid blinking before cessation of attacks, slight jerking of head and mouth.	_	_
16	Female	49	48hrs / 4 attacks	Dizzy spells, felt confused followed by collapse flaccid on floor with eyes open and staring with no significant jerking, unresponsive.	Gabapentin Clobazam	Amitriptiline
17	Female	48	48hrs / 6 attacks	Blank spell, memory problems, bad taste in mouth and funny feeling in head, twitching of arms and right leg jerk around time of entering sleep and waking.	_	Sertraline Amitriptyline
18	Male	36	48hrs / 4 attacks	Déjà vu, muscle twitches with burning smell, pins and needles, tingling in face, arms and legs.	_	_
19	Female	59	48hrs / 3 attacks	Vacant episode, shrugs of shoulder and upper body, tremoring of right arm and a bit of the left arm also, began unresponsive but became intermittently responsive throughout event.	Epilim Levetiracetam	_
20	Female	43	48hrs / 2 attacks	Dizzy spells, unresponsive.	Topiramate	Fluoxetine

MRI = Magnetic resonance imaging; PNES = Psychogenic non-epileptic seizures; EEG = electroencephalography; * Dosages unavailable

Table 4.2. *PNES group characteristics (n = 20).*

Comorbid conditions (n = 16)			
	Number	**Percentage**	**Range***
Depression	13	81.3%	1 - 4
Anxiety	9	56.3%	
Migraine	5	31.3%	
PTSD	2	12.5%	
Panic disorder	2	12.5%	
Agoraphobia	2	12.5%	
OCD	1	6.3%	
Fibromyalgia	1	6.3%	
Number of AEDs taken at time of MRI (n = 12)			
	Number	**Percentage**	
One	6	50%	
Two	5	42%	
Three	1	8%	
Types of traumatic experiences (n = 10)			
	Number	**Percentage**	**Range***
Sexual abuse	4	40%	1 - 5
Physical abuse	3	30%	
Psychological abuse	2	20%	
Loss of child (miscarriage, cot death, other)	5	50%	
Suicide attempt	3	30%	
Other trauma unspecified	3	30%	
Other features (n = 20)			
	Number	**Percentage**	
History of head injury	1	5%	
Positive family history of epilepsy	0	0%	

PNES = psychogenic non-epileptic seizures; PTSD = post traumatic stress disorder; OCD = obsessive compulsive disorder; AEDs = anti-epileptic drugs; MRI = magnetic resonance imaging; *Range refers to the minimum and maximum number of instances i.e. some patients had more than one psychiatric comorbid condition and some patients had been exposed to more than one traumatic event.

Table 4.3. *Results of symptom severity scale in PNES patients (n = 20).*

Item	**Number**	**Percentage**	
Ictal loss of consciousness	17	85%	
Ictal incontinence	5	25%	
Ictal tongue-biting	3	15%	
Ictal injury	6	30%	
A&E attendance for seizure episodes	7	35%	
Seizure duration > 30 minutes	6	30%	
Recurrent symptoms without periods of remission	17	85%	
Group scores for symptom severity scale			
	Mean	**Standard deviation**	**Range**
	3.05	1.50	1 - 7

PNES = Psychogenic non-epileptic seizures; A&E = accident and emergency

4.3.2. Morphological analyses

Age-related changes in cortical thickness were first examined after we had controlled for gender. This analysis identified a single significant cluster surviving cluster-wise correction in the right lateral occipital area, where patients with non-epileptic seizures showed greater decreases in cortical thickness with increasing age

compared to controls (Figure 4.1A & B, Table 4.4). Group differences in cortical thickness, controlling for age and gender, were examined next (Figure 4.1C, Table 4.4). Cluster-wise corrected results showed bilateral structural changes in PNES patients compared to controls, with cortical thickness increases in the cuneus bilaterally, the left paracentral, and left lingual regions. Decreases in cortical thickness were observed in PNES patients compared to controls in the inferior frontal gyrus (pars opercularis) bilaterally, right superior temporal region, and the right medial orbitofrontal cortex. Analysis of gyrification patterns revealed no significant group differences surviving cluster-wise correction for age-related *l*GI while controlling for gender or *l*GI group comparisons controlling for gender and age. Due to the number of PNES patients who had reported trauma exposure (see Table 4.2), additional supplementary post-hoc cortical thickness sub-analyses were conducted in the PNES group only (N = 20), between those had reported traumatic experiences and those who had not. The exact same statistical analyses (described above) was conducted for age-cortical thickness interactions (controlling for gender) and cortical thickness controlling for age and gender. The results of these analyses were all non-significant.

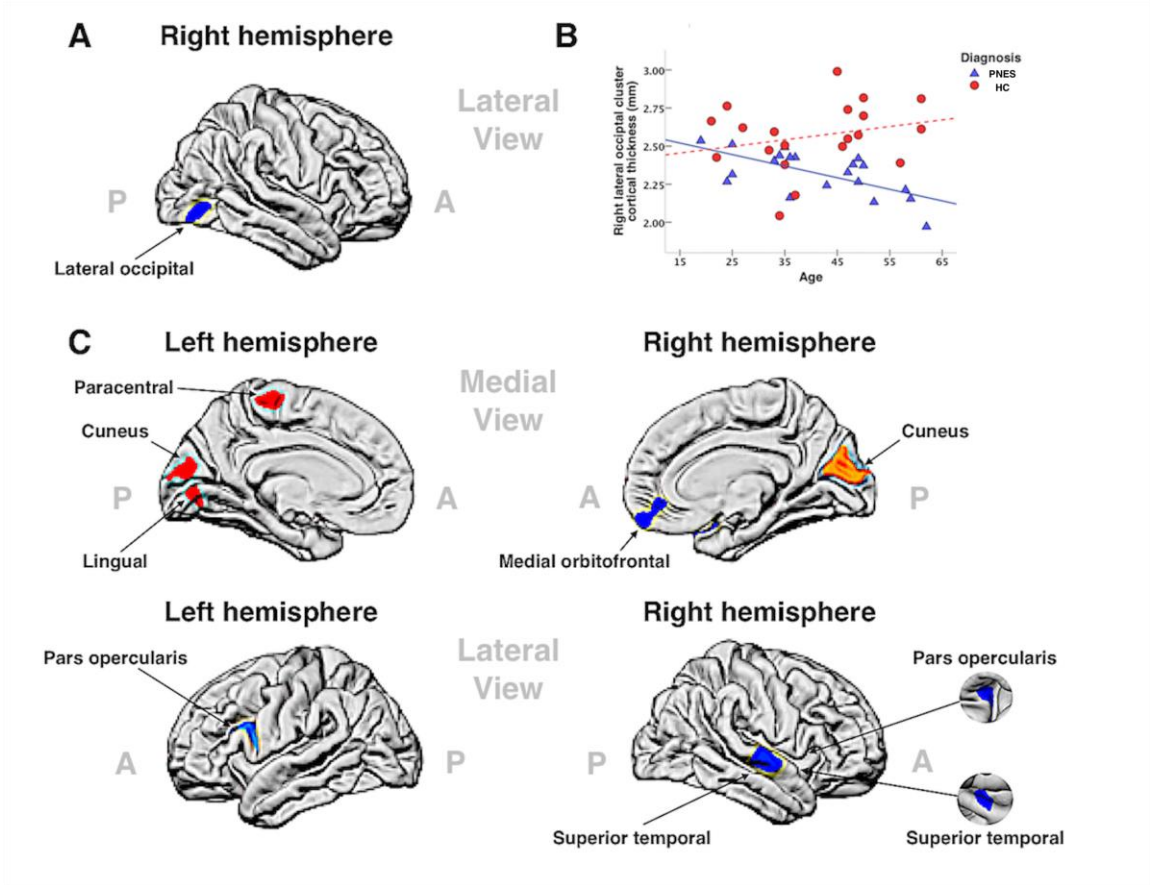


Figure 4.1. Whole-brain group-level analysis of cortical thickness differences between PNES patients and age- and gender-matched healthy controls. Results depict significant clusters surviving cluster forming threshold of $p<0.001$ and cluster-wise correction for multiple comparisons at alpha 0.05. Cortical thickness maps were smoothed using a 10mm full-width at half-maximum (FWHM) Gaussian kernel. Blue and pale blue indicate decreases in cortical thickness. Orange and red indicate increases in cortical thickness. A = anterior, P = posterior. **(A**) Group differences in age-cortical thickness interactions controlling for gender. (**B)** Scatter plot showing age-related changes in average cortical thickness in mm in PNES (blue triangle) and in age- and gender-matched healthy controls (HC, red circle) for the right lateral occipital cluster. (**C)** Group differences in cortical thickness controlling for age and gender.

Table 4.4. *Significant clusters of cortical thickness difference between PNES patients and age- and gender-matched healthy controls for each hemisphere.*

Group differences in age-cortical thickness interactions controlling for gender (DODS)

Cluster No.	Right hemisphere Annotation	Max	Vtx Max	Size (mm^2)	MNIX	MNIY	MNIZ	$P_{cluster}$
1	Lateral occipital	-5.425	41725	238.03	43.2	-75.4	-7.2	0.01236

Group differences in cortical thickness controlling for age and gender (DOSS)

Cluster No.	Left Hemisphere Annotation	Max	Vtx Max	Size (mm^2)	MNIX	MNIY	MNIZ	$P_{cluster}$
1	Pars opercularis	-5.217	47493	530.08	-45.6	16.8	21.2	0.00020
2	Paracentral	4.542	116966	174.58	-8.2	-24.6	62.7	0.04996
3	Cuneus	3.938	112494	268.99	-4.4	-84.2	17.8	0.00699
4	Lingual	3.717	114676	193.57	-10.5	-77.7	-4.9	0.03233
Cluster No.	**Right Hemisphere Annotation**	**Max**	**Vtx Max**	**Size (mm^2)**	**MNIX**	**MNIY**	**MNIZ**	$P_{cluster}$
1	Superior temporal	-7.052	17325	271.41	44.8	4.4	-23.5	0.00539
2	Superior temporal	-6.300	149014	335.88	64.3	-17.9	0.3	0.00160
3	Pars opercularis	-5.754	24955	208.01	37.8	19.2	11.3	0.02484
4	Cuneus	5.013	125855	619.63	5.7	-86.9	11.5	0.00020
5	Medial orbitofrontal	-3.788	12607	207.47	9.8	43.7	-7.4	0.02544

Results of significant clusters surviving cluster forming threshold ($p < 0.001$) and cluster-wise correction for multiple comparisons (alpha = 0.05). DODS = different offset different slope; DOSS = different offset same slope; Max = maximum – log10 (p-value) in the cluster with positive and negative values indicating increases or decreases in cortical thickness in patients with PNES compared to controls; Vtx Max = vertex number of the maximum; MNI = Montreal Neurological Institute; MNIX, MNIY, MNIZ = MNI305 coordinates of maximum; $P_{cluster}$ = cluster-wise probability

4.3.3. Clinical features

Symptom severity positively correlated with cortical thickness in both the left cuneus ($r_s = 0.497$, $p = 0.02$) and right cuneus ($r_s = 0.451$, $p = 0.04$). However, these correlations were not significant following Bonferroni correction for multiple comparisons. No other uncorrected significant correlations were found between cortical thickness results and age at onset, duration of symptoms, or number of antiepileptic drugs taken (see Supplementary Table 4.5). Due to the small number of patients comprising each PNES subtype, it was not feasible to conduct additional analyses based on semiology.

4.4. Discussion

The first finding of this study concerns age-related changes in cortical thickness. We observed cortical thickness differences between groups in the right lateral occipital area where, compared to controls, patients with PNES showed greater cortical thickness decreases with increasing age. This is in keeping with a previous cortical thickness study which found that, compared to healthy controls, PNES patients showed decreased cortical thickness in this area of the brain (Ristić et al., 2015).

In the second group-level analysis controlling for age and gender, we found that patients with PNES showed decreased cortical thickness compared to controls in the right superior temporal gyrus associated with multisensory integration (Karnath, 2001) and the right medial orbitofrontal cortex associated with emotion processing (Northoff, 2000), although the direction of the differences with regard to the right medial orbitofrontal cortex was the opposite of the findings in a previous study (Ristić et al., 2015). However, PNES is highly heterogeneous and therefore, it is possible that this heterogeneity may be the reason for consistent or inconsistent findings across studies that use similar methodological approaches. Additionally, the lack of well-defined and established categorical or dimensional characterizations of specific sub-types of PNES makes it difficult to interpret differing results across studies. We also observed decreased cortical thickness in regions associated with response inhibition (Aron, Robbins, & Poldrack, 2014; Swick, Ashley, & Turken, 2008), namely the left and right pars opercularis. Interestingly, Labate et al. (2012) found that cortical thickness in the left pars opercularis in PNES patients negatively correlated with dissociation scores, suggesting that higher dissociation scores were associated with decreases in cortical thickness in this region of the brain. However, it is difficult to make an equivalent

inference between dissociation and our results, as we were unable to directly measure the tendency to dissociate in our study.

Increased cortical thickness in PNES patients compared to controls was observed in the left paracentral lobule, with the significant cluster spanning both the primary motor cortex and primary somatosensory cortex. The paracentral lobule has been associated with, amongst other things, the planning, control and execution of motor function (Borich, Brodie, Gray, Ionta, & Boyd, 2015). However, this finding differs in terms of both direction and laterality to the findings of Labate et al. (2012), who observed cortical thickness decreases in the right paracentral lobule in patients with PNES. Again, differences in group characteristic and PNES heterogeneity may be a plausible explanation for these inconsistencies. Yet, the role of cortical thickness changes in brain regions involved in motor function is of significant interest in PNES (Labate et al., 2012; Ristić et al., 2015). Cortical thickness increases in PNES were also observed in occipital regions involved in visual processing (Macaluso, Frith, & Driver 2000; Vanni, Tanskanen, Seppa, Uutela, & Hari, 2001), namely the cuneus bilaterally and the left lingual gyrus. A recent imaging study (Ding et al., 2014) found that increased long-range functional connectivity density of occipital regions (right calcarine fissure and bilateral lingual gyri) correlated with disease duration in patients with PNES. The authors proposed that changes in functional connectivity in this region may reflect long-term hypervigilance and increased sensitivity to external stimuli. While the present study does provide some support for their findings, we did not find a significant correlation between cortical thickness results in occipital regions and duration of PNES.

Furthermore, no significant correlations surviving correction for multiple comparisons were found between clinical features in PNES and cortical thickness results, nor did we find cortical thickness differences between patients who had reported

traumatic experiences and those who had not. However, the lack of significant correlations is not altogether surprising. A number of previous neuroimaging studies have failed to find any significant relationship between imaging results and clinical features in this disorder, and those that did reported inconsistent findings (Mcsweeney, Reuber, & Levita, 2017). Perhaps more importantly, clinical features derived from medical records or indeed self-report measures may not be that reliable, especially if they are applied cross-sectionally in small studies. We must also consider the possibility that changes in cortical thickness may reflect other factors not accounted for in the present study (Draganski et al., 2006; Zatorre, Fields, & Johansen-Berg, 2012), especially comorbidities often associated with PNES such as anxiety, depression, posttraumatic stress or personality disorders (Diprose, Sundram, & Menkes, 2016). Disorders such as these could play an aetiological role in PNES on the one hand and be related to changes in cortical thickness on the other. Therefore, it is not clear whether cortical thickness changes associated with PNES in our study are indeed responsible for PNES or whether changes in cortical thickness reflect other factors not necessarily associated with this disorder. This is a critical consideration which has not been sufficiently addressed by our study, nor indeed most other studies which implicate structural and/or functional brain changes in PNES (Mcsweeney, Reuber, & Levita, 2017). This is due to the high levels of co-existing psychiatric disorders, small sample sizes and lack of psychiatric controls free of PNES. However, the high level of psychiatric comorbidity observed in our patient group is in keeping with that observed in most other studies of this disorder and suggests that we have studied a typical patient sample (Diprose, Sundram, & Menkes, 2016).

In addition to looking at cortical thickness, we also conducted a group analysis of gyrification patterns using *l*GI. However, contrary to our prediction that gyrification

may differentiate PNES patients from controls, the results suggest that atypical gyrification patterns may not be a contributor to PNES, at least in our sample. Whereas our study therefore provides some support for the idea that PNES may represent an adaptive (or maladaptive) process reflected by plastic structural brain changes in frontal, sensorimotor, temporal and occipital brain regions, we did not find any evidence of abnormalities on a measure thought to reflect prenatal and early childhood cortical development and organization (Armstrong, Schleicher, Omran, Curtis, & Zilles, 1995). This finding may be surprising. A range of observations provide indirect evidence for the relevance of neglect and trauma in early life to the development of PNES (Bewley, Murphy, Mallows, & Baker, 2005; Holman, Kirkby, Duncan, & Brown, 2008; Kooiman et al., 2004; Novakova, Howlett, Baker & Reuber, 2015). Additionally, atypical gyrification patterns have been observed in major depressive disorder, bipolar disorder, and schizophrenia (Cao et al., 2017). Animal studies provide evidence of life-long structural changes in the brain, neuro-endocrine and behavioural abnormalities after neglect / trauma in early life, which could underpin these findings in humans (Cirulli & Alleva, 2009; Lupien, McEwen, Gunnar, & Heim, 2009). It is possible that the neglect or trauma which may be relevant to PNES affects individuals after the developmental phase in which gyrification patterns are determined. In addition, neglect or trauma in early life are not considered an obligatory precondition to the subsequent development of PNES, but only an important risk factor (Brown & Reuber, 2016b). Alternatively, our sample may have been too small or too heterogeneous to pick up relevant structural abnormalities of early brain development.

In conclusion, our findings of cortical thickness differences between patients with PNES and healthy controls partly corroborate, but also differ from, morphometry-based MRI findings in PNES previously described (Labate et al., 2012; Ristić et al.,

2015). Possible reasons for these variable findings may include sample size, anatomical variation, and likely differences in group characteristics in terms of genetic makeup, medical history, life experiences, semiology, duration of the disorder, personality characteristics, and co-existing psychopathology. Nonetheless, the key take home message is that these findings support the growing body of evidence suggesting that PNES, rather than being a condition that is medically unexplained, may indeed have physical substrates in the brain. The results of the current study and previous neuroimaging studies of PNES have important implications for the way we think about and treat individuals with PNES and how diagnosis may be better communicated to patients. However, longitudinal morphometric studies prospectively capturing a wide range of demographic, developmental and clinical data are needed to better address the role of ageing and whether changes in cortical thickness represent a predisposition to, or consequence of PNES. Furthermore, PNES are paroxysmal events, which are difficult to investigate through the use of sMRI data alone. As such, interictal data provides only part of the picture and future studies should attempt to map electroencephalography (EEG) ictal data acquired during non-epileptic events to the underlying structure, connectivity and folding patterns of the cerebral cortex. This may shed more light on the pathophysiological mechanisms of PNES. Future studies also need to be large enough and involve relevant control groups to allow a better distinction between the likely associations of PNES itself and of concurrent psychopathology and or trauma exposure. The interpretation of such datasets would be greatly aided by a better categorical or dimensional characterization of PNES, a highly heterogeneous disorder in terms of phenomenology, outcome, and presumably aetiology.

Chapter 5. General discussion

5.1. Introduction

The idea that emotions can find expression through bodily sensations, actions or bodily symptoms is not new. In fact, the association between emotion and physical symptoms has been hypothesised since the early 19th Century (Breuer & Freud, 1955). The psychodynamic or psychoanalytical approach to emotion-motor interactions posits that emotional distress, resulting from unconscious conflict, finds expression in bodily symptoms. While this approach has proved to be immensely influential, it has also been immensely difficult to test empirically. However, over the last two decades or so, empirical research into the neurobiological basis of PNES and other FNDs, represents somewhat of a shift away from the psychodynamic perspective and has afforded valuable insights into the link between emotion and motor function in functional neurological symptomology (Voon, Brezing, Gallea, & Hallett, 2011). Notwithstanding the advances made in this area, there is still a considerable need to investigate further the association between psychosocial and biological factors underpinning conditions like PNES, with more of a focus on how emotion-motor interactions may be altered, or indeed not altered, in PNES.

Therefore, the primary aim of this PhD was to further our understanding of emotion-motor interactions. The first aim of this PhD was to investigate emotion-motor interactions in a typically developing healthy population (Chapter 2). Apart from this being, to the best of my knowledge the first study to attempt this with three different age groups at different stages of brain development, I also wanted to conduct this study as a first step to developing an experimental paradigm that may be suitable for use with patients experiencing non-epileptic seizures. I was particularly interested in including an early adolescent group in this EEG experiment given that adolescence is a developmental period in which emotional experiences and expression may be at their

most prominent (Casey & Jones, 2010, Steinberg, 2005), due in part to a maturational mismatch between lower-order limbic regions and higher-order prefrontal regions of the brain (Casey & Jones, 2010; Gogtay et al., 2004; Somerville, Jones, & Casey, 2010). The argument being, that if emotional experiences and expressions are indeed heightened in adolescence, and motor function is modulated by emotional stimuli, be they positive or negative/threat-related, then I would expect to see a greater degree of modulation of preparatory motor activity and subsequent action by emotional stimuli at this stage of brain development. This would tell us whether anticipation of positive or negative outcomes, relative to neutral outcomes, would differentially modulate motor preparation and action not only in an adult neurotypical sample but also in an adolescent neurotypical sample more prone to heightened emotional experiences and expression. If so, this might be a useful approach to adopt when investigating, for example, emotion-motor interactions in PNES given that individuals experiencing PNES may have deficits in emotion processing (Bakvis et al., 2009; Bakvis et al., 2010; Bakvis, Spinhoven, Zitman, & Roelofs, 2011; Pick, Mellers, & Goldstein, 2016; Reuber, 2009).

The second aim of this PhD was to investigate how brain structures and related functions associated with emotion-motor interactions may be affected in a clinical population for which impairments in emotion processing and motor control are notable characteristics, namely PNES. To do this, I first adopting a systematic meta-analytical approach to the imaging literature in PNES (Chapter 3), and second employed whole-brain cortical surface morphometric analyses of T1-weighted sMRI brain scans in a patient population with PNES and age- and gender-matched healthy controls (Chapter 4).

This chapter summarises the key findings from each study (Section 5.2), then draws conclusions from these key findings (Section 5.3). A discussion of the limitations

and strengths of this doctoral work is provided in Section 5.4 and Section 5.5 respectively. This is followed by recommendations for future directions (Section 5.6). Lastly, I will draw some final conclusions form this body of work (Section 5.7).

5.2. Summary of key findings

Study 1 presented in Chapter 2 examined the relationship between emotion and motor function by recording electrophysiological changes in the brain during the anticipation of angry, happy and neutral faces in 18 early adolescents (9 males aged 13-15), 18 late adolescents (9 males aged 18-20) and 18 young adults (9 males aged 25-27). This study used an electrophysiological index of anticipation and motor preparedness, the contingent negative variation (CNV), as well as behavioural measures in the form of reaction time data to investigate motor output. In addition, I also investigated whether viewing angry, happy and neutral facial expressions would differentially modulate visual P1 peak amplitude and N170 amplitudes (as measured by peak to peak amplitude) within and between age groups. The main aim of this study was to gain an insight into how emotion-motor interactions may change at different stages of brain development, particularly during adolescence, a period of proposed heightened emotional reactivity. Again, to the best of my knowledge, this is the first EEG study that has attempted to do this in a neurotypical population involving 13-15 year olds.

The results of this EEG study are largely consistent with previous studies showing developmental differences in electrophysiology (ERPs) and task performance (RTs) between early adolescents and the two older age groups (Bender, Weisbrod, Bornfleth, Resch, & Oelkers-Ax, 2005; Itier & Taylor, 2004a; Klein & Feige, 2005; Kuefner, De Heering, Jacques, Palmero-Soler, & Rossion, 2010; Perchet & Garcia-Larrea, 2005; Segalowitz & Davies, 2004). However, contrary to my hypothesis that CNV amplitudes would be condition dependent, there were no significant effects of

emotion on early/initial phases, late/terminal phases or total CNV during the anticipatory period, either within or between age groups. As noted in Section 2.4.2, it is unlikely that the CNV represent a unitary preparatory state encompassing all of the processes involved in action selection and movement preparation, and this may help explain why CNV amplitudes did not differ significantly between conditions.

Notably, for the early adolescent group only, mean RTs were found to be significantly faster to happy faces relative to neutral faces, suggesting that for early adolescents a relatively faster and more accurate identification of happy faces may have occurred resulting in faster reaction times in happy trials compared to neutral trials. There were no significant differences in mean RTs in response to angry faces relative to neutral faces in any of the three age groups. This may suggest that, for the early adolescent group only, the observed heightened emotional responses to happy faces, in terms of faster mean RTs, may reflect increased reward seeking behaviours in this age group compared to the two older age groups. Supplementary post-hoc analyses revealed a weak relationship between tCNV amplitudes and mean RTs, but only in the neutral condition while controlling for other variables in the model. This again is consistent with previous studies showing a weak relationship between CNV amplitude and reaction time data (Rebert & Tecce, 1973; Smith, Johnstone, & Barry, 2006), which suggests the CNV may be a poor predictor of the speed of motor responses.

Consistent with my hypothesis that viewing angry faces would lead to potentiation of early visually evoked potentials, both the visual P1 and N170 ERP components were found to be larger in response to angry faces relative to neutral faces but not happy faces, suggesting that early non-conscious automated attentional capture was facilitated by negative facial expressions over and above neutral facial expressions but not happy facial expressions (Batty & Taylor, 2003; Carretié, Hinojosa, Martín-

Loeches, Mercado, & Tapia, 2004). However, no significant condition by age group effect was found.

Study 2 presented in Chapter 3 used a systematic meta-analytical approach to critically appraise the evidence for the neurobiological correlates in PNES. The reasons for conducting this systematic meta-review were two-fold. First, at the time of this review, most of the available literature focusing on neuroimaging in PNES was not systematic and may have therefore not included important studies in this area. Additionally, no previous review into the neurobiological correlates in PNES had sought to examine, in any statistical way, convergence across the available studies to get a clearer understanding of the organic (rather than non-organic) correlates of this condition. I felt this was a useful and important approach which may have aided in the resolving of conflict between the studies reviewed, in addition to more clearly defining brain regions of interest for future studies. Second, traditional accounts of PNES have largely focused on psychosocial risk factors that might drive "psychosomatic" or "psychogenic" causation, largely ignoring the structural and/or functional substrates of PNES in the brain. This has accentuated psychological aspects over the physical correlates, and given that not all patients presenting with PNES have co-existing psychological issues or psychiatric disorders in addition to the fact that many patients with psychiatric disorders do not experience non-epileptic seizures, this suggests that psychological issues and/or psychiatric comorbidity is neither necessary nor sufficient for PNES (Edwards, Fotopoulou, & Pareés, 2013). While neuroimaging studies in PNES and other FNDs are still at a relatively early stage, these investigations are important to help reduce the stigma associated with these conditions and in clinical settings to better facilitate the communication of the relevant diagnosis.

In summary, the results of this systematic meta-review suggest that PNES may be associated with pathological brain abnormalities (in a relatively small percentage of patients) and/or changes in brain morphology and/or persistent or recurrent functional changes in the brain. These neurobiological correlates may act as predisposing, precipitating and/or perpetuating factors in PNES. For example, studies investigating brain pathology in PNES only patients (free of co-existing epilepsy) observed signs of brain disease or injury in roughly 25% to 34% of patients (Bolen, Koontz, & Pritchard, 2016; Devinsky, Mesad, & Alper, 2001; Reuber, Fernandez, Helmstaedter, Qurishi, & Elger, 2002). Brain morphometry studies identified significant differences in cortical thickness and cortical volume between PNES patients and healthy controls in motor and premotor regions, the cerebellum, insula, orbitofrontal, enthorinal, and lateral-occipital regions (Labate et al., 2012; Ristić et al., 2015). Structural connectivity studies observed significant differences in the uncinate fasciculus which connects prefrontal and limbic regions, as well as the corona radiata and internal and external capsules associated with motor function (Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer, 2015; Lee et al., 2015). PET studies investigating brain activation patterns in PNES patients compared to controls reported significant hypometabolism (lower glucose uptake) in the right inferior parietal/central brain region as well as bilateral anterior cingulate (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015) while SPECT studies investigating regional cerebral blood flow in PNES patients observed significant abnormalities in roughly 15%-30% of patients (Ettinger et al., 1998; Neiman, Noe, Drazkowski, Sirven, & Roarke, 2009; Varma et al., 1996). Brain activation and functional connectivity studies using PET and fMRI reported significant differences between PNES patients and healthy controls in brain regions associated with attention and regulatory processes, memory, emotion processing and sensory and motor function

(Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015; Ding et al., 2013, 2014; Li et al., 2014, 2015) while other resting state fMRI studies observed significant increases in functional connectivity in resting state networks associated with fronto-parietal activation, executive control, sensorimotor functions, emotion processing as well as the default mode network associated with self-awareness/sense of agency and consciousness (van der Kruijs et al., 2012, 2014).

This systematic meta-review puts forward the evidence for an association between structural and functional brain abnormalities in patients with PNES which may contribute toward a biopsychosocial account of this condition. Indeed, the identification of such neurobiological correlates does not sit well with the understanding of PNES as being a purely "psychogenic" condition without any discernible "physical" correlates. A number of plausible psychophysiological hypotheses were put forward for the aetiology and maintenance of non-epileptic seizures, the most prominent involving emotion dysregulation (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015; Devinsky, Mesad, & Alper, 2001; Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer, 2015; Labate et al., 2012; Lee et al., 2015; Ristić et al., 2015), the role of attention/hyper-vigilance (Ding et al., 2014; Li et al., 2014, 2015), self-awareness/consciousness (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015) and dissociative traits (Ristić et al., 2015; van der Kruijs et al., 2012, 2014).

Study 3 presented in Chapter 4, employed whole-brain cortical surface morphometric analyses of T1-weighted sMRI brain scans of 20 individuals with PNES free of co-existing epilepsy (14 females, mean age 41.05, range 19 – 62) and 20 age- and gender-matched healthy controls (14 females, mean age 40.65, range 21 - 61). First, I examined whether age-related changes in cortical thickness (controlling for gender)

differed between PNES and controls. Second, I examined whether group level cortical thickness results (controlling for age and gender) differed between PNES and controls. Third, I utilized a local Gyrification Index (*l*GI) to measures the degree of gyrification (gyral and sulcal formations) of the cerebral cortex in both PNES and controls. Finally, I conducted correlational analyses to explore the relationship between PNES clinical features and cortical thickness results. Again, the main reason for conducting this study partly mirrors the reasons given for conducting the systematic meta-review into the neurobiological correlates in PNES with regard to addressing the "dualistic" framework in which this condition is often framed and the stigma that is too often attached to PNES, both for patients and clinicians alike. Additionally, given the sparseness of replication in neuroimaging studies in PNES, I was interested to see whether I would observe similar results to those previously reported by Labate et al. (2012) and Ristić et al. (2015), reviewed in Chapter 3.

In agreement with my hypothesis that I would find significant differences in cortical thickness in motor, frontal, occipital brain regions and brain regions associated with emotion processing in PNES compared to controls, the results of this study showed that, PNES patients showed increased cortical thickness in the left paracentral lobule, with the significant cluster spanning the primary motor and somatosensory cortex. These brain regions are involved in the planning, control and execution of movement. Cortical thickness increases were also found in occipital regions including the cuneus bilaterally and the left lingual gyrus. Cortical thickness decreases in PNES compared to controls were found in the right superior temporal gyrus, a brain region associated with multisensory integration (Karnath, 2001), the right medial orbitofrontal cortex, a brain region associated with emotion processing (Northoff, 2000), as well as the left and right pars opercularis (parts of the inferior fontal gyri). The inferior frontal gyri have been

associated with response inhibition and down-regulation of emotional responses (Aron, Robbins, & Poldrack, 2014; Morawetz, Bode, Baudewig, & Heekeren, 2017; Swick, Ashley, & Turken, 2008). These findings support the growing body of evidence suggesting that neurophysiological substrates are observable in PNES and that these neurophysiological substrates are localized to, amongst other regions, brain regions associated with sensory processing and sensory integration, emotion processing and the planning, control and execution of movement. This again implies the involvement of aberrant emotion-motor interactions in PNES. However, there was no evidence of atypical local gyrification patterns in PNES compared to controls, showing that in our sample, this additional facet of cortical structure was not associated with PNES. This will be discussed in more detail in Section 5.3.

5.3. Conclusions from key findings

The doctoral work presented in this thesis found plausible evidence for the neurophysiological underpinnings of aberrant emotion-motor interactions in patients with PNES (Chapter 3 and Chapter 4) but failed to find any significant developmental differences in motor preparation as indexed by the CNV during the anticipation of negative, positive and neutral outcomes (emotional facial expressions) (Chapter 2). Although no significant differences were found for the CNV in Chapter 2, the ERP results were mostly in agreement with previous ERP studies investigating the developmental trajectories of the visual P1 and the N170 ERP components. In addition to which both the visual P1 and the N170 were found to be larger in amplitude in response to the angry faces compared to the neutral faces. This suggests that threat detection can occur as early as 95 ms post stimulus onset, but again no significant age-group differences were found.

Because the CNV results are not in agreement with previous studies showing the modulation of motor preparation and action by emotion (Carretié, Mercado, Hinojosa, Martın-Loeches, & Sotillo, 2004; Casement et al., 2008; Coombes, Cauraugh & Janelle., 2006; Coombes et al., 2009; Hart, Lucena, Cleary, Belger, & Donkers, 2012; Nogueira-Campos et al., 2014; Perri et al., 2014; Schutter, Hofman, & Honk., 2008), it is possible that this study may have failed to tap into affective biases during the anticipatory period/preparatory state resulting in little to no modulation of anticipatory behaviour as indexed by the CNV. This may have occurred principally for the following reason and this may be the most plausible explanation. Again, as noted in Section 2.4.2., the CNV may not represent all aspects of motor preparation. It is possible that while the CNV is generally believed to be an index of motor preparation when an action is required, that the CNV as measured in this study (stimulus locked to S_1), may have failed to capture other aspects of motor preparedness, namely action selection and other processes involved in emotion processing for example. This might help to explain why I did not find significant differences between conditions in addition to the weak relationship between mean CNV amplitudes and mean RTs. As suggested in Section 2.4.2, incorporating a complementary approach to try and answer these hypotheses regarding emotion-motor interactions would be useful. This could be done by using multiple indices of motor preparation and action, one stimulus-locked to S_1, a second stimulus-locked to S_2 and the third stimulus-locked to the participants response (button press) in addition to measures of the accuracy (button press errors) and the speed of motor responses (RTs).

There are however two other plausible explanations for these findings. First, the timing of the stimuli presentations may not have been effectual in modulating motor preparation. For example, displaying the affective pictures during the anticipatory

period between the cue/warning stimulus (S_1) and the target/imperative stimulus (S_2) may have had more of an effect on motor preparation. In addition, this would remove the need for robust associative learning between S_1 and S_2 which may not have been sufficiently strong in this study to incite changes in the CNV. The second alternative reason for these non-significant findings could be due to the emotional face stimuli used in this experiment and the nature of the experiment itself. While one cited study observed significant differences in motor function with the use of emotional faces compared to neutral faces (Schutter, Hofman, & Honk, 2008), in my experiment no such effect was found either within or between age groups. Furthermore, previous fMRI studies have observed greater amygdala activity in adolescents compared to children and adults when viewing emotional facial expressions compared to neutral facial expressions (Hare et al., 2008; Monk et al., 2003). However, and again, this may not have occurred in this study resulting in similar amygdala activation patterns across the three age groups. This was further confirmed by non-significant age group differences for the visual P1 and N170 in response to angry, happy or neutral facial expressions. Therefore, it is possible that the use of faces in this experiment did not garner the sufficient arousal needed to alter, in any significant way, brain circuitry involved in motor preparation as indexed by the CNV (possibly via amygdala-SMA connectivity), and therefore CNV amplitude at the within- or between-subjects level did not differ significantly between conditions.

In addition, it is also possible that the use of predictive cues forewarning the nature of the upcoming stimuli, in this case angry, happy, and neutral facial expressions, may have actually reduced responses of the emotional network, especially given the three seconds delay between the predictive cue offset and the onset of the faces. Responses of the emotional network may have been further reduced through habituation

effects over the course of experiment. Therefore, future work is needed to better delineating valance dependent changes in motor preparation and action by either using images that elicit greater arousal in participants or by presenting the emotional facial expression during the anticipatory period rather than at the end of said period. In summary, future studies investigating the effects of emotion on motor function may want to consider designing a series of experiments using different emotional stimuli onsets (S_1 onset, S_2 onset, and between S_1 and S_2) in addition to multiple indices of motor preparation and action spanning the time period from perception (S_1 onset) to action (button press response), as has been suggested above.

However, the findings presented in Chapter 3 and Chapter 4 did find evidence of atypical neurophysiology in PNES. This may help to explain how altered emotion-motor interactions in PNES facilitate and perpetuate symptoms. A number of key themes emerged from the findings presented in Chapter 3. The key themes pointed to the possible involvement of psychopathology and psychophysiological factors in the aetiology and maintenance of PNES. The psychophysiological factors implicated represent the neurobiological underpinnings of emotion dysregulation, maladaptive attention allocation and hyper-vigilance, impairments in self-awareness/self-consciousness, and/or dissociative states in PNES. How these relate to emotion-motor interactions will be discussed next with the emphasis being on emotion processing and limbic-motor interactions.

In terms of altered emotion processing in PNES, a number of studies observed greater structural and functional connectivity between limbic (insula/amygdala) and prefrontal regions (dorsolateral, orbitofrontal, anterior cingulate cortex) in PNES compared to controls (Hernando, Szaflarski, Ver Hoef, Lee, & Allendorfer, 2015; Lee et al., 2015; van der Kruijs et al., 2012, 2014). This may represent an underlying

vulnerability to emotion dysregulation in this group whereby altered prefrontal-limbic connectivity results in less effective top-down control of sensorimotor and affective responses. Additionally, a number of studies observed atypical activation patterns in PNES patients in cingulate, insular and/or amygdalar regions which may further disrupt the regulatory functions of the prefrontal cortex (Arthuis, Micoulaud-Franchi, Bartolomei, McGonigal, & Guedj, 2015; Neiman, Noe, Drazkowski, Sirven, & Roarke, 2009), thereby making it more difficult to either down-regulate or in other instances up-regulate behavioural responses in PNES. Interestingly, a recent task-based fMRI study in which participants were required to either up-regulate or down-regulate their emotional responses to aversive and neutral pictures noted that successful down-regulation of emotions was predictive of increased coupling between left inferior frontal gyrus and dorsal prefrontal regions while successful up-regulation of emotions was associated with increased coupling of the left amygdala, orbitofrontal cortex, anterior cingulate and other prefrontal regions (Morawetz, Bode, Baudewig, & Heekeren, 2017). It is possible that in PNES, alterations in these networks represent a vulnerability to disruption of the regulatory functions of the prefrontal lobe thereby perpetuating emotion dysregulation.

Moreover, atypical activation patterns in cingulate, insular and/or amygdalar regions may reflect greater attention allocation to external threat (via amygdala) and/or an altered recognition of internal bodily states (via insula). This may help to explain why studies have observed greater attentional bias to negative stimuli in PNES (Bakvis et al., 2009), while other studies have observed reduced awareness of internal bodily signals in functional movement disorders (Ricciardi et al., 2016). Further, atypical cortical thickness and cortical volume in prefrontal, motor, occipital and limbic regions of the brain observed in PNES may represent, again, a vulnerability to less effective

control by higher-order prefrontal brain regions over lower-order limbic activation patterns and motor functions of the brain (Labate et al., 2012; Ristić et al., 2015). For example, Labate et al. (2012) reported negative correlations between depression scores and atrophy of the right superior frontal gyrus and orbitofrontal sulcus and negative correlations between dissociation scores and atrophy in the left inferior frontal gyrus. Ristić et al. (2015) reported negative correlations between disease onset and cortical thickness in the left insula. Again, these findings further implicate alterations in prefrontal and limbic structures in PNES which may lead to depressive episodes and/or represent a vulnerability to PNES onset.

Interestingly, the findings presented in Chapter 4 partly corroborate but also differ from the morphometry-based MRI findings in PNES previously described by Labate et al., 2012 and Ristić et al., 2015. Results are partly similar in terms of the affected brain areas but differ somewhat in terms of the direction of the results. In this study I found that patients with PNES showed decreased cortical thickness compared to controls in the right superior temporal gyrus associated with multisensory integration (Karnath, 2001) and the right medial orbitofrontal cortex associated with emotion processing (Northoff, 2000). This study also found that compared to healthy controls, patients with PNES showed decreased cortical thickness in regions associated with response inhibition and emotion regulation (Aron, Robbins, & Poldrack, 2014; Morawetz, Bode, Baudewig, & Heekeren, 2017; Swick, Ashley, & Turken, 2008), namely the left and right pars opercularis (inferior frontal gyri). Again, Labate et al. (2012) found that cortical thickness in the left pars opercularis in PNES patients negatively correlated with dissociation scores, suggesting that higher dissociation scores were associated with decreases in cortical thickness in this region of the brain. However, it is difficult to make an equivalent inference between dissociation and the

results presented in Chapter 4, as I was unable to directly measure the tendency to dissociate in this study. I also observed increased cortical thickness in PNES patients compared to controls in the left paracentral lobule, with the significant cluster spanning both the primary motor cortex and primary somatosensory cortex. Again, these areas are associated with, amongst other things, the planning, control and execution of movement (Borich, Brodie, Gray, Ionta, & Boyd, 2015).

The study presented in Chapter 4 also found cortical thickness increases in PNES in occipital regions involved in visual processing (Macaluso, Frith, & Driver 2000; Vanni, Tanskanen, Seppa, Uutela, & Hari, 2001), namely the cuneus bilaterally and the left lingual gyrus. A recent fMRI study conducted by Ding and colleagues (2014) found that increased long-range functional connectivity density of occipital regions (right calcarine fissure and bilateral lingual gyri) correlated with disease duration in patients with PNES. The authors proposed that changes in functional connectivity in this region may reflect long-term hypervigilance and increased sensitivity to external stimuli. While the study presented in Chapter 4 does provide some support for their findings, I found a positive trend towards increases in cortical thickness in the cuneus bilaterally and symptom severity in PNES, no significant correlation was found between cortical thickness results in occipital regions and duration of PNES. Taken together, the observed differences in cortical thickness and cortical volume between patients with PNES and healthy controls in the three morphometric studies described above suggests that atypical neuroplasticity in (pre)frontal and limbic cortical regions may be implicated in precipitating and/or perpetuating PNES symptomatology. These changes in brain morphology may be the result of prolonged periods of stress or historical trauma exposure (Cohen et al., 2013; Kelly et al., 2013; Labate et al., 2012; Perez et al., 2015).

It must be noted here that I failed to find any significant differences in the degree of gyrification in PNES compared to controls in addition to finding no significant differences in cortical thickness between PNES patients with reported trauma exposure and PNES trauma naïve patients. As stated in Section 4.4, this finding may be surprising given the relevance of neglect and trauma in early life to the development of PNES (Bewley, Murphy, Mallows, & Baker, 2005; Holman, Kirkby, Duncan, & Brown, 2008; Kooiman et al., 2004; Novakova, Howlett, Baker, & Reuber, 2015) and that atypical gyrification patterns have been observed in children exposed to maltreatment (Kelly et al., 2013). Again, as stated in Section 4.4, it is possible that the neglect or trauma which may be relevant to PNES affects individuals after the developmental phase in which gyrification patterns are largely determined. And again, neglect or trauma in early life is not a necessary precondition to the development of PNES (Brown & Reuber, 2016b).

In addition, from a purely neurodevelopmental perspective, one might have expected to see a greater degree of gyrification in regions/clusters of the cortex neighbouring the gyri that showed decreases in cortical thickness in PNES compared to controls. This is also the case for regions of the cortex that showed increases in cortical thickness in PNES compared to controls but this time in the opposite direction, i.e., greater cortical thickness equals lower *l*GI indices. Studies have shown that decreases in cortical thickness are generally associated with increases in cortical folding patterns, which in turn show a positive relationship with surface area and brain volume (Hogstrom, Westyle, Walhovd, & Fjell, 2013; Gautam, Anstey, Wen, Sachdev, & Cherbuin, 2015). This suggests that the processes involved in changes to brain morphology during early developmental stages are geared toward increasing computational power by maximizing surface area and cortical folding patterns rather

than increasing cortical thickness (Hogstrom, Westyle, Walhovd, & Fjell, 2013). In fact, it has been noted elsewhere that thicker cortices are often found in gyral crowns compared to sulcal walls and fundi (Welker, 1990; Dubois et al., 2019).

The folding patterns observed in both human and animal brains are the result of a complex process driven by forces that directly affect the structure and connectedness of the cerebral cortex (Hogstrom, Westyle, Walhovd, & Fjell, 2013; Zilles, Palomero-Gallagher, & Amunts, 2013). This involves changes to microstructural properties such as neuronal migration and orientation, axonal proliferation, synaptogenesis, glial growth and synaptic pruning during different stages of early cortical development and organization (Dubois et al., 2019). Mechanical models of cortical folding patterns have proposed that gyrification patterns may represent the differential growth trajectories of the gyri versus the sulci (Lefèvre & Mangin, 2010) or the outer cortical layers (I-III) versus inner cortical layers (V-VI)(Armstrong et al., 1991) or the tension applied by glial and axonal fibers on the cortical surface during development resulting in functionally similar regions coming together to facilitate cortico-cortical connections (Van Essen, 1997). With this in mind and because I observed significant differences in cortical thickness but not gyrification patterns between PNES and controls, these findings again suggest that early stages of cortical development may not be important in PNES, but that plastic changes in the cortical mantle may be of significant importance to this condition.

Further, in relation to emotion-motor interactions in PNES, a number of studies presented in Chapter 3 observed greater functional connectivity between limbic (insula/amygdala), anterior cingulate, motor and somatosensory regions (SMA, pre- and postcentral gyri, paracentral lobule) in PNES compared to controls (Ding et al., 2014; Li et al., 2014, 2015; van der Kruijs et al., 2012, 2014). This coupling between limbic and

motor regions, particularly the insula, amygdala, precentral and SMA, may mediate the effects of emotion on motor function, whereby actions are performed or inhibited in an automated bottom-up way. For example, the SMA is associated with the sequential selection of motor programs in response to internally generated cues or triggers, the urge to move (Passingham, 1993) while the precentral gyrus is involved in the control of voluntary movements in response to external stimuli (Debaere, Wenderoth, Sunaert, Van Hecke, & Swinnen, 2003). The insula is associated with interoceptive signals, emotional experiences and self-awareness (Craig, 1996, 2009, 2010) while the amygdala is associated with the processing of emotionally salient external stimuli (Anderson & Phelps, 2001). Therefore, increased activity in amygdalar and insular regions due to either hyper-arousal or hyper-vigilance combined with hyper-connectivity between these regions and motor cortical areas, coupled with less effective top-down control from prefrontal regions, may represent both predisposition to, and perpetuation of, aberrant emotion-motor interactions in PNES.

However, conclusions derived from Chapters 3 and Chapter 4 should be tempered by a number of limitations. First, none of the studies reviewed in Chapter 3 were rated as being of high quality. Second and a related point, only 17.6% of the studies reviewed in Chapter 3 had sample sizes that were considered good (comparative group sizes ≥ 50). This issue was replicated in Chapter 4 and although significant attempts were made to increase sample size, these attempts were not successful in the end. I will discuss this further in the limitations section below (Section 5.4). Additionally, many of the studies presented in Chapter 3, and this includes, although less so, Chapter 4 as well, hypothesised that emotion dysregulation may be a key component of PNES. However, no study attempted to directly measure emotion processing in PNES, either through psychometrics, physiological recordings (skin

conductance response (SCR) or heart rate variability (HRV)) or in the majority of studies by using task-based methods. Therefore, a certain degree of reverse inference was often used when interpreting the results. An attempt was made to incorporate questionnaire based measures of emotional and functional well-being in Chapter 4, but again this was unsuccessful. Again, I will discuss this further in the limitations section below (Section 5.4). Furthermore, there was no clear agreement across the studies presented in Chapter 3 and in Chapter 4 in terms of laterality effects (left versus right hemisphere). Further, based on the results presented in Chapter 3 and Chapter 4, it is unclear whether the imaging results represent pathophysiology in PNES alone or whether the imaging results are more closely related to comorbid psychopathology. Indeed, more than half of the studies presented in Chapter 3 failed to exclude PNES patients with co-existing psychopathologies and/or didn't control for this in their analyses nor did they include a control group with psychiatric conditions free of co-existing PNES. This was also the case for Chapter 4.

A final point relates to the participants involved in the imaging studies discussed above. It is likely that many if not most of the participants recruited or whose data was retrieved retrospectively, came from highly specialised centres and may have therefore represented the most severely affected PNES patients, so the prevalence of atypical patterns observed in brain structure and/or function may not be truly representative of the wider PNES population. Furthermore, these patients may have been taking anticonvulsants and/or other psychopharmaceuticals for longer periods and at higher dosages than other PNES patients not attending these specialist centres and many of the studies reviewed in Chapter 3 in addition to the study presented in Chapter 4 did not attempt to control for this in their analyses. Lastly, the observed lack of convergence found across the studies presented in Chapter 3 points to weak associations between

brain structure and function and PNES symptomatology. However, atypical structural and functional correlates were observed in PNES and while differences between studies are evident, these inconsistencies are likely to reflect divergent aetiological factors and heterogeneity in patients with PNES.

5.4. Limitations

There are several limitations which need to be considered when interpreting the conclusions presented in this thesis. Some of these are methodological and others are theoretical. These will be discussed next.

The first methodological limitation relates to underpowered sample sizes, both for Chapter 2 and for Chapter 4. In relation to Chapter 2, every attempt was made to increase the sample size by using a diverse range of recruitment methods including online social media platforms, flyers distributed to local schools, cafes, and businesses, and via email invitation to online staff and student volunteer lists maintained by the UoS Psychology Department. However, recruiting additional participants was challenging, particularly with regards to those aged between thirteen and fifteen. This meant that the final sample (n = 18 per group) was likely underpowered. Further, this made it difficult to have any degree of confidence if I conducted additional analyses by entering additional variables into the regression models, where sample sizes larger than 30 are generally required, or in the mixed analyses of variance where it is generally recommended that the sample size should be several times the magnitude of the number of variables entered. However, it should be noted that the sample in this EEG study is comparable to other similar studies in this area.

A related point, and a further limitation of the EEG study presented in Chapter 2 relates to the potential impact of pubertal development on anticipatory processes linked

to motor preparation and the potential impact of pubertal development on early visually evoked potentials. This is because in addition to age-related changes in total brain volume and age-related changes in the underlying tissue types, sex-specific developmental changes in the brain have also been reported (Dennison et al., 2013; Giedd et al., 1996; Giedd, 2004; Lenroot et al., 2007; Neufang et al., 2008; Sowell, Trauner, Gamst, & Jernigan, 2002). These findings suggest that puberty and sex-specific hormone levels may result in significant sex-related changes to cortical and subcortical brain development and organization. Therefore, when comparing the early adolescent group to the two older age groups, the potential mismatch between male and female brain maturation in the early adolescent group, with females generally shown to mature earlier than males (Giedd, 2004; Geidd et al., 1996; Lenroot., 2007; Sowell, Trauner, Gamst, & Jernigan, 2002), may have resulted in an obscuration of significant differences which may have been found if I restricted analyses to one sex only or if I entered sex as a covariate. However, this limitation was to some degree tempered by having gender-matched groups in this study.

In relation to the sMRI study in PNES presented in Chapter 4, again every attempt was made to increase the sample size by setting up of an additional NHS data collection site in Wolverhampton. This prolonged data collection by roughly four to six months and although everything had been agreed and ethical approval had been granted, unfortunately final compliance was not given due to the lack of sufficient funds to cover the costs. During this time many attempts were made to identify additional potential participants from the Royal Hallamshire Hospital through contacts in the electrophysiology Department and meetings with consultant neurologists to whom I provided information about the study. While fifty-three 3T T1-weighted MRI brain scans of patients with PNES were retrieved from the Royal Hallamshire Picture

Archiving and Communications System (PACS) office, many had to be excluded due to parts of the brain not being captured in the field of view (FOV), others excluded due to lack of video-EEG recordings and others had to be excluded due to subsequent identification of co-existing epilepsy in these patients. The original sample size for this study was one hundred, fifty PNES and fifty age- and gender-matched healthy controls.

In addition to the attempts made to increase sample size, attempts were also made to include additional demographics and measures pertaining to clinical outcome and emotional well-being. For each participant whose PNES diagnosis was confirmed and for whom I had a suitable MRI brain scan, a questionnaire pack was posted to that participant. If there was no response, a follow up reminder letter with a second copy of the questionnaire pack was posted one month later. Participants who responded received a £10 shopping voucher. The demographic questionnaire enquired about age, gender, employment status and level of education, the onset and duration of their disorder. Additional measures included - The Work and Social Adjustment Scale (WSAS), a self-report scale of functional impairment attributable to an identified problem, in this study PNES (Mundt, Marks, Shear, & Greist, 2002), The Difficulties in Emotion Regulation Scale (DERS), a brief 36-item self-report questionnaire designed to assess multiple aspects of emotion dysregulation (Gratz & Roemer, 2008), The State-Trait Anxiety Inventory (STAI, Spielberger, Gorsuch, & Lushene, 1970), The PHQ-9, a 9-item depression module from the full PHQ, a reliable and valid measure of depression severity (Arroll et al., 2010; Kroenke, Spitzer, & Williams, 2001), The Reuter and Montag's revised Reinforcement Sensitivity Theory Questionnaire (rRST-Q), a self-report inventory measuring individual differences in the revised behavioral inhibition system (BIS), behavioral activation system (BAS) and the fight, flight, freezing system (FFFS) (Reuter, Cooper, Smillie, Markett, & Montag, 2015), and lastly The Lifespan

Inventory of Affect and Trauma, a measure of subjective experiences of trauma, attachment, and affect across the entire lifespan, dividing experiences into the developmental stages of childhood, adolescence, and adulthood. Unfortunately, of the twenty posted, only two completed questionnaires were returned and therefore the planned multiple regression analysis (PNES group only) including these measures and the significant cortical thickness differences found between PNES and healthy controls was not conducted.

In relation to the systematic meta-review in Chapter 3, a very small number of imaging studies were entered into the coordinate-based ALE meta-analyses. The number of studies entered into the coordinate-based ALE meta-analyses would of course been larger if I had included imaging studies of other FNDs. However, for the systematic meta-review I wanted to focus solely on PNES rather than including additional imaging studies of other FND sub-groups, particularly conditions in which symptoms were either of a negative nature (functional limb weakness or paralysis, hearing or visual impairments) or predominantly characterised by somatic symptoms (fibromyalgia, irritable bowel syndrome). Including additional sub-types would have made interpretations more difficult. Also, imaging studies in FND often do not report the full range of symptoms included in the sample and so additional stratified subgroup analysis based on semiology may not have been possible in any case.

The theoretical limitation relates to the inferences made about emotion dysregulation in PNES. While the psychophysiological evidence underpinning altered emotion processing in PNES was found, both in the systematic meta-review and the sMRI study, suggestions that changes in brain structure and/or function represents a vulnerability to emotion dysregulation in PNES is not yet conclusive. This is because conclusions drawn about emotion dysregulation are not completely justified given that

most studies supporting this view did not use any additional measures of emotion processing, i.e., task-based methods with simultaneous physiological recordings such as SCR, HRV, and/or collected psychometric data on the use of emotion regulation strategies. Therefore, future studies will need to address these limitations by adopting multimodal approaches in conjunction with a detailed medical history when dealing with individuals who have PNES. Advances in these areas will allow for a better and more detailed understanding of the neurobiological correlates of this disorder, which may have implications for both diagnosis and better treatment options.

A final limitation relates to structural imaging techniques used in research, in this case PNES and brain development, from which conclusions are derived. In brief, structural MR images of the brain are based on signal intensity values and tissue contrasts derived from proton density (number of hydrogen protons in a given imaged voxel of tissue) and T1 and T2 relaxation, two independent processes that describe the time constant of recovery (T1 relaxation) and decay (T2 relaxation) of the MR signal following the application of multiple radio-frequency pulses and the selected slice encoding and frequency encoding gradients. White matter tissue has a short T1 which results in a high MR signal and appears bright on a T1-weighted image and dark on a T2-weighted image. Grey matter tissue has a long T2 and appears dark on a T1-weighted image and bright on a T2-weighted image. It is these differences in the electromagnetic properties of tissue in the brain (particularly water and fat content) which allow for the segmentation of different tissue types. Therefore, changes in signal intensity values and contrasts undoubtedly effect the measurement of grey matter. The possibility for misclassification of tissue in the brain and subsequent erroneous measurement and interpretation remains an important consideration (Mills et al., 2016). In addition, the use of different automated brain segmentation algorithms may also lead

to heterogeneity of results across studies (Walhovd, Fjell, Giedd, Dale, & Brown, 2016). Perhaps more importantly, MR images of the brain are only a representation of the underlying anatomy, representations which require interpretation. That is to say, anatomical measurements derived from the MR signal are at best an approximation based on signal intensity values and contrast properties which are prone to influence during the MR scan itself (magnetic field inhomogeneities and subject motion – particularly relevant in young children or toddlers, or patients) and subsequent segmentation of tissue types (Lerch et al., 2017; Walhovd, Fjell, Giedd, Dale, & Brown, 2016).

5.5. Strengths

One of the main strengths of this doctoral work is that it examines emotion-motor interactions in two populations which, until very recently, have been largely overlooked, namely during neurotypical adolescence and in patients experiencing non-epileptic seizures. This is true of both the affective neuroscience community and also in the realm of movement neuroscience. A further strength of this doctoral work is it's use of distinct brain imaging techniques and behavioural measures (EEG, RTs, sMRI) and the use of a valuable tool to investigate the strengths and limitations of the existing literature into the structural and functional correlates in PNES, namely the coordinate-based activation likelihood estimation method or ALE that I used in the systematic meta-review presented in Chapter 3.

While the results presented in Chapter 2 are somewhat inconclusive, the methodological approach adopted for this study does have its strengths. As noted in Section 1.4.2, many neurodevelopmental studies have a number of limitations. First, from a developmental perspective, age categorization has certainly been a significant limitation in previous EEG developmental studies. Given the numerous brain changes

that occur in the transition from adolescence to adulthood, in this study I restricted the age ranges to a minimum of two years between participants in each age group thereby avoiding the confound of having different developmental stages (e.g. child and adolescent) in the same age group. Further, with respect to measuring ERP components from a developmental perspective, given that children, adolescents and adults appear to show different brain activation patterns due to differential recruitment of brain regions (Segalowitz & Davies, 2004), it is likely that when completing the same task, possibly involving taxing cognitive demands, children, adolescents and adults may employ different strategies and engage different cortical and subcortical regions of the brain in order to accomplish the same goal (Flores, Digiacomo, Meneres, Trigo, & Gómez, 2009; Killikelly & Szűcs, 2013; Segalowitz & Davies, 2004). Therefore, the experimental paradigm used in this EEG experiment was both simple in terms of low cognitive demands and effective in capturing the components under investigation and therefore this improved the likelihood of capturing any real electrophysiological differences between the age groups. In addition to which, each age group was matched for gender, so although I did not enter gender as an additional between subjects' factor in the analyses, having the same ratio of females to males helped to alleviate further confounds in this study. Further, because in this study the ERP components were found to be mostly consistent with previous studies in this area, the findings of this study can be considered to be fairly robust and therefore inferences drawn can be viewed with a certain degree of confidence.

Again, while the systematic meta-review into the neuroimaging studies in PNES presented in Chapter 3 was inconclusive, this study has added to our knowledge and understanding of the existing neuroimaging literature in PNES and in turn has added to our understanding of the neurobiological substrates of emotion-motor interactions in

this patient population and contributed toward a biopsychosocial account of this condition. In addition, Chapter 3 put forward a number of key considerations and possible future directions that may prove useful for other researchers investigating the neurobiological basis of PNES in the future. In fact, the sMRI study presented in Chapter 4 attempted to address some of the limitations highlighted in Chapter 3. These included making sure that the groups were matched for age and gender, excluding MRI scans of patients who presented with other FNDs, making sure that each MRI scan included in the analysis had come from a patient with a PNES diagnosis confirmed by video-EEG recordings of typical attacks, a confirmed PNES diagnosis by a Consultant Neurologist at the Royal Hallamshire Hospital, and the exclusion of any MRI scans that had come from patients with a mixed seizure disorder (PNES and co-existing epilepsy) or MRI scans that showed signs of clinically significant brain abnormalities, for example hippocampal reductions suggestive of mesial temporal sclerosis or T2 hyperintensities which may have reflected mini strokes. A further concern I became aware of when reviewing the literature for the systematic meta-review presented in Chapter 3, and this has been raised by others also when it comes to FND and somatic symptoms research more generally (Bègue, Adams, Stone, & Perez., 2019), was the lack of additional analyses involving the imaging results and clinically relevant characteristics of the patient population involved, for example symptom severity, duration of illness, age at onset etc. Therefore, in the study presented in Chapter 4, I conducted correlational analyses to investigate the relationship between brain regions that showed increases or decreases in cortical thickness in patients with PNES compared to controls with clinical features in patients with PNES (age at onset, duration of symptoms, symptom severity, and number of antiepileptic drugs taken). An additional strength of this study is that I also conducted additional within-subjects

analysis of both cortical thickness and gyrification patterns between PNES patients who had reported traumatic experiences and PNES patients who had not. This was important as trauma exposure, historical neglect, and abuse is often cited as a significant risk factor in PNES (Bakvis et al., 2009; Brown & Reuber, 2016a; Kaplan et al., 2013; Labate et al., 2012; Perez et al., 2015).

5.6. Future directions

In both Chapter 3 and Chapter 4 I highlight the heterogeneous nature of PNES, a multifactorial biopsychosocial condition for which we still have no clear and easily demonstrable cause. In fact, to date no single psychosocial variable has proved either necessary nor sufficient to the aetiology and maintenance of PNES (Edwards, Fotopoulou, & Pareés, 2013) and inconsistencies in the literature regarding the neurobiological substrates is evident. Furthermore, while much progress has been made in the last decade or so, we still lack a clear understanding of whether structural and/or functional changes found in the brain in PNES represent a predisposition to, or are a consequence of the disorder (Mcsweeney, Reuber, & Levita., 2017; Mcsweeney, Reuber, Hoggard, & Levita., 2018). In an attempt to address these limitations, in particular the question regarding predisposition to or consequence of, future neuroimaging studies investigating the neurobiological basis of PNES should adopt longitudinal methods capturing a wide range of demographic, neurodevelopmental, and clinical data and be of sufficient size and scope to involve relevant control groups, primarily psychopathology controls free of PNES and groups of individuals with trauma exposure who are not also experiencing PNES. In addition, larger sample sizes would allow for both between-subjects analyses (patients > controls) and within-subjects analysis (FND sub-types or PNES sub-types). This would allow for a better understanding of the relationship between any significant neuroimaging findings and

clinical features. There is however one caveat related to how we measure clinical features and I will highlight this in the next paragraph.

One criticism of the studies reviewed in Chapter 3, and this is true of Chapter 4 also, relates to how inferences are made about hypothesised emotion regulation deficits and maladaptive emotion-motor interactions and/or disturbances in information processing and dissociative states in PNES. These inferences are often made primarily based on group-level analyses which show alterations in the structure and/or function of the brain in PNES, yet these studies often fail to empirically test the theories proposed. Rather, these studies often rely on correlates between the imaging results and self-report measures of symptom severity and occurrence, emotion processing and/or levels of dissociative symptoms. However, we know that self-report measures may not be that reliable because prior beliefs and expectations about illness can, in some instances, lead to the overreporting of symptoms and symptom severity (functional tremor versus organic tremor for example; Pareés et al., 2012). This may be especially true when applied cross-sectionally in small sample sizes. Due to the inherent anatomical variation across participants and questions surrounding the validity of clinical measures based on self-reports, it is of course possible that some of the findings presented in this thesis may be incidental to PNES and therefore serendipitous, i.e. false positives.

Therefore, I propose that future work in PNES, and indeed other functional movement disorders, should focus on using experimental paradigms that directly test the theories put forward in this thesis. This is not to negate the relevance of psychosocial factors as they are of course of great importance in formulating both future testable hypotheses and clinical diagnosis (Bodde et al., 2009), but rather this can represent a shift in emphasis from the psychological to a more integrative approach while still allowing for the inclusion of psychosocial risk factors.

A number of neuroimaging studies cited in this thesis have taken such an approach, i.e., employed experimental paradigms to investigate emotion-motor interactions using emotional stimuli in patients with FNDs (Aybeck et al., 2015; Blakemore, Sinanaj, Galli, Aybek, & Vuilleumier., 2016; Fiess, Rockstroh, Schmidt, & Steffen, 2015; Hassa et al., 2017; Szaflarski et al., 2018; Voon et al., 2010a). While these studies are limited in number, they have been informative and further our understanding of emotion-motor interactions in FNDs. However, they all suffer from small sample sizes (20 or less patients per group), in most cases are of low temporal resolution (fMRI studies), and are not so easily comparable given the diverse FND sub-types included in the different studies, and only one study specifically included patients with PNES with generalized motor (tonic–clonic-like) symptoms (Szaflarski et al., 2018). Therefore, further work is needed in this area in an attempt to add to our knowledge base, particularly in patients with PNES.

I suggest that although I failed to find significant condition dependent changes in the CNV in Chapter 2 (possibly due to the neurotypical sample and the way in which I analysed the CNV data), EEG measures of emotion-motor interactions in PNES and other functional movement disorders may provide an important direction for future studies. One possible way forward would be to use experimental paradigms similar to the one I used in Chapter 2, i.e., an emotional variant of the $S_1 – S_2$ CNV paradigm which takes into account multiple complementary indices of motor preparation and action, as suggested in Section 2.4.5. Such future studies could adopt a multimodal approach encompassing both electrophysiological recordings and behavioural measures with concurrent fMRI in addition to questionnaire based measures. The advantage of using such an approach would allow for the investigation of pre-movement-related neural activity in addition to movement instigation and subsequent action with better

temporal resolution and source localisation during the anticipation of, or in response to, emotionally salient stimuli.

As stated in Section 1.4, the intention to produce a movement and the subsequent action is likely to unfold in a sequential manner on a perception-action continuum. EEG is ideally suited to capture the temporal characteristics of emotion-motor interactions. fMRI on the other hand is ideally suited to localising the generators of motor preparation and action (Nagai et al., 2004). Using EEG and fMRI simultaneously during voluntary movements in patients with PNES or other functional movement disorders with emotionally salient stimuli as instigators of action may allow researchers to pinpoint where on the perception-action continuum abnormalities may occur (during perception, motor preparation, movement instigation or movement execution) and localise these abnormalities to the specific brain regions involved, with the SMA, primary motor cortex, insula and amygdala being just four of the possible regions of interest. Such an approach could be equally applicable to studies involving patients with PNES symptoms and patients experiencing functional weakness or functional tremors in addition to clinical or non-clinical controls with similar risk factors to PNES, be they psychiatric controls free of co-existing PNES or controls who have experienced traumatic events with a diagnosis of PNES or without. There is some evidence that such an approach may be useful. For example, attenuation in pre-movement-related potentials like the CNV have been found in functional weakness compared to controls (Blakemore, Hyland, Hammond-Tooke, & Anson, 2015) while spectral power changes in EEG (event-related desynchronization in the beta band - 13-30 Hz) have been observed in PNES prior to the onset of non-epileptic events (Meppelink et al., 2017). These studies suggest that pre-movement-related neural activity in PNES or other functional movement disorders may be used in the future as a

positive auxiliary marker of the disorder and aid in the differential diagnosis of non-epileptic seizures or other functional movement disorders.

Additional use of existing experimental paradigms (Edwards, 2011; Kranick et al., 2013) that incorporate theories of motor control and motor learning (Kawato, Furukawa & Suzuki, 1987; Miall & Wolpert, 1996) would also prove to be very informative. For example, Edwards. (2011), Edwards, Fotopoulou, & Pareés. (2013), Kranick et al. (2013) and Voon et al. (2011, 2016) have implicated the involvement of motor predictions and sensory consequences of action in precipitating events in FNDs. Indeed, the ability to correct orientation or posture at a moment's notice, either consciously or unconsciously, is largely dependent on motor predictions. Motor predictions are an important aspect of sensorimotor control and are facilitated by internal "forward models" that predict the causal relationship between action and consequence (Jordan & Rumelheart, 1992; Frith, Blakemore, & Wolpert, 2000; Wolpert & Flanagan, 2001). This has already been eluded to in Section 1.5.1 with respect to the TPJ as a controller or comparator that compensates for motor predictions and sensory feedback. Alterations to this controller or comparator may partly help to explain why patients with FND experience their symptoms as involuntary, beyond their conscious control. With this in mind, future experimental studies designed to investigate how anticipatory representations of the effects of an action differ in PNES or other FNDs compared to controls is a plausible way forward. Therefore, future studies investigating feed-forward models of motor control in FNDs and in PNES specifically may allow us to better understand how the causal relationship between action and consequence gives rise to a sense of agency or lack of in PNES or other FNDs.

A final suggestion for future studies concerns change. As noted in Section 1.5, the structure and functioning of the brain is not static. We know for example that

learning new skills can lead to experience-dependent subtle yet demonstrable changes to grey and white matter in the brain (Zatorre, Fields, & Johansen-Berg, 2012). I proposed in Chapter 3 and Chapter 4 that neuroplasticity in brain regions associated with emotion processing and motor function may either represent a predisposition to, or result from PNES. Therefore, it is plausible to suggest that future studies should attempt to track changes in the functioning of these brain regions at different stages of a functional disorder, in addition to, and this is an important point, before, during, and after treatment. For example, if symptom occurrence and/or severity is associated with underlying pathophysiology in PNES or other functional movement disorders and if this underlying pathophysiology can be shown to manifest as alterations in pre-movement-related potentials for example, one might expect to see that the alleviation of symptoms should be accompanied by a normalization of the function in circuits in the brain associated with movement. This would be an important finding, one that would may have significant implications not only for treatment options and the management of symptoms, but also for how both patients and clinicians view functional disorders. This may have the further advantage of supporting the hypothesis that patients with PNES and other FNDs are not feigning or malingering (Edwards et al., 2011). In terms of causality and prognosis such findings may offer a sense of hope, a sense that things can change.

In summary, based on the work presenting in this thesis, future studies investigating the neurobiological correlates in PNES and FNDs more generally should consider the following:

1. Future neuroimaging studies investigating structural and/or functional characteristics of the brain should adopt longitudinal approaches employing multi-modal methods and be of sufficient size and scope to involve relevant control groups, in

addition to investigating concurrence or disagreement between FND or PNES sub-types based on semiology.

2. Future studies should focus on using experimental paradigms that directly test the theoretical models which have been proposed. For example, in this thesis, it has been hypothesized that aberrant emotion-motor interactions may be a key characteristic of PNES and other functional movement disorders. Studies have shown that preparatory functions or pre-movement-related neural activity (CNV and ERD) may be a useful indicator of PNES and other functional movement disorders. The use of an emotional variant of the $S_1 – S_2$ CNV paradigm which takes into account multiple complementary indices of motor preparation and action may be a useful and informative experimental approach to take when investigating emotion-motor interactions in PNES and other functional movement disorders. Again, using multi-modal methods may allow researchers to pinpoint the temporal and spatial characteristics of emotion-motor interactions in this patient population.

3. Future studies investigating feed-forward models of motor control in PNES may allow us to better understand how disruptions in the causal relationship between action and consequence gives rise to a sense of agency or lack of in PNES.

4. Future studies should attempt to track changes in the structural and/or functional characteristics of the brain at different stages of a functional disorder, primarily before, during, and after treatment.

5.7. Final conclusions

This doctoral work furthers our understanding of emotion-motor interactions by investigating the involvement of brain regions associated with motor function and emotion processing in a clinical population in which aberrant motor function has been linked to emotion processing difficulties and by charting the maturational trajectory of

emotion-motor interactions in neurotypical brain development. This contribution is an important one given the paucity of studies investigating brain maturation, motor preparation and action at different stages of development and while PNES has certainly gained much attention from the neuroscientific community over the past two decades much work is still needed if we are to better understand the association between risk factors, psychological state and trait characteristics, semiology and the neurobiological substrates of symptom formation and symptom expression.

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Appendices

Appendix 1

The University Of Sheffield.

Downloaded: 28/02/2020
Approved: 01/03/2018

Marco Mcsweeney
Registration number: 150123875
Psychology
Programme: PhD

Dear Marco

PROJECT TITLE: Emotion and motor function
APPLICATION: Reference Number 018026

On behalf of the University ethics reviewers who reviewed your project, I am pleased to inform you that on 01/03/2018 the above-named project was **approved** on ethics grounds, on the basis that you will adhere to the following documentation that you submitted for ethics review:

- University research ethics application form 018026 (form submission date: 20/02/2018); (expected project end date: 01/10/2018).
- Participant information sheet 1040106 version 1 (20/02/2018).
- Participant information sheet 1040105 version 1 (20/02/2018).
- Participant consent form 1040108 version 1 (20/02/2018).
- Participant consent form 1040107 version 1 (20/02/2018).

The following optional amendments were suggested:

As this study is testing children under 18, I am unsure if researchers, or at least one of the researchers, should hold a valid DBS certification to allow them to work with this age group. The ethics committee should advise on this. Note that the FACES database is held at the Max Planck institute and this should be referenced correctly in any associated output. I would encourage the researcher to ask participants' permission to make their anonymised data available for secondary analysis in a public data repository (such as osf.io) Note the wording in the University ethics policy "all researchers are strongly encouraged to consider the possibility of secondary research and data sharing at the outset, before the primary data collection begins, and to build this in to the informed consent process." Suggest removing the phrase "healthy young adults " from the information to be given to participants. We know what is meant by the word healthy here, but a lay-person would probably understand this in a different way. What was the justification for deciding to reimburse £5 or £10? Level of reimbursement should be equivalent for all participants, unless there is a clear justification not to do so.

If during the course of the project you need to deviate significantly from the above-approved documentation please inform me since written approval will be required.

Your responsibilities in delivering this research project are set out at the end of this letter.

Yours sincerely

Thomas Webb
Ethics Administrator
Psychology

Please note the following responsibilities of the researcher in delivering the research project:

- The project must abide by the University's Research Ethics Policy: https://www.sheffield.ac.uk/rs/ethicsandintegrity/ethicspolicy/approval-procedure
- The project must abide by the University's Good Research & Innovation Practices Policy: https://www.sheffield.ac.uk/polopoly_fs/1.671066!/file/GRIPPolicy.pdf
- The researcher must inform their supervisor (in the case of a student) or Ethics Administrator (in the case of a member of staff) of any significant changes to the project or the approved documentation.
- The researcher must comply with the requirements of the law and relevant guidelines relating to security and confidentiality of personal data.
- The researcher is responsible for effectively managing the data collected both during and after the end of the project in line with best practice, and any relevant legislative, regulatory or contractual requirements.

Appendix 2

NHS
Health Research Authority
South West - Exeter Research Ethics Committee
Whitefriars
Level 3
Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 342 1335
Fax:0117 342 0445

25 February 2016

Prof Markus Reuber
Senior Clinical Lecturer
University of Sheffield
Royal Hallamshire Hospital
Glossop Road
Sheffield
S10 2JF

Dear Prof Reuber

Study title:	**Destigmatizing Nonepileptic Attack Disorder**
REC reference:	**16/SW/0008**
IRAS project ID:	**180632**

Thank you for responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mrs Naazneen Nathoo, nrescommittee.southwest-exeter@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Appendix 3

Participant Information Sheet

Project title. Emotion and motor function.

You are being invited to take part in a research study. Before you decide whether you want to take part in this study, you need to understand why we are doing this research and what it will involve. Please read the information below carefully and feel free to ask questions if there is anything you don't understand or if you would like more information.

What is this study about?

We would like to learn more about the brain's electrical activity in response to emotional stimuli. Previous research has indicated that our attention is easily drawn to external emotional stimuli and that this can impact on our motor responses. The findings derived from this study may be helpful for understanding how we self-regulate our emotions during goal-directed behaviours. Your participation is completely voluntary and if you decide to take part you can leave at any time.

Who is being invited to take part?

We are asking adolescents aged between 13 and 14 and young adults aged between 18 and 26 to take part in this study. To take part you need to be right handed. If you have a current diagnosis of any developmental, neurological or psychiatric condition you are unable to take part in this study. Before taking part, you will need to complete the brief screening form accompanying this information sheet to make sure you are able to participate.

What will you be asked to do?

If you decide to take part in this study, we will ask you to come to the Psychology department at the University of Sheffield located in Cathedral Court at a convenient time to complete a computer task and some questionnaires. Your visit to the Psychology department will take about two hours. You will be given £10 to compensate you for your time with us.

What does the study involve?

1. **Questionnaires:** You will also be asked to complete a number of questionnaires which ask about your emotional and functional well-being. Examples of questions

include “S/he finds social situations easy”, “I found myself getting agitated”, “I control my feelings by not showing them”, “I worry about making mistakes” etc.

2. **Computer task:** The computer task will ask you to make a response (button press) to a positive, angry and neutral facial expression presented on the computer screen.
3. **Brain activity:** We will use a technique called electroencephalography (EEG). EEG is a non-invasive and is a very safe technique with no direct known health risk. Throughout the computer task you will wear a cap of electrodes, which will record the electrical activity from your brain. The electrodes will be filled with a salt-based gel, which can be easily washed out with shampoo. The electrode cap will take approximately 20 minutes to set up and the computer task will last around 1 hour. There will be opportunities to take breaks every 10 minutes. Overall, the study will last approximately 2 hours. You are free to withdraw from this experiment at any time and do not need to give a reason for doing so. For more information about EEG, please see the accompanying EEG information sheet.

   Remember that you can withdraw from the study at any time and you can skip any questions you do not wish to answer. Also if you are unhappy or uncomfortable you don't need to answer any of the questions at all. If you have any questions or concerns about the experiment, please do not hesitate to ask.

What are the benefits of taking part?

You will receive £10 for your participation in this research.

What are the risks of taking part?

There are no known risks to taking part in this experiment. However, if, at any point during the experiment, you decide that you do not want to carry on, we will stop the experiment and you are free to withdraw from the study without giving a reason. You will also be asked to complete a number of questionnaires. If completion of these questionnaires raises any issues or concerns for you, please let the research team know and we will also provide you with details of services and organizations you can contact for further support. Contact details for a member of the research team is listed at the bottom of this document.

What will the information be used for?

Before taking part in this study, you will be asked to sign a Consent Form and provide your name, gender, date of birth and contact details. Your personal details will be stored in a locked filing cabinet and on a password-protected computer. All the information

that is collected about you during this study will be kept strictly confidential. We will make sure that your information is kept confidential by using identification numbers in place of your name. This will make sure that your name will not be associated with, or traceable to, any of the collected data. The results from this study may be used anonymously at conferences and written up in scientific journals. The anonymised data may be made available for secondary analysis in a public data repository.

Thank you for taking the time to read this information sheet.

If you have any questions, please do ask!

If you would like to take part in this study or if you would like to speak to someone before deciding whether or not to take part, please contact Marco Mcsweeney (mmcsweeney1@sheffield.ac.uk) or Dr Liat Levita. (l.levita@sheffield.ac.uk / 0114 222 6651).

Appendix 4

Participant Information Sheet

Project title. Emotion and motor function.

We would like to invite your child to take part in a research study. Before you decide whether you want your child to take part in this study, you need to understand why we are doing this research and what it will involve. Please read the information below carefully and feel free to ask questions if there is anything you don't understand or if you would like more information. Contact details are below.

What is this study about?

We would like to learn more about the brain's electrical activity in response to emotional stimuli at different stages of brain development. Previous research has indicated that our attention is easily drawn to external emotional stimuli and that this can impact on our motor responses. The findings derived from this study may be helpful for understanding how we self-regulate our emotions during goal-directed behaviours.

Who is being invited to take part?

We are asking adolescents aged between 13 and 14 and young adults aged between 18 and 26 to take part in this study. To take part your child needs to be right handed. If your child has a current diagnosis of any developmental, neurological or psychiatric condition they are unable to take part in this study. Before allowing your child to take part, you will need to complete the brief screening form accompanying this information sheet to make sure they are able to participate.

What will you be asked to do?

If you decide to let your child take part in this study, we will ask you and your child to come to the Psychology department at the University of Sheffield located in Cathedral Court at a convenient time to complete a computer task and some questionnaires. Your visit to the Psychology department will take about two hours. Your child will be given £10 to compensate them for their time with us.

What does the study involve?

1. **Questionnaires:** Your child will be asked to complete a number of questionnaires which ask about their emotional and functional well-being. Examples of questions include "S/he finds social situations easy", "I found myself getting agitated", "I

control my feelings by not showing them", "I worry about making mistakes" etc. Other questions will ask about your child's stage of development, such as "Have you noticed any skin changes, especially pimples?", "Have you begun to menstruate?" for girls, "Have you begun to grow hair on your face?" for boys etc. We are asking these questions because whilst the teenagers in this study might be the same age, they may be at different stages of puberty. Because these different stages of puberty can interact with the results of this study, we would like to try to keep a record of which stage they are at.

2. **Computer task:** The computer task will ask your child to make a response (button press) to a positive, angry and neutral facial expression presented on the computer screen.
3. **Brain activity:** We will use a technique called electroencephalography (EEG). EEG is a non-invasive and is a very safe technique with no direct known health risk. Throughout the computer task your child will wear a cap of electrodes, which will record the electrical activity from their brain. The electrodes will be filled with a salt-based gel, which can be easily washed out with shampoo. The electrode cap will take approximately 20 minutes to set up and the computer task will last around 1 hour. There will be opportunities to take breaks every 10 minutes. Overall, the study will last approximately 2 hours. Of course, your child is free to withdraw from the experiment at any time and do not need to give a reason for doing so. For more information about EEG, please see the accompanying EEG information sheet.

   Remember that your child can withdraw from the study at any time and can skip any questions they do not wish to answer. Also if they are unhappy or uncomfortable, they don't need to answer any of the questions at all. If you have any questions or concerns about the experiment, please do not hesitate to ask.

What are the benefits of taking part?

Your child will receive £10 for their participation in this research.

What are the risks of taking part?

There are no known risks to taking part in this experiment. However, if, at any point during the experiment, your child decides that they do not want to carry on, we will stop the experiment immediately. Your child is free to withdraw from the study without giving a reason. Your child will also be asked to complete a number of questionnaires. If completion of these questionnaires raises any issues or concerns for you, please let the research team know and we will also provide you with details of services and

organizations you can contact for further support. Contact details for a member of the research team is listed at the bottom of this document.

What will the information be used for?

Before taking part in this study, you will be asked to sign a Consent Form and your child will be asked to provide their name, gender, date of birth and a contact detail (Parent/Guardian). All personal details will be stored in a locked filing cabinet and on a password-protected computer. All the information that is collected during this study will be kept strictly confidential. We will make sure that any information provided is kept confidential by using identification numbers in place of your child's name. This will make sure that their name will not be associated with, or traceable to, any of the collected data. The results from this study may be used anonymously at conferences and written up in scientific journals. The anonymised data may be made available for secondary analysis in a public data repository.

Thank you for taking the time to read this information sheet.

If you have any questions, please do ask!

If you would like to take part in this study or if you would like to speak to someone before deciding whether or not to take part, please contact Marco Mcsweeney (mmcsweeney1@sheffield.ac.uk) or Dr Liat Levita. (l.levita@sheffield.ac.uk / 0114 222 6651).

Appendix 5

Consent Form

Study: Emotion and motor function

The individual taking part in this study should complete this consent form.

Please read the following statements and circle the appropriate answer.

I confirm that I have read and understood the information sheet explaining the above research project and I have had the opportunity to ask questions about the project.	Yes	No
I understand that any personal details that I provide on the screening form (E.g. phone number, email address) will be treated confidentially and stored in a locked filing cabinet and on a password-protected computer.	Yes	No
I understand that my brain activity, physiological and behavioural responses will be recorded during the experiment.	Yes	No
I understand that my brain activity, physiological and behavioural responses will be anonymous. I give permission for members of the research team to have access to my anonymised data. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research.	Yes	No
I understand that my anonymised data may be used for future studies and that my anonymised data may be made available for secondary analysis in a public data repository.	Yes	No
I understand that I am free to withdraw from the study at any time and without having to give a reason for withdrawing.	Yes	No
I agree to take part in this study	Yes	No

Signed: ..... Date ……. (Participant)

Print name: ..... (Participant)

_____	_____	_____
Lead Researcher	Date	Signature

To be signed and dated in presence of the participant

I would like to be informed of other studies that you are running	Yes	No

If Yes, how would you like to be contacted (E.g. Email/Phone)?

_____

Appendix 6

Consent Form

Study: Emotion and motor function

The parent/guardian of participants under the age of 18 should complete this consent form.
Please read the following statements and circle the appropriate answer.

I confirm that I have read and understood the information sheet explaining the above research project and I have had the opportunity to ask questions about the project.	Yes	No
I understand that any personal details that I provide on the screening form (E.g. phone number, email address) will be treated confidentially and stored in a locked filing cabinet and on a password-protected computer.	Yes	No
I understand what my child will be asked to do during this study, and that my child's brain activity, physiological and behavioural responses will be recorded during the experiment.	Yes	No
I understand that my child's brain activity, physiological and behavioural responses will be anonymous. I give permission for members of the research team to have access to my child's anonymised responses. I understand that my child's name will not be linked with these research materials, and that my child will not be identified or identifiable in the report or reports that result from the research.	Yes	No
I understand that my child's anonymised data may be used for future studies and I understand that my child's anonymised data may be made available for secondary analysis in a public data repository.	Yes	No
I understand that I am free to withdraw my child from the study at any time and without having to give a reason for withdrawing. Likewise that my child is free to withdraw at any time.	Yes	No
I agree to let my child participate in this study	Yes	No

Signed: ..... Date …… (Parent/Guardian) Print name: ..... (Parent/Guardian) _____ _____ _____ Lead Researcher Date Signature To be signed and dated in presence of the participant		
I would like to be informed of other studies that you are running	Yes	No
If Yes, how would you like to be contacted (E.g. Email/Phone)? _____		

Appendix 7

The University Of Sheffield.

PARTICIPANT SCREENING FORM

If you are over 18 years old, please complete this information yourself. If you are under 18 years old, a parent/guardian needs to complete this form on behalf of you.

CONFIDENTIALITY - This form and the information contained within it will be treated as a confidential document.

Please answer ALL of the following questions.
If you are a parent/guardian, please answer these questions with respect to YOUR CHILD.

Personal Details	
First name	
Last name	
Parent/Guardian name (if participant is under 18)	
Phone number	
Email address	
Date of birth	
Gender (Male, Female)	
Handedness (Right, Left, Ambidextrous)	

Medical History		
Do you suffer from epilepsy, fits, blackouts, fainting turns or unexplained loss of consciousness, recurrent headaches or migraines?	Yes	No
Have you suffered a head injury leading to loss of consciousness requiring a hospital admission?	Yes	No
Do you suffer from any other medical condition, including heart problems?	Yes	No
Details:		

Appendix 8

The University Of Sheffield.

Debriefing Sheet

Project title. Emotion and motor function.

Thank you so much for taking part in this study!

The general purpose of the study was to help explain how emotional stimuli can impact on attentional and physical responses. More specifically our aim was to examine the neural correlates of orienting activities and response preparation, how this is modulated by emotion and gender at different stages of brain development. We invited people who were right-handed, between 13 and 26 years of age free of neurological or psychiatric disorders.

We were interested in measuring early and late phases of the contingent negative variation (CNV), an event-related potential (ERP) associated with orienting activities and motor response preparation to external stimuli. Previous studies have indicated that the CNV can differ substantially in different populations, particularly during development. However most of these studies have not examined how early and late phases of this ERP component is modulated by emotion, and how this may differ in males and females.

You were also asked to complete a number of questionnaires. We asked you to this because we wanted to compare the electrical brain activity recorded with emotional and functional well-being scores. Previous studies have shown that emotional difficulties can result in a number of psychopathological and psychophysiological symptoms.

We hope that you enjoyed taking part in this study. However, we understand that some of the questions asked might have been upsetting. If you have any concerns resulting from your participation in this study please feel free to contact a member of the research team listed below, or alternatively we have provided contact details for two relevant organizations below. If you require more urgent support, please contact your GP or the emergency services.

If you have any questions do not hesitate to contact Marco Mcsweeney, who is a member of the research team (Email: mmcsweeney1@sheffield.ac.uk).

If participation in this study has sparked your interest in the topic, we have provided some references below.

Samaritans
Helpline: 08457 90 90 90
www.samaritans.org

Mind
Helpline: 0300 123 3393 (9am-6pm, Monday to Friday)
www.mind.org.uk

Appendix 9

NHS National Institute for Health Research

AQ-10 (Adolescent Version)

Autism Spectrum Quotient (AQ)

A quick referral guide for parents to complete about a teenager aged 12-15 years old with suspected autism who does not have a learning disability.

Please tick one option per question only:		**Definitely Agree**	**Slightly Agree**	**Slightly Disagree**	**Definitely Disagree**
1	S/he notices patterns in things all the time				
2	S/he usually concentrates more on the whole picture, rather than the small details				
3	In a social group, s/he can easily keep track of several different people's conversations				
4	If there is an interruption, s/he can switch back to what s/he was doing very quickly				
5	S/he frequently finds that s/he doesn't know how to keep a conversation going				
6	S/he is good at social chit-chat				
7	When s/he was younger, s/he used to enjoy playing games involving pretending with other children				
8	S/he finds it difficult to imagine what it would be like to be someone else				
9	S/he finds social situations easy				
10	S/he finds it hard to make new friends				

Appendix 10

A Self-Administered Rating Scale for Pubertal Development

Introduction: The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. Since they may have something to do with your sleep patterns, do your best to answer carefully. If you do not understand a question or do not know the answer, just mark "I don't know."

Question	Response Options	Point Value
1. Would you say that your growth in height:	has not yet begun to spurt[2]	1
	has barely started	2
	is definitely underway	3
	seems completed	4
	I don't know	
2. And how about the growth of your body hair? ("Body hair" means hair any place other than your head, such as under your arms.)		
Would you say that your body hair growth:	has not yet begun to grow	1
	has barely started to grow	2
	is definitely underway	3
	seems completed	4
	I don't know	
3. Have you noticed any skin changes, especially pimples?		
	skin has not yet started changing	1
	skin has barely started changing	2
	skin changes are definitely underway	3
	skin changes seem complete	4
	I don't know	
FORM FOR BOYS:		
4. Have you noticed a deepening of your voice?		
	voice has not yet started changing	1
	voice has barely started changing	2
	voice changes are definitely underway	3
	voice changes seem complete	4
	I don't know	
5. Have you begun to grow hair on your face?		
	facial hair has not yet started growing	1
	facial hair has barely started growing	2

facial hair growth has definitely started	3
facial hair growth seems complete	4
I don't know	

FORM FOR GIRLS:

4. Have you noticed that your breasts have begun to grow?

have not yet started growing	1
have barely started growing	2
breast growth is definitely underway	3
breast growth seems complete	4
I don't know	

5a. Have you begun to menstruate (started to have your period)?

yes	4
no	1

5b. If yes, how old were you when you started to menstruate?

age in years

Appendix 11

HOW-I-FEEL QUESTIONNAIRE

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide how you feel *right now*. Then put a circle around the word or phrase that best describes how you feel. There are no right or wrong answers. Don't spend too much time on any one statement. Remember, find the word or phrase which best describes how you feel right now, *at this very moment.*

1. I feel……1) very calm 2) calm 3) not calm
2. I feel……1) very upset 2) upset 3) not upset
3. I feel……1) very pleasant 2) pleasant 3) not pleasant
4. I feel……1) very nervous 2) nervous 3) not nervous
5. I feel……1) very jittery 2) jittery 3) not jittery
6. I feel……1) very rested 2) rested 3) not rested
7. I feel……1) very scared 2) scared 3) not scared
8. I feel……1) very relaxed 2) relaxed 3) not relaxed
9. I feel……1) very worried 2) worried 3) not worried
10. I feel……1) very satisfied 2) satisfied 3) not satisfied
11. I feel……1) very frightened 2) frightened 3) not frightened
12. I feel……1) very happy 2) happy 3) not happy
13. I feel……1) very sure 2) sure 3) not sure
14. I feel……1) very good 2) good 3) not good
15. I feel……1) very troubled 2) troubled 3) not troubled
16. I feel……1) very bothered 2) bothered 3) not bothered

17. I feel……1) very nice 2) nice 3) not nice

18. I feel……1) very terrified 2) terrified 3) not terrified

19. I feel……1) very mixed-up 2) mixed-up 3) not mixed-up

20. I feel……1) very cheerful 2) cheerful 3) not cheerful

HOW-I-FEEL QUESTIONNAIRE

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide if it is *hardly ever*, or *sometimes*, or *often* true for you. Then for each statement, put a circle around the answer that seems to describe you best. There are no right or wrong answers. Don't spend too much time on any one statement. Remember, choose the word which seems to describe how you usually feel.

1. I worry about making mistake	hardly ever	sometimes	often
2. I feel like crying	hardly ever	sometimes	often
3. I feel unhappy	hardly ever	sometimes	often
4. I have trouble making up my mind	hardly ever	sometimes	often
5. It is difficult for me to face my problems	hardly ever	sometimes	often
6. I worry too much	hardly ever	sometimes	often
7. I get upset at home	hardly ever	sometimes	often
8. I am shy	hardly ever	sometimes	often
9. I feel troubled	hardly ever	sometimes	often
10. Unimportant thoughts run through			

my mind and bother me	hardly ever	sometimes	often
11. I worry about school	hardly ever	sometimes	often
12. I have trouble deciding what to do	hardly ever	sometimes	often
13. I notice my heart beats fast	hardly ever	sometimes	often
14. I am secretly afraid	hardly ever	sometimes	often
15. I worry about my parents	hardly ever	sometimes	often
16. My hands get sweaty	hardly ever	sometimes	often
17. I worry about things that may happen	hardly ever	sometimes	often
18. It is hard for me to fall asleep at night	hardly ever	sometimes	often
19. I get a funny feeling in my stomach	hardly ever	sometimes	often
20. I worry about what others think of me	hardly ever	sometimes	often

Appendix 12

ERQ-CA

Instructions and Items

We would like to ask you some questions about how you control your feelings. The questions below are about two areas of feelings. One is what you feel like inside. The other is how you show your feelings in the way you talk or act. Here's an example question:

e.g. "My favourite ice cream is strawberry."

(1. strongly disagree, 2. disagree, 3. half and half, 4. agree, 5. strongly agree)

1. ____When I want to feel happier, I think about something different

2. ____I keep my feelings to myself.

3. ____When I want to feel less bad (e.g., sad, angry or worried). I think about something different.

4. ____When I am feeling happy, I am careful not to show it.

5. ____When I'm worried about something, I make myself think about it in a way that helps me feel better.

6. ____I control my feelings by not showing them.

7. ____When I want to feel happier about something, I change the way I'm thinking about it.

8. ____I control my feelings about things by changing the way I think about them.

9. ____When I'm feeling bad (e.g. sad, angry, or worried). I'm careful not to show it.

10. ___When I want to feel less bad (e.g. sad, angry, or worried) about something, I change the way I'm thinking about it

Appendix 13

DASS21

Name: Date:

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree or a good part of time
3 Applied to me very much or most of the time

1 (s)	I found it hard to wind down	0	1	2	3
2 (a)	I was aware of dryness of my mouth	0	1	2	3
3 (d)	I couldn't seem to experience any positive feeling at all	0	1	2	3
4 (a)	I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5 (d)	I found it difficult to work up the initiative to do things	0	1	2	3
6 (s)	I tended to over-react to situations	0	1	2	3
7 (a)	I experienced trembling (e.g. in the hands)	0	1	2	3
8 (s)	I felt that I was using a lot of nervous energy	0	1	2	3
9 (a)	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10 (d)	I felt that I had nothing to look forward to	0	1	2	3
11 (s)	I found myself getting agitated	0	1	2	3
12 (s)	I found it difficult to relax	0	1	2	3
13 (d)	I felt down-hearted and blue	0	1	2	3
14 (s)	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15 (a)	I felt I was close to panic	0	1	2	3
16 (d)	I was unable to become enthusiastic about anything	0	1	2	3
17 (d)	I felt I wasn't worth much as a person	0	1	2	3
18 (s)	I felt that I was rather touchy	0	1	2	3
19 (a)	I was aware of the action of my heart in the absence of physical exertion (e.g. sense of heart rate increase, heart missing a beat)	0	1	2	3
20 (a)	I felt scared without any good reason	0	1	2	3
21 (d)	I felt that life was meaningless	0	1	2	3

Appendix 14

NHS
National Institute for Health Research

AQ-10

Autism Spectrum Quotient (AQ)

A quick referral guide for adults with suspected autism who do not have a learning disability.

Please tick one option per question only:		Definitely Agree	Slightly Agree	Slightly Disagree	Definitely Disagree
1	I often notice small sounds when others do not				
2	I usually concentrate more on the whole picture, rather than the small details				
3	I find it easy to do more than one thing at once				
4	If there is an interruption, I can switch back to what I was doing very quickly				
5	I find it easy to 'read between the lines' when someone is talking to me				
6	I know how to tell if someone listening to me is getting bored				
7	When I'm reading a story I find it difficult to work out the characters' intentions				
8	I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant etc)				
9	I find it easy to work out what someone is thinking or feeling just by looking at their face				
10	I find it difficult to work out people's intentions				

Appendix 15

SELF-EVALUATION QUESTIONNAIRE

STAI FORM Y-1

S ____

T ____

Directions

A number of statements which people have used to describe themselves are given below. Please read each statement and then circle the appropriate value to the right of the statement to indicate how you feel r*ight now*, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feeling best

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I feel tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I feel jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

S ____

T ____

Directions

A number of statements which people have used to describe themselves are given below. Please read each statement and then circle the appropriate value to the right of the statement to indicate how you *generally feel*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix 16

Emotion Regulation Questionnaire (ERQ)
Gross & John
9/03

The Emotion Regulation Questionnaire is designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression.

Citation

Gross, J.J., & John, O.P. (2003). Individual differences in two emotion regulation processes: Implications for affect, relationships, and well-being. Journal of Personality and Social Psychology, 85, 348-362.

Instructions and Items

We would like to ask you some questions about your emotional life, in particular, how you control (that is, regulate and manage) your emotions. The questions below involve two distinct aspects of your emotional life. One is your emotional experience, or what you feel like inside. The other is your emotional expression, or how you show your emotions in the way you talk, gesture, or behave. Although some of the following questions may seem similar to one another, they differ in important ways. For each item, please answer using the following scale:

1	2	3	4	5	6	7
strongly disagree			**neutral**			**strongly agree**

1. ____ When I want to feel more *positive* emotion (such as joy or amusement), I *change what I'm thinking about*.
2. ____ I keep my emotions to myself.
3. ____ When I want to feel less *negative* emotion (such as sadness or anger), I *change what I'm thinking about*.
4. ____ When I am feeling *positive* emotions, I am careful not to express them.
5. ____ When I'm faced with a stressful situation, I make myself *think about it* in a way that helps me stay calm.
6. ____ I control my emotions by *not expressing them*.
7. ____ When I want to feel more *positive* emotion, I *change the way I'm thinking* about the situation.
8. ____ I control my emotions by *changing the way I think* about the situation I'm in.
9. ____ When I am feeling *negative* emotions, I make sure not to express them.
10. ____ When I want to feel less *negative* emotion, I *change the way I'm thinking* about the situation.

Appendix 17

The University Of Sheffield.

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Date:
Dear Sir/Madam,

Re: Destigmatizing Nonepileptic Attack Disorder

We are currently conducting a research study at the Royal Hallamshire Hospital examining the emotional and physical well being of people that suffer from seizures. The study will involve filling in questionnaires about the way you feel.

You have been identified as someone who could take part in this study because you have previously seen a neurologist for help with seizures at the Royal Hallamshire Hospital.

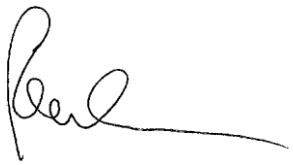
A participant information sheet is enclosed with this letter. Please read the information sheet to help you to understand what the study will involve and to think about whether you would like to take part.

If you wish to take part please fill in the reply slip below, consent form and the questionnaires attached, and once done post all using the prepaid enveloped enclosed. Once we receive your responses we will post you £5 voucher as thank you for helping with this research and as a compensation of the time you have spent on this.

If you have any questions do not hesitate to contact Marco Mcsweeney who is a member of the research team (Email: mmcsweeney1@sheffield.ac.uk). Also feel free to also contact the research supervisors (Dr Liat Levita, 0114 222 6651; Dr Markus Reuber, 0114 226 8763) if you have any further questions.

If you do decide to take part in the study you will be free to withdraw at any time.

Kind Regards



Professor Markus Reuber,
Honorary Consultant Neurologist

Reply Slip

Full Name: _____

Address:_____

_____

_____

_____

_

Contact Telephone No: _____

Email Address (*if available*):_____

I am willing to part in this study (*please initial box if appropriate*):

☐

I am unwilling to part in this study (*please initial box if appropriate*):

☐

Appendix 18

The University Of Sheffield.

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

STH18940

PARTICIPANT INFORMATION SHEET

Title of Project: Seeking a better understanding of Nonepileptic Attack Disorder

Name of Researchers: Dr Liat Levita, Prof Markus Reuber

We would like to invite you to take part in a research study. Before you decide whether to take part, you should understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Please contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

Background

Nonepileptic attack disorder (NEAD) can be a disabling condition. The lives of people with this disorder can therefore be more stressful than the lives of people who do not experience seizures. We would like to study this and see how people who either still experience seizures or have had them in the past feel. In order to do this, we need to recruit 50 participants diagnosed with NEAD and 50 healthy participants. Both groups will be used for brain scan analysis. Only the NEAD group will be asked to provide information about their social, emotional, and physical well-being.

This study is being carried out as part of a research project based at the University of Sheffield and has been reviewed by the South West – Exeter Research ethics Committee.

What is the purpose of the study?

This is a part of a larger study where we are examining the physical basis of having seizures by analyzing brain scans of people who have or have had seizures. In this part of the study we are asking people who have had brain scans in the Royal Hallamshire hospital in the past to let us know how they feel.

Why have I been asked to take part?

We are approaching people who have been diagnosed with NEAD by a neurologist at the Royal Hallamshire Hospital in Sheffield. We are specifically approaching people with this seizure disorder who have had an MRI scan of the head. The reason for this is that we want to examine if there is a relationship between the size of certain areas of the brain that process emotions and certain emotions that people with seizures sometimes feel. We hope that this study will allow us to better understand how a physical problem such as a seizure could have

anything to do with emotions. Hence, the aim of this work is to explain NEAD by exploring its physical basis in the brain.

Do I have to take part?
It is up to you to decide whether or not to take part. If you have any questions about this study at any time, you can contact us. If you do decide to take part you are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive in any way. If you do decide to take part you will be required to sign a consent form. You will be provided with your own copy of the signed consent form along with a copy of the participant information sheet, and your GP will be informed of your participation.

What will happen to me if I take part?
With this letter you will have a pack of questionnaires to fill in. Filling in these questionnaires should take about 30 min. Once you are done, please place post these to us using the prepaid envelope provided in this package. We will then send you £5 voucher for taking part.

What are the possible benefits of this study?
This study will help understand the way people suffering or having suffered from seizures in the past feel both emotionally and physically, which will help provide better support for people who suffer from these conditions in the future. However, there will be no direct benefit to participants for taking part in this study.

What are the possible risks of taking part in this study?
There are no significant risks associated with taking part in the study. However, please note that we will ask you to complete some self-report scales that include measures of anxiety and depression and some of the questions ask about sensitive topics. Also, some of the questions ask about what you felt or experienced in the past, which you might find difficult to remember, but we would be grateful if you could answer each question to the best of your ability. If completion of these questionnaires raises any issues or concerns for you, please let the research team know and we will also provide you with details of services and organisations you can contact for further support. Contact details for a member of the research team is listed at the bottom of this document. If you have additional unresolved concerns regarding your well-being, available services and organisations are provided at the beginning of the questionnaire pack.

Will my taking part in this study be kept confidential?
The research team will only have access to study ID and not your names or any identifying information. All the information that is collected about you during this study will be kept strictly confidential. Your medical notes and medical data will be accessed. We will keep your personal details, such as name, address and telephone number, separately and locked in a secure location. This means that your identity will be kept private. Any personal details held by us will be destroyed once the study has finished. However, confidentiality might need to be broken if concerns arise about a participants' or any other's safety, in which case their GP will be contacted, and any necessary assistance will then be provided.

What will happen to the results of the study?
The results of this study will contribute to a research study. We will also publish the results of the study in a scientific journal. You will not be identified individually in the write-up. If you would like a summary of the results of the study once it is complete, please let us know.

What if I change my mind?
You do not have to take part in this study. If you have agreed to take part, you can stop at any time without giving your reasons.

Who should I contact if I have a question or need more information?
Marco Mcsweeney, Research Assistant, contact details (Email: mmcsweeney1@sheffield.ac.uk)

What if something goes wrong?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact Sheffield Patient Services Team (previously known as PALS) on 0114 2712400 or Dr Philip Harvey (Registrar and Secretary, University of Sheffield) on registrar@sheffield.ac.uk or 0114 222 1101.

Appendix 19

The University Of Sheffield.

Sheffield Teaching Hospitals NHS
NHS Foundation Trust
Excellence as standard

Consent Form

Seeking a better understanding of Nonepileptic Attack Disorder

Name of Researchers: Dr Liat Levita, Professor Markus Reuber

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to give consent for my medical data to be accessed. ☐
4. I agree to give my consent to inform my GP of my participation. ☐
5. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from The University of Sheffield, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
6. I agree to take part in the above study and understand that the data will be used as part of a research study. ☐

_____ _____
Name Signature

_____ _____
Today's date Your date of birth

Appendix 20

Sheffield Teaching Hospitals NHS
NHS Foundation Trust
Excellence as standard

Thank you so much for taking part!

Once you have completed this questionnaire package could you please place this along with the consent form and reply slip in the paid for envelope enclosed and post it to us.

Thank you once again for taking part!

If you have any questions do not hesitate to contact Marco Mcsweeney, who is a member of the research team (Email: mmcsweeney1@sheffield.ac.uk).

Also feel free to contact the research supervisors (Dr Liat Levita, 0114 222 6651; Markus Reuber, 0114 226 8763) if you have any further questions.

For more information about Non-Epileptic Attacks please visit our website: www.nonepilepticattacks.info.

We hope that you have not found completing this survey upsetting. However, we understand that some of the questions might be upsetting for some people. Hence, case you are feeling distressed and would like support, we have listed the contact details for two relevant organisations below. If you need more urgent support, please contact your GP or the emergency services.

Samaritans

Helpline: 08457 90 90 90

www.samaritans.org

Mind

Helpline: 0300 123 3393 (9am-6pm, Monday to Friday)

www.mind.org.uk

Appendix 21

Demographic Questionnaire

Personal Information

Please answer the following questions about yourself. The information you are giving us will be treated as confidential and will be anonymised. Do not put your name on the questionnaire.

1. Date of birth: ……..

2. Gender: *(please tick the correct option)*

Male ☐ Female ☐ ☐ Other please specify _____

4. How would you describe your ethnic background?
(Please tick the box that applies to you, or write an answer in the space provided)

White – English/Welsh/Scottish/Northern Irish/British □
White – Irish □
White – Gypsy or Irish Traveller □
White - Any Other White background □
Mixed/Multiple ethnic group - White and Black Caribbean □
Mixed/Multiple ethnic group - White and Black African □
Mixed/Multiple ethnic group - White and Asian □

Mixed/Multiple ethnic group -
Any Other Mixed/multiple ethnic background □
Asian/Asian British – Indian □
Asian/Asian British – Pakistani □
Asian/Asian British – Bangladeshi □
Asian/Asian British – Chinese □
Asian/Asian British - Any other Asian background □
Black/African/Caribbean/Black British – African □
Black /African/Caribbean/Black British – Caribbean □

Black/African/Caribbean/Black British –
Any other Black / African / Caribbean background □
Other ethnic group – Arab □

Any other ethnic group; Please specify:

_____

5. How would you describe your current employment status? (Please tick the box/(es) that applies/(apply) to you or write an answer in the space provided)

In full-time paid work □
In part-time paid work □
In full-time education □
In part-time education □
Full-time carer/homemaker □

On leave/out of work due to illness or disability □
Retired □

Other; Please specify:_____

6. What is your highest educational qualification? (Please tick the box/(es) that applies/(apply) to you or write an answer in the space provided)

No educational qualifications □
Standard grades, O grades, O levels, GCE/GCSEs □
Highers, advanced highers, A levels □
Vocational qualification (e.g. SVQ, NVQ, SCOTVEC) □
HNC/HND □
Degree (e.g., BA, BSc) □
Postgraduate qualification (e.g. MSc, PhD) □
Professional qualification (e.g. CAEW, CIIA) □

Other; Please specify:_____

7. When did you first have a seizure?
(For example, 6 months ago or 3 years ago)

…… months ago

…… years ago

8. When did you have your last seizure? *(e.g., 1 day ago or 3 months ago)*

……

9. Are you currently on any medication? *(please tick as appropriate)*

☐ YES

☐ NO

If yes, please list your medication below:

……
……
……
……
……
……
……
……

10. Have you received or are receiving any form of psychological therapy

☐ YES

☐ NO

Appendix 22

Work and Social Adjustment Scale

Rate each of the following questions on a 0 to 8 scale in the box alongside each item - 0 indicates no impairment at all and 8 indicates very severe impairment

1. Because of my nonepileptic attack disorder, my ability to work is impaired.

   ☐

   If you are retired or choose not to have a job for reasons unrelated to your problem, please write N/A - not applicable

   _____

2. Because of my Nonepileptic Attack Disorder, my home management (cleaning, tidying, shopping, cooking, looking after home or children, paying bills) is impaired.

   ☐

3. Because of my nonepileptic attack disorder, my social leisure activities (with other people, such as parties, bars, clubs, outings, visits, dating, home entertainment) are impaired. 0 means not at all impaired and 8 means very severely impaired.

   ☐

4. Because of my nonepileptic attack disorder, my private leisure activities (done alone, such as reading, gardening, collecting, sewing, walking alone) are impaired.

   ☐

5. Because of my nonepileptic attack disorder, my ability to form and maintain close relationships with others, including those I live with, is impaired.

   ☐

Appendix 23

Difficulties in Emotion Regulation Scale (DERS)

1	2	3	4	5
Almost never	**Sometimes**	**About half the time**	**Most of the time**	**Almost always**
(0-10%)	**(11-35%)**	**(36-65%)**	**(66-90%)**	**(91-100%)**

Please indicate how often the following 36 statements apply to you by ticking the appropriate number from the scale above (1 – 5) in the boxes alongside each item

		1	2	3	4	5
1.	I am clear about my feelings					
2.	I pay attention to how I feel					
3.	I experience my emotions as overwhelming and out of control					
4.	I have no idea how I am feeling					
5.	I have difficulty making sense out of my feelings					
6.	I am attentive to my feelings					
7.	I know exactly how I am feeling					
8.	I care about what I am feeling					
9.	I am confused about how I feel					
10.	When I'm upset, I acknowledge my emotions					
11.	When I'm upset, I become angry with myself for feeling that way					
12.	When I'm upset, I become embarrassed for feeling that way					
13.	When I'm upset, I have difficulty getting work done					
14.	When I'm upset, I become out of control					
15.	When I'm upset, I believe that I will remain that way for a long time					
16.	When I'm upset, I believe that I'll end up feeling very depressed					
17.	When I'm upset, I believe that my feelings are valid and important					
18.	When I'm upset, I have difficulty focusing on other things					
19.	When I'm upset, I feel out of control					
20.	When I'm upset, I can still get things done					
21.	When I'm upset, I feel ashamed with myself for feeling that way					
22.	When I'm upset, I know that I can find a way to eventually feel better					
23.	When I'm upset, I feel like I am weak					
24.	When I'm upset, I feel like I can remain in control of my behaviours					
25.	When I'm upset, I feel guilty for feeling that way					
26.	When I'm upset, I have difficulty concentrating					
27.	When I'm upset, I have difficulty controlling my behaviours					
28.	When I'm upset, I believe that there is nothing I can do to make					
29.	When I'm upset, I become irritated with myself for feeling that way					
30.	When I'm upset, I start to feel very bad about myself					
31.	When I'm upset, I believe that wallowing in it is all I can do					
32.	When I'm upset, I lose control over my behaviours					
33.	When I'm upset, I have difficulty thinking about anything else					
34.	When I'm upset, I take time to figure out what I'm really feeling					
35.	When I'm upset, it takes me a long time to feel better					
36.	When I'm upset, my emotions feel overwhelming					

Appendix 24

SELF-EVALUATION QUESTIONNAIRE

STAI FORM Y-1

S ____

T ____

Directions

A number of statements which people have used to describe themselves are given below. Please read each statement and then circle the appropriate value to the right of the statement to indicate how you feel r*ight now*, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feeling best

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
21. I feel calm	1	2	3	4
22. I feel secure	1	2	3	4
23. I feel tense	1	2	3	4
24. I feel strained	1	2	3	4
25. I feel at ease	1	2	3	4
26. I feel upset	1	2	3	4
27. I am presently worrying over possible misfortunes	1	2	3	4
28. I feel satisfied	1	2	3	4
29. I feel frightened	1	2	3	4
30. I feel comfortable	1	2	3	4
31. I feel self-confident	1	2	3	4
32. I feel nervous	1	2	3	4
33. I feel jittery	1	2	3	4
34. I feel indecisive	1	2	3	4
35. I am relaxed	1	2	3	4
36. I feel content	1	2	3	4
37. I am worried	1	2	3	4
38. I feel confused	1	2	3	4
39. I feel steady	1	2	3	4
40. I feel pleasant	1	2	3	4

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

S ____

T ____

Directions

A number of statements which people have used to describe themselves are given below. Please read each statement and then circle the appropriate value to the right of the statement to indicate how you *generally feel*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
41. I feel pleasant	1	2	3	4
42. I feel nervous and restless	1	2	3	4
43. I feel satisfied with myself	1	2	3	4
44. I wish I could be as happy as others seem to be	1	2	3	4
45. I feel like a failure	1	2	3	4
46. I feel rested	1	2	3	4
47. I am "calm, cool and collected"	1	2	3	4
48. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
49. I worry too much over something that really doesn't matter	1	2	3	4
50. I am happy	1	2	3	4
51. I have disturbing thoughts	1	2	3	4
52. I lack self-confidence	1	2	3	4
53. I feel secure	1	2	3	4
54. I make decisions easily	1	2	3	4
55. I feel inadequate	1	2	3	4
56. I am content	1	2	3	4
57. Some unimportant thought runs through my mind and bothers me	1	2	3	4
58. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
59. I am a steady person	1	2	3	4
60. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix 25

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

Appendix 26

Life Span Inventory of Affect and Trauma

Instructions: In each of the following sections, you will be asked to rate how often you had some particular experiences and had certain feelings. You will be asked to rate the same items several times, in order to find out about your experiences during three different stages of your life (childhood, adolescence, and adulthood).

SECTION 1

PART A: In your ***childhood*** to what degree did you experience…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Physical illness(es)							
Stressful experiences							
Poverty							
Traumatic events							
Physical neglect							
Physical abuse							
Emotional neglect							
Emotional abuse							
Sexual abuse							
Mental health difficulties							

PART B: During your ***childhood*** to what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Sad							
Happy							
Angry							
Afraid							
Carefree							
Stressed							
Content							
Guilty							
Depressed							
Worried							
Anxious							
Untroubled							

PART C: During your ***childhood*** to what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Secure							
Lonely							
Loved							
Confident							
Ignored							
Supported							
Disliked							
Bullied							
Used							
Smothered							

SECTION 2

PART A: In your ***adolescence*** to what degree did you experience…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Physical illness(es)							
Stressful experiences							
Poverty							
Traumatic events							
Physical neglect							
Physical abuse							
Emotional neglect							
Emotional abuse							
Sexual abuse							
Mental health difficulties							

PART B: During your ***adolescence*** to what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Sad							
Happy							
Angry							
Afraid							
Carefree							
Stressed							
Content							
Guilty							
Depressed							
Worried							
Anxious							
Untroubled							

PART C: During your ***adolescence*** to what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Secure							
Lonely							
Loved							
Confident							
Ignored							
Supported							
Disliked							
Bullied							
Used							
Smothered							

SECTION 3

PART A: In your ***adulthood to*** what degree did you experience…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Physical illness(es)							
Stressful experiences							
Poverty							
Traumatic events							
Physical neglect							
Physical abuse							
Emotional neglect							
Emotional abuse							
Sexual abuse							
Mental health difficulties							

PART B: During your ***adulthood*** to what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Sad							
Happy							
Angry							
Afraid							
Carefree							
Stressed							
Content							
Guilty							
Depressed							
Worried							
Anxious							
Untroubled							

PART C: During your ***adulthood to*** what extent did you feel…?

	Not at all			Some			A lot
	0	1	2	3	4	5	6
Secure							
Lonely							
Loved							
Confident							
Ignored							
Supported							
Disliked							
Bullied							
Used							
Smothered							

Appendix 27

Reuter and Montag's rRST-Q

A number of statements which people have used to describe themselves are given below. Please read each statement and then circle the appropriate value to the right of the statement to indicate how this statement applies to you. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer that seems to describe you best

	Strongly disagree	Disagree	Agree	Strongly agree
1. I'm a spontaneous person				
2. I'm often glad if someone makes decisions for me				
3. I often feel paralyzed when in a dangerous situation				
4. I often doubt if my efforts will pay off				
5. Most of the time I have a thirst for action.				
6. When faced with danger, I tend to flee				
7. If I have the choice between two appealing options, I have difficulty deciding on one				
8. My friends think of me as an indecisive person				
9. I usually approach unpleasant tasks without hesitation				
10. I will gladly let unpleasant tasks slip by				
11. I find it hard to bear uncertainty				
12. I often take risks				
13. I'm easily inspired by new things				
14. I like sitting unpleasant things out				
15. Most of the time, I cannot defend myself if I am criticized				
16. To avoid worse things happening, I would rather give in				
17. Attack is the best form of defense				
18. Whoever dares wins				
19. I usually avoid confrontations				
20. When an unpleasant event is inevitable, I'm thrown into a state of panic				
21. I don't have problems deciding on a dish in a restaurant				
22. I am a rather quick-witted person				
23. I often don't know what I want				
24. I get fired up when I see the chance to achieve something				
25. I am an outgoing person				
26. When faced with two unpleasant alternatives, it is difficult for me to decide on the lesser of two evils				

Appendix 28

Group differences on self-report measures for Study 1 (Chapter 2)

To determine whether responses on self-report measures differed significantly between age groups, first, a Shapiro-Wilk test was used to test the assumption of normality. The results indicated that STAI-S (percentage scores) and the three DASS-21 sub-scales (depression, anxiety, stress) had non-normal distributions. For these measures a Kruskall-Wallis H-test was conducted. For the AQ-10 child and adult versions, the STAI-T (percentage scores), CR (percentage scores) and ES (percentage scores), a one-way MANOVA was used. Secondly, scores for each age-group were inspected for extreme outliers. Three extreme outliers were present in the young adult group on the DASS-21 anxiety sub-scale only.

The results of the Kruskall-Wallis H-test revealed significant group differences for STAI-S scores ($\chi 2$ (2) = 34.14, $p < 0.001$) but not for depression ($\chi 2$ (2) = 1.11, $p = 0.57$), anxiety ($\chi 2$ (2) = 4.40, $p = 0.11$) nor stress ($\chi 2$ (2) = 0.42, $p = 0.80$) DASS-21 subscales. A series of Mann-Whitney U tests were then conducted. To ameliorate inflated Type-I error rates a Bonferroni correction was applied (alpha = 0.016). The Mann-Whitney U tests revealed that the early adolescent group had significantly higher STAI-S scores compared to the late adolescent group ($p < 0.001$) and the young adult group ($p < 0.001$). No significant differences were found between the late adolescent group and the young adult group ($p = 0.84$).

The results of the one-way MANOVA revealed a significant main effect of group for STAI-T scores $F(2, 51) = 5.58$, $p < 0.01$, $\eta_p^2 = 0.180$ and CR percentage scores $F(2, 51) = 8.36$, $p < 0.01$, $\eta_p^2 = 0.247$. Post-hoc tests revealed that early adolescents had significantly higher STAI-T scores compared to late adolescents ($p < 0.05$) and young adults ($p < 0.01$) and significantly lower CR scores compared to late adolescents ($p < 0.01$) and young adults ($p < 0.001$). No significant differences were found between late adolescents and young adults for STAI-T scores nor CR scores. No significant differences were found between groups for ES scores $F(2, 51) = 0.850$, $p = 0.43$, $\eta_p^2 = 0.032$ nor AQ-10 scores $F(2, 51) = 1.74$, $p = 0.18$, $\eta_p^2 = 0.064$. Levene's test of homogeneity of variances was non-significant ($p > 0.05$) and the Box's M test for equality of covariance matrices was non-significant ($p = 0.724$). The results are presented in Supplementary Table 2.3.

Appendix 29

Number of channels removed, head size, room temperature and button press errors for Study 1 (Chapter 2)

Across all age groups, on average 6 channels were removed prior to re-referencing to the average (median = 6, mode = 5, min = 1, max = 16). In the early adolescent group, the mean number of channels removed was 7.05 (*SD* = 4.07, median = 5, mode = 5, min = 1, max = 16). In the late adolescent group, the mean number of channels removed was 6.88 (*SD* = 3.44, median = 6.5, mode = 8, min = 2, max = 14). In the young adult group, the mean number of channels removed was 5.27 (*SD* = 3.04, median = 4.5, mode = 2, min = 1, max = 12). A Kruskall-Wallis H-test was conducted to examine age group differences in the number of channels removed. This resulted in a non-significant finding ($\chi 2$ (2) = 2.56, p = 0.277) demonstrating that there were no significant group differences in the number of channels removed. A one-way ANOVA revealed a non-significant main group effect for head size circumference $F(2, 51)$ = 1.08, p = 0.34, η_p^2= .081. Exploration of the room temperature reported at the time of recording the EEG revealed that across age groups room temperatures ranged from 21 to 26 degrees Celsius. Mann-Whitney U tests revealed a significant difference in room temperatures (Bonferroni correction alpha = 0.016) during EEG recordings between late adolescents and young adults ($p < 0.001$). The group means and standard deviations for head size and room temperature as well as the total number of trials averaged to compute the ERPs (CNV, N170, P1) are presented in Supplementary Table 2.4.

Across all age groups, on average 8 trials were removed due to button press errors (median = 6, mode = 3, min = 0, max = 36). A one-way ANOVA using the univariate function is SPSS was conducted to examine group differences in the number of button press errors that occurred during trials. A Shapiro-Wilk test revealed normal distributions of scores for each age group ($p > 0.05$) and no extreme outliers were present in the data. However, the Levene's test of equality of error variances was significant ($p < 0.01$), violating the assumption of homogeneity of variance. A significant main effect for group was found $F(2, 51) = 7.66$, p = .001, η_p^2= .231. Bonferroni corrected pairwise comparisons revealed that the early adolescent group performed significantly worse, making more button press errors compared to the late adolescent group (p = .009) and the young adult group (p = .002), this being the primary reason for the exclusion of more trials in this age group compared to the other two age

groups. There were no significant differences in the number of button press errors between the late adolescent group and the young adult group ($p > .05$). Button press errors means and standard deviations for each age-group are presented in Supplementary Table 2.4.

Appendix 30

Trials included in analyses for Study 1 (Chapter 2)

Across all age groups, on average 48 CNV/P1 angry condition trials locked to S_1 onset were retained (median = 48, mode = 48, min = 39, max = 54), 47/P1 CNV happy condition trials locked to S_1 onset were retained (median = 48, mode = 47, min = 37, max = 54), and 47 CNV/P1 neutral condition trials locked to S_1 onset were retained (median = 48, mode = 48, min = 38, max = 53). The total number of trials in each age-group per condition included in the analyses are presented in Supplementary Table 2.5. A Shapiro-Wilk test revealed non-normal distributions in the young adult group for happy and neutral conditions (negative skew) while the distribution of scores for CNV/P1 trials in the other two age groups were normally distributed in all conditions. No extreme outliers were present. To examine group differences in the number of trials included in the analyses for each group for each condition, a one-way ANOVA was conducted for the angry condition and a Kruskall-Wallis H-test was used to examine group differences for happy and neutral conditions. The one-way ANOVA for trial numbers included in the angry CNV/P1 condition locked to S_1 onset revealed a significant main effect of group $F(2, 51) = 8.10$, $p < 0.01$, $\eta_p^2 = .241$. Levene's test of equality of error variances was non-significant. Bonferroni corrected pairwise comparisons revealed that the early adolescent group contained a significantly lower number of CNV/P1 trials in the angry condition compared to the late adolescent group ($p = .009$) and the young adult group ($p = .001$) only. There was no statistically significant difference in the number of CNV/P1 trials in the angry condition between the late adolescent group and the young adult group ($p > .05$). The Kruskall-Wallis H-test resulted in a significant finding for the number of CNV/P1 trials in the happy condition ($\chi2$ (2) = 12.89, $p < 0.01$) and the number of CNV/P1 trials in the neutral condition ($\chi2$ (2) = 15.46, $p < 0.001$). A series of Mann-Whitney U tests were then conducted. To ameliorate inflated Type-I error rates a Bonferroni correction was applied (alpha = 0.016). The Mann-Whitney U tests revealed a significantly lower number of CNV/P1 happy condition trials in the early adolescent group compared to the young adult group ($p < 0.01$). Significantly fewer CNV/P1 happy condition trials were also present in the late adolescent group compared to the young adult group ($p < 0.01$). Again, a significantly lower number of neutral CNV/P1 condition trials were present in the early adolescent group compared to the young adult group ($p < 0.01$) and a

significantly lower number of CNV/P1 neutral condition trials were present in the late adolescent group compared to the young adult group ($p < 0.01$).

Group differences in the number of N170/P1 trials locked to S_2 onset for each condition were also examined. Across all age groups, on average 48 N170/P1 angry condition trials locked to S_2 onset were retained (median = 48, mode = 48, min = 41, max = 54), 47 N170/P1 happy condition trials locked to S_2 onset were retained (median = 48, mode = 47, min = 37, max = 54), and 48 N170/P1 neutral condition trials locked to S_2 onset were retained (median = 48, mode = 48, min = 40, max = 53). A Shapiro-Wilk test revealed non-normal distributions in the late adolescent group for the neutral condition (positive skew) and non-normal distributions in the young adult group for the happy and neutral conditions (both negative skew). One extreme outlier was present in the late adolescent group for the neutral condition. A one-way ANOVA for the number of N170/P1 trials in the angry condition revealed a significant main effect of group $F(2, 51) = 7.38$, $p < 0.01$, $\eta_p^2 = .225$. Levene's test of equality of error variances was non-significant. Post-hoc tests revealed that the early adolescent group contained a significantly lower number of N170/P1 trials in the angry condition compared to the late adolescent group ($p < 0.01$) and the young adult group ($p < 0.01$) only. The Kruskall-Wallis H-test resulted in a significant finding for the number of N170/P1 trials in the happy condition ($\chi 2\ (2) = 10.64$, $p < 0.01$) and the number of N170/P1 trials in the neutral condition ($\chi 2\ (2) = 15.57$, $p < 0.001$) across the three groups. Again, a series of Mann-Whitney U tests were then conducted, and again to ameliorate inflated Type-I error rates a Bonferroni correction was applied (alpha = 0.016). The Mann-Whitney U tests revealed a significantly lower number of N170/P1 happy condition trials in the early adolescent group compared to the young adult group ($p < 0.01$). Again, a significantly lower number of N170/P1 neutral condition trials were present in the early adolescent group compared to the young adult group ($p < 0.01$) and a significantly lower number of N170/P1 neutral condition trials were present in the late adolescent group compared to the young adult group ($p < 0.01$).

Appendix 31

Assumption testing for ANOVAs conducted on RT and ERP data for Study 1 (Chapter 2)

Assumption testing for mean reaction time ANOVA analyses

There were no extreme outliers in the data. Two of the nine cells of the design (angry condition) were not normally distributed, one for the early adolescent group and one for the young adult group, as assessed by Shapiro-Wilk's test of normality ($p < .05$), both showing moderate positive skew. The assumption of homogeneity of variances was met, as assessed by Levene's test of homogeneity of variance ($p > .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices ($p > .001$). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(2) = 1.701$, $p = .427$.

Assumption testing for iCNV ANOVA analysis

One extreme outlier was identified in the late adolescent group (participant 11). This participant was removed from the analysis. Two of the nine cells of the design (happy condition) were not normal distributed for the late adolescent and young adult group, as assessed by Shapiro-Wilk's test of normality ($p < .05$), both showing moderate negative skew. The assumption of homogeneity of variances was met, as assessed by Levene's test of homogeneity of variance ($p > .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices ($p = .176$). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(2) = 3.156$, $p = .206$.

Assumption testing for tCNV ANOVA analysis

Participant 11 was identified as a significant outlier with a studentized residual value of -3.83 for the neutral condition. This participant was removed from the analysis. One of the nine cells of the design was not normally distributed for the late adolescent group (angry condition), as assessed by Shapiro-Wilk's test of normality ($p < .05$), showing moderate negative skew. The assumption of homogeneity of variances was met, as assessed by Levene's test of homogeneity of variance ($p > .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance

matrices (p = .255). Mauchly's test of sphericity indicated that the assumption of sphericity was not met for the two-way interaction, $\chi^2(2) = 6.356$, p = .042.

Assumption testing for Total CNV ANOVA analysis

There were no extreme outliers in the data. Three of the nine cells of the design (angry, happy, and neutral) were not normal distributed for the late adolescent group only, as assessed by Shapiro-Wilk's test of normality ($p < .05$), all showing moderate negative skew. The assumption of homogeneity of variances was not met for the angry condition (p = .008), as assessed by Levene's test of homogeneity of variance ($p < .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices (p = .268). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(2) = 5.652$, p = .059.

Assumption testing for the Visual P1 to S_1 ANOVA analyses

Three extreme outliers were identified, one in each age group. These outliers were removed prior to running the analysis. For each group, all conditions measured at O1/O2 were normally distributed as assessed by Shapiro-Wilk's test ($p > .05$). The assumption of homogeneity of variances was not met for all repeated measures of the design, as assessed by Levene's test of homogeneity of variance ($p < .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices (p = .129). Mauchly's test of sphericity indicated that the assumption of sphericity was met, $\chi^2(2) = .009$, p = .996.

Assumption testing for the Visual P1 to S_2 ANOVA analyses

No extreme outlier was present in the data. One of the eighteen cells of the design was not normally distributed, as assessed by Shapiro-Wilk's test of normality (p = .030), showing a moderate positive skew. The assumption of homogeneity of variances was not met for four of the repeated measures, as assessed by Levene's test of homogeneity of variance ($p < .05$), with the exception of the O2/PO8 electrode cluster in the angry condition and the O2/PO8 electrode cluster in the happy condition ($p > .05$). The assumption of homogeneity of covariances was met, as assessed by Box's test of equality of covariance matrices ($p > .001$). Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the three-way interaction, $\chi^2(2) = 16.232$, p = .019.

Assumption testing for the N170 to S_2 ANOVA analyses

Three extreme outliers were identified, one in each age group. These outliers were removed prior to running the analysis. All eighteen cells of the design were normally distributed, as assessed by Shapiro-Wilk's test of normality ($p > .05$). The assumption of homogeneity of variances was not met for two of the six repeated measures (P10 in the angry condition and in the happy condition), as assessed by Levene's test of homogeneity of variance ($p < .05$). The assumption of homogeneity of covariances was met, as assessed by Box's test of equality of covariance matrices ($p = .420$). Mauchly's test of sphericity indicated that the assumption of sphericity was met for the three-way interaction, $\chi^2(2) = .503$, $p = .778$.

Appendix 32

The relationship between the contingent negative variation, state anxiety, and reaction time data post-hoc analyses for Study 1 (Chapter 2).

Given that significant group differences were found in mean reaction time but not in CNV amplitudes in addition to the findings that mean reaction times were faster to happy faces compared to neutral faces for early adolescents, the lack of significant within and between subjects' findings with regards to CNV mean amplitude was somewhat surprising. Therefore, in addition to the mixed methods analyses described above I conducted a series of post-hoc standard multiple regressions to assess the relationship between a number of predictors and mean reaction times. The following predictors were entered into the regression model - iCNV at FCz, tCNV at Cz, state anxiety levels, and dummy coded age groups with mean reaction times as the outcome variable. This was repeated three times, once for each condition. State anxiety was chosen to be entered into the model as previous work has shown a relationship between state anxiety and iCNV amplitudes (Carretié, Mercado, Hinojosa, Martın-Loeches, & Sotillo, 2004) whereby participants with high state anxiety scores showed significantly greater iCNV amplitudes during the anticipation of negative stimuli. This suggests that individuals with higher levels of state anxiety demonstrate valence-related bias towards negative stimuli. However, and somewhat counterintuitively, there is evidence to suggest that high-anxious individuals demonstrate delayed reaction times to emotional and non-emotional facial expressions compared to low-anxious individuals (Bar-Haim, Lamy & Glickman, 2005). Moreover, in this study I found that early adolescents showed significantly higher levels of state anxiety which may help to account for differences found in the reaction time data. Table 32.1 shows the zero order correlations between the predictor variables used for each regression model.

Table 32.1. *Pearson correlation coefficients of predictor and outcome variables.*

Angry condition	Mean RT	iCNV FCz	tCNV Cz	Total CNV	STAI - S
iCNV at FCz	.208	-			
tCNV at Cz	.265*	.276*	-		
STAI – S%	.554***	-.048	.203	-.037	-
Happy condition	**Mean RT**	**iCNV FCz**	**tCNV Cz**	**Total CNV**	**STAI - S**
iCNV at FCz	.081	-			
tCNV at Cz	.253*	.336**	-		
STAI – S%	.546***	-.092	.174	.173	-
Neutral condition	**Mean RT**	**iCNV FCz**	**tCNV Cz**	**Total CNV**	**STAI - S**
iCNV at FCz	.290*	-			
tCNV at Cz	.439***	.698***	-		
STAI – S%	.584***	.008	.244*	.172	-

Note. STAI – S% = state anxiety percentage scores; * Significance at $p < .05$; ** Significance at $p < .01$; *** Significance at $p < .001$

Total CNV was highly correlated with both iCNV and tCNV and was therefore not entered into the model over concerns of multicollinearity. In addition, total CNV was a poor predictor of mean reaction times. Two separate sets of standard multiple regressions were conducted, one with iCNV and the other with tCNV included as predictors. Prior to running the final standard multiple regression analyses the data was checked for outliers, as assessed by inspection of the casewise diagnostic output in SPSS and studentized deleted residual values being +/- 3 standard deviations, high leverage points as assessed by leverage values being above .2 (Huber, 1981), and influential points as assessed by Cook's Distance values being above 1 (Cook & Weisberg, 1982). No outliers or influential points were found. However, a number of participants were found to have leverage values above .2 but below .35. The multiple regression analyses were run again with and without these participants. For the multiple regressions with iCNV included as a predictor of RTs in response to angry and neutral faces, the results changed materially and therefore participants with high leverage values (2 late adolescents) were removed from all analyses which included iCNV as a predictor variable. For the multiple regressions with tCNV included as a predictor of RTs in response to angry, happy and neutral faces, five participants had leverage values above .2 (1 early adolescent, 3 late adolescents, 1 young adult). However, the results did not materially change as a consequence of inclusion or exclusion of these participants from the regression models and therefore these participants were not removed from these analyses.

For each multiple regression model, visual inspection of partial regression plots

and plots of studentized residuals against the predicted values indicated that the assumption of linearity was met. There was independence of residuals, as assessed by Durbin-Watson statistics. For each multiple regression model there was homoscedasticity, as assessed by visual inspection of plots of the standardized residuals versus standardized predicted values. For each multiple regression model residuals were normally distributed as assessed by visual inspection of histograms and normal probability plots. All tolerance values were greater than 0.1. The "Enter" method was selected, a forced entry method which enters all of the specified predictors into the regression equation regardless of their level of significance, all weighted equally.

Multiple regression analyses

The regression model for mean RT in the angry condition with iCNV, STAI-S scores and dummy coded age groups as predictors was significant $F(4, 51) = 7.233$, $p < .001$ with an R^2 of 38.1%. This accounted for roughly 38% of the proportion of variance in mean RT predicted by the linear combination of the predictor variables. However, only iCNV amplitude approached significance and uniquely explained 4.3% of the variability in mean RT, while STAI-S accounted for only .6%. These percentages and those listed below are based on semipartial correlations for each predictor once the model was finalized. The regression model for mean RT in the happy condition with iCNV, STAI-S scores and dummy coded age groups as predictors was also significant $F(4, 51) = 5.653$, $p = .001$ with an R^2 of 32.5%. Again, none of the predictor variables in the model were significant. iCNV amplitude only explained .11% of the variability in mean RT, while STAI-S only accounted for .21%. The regression model for mean RT in the neutral condition with iCNV, STAI-S scores and dummy coded age groups as predictors was the most significant of the three regression models $F(4, 51) = 9.126$, $p = < .001$ with an R^2 of 43.7%. In this instance iCNV amplitude explained 4.4% of the variability in mean RT, while STAI-S only accounted for .07%. Taken together, while the overall model was statistically significant, these results show that while controlling for other variables in the model, none of the predictor variables on their own were significant predictors of mean RT in each of the three conditions. Moreover, the results of the regression analyses show that when iCNV and STAI-S are entered into the model age group is not a significant factor in determining mean RT. Table 32.2 shows the regression coefficients and tests of significance. Again, the zero order correlations are

presented in Table 32.1.

Table 32.2. *Results of regression analyses with iCNV, STAI-S and dummy coded age groups as predictors (N = 52).*

Regression	Significance	*R*	Adjusted R^2	Variable	*B*	S.E. *B*	*β*	t	Significant *p*
Angry RT	< .001	.617	.328	iCNV FCz	5.367	2.959	.211	1.814	.076
				STAI - S	.543	.793	.172	.685	.497
				Early adolescents	39.217	27.455	.364	1.428	.160
				Late adolescents	-11.834	14.613	-.107	-.810	.422
				Constant	286.034	31.978		8.945	<.001
Happy RT	.001	.570	.267	iCNV FCz	.825	2.944	.035	.280	.780
				STAI - S	.286	.751	.100	.381	.705
				Early adolescents	47.418	25.771	.488	1.840	.072
				Late adolescents	1.073	13.953	.011	.077	.939
				Constant	280.826	30.142		9.317	<.001
Neutral RT	<.001	.649	.360	iCNV FCz	7.464	4.417	.279	1.690	.098
				STAI - S	1.165	.718	.355	1.623	.111
				Early adolescents	31.965	26.394	.285	1.211	.232
				Late adolescents	6.413	14.536	.056	.441	.661
				Constant	259.729	30.262		8.583	<.001

Note. The young adult group was used as the reference group for each regression; *B* = unstandardized coefficient; S.E. *B* = standardized error of unstandardized coefficient; *β* = standardized coefficient

The regression model for mean RT in the angry condition with tCNV, STAI-S scores and dummy coded age groups as predictors was significant $F(4, 53) = 6.326$, $p <$ .001 with an R^2 of 34.1%. However, only STAI-S scores approached significance and uniquely explained 4.6% of the variability in mean RT, while tCNV amplitude accounted for only 1.7%. The regression model for mean RT in the happy condition with tCNV, STAI-S scores and dummy coded age groups as predictors was also significant $F(4, 53) = 5.931$, $p < .001$ with an R^2 of 32.6%. Again, only STAI-S scores approached significance and uniquely explained 5.1% of the variability in mean RT, while tCNV amplitude accounted for only 2.1%. The regression model for mean RT in the neutral condition with tCNV, STAI-S scores and dummy coded age groups as predictors was again the most significant of the three regressions $F(4, 53) = 9.914$, $p = <$ .001 with an R^2 of 44.7%. In this instance tCNV amplitude was a statistically significant predictor of mean RT and explained 8.2% of the variability in mean RT, while STAI-S only accounted for 2.7%. Table 32.3 shows the regression coefficients and tests of significance. Again, the zero order correlations are presented in Table 32.1.

Table 32.3. *Results of regression analyses with tCNV, STAI-S and dummy coded age groups as predictors (N = 54).*

Regression	Significance	*R*	Adjusted R^2	Variable	*B*	S.E. *B*	*β*	t	Significant *p*
Angry RT	< .001	.584	.287	tCNV Cz	2.125	1.884	.139	1.128	.265
				STAI - S	1.425	.766	.428	1.859	.069
				Early adolescents	7.810	28.170	.068	.277	.783
				Late adolescents	-10.609	15.331	-.093	-.692	.492
				Constant	249.112	31.378		7.939	<.001
Happy RT	.001	.571	.271	tCNV Cz	2.162	1.724	.155	1.254	.216
				STAI - S	1.385	.717	.499	1.933	.059
				Early adolescents	10.194	26.190	.096	.389	.699
				Late adolescents	3.391	14.353	.032	.236	.814
				Constant	244.695	29.393		8.325	<.001
Neutral RT	<.001	.669	.402	tCNV Cz	4.399	1.634	.301	2.692	.010
				STAI - S	1.089	.706	.322	1.543	.129
				Early adolescents	26.295	25.499	.227	1.031	.307
				Late adolescents	1.790	14.352	.015	.125	.901
				Constant	261.680	29.437		8.890	<.001

Note. The young adult group was used as the reference group for each regression; *B* = unstandardized coefficient; S.E. *B* = standardized error of unstandardized coefficient; *β* = standardized coefficient

In conclusion, while the overall models for mean RT were all statistically significant, these results show that while controlling for other variables in the model, only tCNV amplitude in the neutral condition was a statistically significant predictor of mean RT accounting for roughly 8.2% of the variability in mean RT. The key finding from the results of the regression analyses show that when iCNV, tCNV and STAI-S are entered into the model their respective models, age group is not a significant factor in determining mean RT.

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Appendix 33

Supplementary Tables for Chapter 2

Supplementary Table 2.1. *Descriptive statistics (Mean, SD in brackets) for AQ-10, STAI (state & trait children & adult versions), ERQ (adult & child versions) and DASS-21 questionnaire.*

			Mean (SD)							
			AQ – 10	STAI-S %	STAI-T %	ERQ – CR %	ERQ – ES %	DASS-21 Dep	DASS-21 Anxiety	DASS-21 Stress
Age group	**Gender**	***n***								
Early Adolescents	Male	9	3.44 (1.50)	70.92 (6.29)	58.70 (13.30)	59.25 (14.31)	56.66 (17.67)	4.11 (4.31)	2.66 (2.34)	4.11 (2.84)
	Female	9	2.55 (2.29)	65.92 (4.25)	61.48 (11.62)	57.40 (10.51)	52.77 (22.79)	3.66 (3.46)	4.00 (2.73)	7.00 (3.84)
	Total	18	3.00 (1.94)	68.42 (5.81)	60.09 (12.20)	58.33 (12.22)	54.72 (19.88)	3.88 (3.80)	3.33 (2.56)	5.55 (1.81)
Late Adolescents	Male	9	1.88 (1.53)	37.50 (10.21)	49.72 (13.37)	71.42 (12.31)	53.96 (20.39)	3.55 (4.41)	3.77 (1.30)	5.44 (1.81)
	Female	9	2.22 (1.48)	40.60 (12.24)	51.94 (12.08)	69.04 (14.91)	53.96 (16.88)	2.88 (2.52)	4.22 (4.81)	5.22 (3.49)
	Total	18	2.05 (1.47)	39.09 (11.06)	50.83 (12.41)	70.23 (13.32)	53.96 (18.16)	3.22 (3.50)	4.00 (3.42)	5.33 (2.70)
Young Adults	Male	9	2.77 (1.20)	38.61 (8.03)	42.50 (9.58)	76.45 (8.78)	57.14 (18.47)	1.77 (1.48)	1.11 (0.78)	4.22 (1.71)
	Female	9	1.66 (1.41)	38.75 (7.75)	51.25 (12.91)	73.54 (15.31)	36.90 (17.85)	2.33 (2.64)	3.00 (3.16)	6.11 (4.59)
	Total	18	2.22 (1.39)	38.68 (7.66)	46.87 (11.91)	75.00 (12.20)	47.02 (20.47)	2.05 (2.09)	2.05 (2.43)	5.16 (3.50

Note. AQ = Autism Spectrum Quotient; STAI = State Trait Anxiety Inventory; S = State; T = Trait; ERQ = Emotion Regulation Questionnaire; CR = Cognitive Reappraisal; ES = Emotion Suppression; DASS = Depression Anxiety Stress Scale.

Supplementary Table 2.2. *Descriptive statistics (Mean, SD in brackets) and Cronbach's Alpha for summed scores of STAI, STAI-C, ERQ, ERQ-CA, DASS-21 sub-scales.*

Age Groups	Questionnaire	Sub-scale	*Mean (SD)*	Min	Max	α
Early Adolescents (n = 18)	AQ-10-C	Child version	3.00 (1.94)	0	8	0.58
	STAI-S-C	State	41.05 (3.48)	38	50	0.76
	STAI-T-C	Trait	36.05 (7.32)	24	50	0.85
	ERQ-CA	CR	17.50 (3.66)	11	23	0.78
	ERQ-CA	ES	10.94 (3.97)	5	18	0.89
	DASS-21	Depression	3.88 (3.80)	0	12	0.82
	DASS-21	Anxiety	3.33 (2.56)	0	8	0.49
	DASS-21	Stress	5.55 (3.60)	0	16	0.70
Late Adolescents (n = 18)	AQ-10-A	Adult version	2.05 (1.47)	0	5	0.35
	STAI-S	State	31.27 (8.85)	20	52	0.91
	STAI-T	Trait	40.66 (9.93)	21	60	0.91
	ERQ	CR	29.50 (5.59)	18	38	0.77
	ERQ	ES	15.11 (5.08)	7	25	0.81
	DASS-21	Depression	3.22 (3.50)	0	13	0.81
	DASS-21	Anxiety	4.00 (3.42)	0	14	0.67
	DASS-21	Stress	5.33 (2.70)	2	11	0.41
Young Adults (n = 18)	AQ-10-A	Adult version	2.22 (1.39)	0	5	0.27
	STAI-S	State	30.94 (6.13)	21	41	0.86
	STAI-T	Trait	37.50 (9.53)	24	60	0.93
	ERQ	CR	31.50 (5.12)	23	42	0.71
	ERQ	ES	13.16 (5.73)	4	23	0.89
	DASS-21	Depression	2.05 (2.09)	0	9	0.61
	DASS-21	Anxiety	2.05 (2.43)	0	9	0.75
	DASS-21	Stress	5.16 (3.50)	1	15	0.84

Note. AQ = Autism Spectrum Quotient; STAI = State Trait Anxiety Inventory; S = State; T = Trait; ERQ = Emotion Regulation Questionnaire; CR = Cognitive Reappraisal; ES = Emotion Suppression; DASS = Depression Anxiety Stress Scale.

Supplementary Table 2.3. *Self-report questionnaire scores by group (N=54).*

	Early adolescent	**Late adolescent**	**Young adult**	χ^2/F	***P***
		Mean (SD)			
AQ-10	3.00 (1.94)	2.05 (1.47)	2.22 (1.39)	$F(2, 51) = 1.74$	> 0.10
STAI-S %	**68.42 (5.81)**	**39.09 (11.06)**	**38.68 (7.66)**	**$\chi 2\ (2) = 34.14$**	**< 0.001**
STAI-T %	**60.09 (12.20)**	**50.83 (12.41)**	**46.87 (11.91)**	**$F(2, 51) = 5.58$**	**< 0.01**
ERQ-CR %	**58.33 (12.22)**	**70.23 (13.32)**	**75.00 (12.20)**	**$F(2, 51) = 8.36$**	**< 0.01**
ERQ-ES %	54.72 (19.88)	53.96 (18.16)	47.02 (20.47)	$F(2, 51) = 0.85$	> 0.10
DASS-21-depression	3.88 (3.80)	3.22 (3.50)	2.05 (2.09)	$\chi 2\ (2) = 1.11$	> 0.10
DASS-21-anxiety	3.33 (2.56)	4.00 (3.42)	2.05 (2.43)	$\chi 2\ (2) = 4.40$	> 0.10
DASS-21-stress	5.55 (3.60)	5.33 (2.70)	5.16 (3.50)	$\chi 2\ (2) = 0.42$	> 0.10

Note. AQ = Autism Spectrum Quotient; STAI = State Trait Anxiety Inventory; S = State; T = Trait; ERQ = Emotion Regulation Scale; CR = Cognitive Reappraisal; ES = Emotion Suppression; DASS = Depression Anxiety Stress Scale.

Supplementary Table 2.4. *Group means and standard deviations (SD) for head size, room temperature and errors during trials as well as the total number of trials averaged to compute the ERPs.*

			Mean (SD)				
Age group	**Gender**	***n***	Head size	Room temp*	# Trials (CNV/P1)	# Trials (N170/P1)	Button press errors
Early Adolescents	Male	9	56.66 (2.64)	23.11 (1.45)	135.11 (10.16)	136.33 (9.56)	15.22 (9.95)
	Female	9	55.88 (1.90)	23.11 (1.53)	136.11 (10.97)	139.00 (10.39)	8.77 (5.33)
	Total	18	56.27(2.27)	23.11 (1.45)	135.61 (10.27)	137.66 (9.78)	12.00 (8.42)
Late Adolescents	Male	9	58.38 (1.24)	23.88 (0.60)	137.88 (7.65)	140.66 (8.90)	7.00 (4.15)
	Female	9	55.66 (1.58)	24.11 (0.92)	145.88 (7.11)	147.77 (5.33)	5.22 (3.19)
	Total	18	57.02 (1.96)	24.00 (0.76)	141.88 (8.26)	144.22 (8.00)	6.11 (3.70)
Young Adults	Male	9	59.50 (2.09)	22.77 (1.09)	146.55 (9.86)	147.11 (9.66)	5.33 (2.91)
	Female	9	55.33 (1.41)	22.66 (1.00)	150.11 (4.88)	150.77 (4.52)	5.11 (3.78)
	Total	18	57.41 (2.75)	22.72 (1.01)	148.33 (7.76)	148.94 (7.55)	5.22 (3.28)

Note. Room temp = room temperature in degrees celsius

Supplementary Table 2.5. *Means and standard deviations (SD) for the number trials for each condition for each age group (N = 54).*

		Mean (SD)					
		CNV/P1			N170/P1		
		Angry	Happy	Neutral	Angry	Happy	Neutral
Age Groups	**Gender**						
Early Adolescents (n = 18)	Male	44.88 (4.04)	44.44 (4.03)	45.77 (3.07)	45.33 (3.80)	45.00 (3.87)	46.00 (2.69)
	Female	45.33 (2.82)	45.11 (4.64)	46.77 (3.86)	46.00 (3.27)	45.77 (4.81)	47.22 (3.86)
	Total	45.11 (3.39)	44.77 (4.23)	46.27 (3.42)	45.66 (3.46)	45.38 (4.25)	46.11 (3.29)
Late Adolescents (n = 18)	Male	47.00 (3.74)	44.77 (3.59)	46.11 (1.96)	48.11 (4.13)	45.33 (3.96)	47.22 (1.64)
	Female	49.77 (2.33)	48.66 (2.50)	47.44 (3.46)	50.22 (2.16)	49.11 (2.42)	48.44 (1.87)
	Total	48.38 (3.34)	46.72 (3.61)	46.77 (2.81)	49.16 (3.38)	47.22 (3.73)	47.83 (1.82)
Young Adults (n = 18)	Male	48.66 (3.04)	48.88 (4.16)	49.00 (3.70)	48.66 (3.04)	49.00 (3.87)	49.44 (3.77)
	Female	49.44 (2.29)	50.11 (2.52)	50.55 (1.81)	49.77 (2.16)	50.22 (2.27)	50.77 (1.92)
	Total	49.05 (2.64)	49.50 (3.39)	49.77 (2.94)	49.22 (2.62)	49.61 (3.14)	50.11 (2.98)

Supplementary Table 2.6. *Means and standard errors (SE) for visual P1 peak amplitude in response to the predictive cue stimuli at O1 and O2 (N=51).*

	Early adolescent	**Late adolescent**	**Young adult**	**1***	**2***
Electrode/condition		*Mean (SE)*		*p*	*p*
Left hemisphere					
O1/angry	9.179 (.730)	3.953 (.730)	3.620 (.730)	< .001	< .001
O1/happy	8.880 (.741)	4.756 (.741)	3.336 (.741)	.004	< .001
O1/neutral	8.979 (.859)	4.316 (.859)	3.396 (.859)	.007	.001
Right hemisphere					
O2/angry	10.387 (.787)	3.858 (.787)	3.072 (.787)	< .001	< .001
O2/happy	10.477 (.823)	4.633 (.823)	3.032 (.823)	< .001	< .001
O2/neutral	10.097 (.983)	3.983 (.983)	3.757 (.983)	.002	.001

Note. 1 = early adolescents > late adolescents, 2 = early adolescents > young adults

Appendix 34

Supplementary Tables for Chapter 4

Supplementary Table 4.5. *Uncorrected correlations between clinical features in PNES and clusters surviving cluster-wise correction in patients with PNES (n = 20).*

	Cluster Annotation		Age at onset	Duration of symptoms	Symptom severity	Number AEDs taken
Spearman's rho	**Left pars opercularis**	Correlation Coefficient	-0.17	-0.18	0.16	-0.08
		Sig. (2-tailed)	0.47	0.43	0.47	0.71
	Left paracentral	Correlation Coefficient	-0.10	0.14	0.03	0.15
		Sig. (2-tailed)	0.66	0.55	0.90	0.51
	Left cuneus	Correlation Coefficient	-0.17	0.11	**0.49***	0.19
		Sig. (2-tailed)	0.47	0.63	**0.02**	0.41
	Left lingual	Correlation Coefficient	-0.13	-0.02	0.21	-0.03
		Sig. (2-tailed)	0.59	0.92	0.37	0.90
	Right lateral occipital	Correlation Coefficient	-0.21	-0.02	0.18	0.05
		Sig. (2-tailed)	0.35	0.91	0.44	0.81
	Right superior temporal 1	Correlation Coefficient	-0.29	0.22	0.25	0.39
		Sig. (2-tailed)	0.20	0.33	0.28	0.08
	Right superior temporal 2	Correlation Coefficient	-0.43	0.09	0.17	0.01
		Sig. (2-tailed)	0.05	0.68	0.46	0.94
	Right pars opercularis	Correlation Coefficient	-0.12	-0.18	-0.01	-0.18
		Sig. (2-tailed)	0.61	0.43	0.94	0.42
	Right cuneus	Correlation Coefficient	-0.11	0.06	**0.45***	0.13
		Sig. (2-tailed)	0.63	0.77	**0.04**	0.57
	Right medial orbitofrontal	Correlation Coefficient	0.00	-0.14	-0.17	-0.03
		Sig. (2-tailed)	0.98	0.54	0.45	0.87

PNES = Psychogenic non-epileptic seizures; AEDs = anti-epileptic drugs; *. Correlation is significant at the 0.05 level (2-tailed). Results are uncorrected for multiple comparisons.

Appendix 35

Flow chart for EEG preprocessing (Chapter 2)

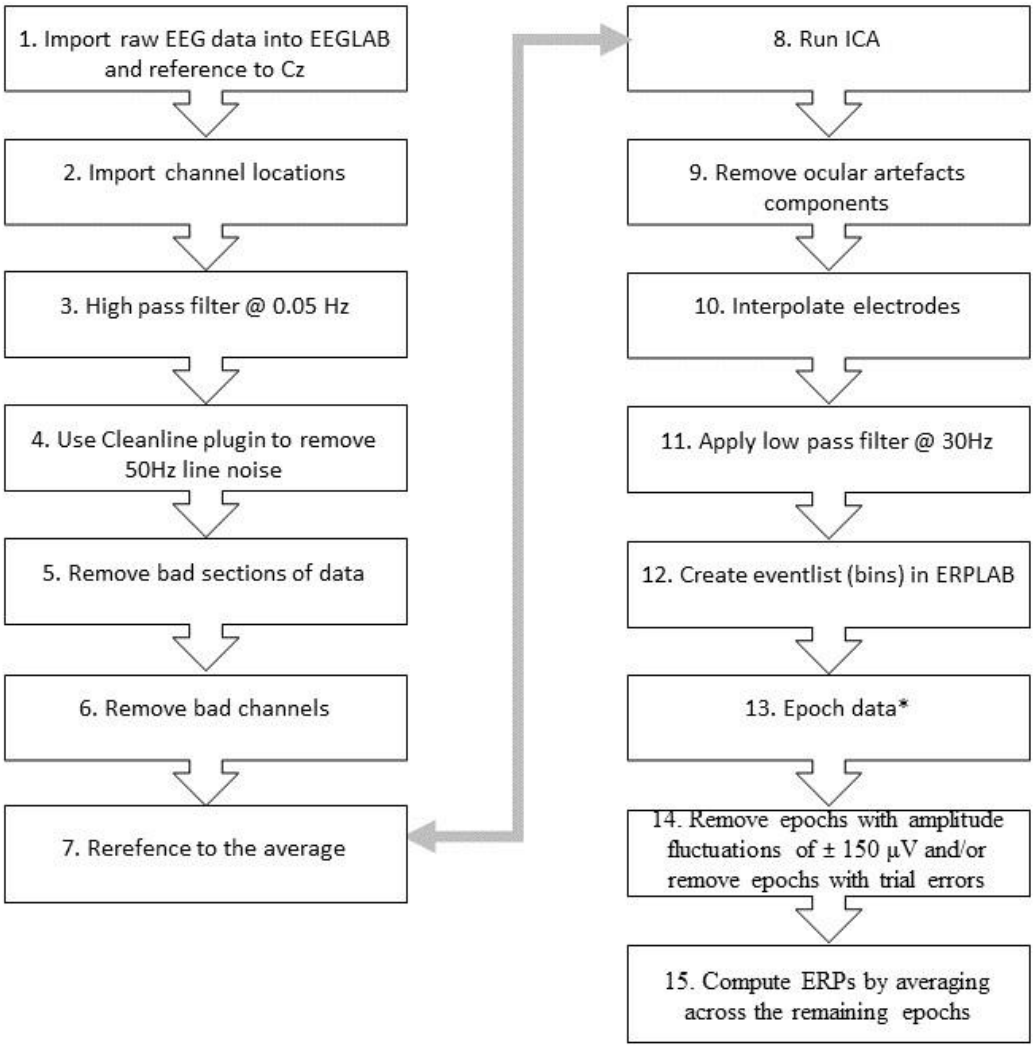


Figure 35.1. Flow chart showing steps taken to preprocess the raw EEG data. *Note.* Step 13. Epoched data*. For CNV analyses, data was epoched -500ms to 5000ms locked to S_1 onset with a -500ms to 0ms baseline correction. For P1 analyses to S_1, data was epoched -200ms to 1000ms, locked to S_1 onset, with a -200ms to 0ms baseline correction. For P1/N170 analyses to S_2, data was epoched -200ms to 1000ms, locked to S_2 onset, with a -200ms to 0ms baseline correction.

Appendix 36

Measuring ERP amplitudes (Chapter 2)

Step 1. Once each ERP was computed for each participant for each condition (CNV locked to S_1, visual P1 locked to S_1, Visual P1 locked to S_2, and N170 locked to S_2), each participants ERP was loaded into the ERPLAB measurement tool (Figure 36.1).

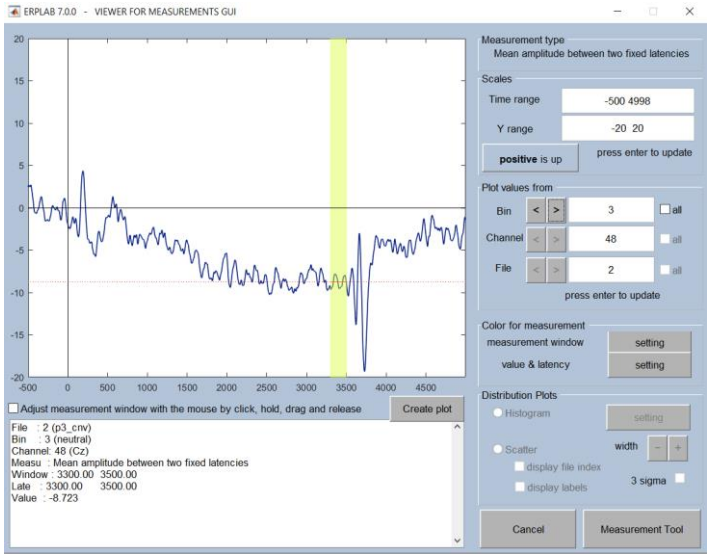


Figure 36.1. Example of taking a measurement of the average mean amplitude in a latency window of 200ms prior to S_2 onset (tCNV) for one participant for one condition (bin 3: neutral condition) at channel Cz.

Step 2. Each measurement for each participant for each condition was saved as a text file and imported into SPSS for further analyses.

Appendix 37

Procedure used for ALE meta-analyses Study 2 (Chapter 3)

Step 1. Create text file containing all of the reported coordinates in Talairach or MNI space in addition to information about the study Authors, year and the number of subjects in each study (Figure 35.1 a).

a) **b)**

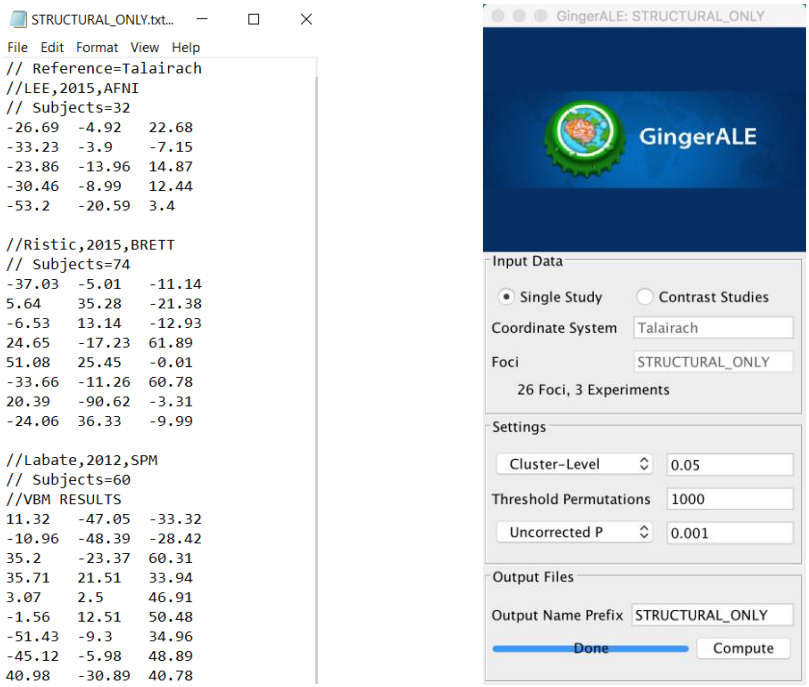


***Figure 37.1*.(a)** Example of text file used in ALE meta-analysis. **(b).** GingerAle interface.

Step 2. Load foci into GingerAle using File > Load foci (Figure 35.1 b).

Step 3. Select Single Study, Coordinates System > Talairach

Step 4. Under Settings, select multiple comparison correction by using either voxelwise (uncorrected values, False Discovery Rate FDR or Family Wise Error FWE), or alternatively by conducting multiple comparison correction on the cluster-level. Select the number of Threshold Permutations to run.

Step 5. Select Compute to implement the coordinate based ALE algorithm. GingerAle will then compute cluster statistics on the brain regions above the selected thresholds. These include volume, bounds, weighted centre and the locations and values of the peaks within the brain regions. The GingerAle output files include an unthresholded ALE score image, an unthresholded *P* value image, a thresholded ALE image, and statistic text files. All images are in NIfTI format (http://nifti.nimh.nih.gov) and can be viewed in any compatible medical imaging/neuroimaging software packages.

Step 6. To view the results, open Mango (rii.uthscsa.edu/mango) and select Open > Open image > select a template (colin1.1.nii). Click on File > Add Overlay and select the thresholded image (E.g. *_p001.nii, *_C05.nii, *_FWE05.nii. etc.). Then choose Edit > Update Image Range (Figure 35.2).

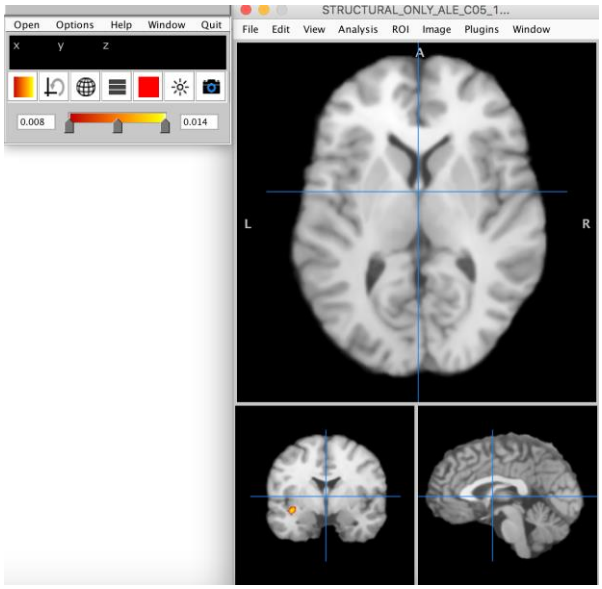


Figure 37.2. Example of thresholded image as viewed in Mango.

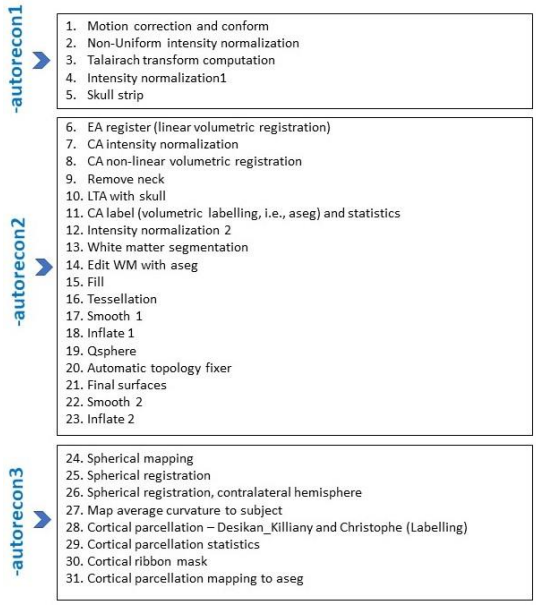
Appendix 38

Procedure used for MRI and *I*GI Study 3 (Chapter 4)

FreeSurfer

FreeSurfer is an open-source software package comprised of automated tools used for the visualisation and analyses of both cortical and subcortical anatomy and functional neuroimaging data. Again, as described in Chapter 4 (Section 4.2.3), the implementation of FreeSurfer algorithms results in two preprocessing streams. The surface-based stream constructs models of the white matter (WM) / gray matter (GM) boundary and the boundary between the GM and cerebralspinal fluid (pial surface) from which cortical thickness, cortical surface area, and cortical folding patterns at each point on the cortical surface can be measured (Dale, Fischl, & Sereno, 1999). The volume-based stream preprocesses MRI volumes and labels subcortical tissue classes allowing for the representation and measurement of subcortical structures (putamen, hippocampus, amygdala, ventricles etc; Fischl et al., 2002, 2004b). Both cortical and subcortical labelling is based on a subject-independent atlas and the subject-specific values. The fully automated FreeSurfer pipeline consists of several stages (Table 38.1). For a visual representation of each step and at which points edits are made (if required) see Figure 38.2 (downloaded from http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingDataV6.0). Steps taken are the same as those used in FreeSurfer Version 5.3.0.

Table 38.1. *Recon-all processing pipeline*



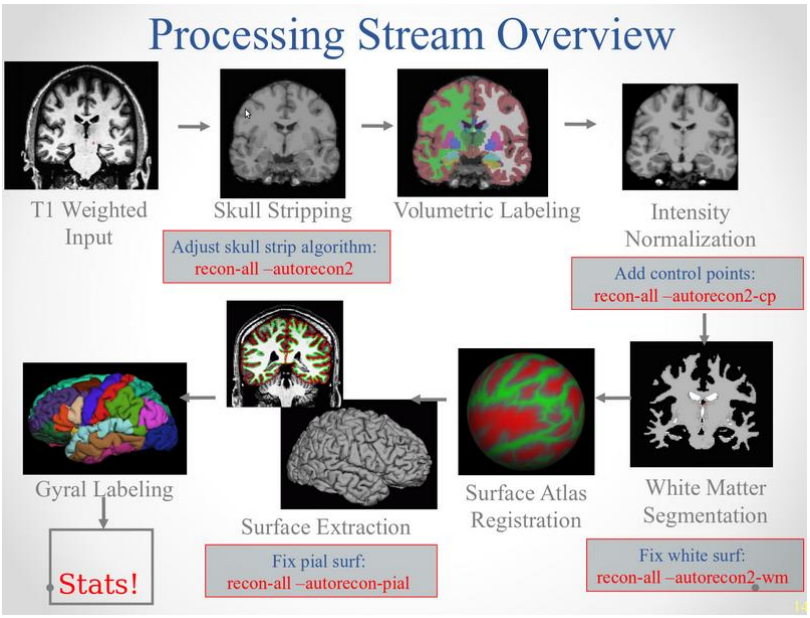


Figure 38.1. Visual depiction of FreeSurfer processing pipeline.

Running automated reconstructions using High Performance Computing cluster (HPC, Iceberg)

Accessing iceberg from an Apple Workstation

1. Access Icberg using Terminal Access - Unix Style "X11-terminal" access.

2. Open a new terminal window and type: ssh -X <username>@iceberg.sheffield.ac.uk
If -*X* parameter in step(2) above does not work try -*Y* instead.

3. Then login with your iceberg password.

Note. When using FreeSurfer, it is advisable to use the /data directory rather than the /home directory to save data, as /data allocates 100G and /home allocates only 10G. To see allocated memory simply type: *quota*. To view the directory, you are working in type: *pwd*. To view contents of that directory type: *ls*

4. Transfers data between MAC workstations and Iceberg using Filezilla (https://filezilla-project.org/). Simply drag and drop files to/from iceberg as required.

Host = sftp://iceberg.shef.ac.uk
Enter username
Enter password
Select port 22

5. To run the entire reconstruction with one command for one subject type: recon-all –autorecon-all -subjid <subject name>

Alternatively, you can run the reconstruction in 3 stages with the following commands:

1. *recon-all –autorecon1 -subjid <subject name>* (Stages 1-5) ~45 min
    - Check talairach transform, skull strip, normalization

2. *recon-all –autorecon2 -subjid <subject name>* (Stages 6-22) ~20 hours
    - Check surfaces
    - 1.Add control points: recon-all –autorecon2-cp (Stages 10-22)
    - 2.Edit wm.mgz: recon-all –autorecon2-wm (Stages 13-22)
    - 3.Edit brain.mgz: recon-all –autorecon2-pial (Stage 19-22)

3. *recon-all –autorecon3 –subjid <subject name>* (Stages 23-29) ~6 hours

6. To run recon-all on all subjects in the study, create a text file containing all the commands, save it as .sh file extension and call it *my_job.sh* (say), and then submit that with qsub: *qsub my_job.sh.* For example, the text file might contain the following commands:

```
#!/bin/bash
#$ -l h_rt=40:00:00 -l mem=4G -l rmem=4G
#$ -M ...@sheffield.ac.uk
#$ -m a
#$ -t 1-5
module load apps/binapps/freesurfer/5.3.0
export SUBJECTS_DIR=/data/<username>/job1/s_MRI_PNES_data
cd $SUBJECTS_DIR
recon-all -autorecon-all -subjid MRI_$SGE_TASK_ID
```

Note: -l h_rt determines run time; *-l mem* determines virtual memory; *-l rmem* determines real memory; The *-t* means run job-tasks numbered 1 to 5. The number of an individual task is stored in the variable *$SGE_TASK_ID*.

To check on job progress log onto Iceberg and enter the following: *qstat –u <username>*

Note. If you enter *qstat* on its own it will report all jobs currently running on Iceberg. If you enter this command and no jobs appear to be running, this might mean that Iceberg has finished the job or that errors occurred during batch processing.

Once Iceberg has finished the job (this could take anything between 10 and 20 hours) you can check to see if everything worked o.k by entering *tail my_job.sh.o118789.1* for example. Use the tail command as this will only display the last job sequence rather than showing you the whole thing.

If you get the message that *recon-all –s s_1* finished without errors, then you can move on to checking the reconstruction for errors.

If you get the message that recon-all exited with errors you will need to check when and where the error occurred. This will be indicated below the exited with errors message when you checked the tail of that job with the command used above (*tail my_job.sh.o118789.1* for example).

Quality control workflow (soft failures)

Soft failures can occur during the FreeSurfer recon-all pipeline. It is very important to check to see if this has occurred, and if so, manual interventions will be required. The most common soft failures are listed below. Each of these was checked for in every T1-weighted brain MRI scan used in Study 3 (Chapter 4). The commands used to check for these errors are presented below. Hard failures occur when FreeSurfer fails to complete all of the processing steps (exits with an error).

Note: Any manual interventions needed can be done all at once (with the exception of

the talairach and the skullstrip). It is important to check how the surfaces appear before and after manual intervention. It is also advisable to check for errors by inspecting the coronal, sagittal and axial planes.

1. Talairach registration – check the transform by loading it visually into freeview or tkregister2. Check for any changes in orientation (the coronal view of the subject should be the coronal view of the talairach transform). Severe changes in positioning and large rotations or twisting will need to be addressed.

2. Skullstrip – In some cases the skullstrip may have failed to remove parts of the skull and/or some of the dura, i.e. extended pial to include parts of dura. To check use the following command:

*freeview –v mri/T1.mgz *
*mri/brainmask.mgz *
*-f surf/lh.white:edgecolor=blue *
*surf/lh.pial:edgecolor=red *
*surf/rh.white:edgecolor=blue *
surf/rh.pial:edgecolor=red

Note. When checking skullstrip errors, load the brainmask.mgz, T1.mgz, (aseg.mgz if you wish) and also lh.pial and rh.pial for each participant and move through the slices (coronal view) from the front of the brain to the back and check for errors (is too much taken away – parts of cerebellum missing etc. – is too little removed i.e. parts of skull still clearly visible). Switch between T1 and brainmask volumes to check for errors and check coronal, sagittal and axial views. The following command is useful when not enough is removed. This reduces the watershed level to make the cuts more aggressive – the default is 25: *recon-all -skullstrip -wsthresh 15 -clean-bm -no-wsgcaatlas –subjid <subject name>*

Once all of the skullstrip errors have been fixed, re-run the recon-all from this point by using the following command: *recon-all -autorecon2 -autorecon3 -subjid <subject name>*

3. White matter segmentation errors – These can occur due to intensity normalization failures or partial voluming. White matter segmentation errors can result in grey matter

(GM) being classified as white matter (WM), WM being classified as GM and topological defects (holes or handles).

1. To check for intensity normalization failures and add control points to fix intensity normalization, use this command:

*freeview –v mri/aparc+aseg.mgz:colormap=lut:opacity=0.75 *
*mri/T1.mgz *
*mri/brainmask.mgz *
*mri/brain.finalsurfs.mgz *
*mri/wm.mgz:colormap=heat:opacity=0.4 *
*-f surf/lh.white:edgecolor=blue:edgethickness=1 *
*surf/lh.pial:edgecolor=red:edgethickness=1 *
*surf/rh.white:edgecolor=blue:edgethickness=1 *
surf/rh.pial:edgecolor=red:edgethickness=1

Note: Control points should be used sparingly and added in WM with values that are close to but not 110. Also, it is important to view each slice in each orientation, with the coronal and sagittal being the most useful. Also, control points should be used sparingly and not be added on every single slice.

Re-run recon-all from this point by using the following command:
recon-all -autorecon2-cp -autorecon3 -subjid <subject name>

2. To check if white matter (WM) is classified as non-WM or grey matter (GM) is classified as WM use the following commands:

*freeview –v mri/brainmask.mgz *
*mri/wm.mgz:colormap=heat:opacity=0.4 *
*-f surf/lh.white:edgecolor=blue *
*surf/lh.pial:edgecolor=red *
*surf/rh.white:edgecolor=blue *
*surf/rh.pial:edgecolor=red *
*surf/rh.inflated:visible=0 *
surf/lh.inflated:visible=0

Click on the **Recon Edit** option and enter a brush size of either 1 or 2. Make sure edits are made to the voxels in the wm.mgz and not the brainmask.mgz. Edits to the brainmask.mgz are made for pial errors and not WM segmentation errors.

Note: Again, it is important to view each slice in each direction. What at first might appear to be an error (coronal view), when viewed from a different angle may in fact not be an error.

Re-run recon-all from this point by using the following command:
recon-all -autorecon2-wm -autorecon3 -subjid <subject name>

3. To check for topological defects use the following command:

*freeview –v mri/brainmask.mgz *
*mri/wm.mgz:colormap=heat:opacity=0.4 *
*mri/T1.mgz:visible=0 *
*mri/aseg.mgz:colormap=lut:opacity=0:visible=0 *
*-f surf/lh.smoothwm.nofix:visible=0 *
surf/rh.smoothwm.nofix:visible=0

Note: Holes refer to segmentation errors where real WM has been excluded from the wm.mgz. Handles refer to segmentation errors in WM reconstruction which results in bridging between sulci (inclusion of non-WM in WM mask). If there are a lot of holes and handles run topological fixer again: *recon-all –fix –s <subject name>*. Once this has finished fix any remaining defects followed by *recon-all –autorecon-wm –autorecon3 –s <subject name>*. If required, edit the wm.mgz using the to **Recon Edit** option to add voxels in the wm.mgz where they are missing (holes) and remove voxels where they are not WM (handles).

4. Editing brainmask.mgz / correcting pial surfaces – in FreeSurfer pial surfaces are generated form the white surfaces and inaccuracies can occur where the pial surface extends beyond its boundaries to include dura, blood vessels etc.

To check to see if the pial surfaces are accurate use following command:

*freeview -v mri/T1.mgz *
*mri/brainmask.mgz *
*-f surf/lh.white:edgecolor=yellow *
*surf/lh.pial:edgecolor=red *
*surf/rh.white:edgecolor=yellow *
surf/rh.pial:edgecolor=red

Once edits to the pial surfaces have been made, use the following command to re-run recon-all: *recon-all -autorecon-pial -subjid <subject name>*

On final recon-all use -qcache flag to 1) resample the data onto the average subject, and 2) smooth the data with a range of FWHM values, 0, 5, 10, 15, 20 and 25mm!

GLM analyses and correction for multiple comparisons (command line)

Step 1. Create FreeSurfer Group Descriptor text file (fsgd) containing your Discrete Factors (Group, Gender) and continuous variables such as age, weight, IQ etc… and save it in your *$SUBJECTS_DIR* directory with an appropriate name: *pnes__hc_ct.txt*

GroupDescriptorFile 1
Class PNES_Male
Class PNES_Female
Class HC _Male
Class HC_Female
Variables Age
Input <subject ID 1> PNES_Male 25
Input <subject ID 2> HC_Female 47
Input <subject ID 3> HC_Male 25
Input <subject ID 4> PNES_Female 25

Step 2. Create a text file to be used as your Contrast (Vector that defines your hypothesis). For example, in the text file input "+1 +1 -1 -1 0" to calculate the difference between PNES and HC groups while controlling for gender and age (different offset, same slope; DOSS) and save it as Conrast.txt. If using different offset, different slope (DODS), the Contrast text file will be different as you will be modelling both the intercept and slope for each group. When modelling the interaction between group and age while controlling for gender i.e., slope and not intercept, input the following into the Contrast text file "0 0 0 0 +1 +1 -1 -1"

Step 3. For cached data (-qcache flag used during recon-all) type in the following in terminal to run the GLM:

mri_glmfit --y lh.thickness.sm10.mgh --fsgd pnes__hc_ct.txt doss --C Contrast.txt --surf fsaverage lh --cortex --glmdir lh_pnes_hc_ct.glmdir

Note: This runs the GLM for the left hemisphere only (*lh*) with a FWHM smoothing kernel of 10mm. Repeat for right hemisphere (*rh*). Output files are saved to the *glmdir lh_pnes_hc_ct.glmdir*. **These are uncorrected results!**

Step 4. Use cluster-wise correction for multiple comparisons (other options include FDR and a more recent version for permutation). This simulation will **1)** synthesise a z map **2)**, smooth z map (using residual FWHM), **3)** threshold z map, **4)** find clusters in thresholded map, **5)** record area of maximum cluster, **6)** repeat over the specified number of iterations (10,000), **7)** apply these thresholds to the original data to determine under the null-hypothesis, the probability of finding clusters in the simulated data that size or larger. To run the simulation, use the following commands:

mri_glmfit-sim --glmdir lh_pnes_hc_ct.glmdir --cache 3.0 abs --cwp 0.05 --2spaces

Note: --cache 3.0 is the cluster-forming threshold ($p < 0.001$). *--cwp 0.05* is the cluster-wise *p* value ($p < 0.05$). To see all cluster this can be set to 0.999. *--2spaces* adjusts the *p* value for the 2 hemispheres (Bonferroni correction).

Step 5. The glmdir will contain numerous files, two of which will be a cluster-wise corrected map (overlay – cache.th30.abs.sig.mgh) and a summary table of the results in text format (clusters – cache.th30.abs.sig.cluster.summary). The summary table can be viewed in the Terminal using the *less* command followed by the path to the file. To view the results in freeview enter the following command:

*freeview -f *
$SUBJECTS_DIR/fsaverage/surf/lh.inflated:overlay= lh_pnes_hc_ct.glmdir
*/lh.cache.th30.abs.sig.mgh/annot=lh_pnes_hc_ct.glmdir/cache.th30.abs.sig.ocn.annot *
Viewport 3d -layout 1

Additional information on the local Gyrification Index (*l*GI) and its measurement

The *l*GI is inspired by the Gyrification Index (GI; Zilles, Armstrong, & Schleicher, & Kretschmann, 1988), a method previously used to compare cortical folding patterns across species. The GI is the ratio of the total folded surface across the perimeter of the cortex as delineated on 2-D coronal slices (Zilles, Armstrong, & Schleicher, & Kretschmann, 1988). However, this approach to the measurement of gyrification is problematic. For example, surfaces are delineated on 2-D coronal slices which do not account for the fact that the cortical surface is in reality a 3-D surface. Perimeter measurements may be affected by slice orientation and may not take into account buried sulci. Manual delineation is often used. This raises concerns about subjectivity and reproducibility, particularly in larger studies which involve the use of many brains (Schaer et al., 2008). The *l*GI attempts to address these issues by quantifying and comparing the local gyrification patterns at thousands of points over the cortical surfaces generated in FreeSurfer for each hemisphere. First an outer surface is created using a morphologically closing algorithm. Then, roughly 800 overlapping 3-D circular regions of interest (ROIs) are created on the outer surfaces. For each of these ROIs, a corresponding ROI is defined for the pial surfaces. In essence, the *l*GI is a ratio of the amount of cortical surface invaginated in the sulci to the amount of cortical surface of corresponding ROI on the pial surfaces. The *l*GI results in an individual map containing one *l*GI value at each point on the cortical surfaces (~150,000 per hemisphere) (Schaer et al., 2012). To following steps were used to compute *l*GI values used in Study 3 (Chapter 4):

Step 1. Following reconstruction and inspection of the brain surfaces output by FreeSurfer, use the following command to compute the *l*GI: *recon-all -s <subject name> -localGI*

*Note: l*GI values for each subject were calculated in Matlab. To do this you need to have the Image Processing Toolbox installed to Matlab and the *?h.pial* surface files for each subject to already exist in the subject's *<subj>/surf* directory. You will also need *$FREESURFER_HOME/matlab* in your matlab path set up in your *~/matlab/starup.m* script.

Step 2. Check the results of the *l*GI computation for each subject in your study**.** Change your current working directory to your subjects directory and load the *l*GI overlay in tksurfer: *tksurfer lh pial -overlay /surf/lh.pial_lgi -fthresh 1*

Note: This checks the *l*GI values at each of the vertices for the left hemisphere (lh). To check the right hemisphere use *rh pial* instead. Typical *l*GI values are between 1 and 5, so setting the minimum threshold to 1 (*-fthresh 1*) allows you to quickly check the results of the *l*GI computation for each subject in the study. *l*GI maps should not show any gray cortical areas. To check your current working directory, use the *pwd* command. To change directory to a subjects directory, use the *cd* command followed by <subjects directory name>.

Step 3. Create the FSGD text file and Contrast text file to be used for the GLM model. These steps are the same as those used for the cortical thickness analyses outlined above.

Step 4. Resample the *l*GI data in the common space (*fsaverage*): *mris_preproc --fsgd FSGD.txt --target fsaverage --hemi lh --meas pial_lgi --out lh.lgi.mgh.*

Step 5. Smooth the data on the cortical surface with the desired smoothing kernel (0, 5, 10, 15, 20, 25mm): *mri_surf2surf --hemi lh --s fsaverage --sval lh.lgi.mgh --fwhm 0 –tval lh.0.lgi.mgh*

Step 6. Run the GLM analyses: *mri_glmfit --y lh.0.lgi.mgh --fsgd PNES__HC_LGI.txt doss --glmdir lh_pnes_hc_lgi.glmdir --surf fsaverage lh --C Contrast.txt*

Step 7. Correct for multiple comparisons: *mri_glmfit-sim --glmdir lh_pnes_hc_lgi.glmdir --cache 3.0 abs --cwp 0.05 --2spaces*

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage*, *9*(2), 179-194.

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., ... & Montillo, A. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron*, *33*(3), 341-355.

Fischl, B., Van Der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D. H., ... & Caviness, V. (2004b). Automatically parcellating the human cerebral cortex. *Cerebral cortex*, *14*(1), 11-22.

Schaer, M., Cuadra, M. B., Tamarit, L., Lazeyras, F., Eliez, S., & Thiran, J. P. (2008). A surface-based approach to quantify local cortical gyrification. *IEEE transactions on medical imaging*, *27*(2), 161-170.

Zilles, K., Armstrong, E., Schleicher, A., & Kretschmann, H. J. (1988). The human pattern of gyrification in the cerebral cortex. *Anatomy and embryology*, *179*(2), 173-179.