Behavioural inhibition as an early marker of anxiety in children at risk for Autism Spectrum Disorders

Ersoy, Mutluhan

Awarding institution: King's College London

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Behavioural Inhibition as an Early Marker of Anxiety in Children at Risk for

Autism Spectrum Disorders

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This thesis is submitted to King’s College London for the degree of Doctor of Philosophy

2019
Declaration

The data presented in this PhD thesis were collected from the Phase 2 (Chapter 2, 3) and Phase 3 (Chapter 4, 5) cohorts of the British Autism Study of Infant Siblings (BASIS) project (www.basisnetwork.org). I joined the BASIS study at the beginning of the 24-month data collection of Phase 3. I contributed the adaption and implementation/oversight of the observational measures the Stranger Approach and the Unpredictable Toy tasks (summarised in Chapter 4). I was also involved in the arranging testing days, testing participants at 24 and 36-month time points, collecting, entering and analysing data. I tested 24- and 36-month-old toddlers on experimental tasks (on eye-tracking, EEG and behavioural tasks), administering clinical (the Autism Diagnostic Observational Schedule) and developmental measures (the Mullen Scale of Early Learning). In Chapter 2 and 3, the data were from Phase 2 of the BASIS project, which was collected by the BASIS team before I started my PhD. However, I got permission from the study PIs (Professor Mark Johnson and Professor Tony Charman) to access and analyse the data under existing ethical permissions. In Chapter 2, I analysed the longitudinal data by using structural equation modelling. In Chapter 3, I developed a novel coding scheme to apply previously collected data to capture temperamental dispositions. I involved in the data collection, entry and analysis of the data that were used in Chapter 5.
Acknowledgements

I would like to sincerely thank my supervisors Professor Tony Charman and Professor Emily Jones for giving me a unique opportunity to work with them on the BASIS project. Without their generous support and encouragements, I would not be able to finish this PhD. I have learnt a lot from them during my PhD, thank you!

I also would like to thank the Ministry of Turkish Education for funding my postgraduate education. Without this huge support, I would not be able to be here.

I must say a huge thank you to all the current and past members of the Autism and Development Team: Tessel Bazelmans, Lauren Taylor, Sophie Carruthers, Bosiljka Milosavljevic, Greg Pasco, Francisca Monteiro, Mary Agyapong, Julian Tillman, Alex Hendry, Sabira Habib, Eleanor Janega. It was amazing to work with you.

I would like to thank my parents Afife and Ismail Ersoy for their encouragement and support on this journey. I am also grateful to my dearest friend Tabita Zorbaz who was there for me at all the time. I must also thank Debbie Harte, she has been so supportive and made me feel at home during the past four years.

Finally, I would also like to thank all families who participated in the BASIS for their collaboration and enormous contribution.
Abstract

Autism Spectrum Disorder (ASD) is a heritable neurodevelopmental condition that is characterised by social communication impairments, restricted and repetitive behaviours, and sensory anomalies. Anxiety is one of the marked co-occurring psychiatric conditions in individuals with ASD and the underlying mechanism of this co-occurrence has not been fully understood. This is because studies have focused on mid-childhood or adolescence when the interplay between genetic and environmental risk factors make it harder to disentangle the overlap between symptoms of anxiety and ASD. Temperament traits in infancy, especially behavioural inhibition (BI) which is a temperament trait that is involved in the aetiology of the childhood anxiety in the general population, may be an informative target to explore roots of this interplay prior to the consolidation of both disorders.

The current thesis employs a multi-method approach to investigate the association between BI and anxiety in two cohorts of infants at high- and low-familial risk for ASD. Participants of Chapter 2 and 3 were drawn from the second phase of the British Autism Study of Infant Siblings (BASIS; this study is complete and outcome grouping is available). In Chapter 2, longitudinal associations between parent-reported BI, effortful control, anxiety and ASD traits were examined using cross-lagged panel models. In Chapter 3, temperament traits were measured during the Autism Observational Schedule for Infants (AOSI; 15 months) by using a new observational coding scheme. Further analyses investigated whether observed individual differences relate to AOSI scores, anxiety and ASD traits at 36 months. In Chapter 4, factor scores for social and non-social BI were generated at 24 months by using observational, parent-reported and global ratings of BI. In Chapter 5, the associations between parental and child anxiety and ASD trait was examined.
Participants of Chapter 4 and 5 were drawn from the third phase of the BASIS project. Both parent-reported and observed BI was associated with higher levels of anxiety but not ASD traits. Parental characteristics (anxiety and ASD traits) were related to child characteristics. Overall, these findings suggest that similar to the general population, BI is involved in the aetiology of early emerging anxiety traits in toddlers at risk of ASD. The consistent association between BI and anxiety across chapters suggest that there may be separate developmental pathways for anxiety and ASD. So, BI may provide a translational target for pre-emptive interventions.
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<th>Description</th>
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<tbody>
<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>ADI-R</td>
<td>Autism Diagnostic Interview-Revised</td>
</tr>
<tr>
<td>ADOS</td>
<td>Autism Diagnostic Observation Schedule</td>
</tr>
<tr>
<td>AOSI</td>
<td>Autism Observation Schedule for Infants</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism Spectrum Disorder</td>
</tr>
<tr>
<td>BAP</td>
<td>Broad Autism Phenotype</td>
</tr>
<tr>
<td>BASIS</td>
<td>British Autism Study of Infant Siblings</td>
</tr>
<tr>
<td>BI</td>
<td>Behavioural Inhibition</td>
</tr>
<tr>
<td>CBCL</td>
<td>Child Behaviour Check List</td>
</tr>
<tr>
<td>CFA</td>
<td>Confirmatory Factor Analysis</td>
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<tr>
<td>CFI</td>
<td>Comparative Fit Index</td>
</tr>
<tr>
<td>CSS</td>
<td>Calibrated Severity Scores</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual</td>
</tr>
<tr>
<td>EC</td>
<td>Effortful Control</td>
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<tr>
<td>ECBQ</td>
<td>Early Childhood Behaviour Questionnaire</td>
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<tr>
<td>GAD</td>
<td>Generalised Anxiety Disorder</td>
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<tr>
<td>HR</td>
<td>High-Risk</td>
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<tr>
<td>IBQ-R</td>
<td>Infant Behaviour Questionnaire-Revised</td>
</tr>
<tr>
<td>LR</td>
<td>Low-Risk</td>
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<tr>
<td>MSEL</td>
<td>Mullen Scales of Early Learning</td>
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<td>PDD</td>
<td>Pervasive Developmental Disorder</td>
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<tr>
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<td>Parent-Report</td>
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<td>Q-Chat</td>
<td>Quantitative Checklist for Autism in Toddlers</td>
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<tr>
<td>Abbreviation</td>
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<tr>
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<tr>
<td>RMSEA</td>
<td>Root Mean Square Error of Approximation</td>
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<tr>
<td>RRB</td>
<td>Restricted Repetitive Behaviours</td>
</tr>
<tr>
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<td>Social Affect</td>
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<tr>
<td>SCQ</td>
<td>Social Communication Questionnaire</td>
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Chapter 1. A General Introduction to Autism Spectrum Disorder, Anxiety and Behavioural Inhibition as an Early Marker for Co-occurring Anxiety

1.1. Introduction

Autism Spectrum Disorder (ASD) is a multifactorial neurodevelopmental condition, which affects approximately 1% of the population (Baird et al., 2006; Brugha et al., 2011; Christensen et al., 2016). The core symptoms of ASD involve varying degrees of impairments in 1) verbal and nonverbal social communication, and 2) the presence of rigid interest, repetitive behaviours, and sensory abnormalities (American Psychiatric Association, 2013). These core diagnostic symptoms emerge in early childhood and influence social life and adaptive functioning (Bishop-Fitzpatrick, Mazefsky, Eack, & Minshew, 2017; Kanne et al., 2011; Klin et al., 2007). ASD is a heterogeneous condition with co-occurring psychiatric and medical conditions (e.g., Rosen, Mazefsky, Vasa, & Lerner, 2018; Soke, Maenner, Christensen, Kurzius-Spencer, & Schieve, 2018). Among co-occurring psychiatric conditions, anxiety problems are highly prevalent and up to 80% individuals with ASD also have these disorders (Salazar et al., 2015; Simonoff et al., 2008; van Steensel, Bogels, & Perrin, 2011). It is now well-established that the presence of anxiety, in addition to ASD, can contribute to difficulties in the daily life of individuals with ASD (Chang, Quan, & Wood, 2012) and consequently, may affect their long-term prognosis. However, the reason for the high overlap between ASD and anxiety and whether anxiety within ASD has the same nature as anxiety among individuals who do not have ASD has remained unclear.

There have been substantial investigations to understand why individuals with ASD also experience higher degrees of anxiety (e.g., Duvekot, van der Ende, Verhulst, & Greaves-Lord, 2018; Kerns & Kendall, 2012; Wood & Gadow, 2010), but the roots
of anxiety in ASD is not clear. Specifically, it has not been understood whether anxiety constitutes true comorbidity or whether it constitutes a different form or condition in ASD. One of the factors that has hampered the exploration of anxiety in ASD could be that these investigations were conducted with children and adolescents who had already received an ASD diagnosis. Once anxiety and ASD symptoms unfold, other genetic, environmental factors or epigenetic modification (due to the interaction between genetic and environmental stimuli) may confound the phenotypic representation of symptoms of both conditions. For example, in mid-childhood and adolescence, when social demand exceeds, environmental factors such as being bullied in the classroom may alter the ASD or anxiety-related genes and subsequently results in anxiety phenotype in children with ASD. Also, during the course of development, the anxiety problems may be more prevalent in mid-childhood and adolescence. Investigations in this period may be difficult because anxiety and ASD related genetic codes may be altered by the overlapping social or non-social difficulties. So, it may be more informative to characterise the developmental processes that underpin anxiety and ASD traits at early developmental periods.

The primary aim of the current thesis is to explore whether behavioural inhibition (BI), a temperamental trait observed in infancy and toddlerhood, is a potential risk factor for co-occurring anxiety traits in these cohorts at low and at high familial risk of ASD. In the general population, developmental and clinical investigations have consistently indicated that BI in early developmental periods is a risk factor for childhood and adolescent anxiety problems (e.g., Degnan & Fox, 2007; Essex, Klein, Slattery, Goldsmith, & Kalin, 2010; Kagan, Reznick, Clarke, Snidman, & Garcia-Coll, 1984; Papachristou, Theodorou, Neophy'tou, & Panayiotou, 2018). However, despite there have been investigations on the link between temperament traits and ASD
symptoms in children with an ASD diagnosis (e.g., Burrows, Usher, Schwartz, Mundy, & Henderson, 2016; De Pauw, Mervielde, Van Leeuwen, & De Clercq, 2011; Macari, Koller, Campbell, & Chawarska, 2017), less is known whether the specific temperament trait, BI underlies co-occurring anxiety traits within the context of prospective longitudinal risk studies. Hence, BI may be an appropriate childhood construct for exploring roots of co-existing anxiety traits in infant and toddlers, who are at familial risk of ASD. In addition to childhood BI tendency, parental factors such as psychopathology (anxiety traits) and parenting behaviours influence both childhood BI and anxiety traits in the general population (e.g., Aktar, Majdandzic, de Vente, & Bogels, 2014; Degnan, Almas, & Fox, 2010). In the ASD population, parental anxiety and ASD traits are also frequent (Crea, Dissanayake, & Hudry, 2016; De la Marche et al., 2015), thus exploring whether parental traits aggregate within the high-risk (HR) for ASD design will be another possible way of exploring co-occurring anxiety and ASD.

The HR for ASD design is a prospective longitudinal method that allows for following infant siblings of an older child with an ASD diagnosis (proband) up until the age of 3, when the most reliable clinical judgement of ASD can be made the earliest. Also, following a low-risk (LR) group of infants, who did not have any first degree relatives with ASD, enables the HR design to compare the findings in the bot groups to explore whether the findings are specific to the HR group or are generalisable to the LR group. This design provides a methodological basis to identify early markers of ASD before the core symptoms fully manifest themselves. HR for ASD research may also provide a basis for investigating the roots of co-occurring anxiety traits. In the realm of the current thesis, this design may be helpful to investigate BI as an early marker of later anxiety problems in infants at risk for ASD.
Moreover, the broader aim of the current thesis is to employ a multi-method investigation to address the potential bias that may arise from measurement methods (summarised in depth in Chapter 3). Bringing together different measurement methods (e.g., observations, parental reporting and globally rated temperament traits) may strengthen the scientific value and generalisability of the findings. Further, the application of various forms of statistical analysis using a multivariate structural equation modelling (SEM) framework enables the relationships between multiple predictors and outcome variables to be tested simultaneously. This method is appropriate especially for testing the primary aim of this thesis because incorporating anxiety and ASD scores into the models as a correlated outcome (e.g., Model 2.3), allows me to understand whether BI establishes shared or distinct pathways for co-occurring anxiety and ASD traits. Overall, the current thesis provides not just fundamental basic scientific value, but also, targets for early interventions that may subsequently influence long-term prognosis.

In the current chapter, I will summarise the history, prevalence, and the aetiology of ASD. I will then discuss evidence for co-occurring anxiety conditions in the ASD literature. Next, I will employ a top-down approach for the conceptualisation of BI by summarising broad childhood temperament and then, BI as a sub-dimension of temperament, as well as its association with childhood anxiety problems. Finally, I will discuss prospective longitudinal investigations of ASD and how this approach provides an advantageous methodology to study early signs of the disorder as well as co-occurring conditions.
1.2. An overview of autism spectrum disorder: history, prevalence and aetiology

The term ‘autism’ was first introduced by Bleuler (1911) as a symptom of schizophrenia for describing disassociation from the external world. Autism continued to be recognised as a form of early-onset schizophrenia until Kanner (1943) distinguished childhood autism from schizophrenia by noting differences in the age of onset and symptom characteristics. Kanner (1943) described eleven clinical cases in the classic paper ‘Autistic Disturbances of Affective Contact’ and highlighted commonly observed features of “infantile autism” as having a lack of motivation to be social, being resistant to change, insisting on sameness, having difficulty in anticipating cues and exhibiting language impairments including delayed speech, echolalia and problems with the use of pronouns (i.e., pronominal reversals). As summarised in an English translation, during the same period as Kanner (1943), Asperger (1991) described his clinical observations of cases who had deficiency in non-verbal communication, eye contact, lack of empathy and extraordinary talent and drew attention to the daily life functioning of these individuals and their ability to pursue a career despite the difficulties in social and non-social interactions. With these works, Kanner (1943) contributed to the conceptualisation of ‘low-functioning’ cases, while Asperger (1991) contributed to the definition of ‘high-functioning’ cases and the heterogeneity of autism was introduced. Both papers summarised the core difficulties of autism and provided a basis for the development of the concept.

Infantile autism was first recognised as a formal disorder in the Diagnostic and Statistical Manual of Mental Disorders, third version (DSM-III; American Psychiatric Association, 1980). Along with the childhood-onset pervasive developmental disorder (PDD), infantile autism constituted the broader criteria of PDDs (American Psychiatric
Association, 1980). The formal consensus on autism symptoms for diagnostic classification helped clinical recognition and enabled autism research to move forward. Subsequently, the diagnostic classification of autism changed substantially from DSM-III (American Psychiatric Association, 1980) to DSM-5 (American Psychiatric Association, 2013). In DSM-IV-TR (American Psychiatric Association, 2000), the term infantile autism was removed and instead PDD was introduced. PDD included following sub-categories: autistic disorder, Asperger's syndrome, pervasive developmental disorder (not otherwise specified), Rett disorder and childhood disintegrative disorder. Due to the overlaps in the presentation of these sub-groups, the categories for PDD were collapsed to a single disorder: Autism Spectrum Disorder in DSM-5 (American Psychiatric Association, 2013). While DSM-IV-TR involved deficits in social interaction, social communication and restricted, repetitive behaviours for symptom classification, in DSM-5 these symptoms have been collapsed into two significant dimensions of symptoms for ASD: social communication and restricted, repetitive behaviours and sensory abnormalities (American Psychiatric Association, 2013).

Problems in social communication and interaction include difficulties around initiating and responding to reciprocal social interactions, sharing interests or affect, modulate verbal and non-verbal communication, lack of eye contact, limited gesture use, difficulties in making friends or being interested in others. Deficits in restricted and repetitive behaviours involve insistence on sameness, rigidity in thinking and routines, idiosyncratic speech, stereotyped motor movements and use of objects, limited interest in objects, limited ability in functional and symbolic play. In contrast to earlier versions, in the DSM-5, hyper- or hypo-sensory difficulties are included in the domain of
restricted, repetitive behaviour (American Psychiatric Association, 2013). These symptoms emerge at the early stages of development and persist across the lifespan.

Recent epidemiologic studies have suggested the estimated prevalence of ASD as 1 in 100 individuals (Baird et al., 2006; Baron-Cohen et al., 2009; Brugha et al., 2011; Christensen et al., 2016). This rate is substantially higher than the earlier studies conducted between 1960 and 1980, which yielded a prevalence rate of around 5 in 10,000 children (Gillberg & Wing, 1999). The increase in the prevalence rate suggests that ASD is noticeably more common than previously recognised (Elsabbagh et al., 2012; Fisch, 2012), but the underlying reason for this change and whether it does constitute a change is not clear. It could be that the increase in the prevalence rate is because of improvements in recognition of ASD, in particular, subsequent improvement in the standardised diagnostic tools given the help from broadening the diagnostic criteria of the DSM-IV-TR (most epidemiologic studies followed DSM-IV-TR classification) and increased awareness of the condition in society (e.g., Lord et al., 2012; Rutter, LeCouteur, & Lord, 2003). Nevertheless, the increased prevalence rate highlights the need for more research to identify the nature of ASD and provide early intervention targets to help the prognosis of individuals with ASD.

ASD has considerable heterogeneity in terms of its aetiology, phenotype and clinical presentation within and between individuals, which challenges early diagnosis and identification of early treatment targets (Bruining et al., 2010; Masi, DeMayo, Glozier, & Guastella, 2017). Heterogeneity in these factors results in diverse manifestations of social communication, behavioural expressions and adaptive functioning. There are also different symptom trajectories in early development (Kim et al., 2018; Kim, Macari, Koller, & Chawarska, 2016; Ozonoff et al., 2018). Some
children who receive ASD diagnosis at the age of 3 present ASD markers as early as 6 months old and after the first birthday, which is consistent with early-onset of ASD. In contrast some of those who receives ASD diagnosis around age of 3 and do not show early ASD markers until the age of 3 is in line with late-onset of ASD (Chawarska et al., 2014; Kim et al., 2016; Ozonoff, Heung, Byrd, Hansen, & Hertz-Picciotto, 2008; Ozonoff et al., 2015; Yoder, Stone, Walden, & Malesa, 2009). In addition to symptom trajectories, genetic variability (e.g., Gaugler et al., 2014), sex differences (e.g., Lai, Lombardo, Au yeung, Chakrabarti, & Baron-Cohen, 2015), temperament (e.g., Mundy, Henderson, Inge, & Coman, 2007) and comorbid conditions have been found to be contributing to the heterogeneity of ASD.

Genetic variability is considered as a major influence on the aetiology of ASD (Gaugler et al., 2014). The genome-wide investigations have not ascertained specific common variants as yet, but sets of rare variants (with 3% liabilities), de novo mutations (with 2.6% liabilities), and common variants (with 49% liabilities), indicated a cumulative risk factor for ASD (Gaugler et al., 2014; Geschwind, 2011; Jeste & Geschwind, 2014; Yuen et al., 2016). While several genes have been identified as being involved in the aetiology of ASD, it is still not clear whether a single gene would lead to multiple phenotypic expressions or in contrast, multiple genes would lead to the same phenotypes between individuals. In addition to this, ASD may share the same pathways with other neurodevelopmental disorders, such as Attention Deficit Hyperactive Disorder (ADHD; Johnson, Gliga, Jones, & Charman, 2015). Also, the small effect size in genome-wide investigations and diverse underlying biological mechanism can be affected by the interplay between gene and environmental factors (e.g., exposure to maternal alcohol consumption; Kim & Leventhal, 2015).
There is a sex difference in the prevalence of ASD, whereby the male to female ratio is 4:1 (Lai et al., 2015; Lai et al., 2017; Loomes, Hull, & Mandy, 2017; Palmer et al., 2017). Being female has been generally considered as a protective factor for ASD, but it has been suggested that the female ASD phenotype diverges from the male one with regards to “camouflaging” the social difficulties that females encounter in social situations (Bargiela, Steward, & Mandy, 2016; Loomes et al., 2017). Masking or altering the responses accordingly in the social context may confound early detection and precede underrecognition of females with ASD. Camouflaging may depend on the intellectual capacity of females. Notably, lower levels of cognitive capacity result in early identification of ASD in females (Giarelli et al., 2010; Lai et al., 2017). This may be partly due to the lack of sensitivity in the diagnostic tools to capture female ASD presentations. A recent investigation suggested that while males received higher scores in the observational measures than females, the latter reported higher symptom severity in the self-reported questionnaires (Charman, Loth, et al., 2017). This might be the result of male bias of the clinicians or the abilities of females to deflect their problems better than males. These findings may be due to the underlying genotypic differences for males and females or solely due to diagnostic biases.

According to Mundy et al. (2007), temperament, which pertains to constitutionally based individual differences in reactivity and regulation (Rothbart & Derryberry, 1981), is one of the modifier processes that interact with ASD symptoms and affect individual variation in the phenotypic expression. Temperament has been explored in children with ASD to map the patterns of its traits and to identify its role in the ASD phenotype. Indeed, children with ASD have been found to be less adaptable to changes in their routines or environments, less persistent and requiring more intense stimulation from the environment to obtain a response, less responsive to small changes
in their environment; they also have more negative emotionality and less regulatory skills than the control groups (Brock et al., 2012; De Pauw, Mervielde, & Van Leeuwen, 2009; Hepburn & Stone, 2006). Temperament traits in toddlerhood have also been associated with the ASD symptoms (Bolton, Golding, Emond, & Steer, 2012) as well as symptom severity (Macari et al., 2017). Schwartz et al. (2009) showed significant correlations between lower levels of surgency and withdrawal, higher levels of social communication difficulties and restricted and repetitive behaviours. Brock et al. (2012) also showed that higher composite scores of hyporesponsiveness, hyperresponsiveness and sensory seeking behaviours were associated with increased withdrawal and heightened negative affectivity among children with ASD. Hence, temperament traits may be another factor that contributes to the symptom severity of ASD and its associated mechanisms, which then results in variations in phenotypic presentation.

Co-occurring medical and psychiatric conditions are common in ASD (Lai, Lombardo, & Baron-Cohen, 2014; Rosen et al., 2018; Salazar et al., 2015; Simonoff et al., 2008) and substantially contribute to the heterogeneity of its phenotype. Children with ASD may have concurrent developmental conditions (e.g., intellectual disability, language disorders), medical conditions (e.g., epilepsy, gastrointestinal problems, sleep disturbances), genetic syndromes (e.g., tuberous sclerosis complex) and/or psychiatric problems (e.g., anxiety, ADHD; Close, Lee, Kaufmann, & Zimmerman, 2012; Lai et al., 2014; Salazar et al., 2015; Simonoff et al., 2008). These co-occurring conditions may mask or modify the presentation of core ASD symptoms, contribute to the variability in symptom profiles and severity, ultimately, hinder the recognition (Close et al., 2012; Soke et al., 2018). Especially, psychiatric conditions, anxiety and ADHD are the most commonly detected co-occurring conditions (Salazar et al., 2015), with the symptoms of
these problems influencing the processing of social information, exacerbating the difficulties in school functioning and peer relationships (Chiang & Gau, 2015; Sokolova et al., 2017). Thus, exploration of the nature of these co-occurring conditions may extend the current understanding on the aetiology of ASD and help to identify whether the findings are specific to ASD phenotype or also shared with other childhood disorders.

Given the main aim of the current thesis is to explore the co-occurring anxiety problems in infants at HR for ASD, in the next section, the focus of the literature review will be on anxiety problems in the general population and then in the ASD sample.

1.3. Anxiety problems

1.3.1. Anxiety in the general population

Anxiety disorders are one of the highly common psychiatric conditions in children and adolescents, with a lifetime prevalence of between 17 and 27% in the general population (Costello, Egger, & Angold, 2005; Somers, Goldner, Waraich, & Hsu, 2006). Anxiety traits can emerge in the preschool period, persist or recur throughout the development (Bufferd, Dougherty, Carlson, & Klein, 2011; Bufferd et al., 2016). In DSM-5 (American Psychiatric Association, 2013), the definition of anxiety disorders involves three common facets: excessive fear, anxiety and associated behavioural disturbances (e.g., worry). Fear refers to a perceived impending threat and it is consistent with physiological arousals (such as fight-or-flight responses), thoughts of immediate danger and contact avoidant behaviours. On the other hand, anxiety refers to the perception of a threat that is likely to happen in the future, that is accompanied by physical reactions (e.g., intense muscle tension), cautious avoidance and hypervigilance for preparation of this future threat.
Anxiety and fear are typically adaptive responses that are executed to avoid behaviours about threatening situations. Both become maladaptive when they start to interfere the daily functioning when the threat response occurs in the absence of any real threat and when they frequently occur with high severity in a persistent manner (American Psychiatric Association, 2013). The distinction between adaptive and pathological anxiety in childhood may not be easy since children have fears and anxieties as part of the developmental process and childhood fears are transient for some cases (Beesdo, Knappe, & Pine, 2009; Broeren, Muris, Diamantopoulou, & Baker, 2013). For example, separation anxiety is highly observed among 12-18 months old infants but becomes less frequent in later childhood. However, when the anxiety interferes with the daily functioning, it may form a pathologic representation in childhood.

In DSM-5 (American Psychiatric Association, 2013) anxiety disorder is a broad category, including the following disorders: separation anxiety disorder, selective mutism, specific phobia, social anxiety disorder (social phobia), panic disorder panic attack specifier, agoraphobia, generalised anxiety disorder, substance/medication-induced anxiety disorder, anxiety disorder due to another medical condition and other specified anxiety disorder unspecified anxiety disorder. While obsessive-compulsive disorder and posttraumatic stress disorder were counted under anxiety disorders in the DSM-IV-TR (American Psychiatric Association, 2000), with the changes in the classification systems, in the DSM-5 (American Psychiatric Association, 2013) these sub-categories were excluded from the anxiety disorder classification and so they will not be covered in the next sections. These sub-categories and their symptom classification are also used for the identification of childhood anxiety problems as well
as among individuals with ASD who exhibit such problems (MacNeil, Lopes, & Minnes, 2009).

1.3.2. Anxiety in ASD

While anxiety does not involve in the phenomenological features of ASD; it is highly common in children and adolescents with ASD (Rosen et al., 2018; Salazar et al., 2015; Simonoff et al., 2008). That is, when compared to peers in the general population, anxiety problems are more prevalent in individuals with ASD (Bellini, 2004; Guttmann-Steinmetz, Gadow, DeVincent, & Crowell, 2010; Soke et al., 2018). The prevalence rate of clinically elevated anxiety problems in children who have had a statement for special educational needs or with ASD is around 42% (Simonoff et al., 2008), whilst in a community sample of children with ASD, the prevalence rate was around 79% (Salazar et al., 2015). A meta-analytic study also suggested that between 11% and 84% of children with ASD experience some degree of anxiety traits (White, Oswald, Ollendick, & Scahill, 2009). Moreover, children and adolescents with ASD, regardless of their other co-occurring psychiatric conditions and IQ levels were found to have some degree of threshold and sub-threshold anxiety traits (Caamaño et al., 2013; Wijnhoven, Creemers, Vermulst, & Granic, 2018).

Similar to ASD symptoms, anxiety traits are moderately heritable (~ 50%; Hallett, Ronald, & Happé, 2009; Scherff et al., 2014) and there are higher levels of anxiety traits in unaffected co-twins of ASD probands (Hallett, Ronald, et al., 2013). The presentation of anxiety in ASD can be complicated. The interplay between underlying mechanisms of both conditions may result in traditional and/or ASD-specific phenotypes (Kerns et al., 2014). Nevertheless, concurrent anxiety problems are
additional burdens in daily life, they can influence social and adaptive functioning of children with ASD (Bellini, 2004).

1.3.2.1. Anxiety in ASD: A true comorbidity or ASD specific Anxiety?

Comorbidity in psychiatric disorders refers to the existence of two or more monomorbid disorders within an individual (Caron & Rutter, 1991). Despite the elevated rates of concurrent anxiety problems in individuals with ASD, because of the ambiguity in the aetiology of ASD and anxiety in ASD, it has not been entirely determined whether anxiety represents “true” comorbidity or, instead, it represents ASD-specific anxiety symptoms (Kerns & Kendall, 2012; Wood & Gadow, 2010). As a result of lack of clarity regarding this matter, rather than using the term comorbidity, I will use the term co-occurrence throughout to refer to accompanying anxiety traits or symptoms.

To conceptualise the nature and the role of anxiety in ASD, Wood and Gadow (2010) proposed the following framework: 1) ASD and anxiety may represent true comorbidity with distinct phenotypes, aetiology and causal pathways; 2) Monomorbid anxiety may be altered by an ASD pathogenic process and result in ASD-specific presentation, which could be considered as a unique syndrome apart from ASD; and 3) individuals with ASD who have additional anxiety symptoms may differ from those with ASD who do not have anxiety due to either phenotypic heterogeneity or a sub-type of ASD, in other words, anxiety can mediate or moderate the severity of ASD.

Investigations into the nature of anxiety in ASD have not supported the true comorbidity. To the contrary, the findings have suggested that presentations of the ASD phenotype have similarities and differences to the anxiety phenotype that were observed in the non-ASD populations (Kerns et al., 2014; Rodgers & Ofield, 2018). Twin studies
have shown that anxiety traits are associated with ASD traits in the general population, thus suggesting a moderate phenotypic overlap (Hallett et al., 2009; Scherff et al., 2014). In more depth, Kerns et al. (2014) assessed traditional, and ASD-specific presentation of anxiety symptoms for 7 to 17 years old individuals with ASD. The results for the overall anxiety symptoms revealed that while 17% of the individuals presented traditional anxiety, 15% presented ASD-specific anxiety and 31% of the sample presented both. In terms of the specific type of anxiety, 30% of the children presents traditionally defined specific phobias/fears, such as spiders, animals or germs (Evans, Canavera, Kleinpeter, Maccubbin, & Taga, 2005; Kerns et al., 2014; Leyfer et al., 2006; van Steensel et al., 2011) whereas around 20% individuals with ASD had ASD-specific presentations characterised by unusual fear of bubbles, being in supermarkets, running water (Kerns et al., 2014), vacuum cleaners, toilets (Mayes et al., 2013) or specific situations, such as the school bus (Leyfer et al., 2006).

After phobias, the second highly co-occurring anxiety problem was GAD, around 22% of individuals with ASD presented traditional representations of GAD, which is defined as general worries about daily life routines, such as school and family (Kerns et al., 2014). On the other hand, 20% exhibited to some extent ASD-specific GAD, which was characterised by the absence of generalised worry, being more in line with worry around changes in routine, such as the daily schedule (Kerns et al., 2014). Social anxiety was the third commonly reported anxiety disorder in ASD and around 17% of individuals with ASD presented traditional social anxiety (Kerns et al., 2014; van Steensel et al., 2011), which was consistent with the worry in social situations owing to social humiliation and performance anxiety. Moreover, approximately 8% of the sample presented ASD-specific symptoms that were different from the classic presentation due to lack of awareness or worry about negative evaluation, but more
consistent with lack of understanding of social rules (Kerns et al., 2014). Overall, these investigations addressed proposed models from Wood and Gadow (2010) and the results appeared to reject true comorbidity and instead, supported the ASD-specific presentations of anxiety as well as the traditionally defined anxiety problems. Nevertheless, the inflated rates of traditional anxiety in these investigations may be evidence of overlapping phenotypic presentation, owing to the measures being developed for non-ASD individuals and these measures may reflect ASD symptoms other than anxiety. To address these issues, in the next section, I will synthesise the phenotypic overlap between ASD and anxiety and the direction of the associations between both conditions.

1.3.2.2, Phenotypic overlap and direction of the association

As mentioned earlier, individuals with ASD have difficulties in social communication, speech abnormalities, problems in understanding non-verbal cues like gestures and they have poor eye contact. These phenotypic characteristics also overlap with anxiety symptoms, especially social anxiety disorder which is characterised by an intense fear of social interactions and worry about being criticised by others in social situations (American Psychiatric Association, 2013). Individuals with social anxiety may have difficulties in initiating conversations with unfamiliar people, may have anxiety in doing things when others are watching due to the extensive worry of being negatively evaluated and may worry about social activities. These patterns of thoughts and feelings may result in poor eye contact and avoidance of social interaction or poor communication skills (e.g., Halls, Cooper, & Creswell, 2014). The phenotypic convergence in symptom presentations may confound differential diagnosis.
Despite this, it is not clear yet whether social anxiety is a morbid condition, or it is evident due to social communication difficulties of ASD. Social anxiety symptoms may have bidirectional effects with the difficulties of social functioning in individuals with ASD. The social communication difficulties that are the result of ASD symptomatology may trigger social anxiety problems and this coexistence may confound the ASD related social impairments. Deficits in social skills may generate negative responses from others and may cause adverse belief about individuals’ own social skills, consequently, influence social isolation (Rapee & Spence, 2004) that escalate the severity of avoidance of social situations, awkward interactions with peers, and thus, promote further isolation. Alternatively, a combination of physiological arousal, which is a characteristic of anxiety disorders, social skill deficits as well as a lower assertion in ASD could contribute to social anxiety symptoms (Bellini, 2004, 2006).

On the other hand, elevated ASD traits are common in individuals with a social anxiety disorder (Kleberg et al., 2017; Puleo & Kendall, 2011). For example, a clinical sample of children and adolescents with this anxiety disorder showed elevated levels of ASD symptoms of social communication difficulties and restricted repetitive behaviours, as measured by the Social Communication Questionnaire (Halls et al., 2014; Rutter, Bailey, & Lord, 2003). Moreover, children with a social anxiety disorder were more likely to score above the cut-off for an ASD diagnosis on this questionnaire and after removing the overlapping symptoms from this tool, the pattern of significance remained the same. In the general population, children and adolescents with anxiety traits presented language problems, which had bidirectional associations with anxiety problems from early childhood and adolescence (Helland, Røysamb, Wang, & Gustavson, 2017; Voci, Beitchman, Brownlie, & Wilson, 2006). Overall, this evidence
suggests that there is no clear distinction between anxiety and ASD phenotypes, which may imply shared underlying mechanisms.

Another domain of core ASD symptoms is restricted, repetitive behaviours and sensory abnormalities (American Psychiatric Association, 2013). This cluster of symptoms may involve talking about a topic repeatedly, repetitive physical movements like body rocking or finger flicking, insistence on sameness such as following the same routine in daily life or doing same activities, stereotyped behaviours, rigid preferences on eating a specific food or wearing certain clothes. Insistence on sameness could be associated with the anxiety around changes in routines, such as taking a new route or alteration to a daily routine (Kerns et al., 2014). Indeed, Gotham et al. (2013) found a modest relationship between anxiety and insistence on sameness in children with ASD, while Factor, Condy, Farley, and Scarpa (2016) found positive correlations between restricted, repetitive behaviours and insistence on sameness.

Another factor associated with anxiety in ASD is intolerance of uncertainty (Wigham, Rodgers, South, McConachie, & Freeston, 2015) which comes with the assumptions that uncertainty is stressful, negative, threatening and should be eluded. (Anderson et al., 2012; Zdebik, Moss, & Bureau, 2017). Intolerance of uncertainty is considered to be a feature of GAD (Anderson et al., 2012) and it is characterised by extreme worry about general issues or situations that are disproportionately perceived as being dangerous and risky (American Psychiatric Association, 2013). Intolerance to uncertainty and insistence on sameness shares some features such as a preference for the same routine and not wanting a change in ASD (Boulter, Freeston, South, & Rodgers, 2014). Individuals with ASD, could simply be disinterested in social interaction and dislike uncertainty because it violates the structure that they need. Vasa, Kreiser,
Keefer, Singh, and Mostofsky (2018) found that intolerance of uncertainty was correlated with anxiety and ASD symptoms, but this was due to presence of the ASD symptoms, so intolerance of uncertainty was more strongly associated with ASD than with anxiety. Moreover, in a clinical sample with GAD, individuals reported higher levels of intolerance of uncertainty in ambiguous situations than a control group (Anderson et al., 2012). For example, people with anxiety disorders avoid social interaction because they fear negative evaluation by others, and they dislike uncertainty because they fear negative outcomes. Thus, intolerance of uncertainty may be a shared aspect of ASD symptoms and anxiety problems. This may suggest that the underlying cognition in ASD and monomorbid anxiety are the same and that the underlying mechanism of anxiety is similar in individuals with and without ASD.

In an attempt to explore whether ASD symptoms influence anxiety problems or in contrast anxiety problems influence the ASD symptoms in school-aged children, longitudinal investigations employed cross-lagged models. For example, Duvekot et al. (2018) researched the reciprocal interaction between social communication, restricted/repetitive behaviours and anxiety separately after controlling for possible confounders, such as IQ, age, gender and externalising problems in children with ASD. The results showed that higher levels of anxiety symptoms in a group of children aged between 2 and 10 years old were associated with social communication difficulties that were measured in two-year intervals, but there was no significant relationship between restricted, repetitive behaviours and anxiety symptoms. Similarly, Pickard, Rijsdijk, Happé, and Mandy (2017) investigated the relationship between social anxiety and ASD related social communication difficulties. The findings showed that social communication difficulties in a group of children aged between 7 and 10 were associated with higher social anxiety symptoms that were measured 3 years later. Green,
Ben-Sasson, Soto, and Carter (2012) examined the relationship between sensory sensitivity and anxiety problems in a group of toddlers aged between 18 and 33 months in the one-year interval and showed unidirectional relationships; higher sensory sensitivity was related to higher anxiety problems, but anxiety scores did not predict sensory over-responsivity over time. Teh, Chan, Tan, and Magiati (2017) also elicited a unidirectional association. The findings indicated that social communication, repetitive speech and stereotyped behaviour symptoms of a group of children aged between 5 and 17 years old with ASD associated with anxiety traits that were measured 10 to 19 months interval. To the contrary, Hallett, Ronald, Rijsdijk, and Happé (2010) showed a bidirectional effect between anxiety and ASD traits in a group of children who were aged between 7 and 12 years old over a five-year interval. Hence, there are inconsistent results in the direction of the association between anxiety and ASD related difficulties over time. This may be due to anxiety symptoms or traits being more pronounced during a specific period, ultimately, making it challenging to disentangle the overlap and to understand the underlying mechanisms of co-occurrence between these two conditions.

The exploration of anxiety problems in ASD can be confounded by the age of the participants, effecting inconsistencies in the outcomes. Longitudinal investigations in the general population showed that age has a significant effect on total anxiety, separation anxiety, GAD, panic disorder, and agoraphobia (Copeland, Angold, Shanahan, & Costello, 2014). While total anxiety disorders were more prevalent at 9- and 10-year olds, this rate drops and reaches a plateau from 11 to 16 years and increases from 16 years up until 26 years. However, the trajectories of specific anxiety disorders may differ based on the sub-types. For example, separation anxiety disorder is higher in mid-school age and decreases from adolescence onwards, arriving at a stable pattern.
until adulthood (Copeland et al., 2014). On the other hand, GAD, panic disorders and agoraphobia are stable during mid-childhood and adolescence whereas prevalence rates increase from late adolescence onwards (Copeland et al., 2014). Latent-class analysis on preschoolers and school-age period anxiety problems showed two stable (high and moderate) and two decreasing groups (high and low), with the highly stable group reporting anxiety diagnosis during preschool period (at 3 and 4) and at the age of 13 (Kertz, Sylvester, Tillman, & Luby, 2017).

A positive association between age of the children and anxiety symptom severity has also been reported in studies conducted within individuals with ASD (e.g., Gotham, Brunwasser, & Lord, 2015; van Steensel et al., 2011). A meta-analytic study about the effect of age on the expression of anxiety problems in individuals with ASD who aged less than 18 years old indicated that higher mean age is associated with higher prevalence rates for general anxiety and GAD, while a lower mean age was related to higher prevalence rates for separation anxiety disorder (van Steensel et al., 2011). Other studies have also suggested that GAD is highly frequent during adolescence and early adulthood in those with ASD (Dubin, Lieberman-Betz, & Lease, 2015; Sonuga-Barke et al., 2017). Anxiety symptoms that present in mid-childhood and adolescence may intertwine with ASD symptoms than earlier presentation due to multiple shared genetic or environmental vulnerabilities. Hence, investigating the roots of anxiety in ASD during mid-childhood and adolescence limits research being able to untangle the overlap between anxiety and ASD phenotype.

Kerns and Kendall (2012) suggested that in order to understand pathways to co-occurring anxiety in ASD, methodologically, it may be more informative to investigate risk factors in infancy and toddlerhood before their phenotypic presentations are
confounded by each other as well as other factors such as symptom onset, genetic or environmental influences. In the general population, regarding the temperament traits, BI in infancy and toddlerhood has been found to be a robust predictor of childhood anxiety problems (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Kagan, Snidman, Zentner, & Peterson, 1999). To my knowledge, there have been very few investigations on the associations between BI and anxiety traits in ASD research and HR for ASD sibling design (Shephard et al., 2018). Thus, studying BI will provide a novel understanding as to whether this underpins anxiety as seen in those in the general population and it can show whether it is a shared or distinct predictor of anxiety and ASD traits. In the next section, I will summarise temperament and BI, following which, I will discuss HR for ASD sibling design as a methodological alternative to the studies mentioned above.

1.4. BI as a temperamental risk factor for anxiety

1.4.1. Broad temperament construct

Temperament refers to individual differences in emotional and behavioural tendencies that emerge in infancy and it comprises biological and heritable foundations (Rothbart & Derryberry, 1981). This psychological construct of childhood has received a great deal of research interest from developmental scientists, as temperament traits in infancy and toddlerhood provide the foundation for studying emotional and behavioural adjustment (Chess & Thomas, 1987; Goldsmith, 1996; Rothbart & Derryberry, 1981). Temperament research dates back to the classic New York Longitudinal Study (NYLS) (Chess & Thomas, 1987). In NYLS, Chess and Thomas (1987) explored the variations in behavioural styles of infants and argued that temperament is a stylistic component of behaviours along with skills and motivation, but it constitutes a distinct psychological
construct from cognition and motivation. Moreover, Goldsmith (1996) highlighted the importance of affective features in the organisation of child temperament and suggested that temperament influences individual differences in children’s internal experiences of expressions of primary emotions. Based on these two approaches, Rothbart and Hwang (2002) conducted a factor analytic study with emotional and behavioural characteristics of temperament traits, identifying three factors for temperament construct: surgency, negative affect and effortful control (EC). The surgency and negative affect constitute the reactivity domain, which is characterised by arousability of behavioural and physiological responses to stimuli. EC refers to regulatory processes, which modulate reactivity through attentional shifting, and inhibitory control.

How can we identify temperament in children? Chess and Thomas (1987) argued that children may have similar motivation and abilities, but may differ regarding the temperamental style. In contrast, they may have similar temperamental style but differ in terms of motivation, abilities and consequently, this influence the variations in behavioural manifestation. So, what is the difference between fearful facial expression and temperamental fear? Possibly, an individual’s facial fear as a response to a threatening situation cannot be referred as temperamental, but instead, it is an emotional reaction. However, when compared this reaction to other children, the differences in intensity of these expressions constitute temperament. In other words, most children will probably react to a loud sound (e.g., hand drier, vacuum cleaner) to some degree, but some of them, for example, will show facial fear for a short duration and manage to regulate their responses easily, while others will present more intense facial fear and will cry or go to a parent for a cuddle. So, the individual differences in these reactions represent temperamental traits.
Temperament has been widely studied due to its well-established link with internalising and externalising problems (e.g., Forbes, Rapee, Camberis, & McMahon, 2016; Nigg, 2006; Wichstrom, Penelo, Rensvik Viddal, de la Osa, & Ezpeleta, 2017). In infancy and toddlerhood, temperamental dispositions can be considered a risk factor for childhood psychopathology. For example, lower levels of temperamental impulsivity, inhibitory control, frustration and surgency have been associated with externalising problems (Scheper et al., 2017) whereas temperamental BI has been linked to anxiety problems in preschoolers and school-age children (e.g., Chronis-Tuscano et al., 2009; Kagan et al., 1999; Kiel & Buss, 2014).

1.4.2. BI and anxiety problems

BI is a temperamental feature identified in early childhood and characterised by increased wariness, fearfulness and avoidance towards unfamiliar people and novel situations (Fox et al., 2001; Kagan et al., 1984). While the fundamental characteristic of BI is the reaction to novelty, it can manifest itself differently during the course of development. In infancy, children with BI display heightened reactions and sensitivity to novelty (Calkins, Fox, & Marshall, 1996), whilst in toddlerhood they withdraw from novel, unfamiliar social or non-social stimuli (Brooker, Kiel, & Buss, 2016; Fox et al., 2001; Kagan et al., 1999). Moreover, in childhood, when inhibited children withdraw, this tendency leaves them socially less competent and assertive (Frenkel et al., 2015; Kiel & Buss, 2011; Rubin, Burgess, & Hastings, 2002; Walker, Henderson, Degnan, Penela, & Fox, 2014). BI tendencies during the early developmental period have also been associated with later negative social experiences, poor peer relationships and subsequently, internalising problems and more specifically anxiety problems (Clauss & Blackford, 2012; Hudson, Dodd, & Bovopoulos, 2011).
BI can be measured via observational standardised laboratory tasks enriched with novel stimuli (Fox et al., 2001; Kagan et al., 1984; Planalp, Van Hulle, Gagne, & Goldsmith, 2017). These observational measures generally include an unfamiliar adult (social context) and a toy that makes an unpredictable noise or movements (non-social context). While the coding schemes vary between these studies, children who were characterised by BI exhibited higher levels of intensity of facial fear, longer latencies to approach, longer withdrawals, increased proximity to parents and negative vocalisations consistently. Other studies employed parent reports of BI (e.g., Wolfe, Zhang, Kim-Spoon, & Bell, 2014) and generally used the shyness or fear subscales of temperament questionnaires (Biederman et al., 2001; Goldsmith, 1996; Hudson, Dodd, Lyneham, & Bovopoulous, 2011; Putnam, Helbig, Gartstein, Rothbart, & Leerkes, 2014). The complexity of identifying individual differences may obscure the measurement of BI. So, several response parameters, such as intensity, duration and frequency have been implemented to observe these behaviours in a novel context (e.g., Brooker et al., 2013; Buss, 2011). Furthermore, to overcome possible bias, studies have also used multi-method approaches for generating more reliable BI scores (Barker et al., 2015; Walker et al., 2014). For instance, Brooker et al. (2013) created factor scores by submitting those yielded from parent-reports and standardised observational tasks. Barker et al. (2015) created a global measure of BI by averaging observed and parent-reported data. However, it may be important to consider parent and observer agreement regarding a child’s BI scores to understand whether the child’s responses reflect trait level or observation specific responses and to overcome the informant contrast effect which is arisen distorted parental reports of the child temperament. In sum, the measurement of BI in developmental research plays a crucial role to generate more accurate data.
BI in infancy and toddlerhood has been well-studied and it represents a clinically relevant risk marker for anxiety problems in the general population (e.g., Buss et al., 2013; Clauss, Avery, & Blackford, 2015; Clauss & Blackford, 2012; Dougherty et al., 2011; Scheper et al., 2017). BI shows moderate continuity (Fox, Henderson, Marshall, Nichols, & Ghera, 2005), with children who present increased fear as well as withdrawal reactions to novelty in infancy and toddlerhood being more likely to develop anxiety in preschool (Kiel, Premo, & Buss, 2016), school-age periods (Barker et al., 2015; Chronis-Tuscano et al., 2009; Hirshfeld-Becker et al., 2007; Hudson, Dodd, & Bovopoulos, 2011) and adolescence (Frenkel et al., 2015) in the general population. Similarly, BI was linked to anxiety disorders in a clinical sample of 3-year-old toddlers who received any type of anxiety diagnosis (Dougherty et al., 2011; Dougherty et al., 2013). Moreover, BI that was assessed by observational and parent-report measures in the same cohort of children were significantly related to persistence/recurrence anxiety disorders at the age of 6 (Bufferd et al., 2016). Likewise, in children who were clinically referred due to either internalising and externalising problems (aged 3 and 7), BI was significantly related to anxiety problems after controlling for the latter type of problem (Scheper et al., 2017). Thus, this well-established link to anxiety problems makes BI a salient risk marker.

1.4.3. BI as a construct

In the temperament literature, the constructs of BI, fear and shyness are used to refer to similar reactions: initial emotional and behavioural responses to novelty and unfamiliarity. For instance, Eggum et al. (2009) referred to reactivity to novelty as fear and referred to fear as an umbrella term encompassing shyness and BI. In contrast, Brooker et al. (2016) referred to shyness as social fearfulness. These studies used standardised observational temperament measures that were designed to measure
reactivity to novelty. However, these studies commonly applied similar coding schemes and commonly generated components that reflected fearfulness, shyness and withdrawal (e.g., Buss, 2011; Kiel & Buss, 2012). So, given the evidence, BI constitutes a broader construct for fear, shyness and withdrawal. Differences between these studies may be evident due to situational differences in the observational schemes used.

Factor analyses of parent-report temperament instruments supported this view and have suggested that the responses to novelty depend on the context of the observations (Putnam, Gartstein, & Rothbart, 2006). These tools do not measure BI directly but have separate subscales for reactive fear and shyness (Putnam et al., 2006) which have been widely used as a proxy of BI (Gagne, Van Hulle, Aksan, Essex, & Goldsmith, 2011; Geng, Hu, Wang, & Chen, 2011). Shy and fearful reactions to novelty formed a single factor (fear) in infancy (Gartstein & Rothbart, 2003) but began to constitute two distinct but related constructs in toddlerhood (Putnam, Gartstein, & Rothbart, 2006), probably due to development over this period in the domain of social cognition that can contribute to development of self-conscious emotions (shyness). Shyness consists of withdrawal towards unfamiliar adults or peers in a social context whereas fear is consistent with reactions to novel non-social objects or environments. Even though shyness and fear are two distinct constructs, in parent-report instruments, both constructs have their primary loadings on the same broader negative affect factor (Putnam et al., 2006). This suggests that these temperament traits could form a broader construct of BI that consists of both social (shyness) and non-social components.

Despite the evidence, previous research has not explored whether BI constitutes a multidimensional or homogenous, unitary structure. Responses to novelty in a social and non-social context may be organised in more than one way. For example, moving
closer to a parent could be due to a fearful reaction to an object in a non-social context, or shyness in response to an unfamiliar person in a social context. However, although the organisation of these responses may be based on the context of a novel situation, both fearful, shy and withdrawal tendencies may be part of a broader pattern of BI that could relate to a lack of motivation to engage with unfamiliar situations. There need to be more investigations on the factor structure of these constructs.

1.4.4. Factors that affect BI tendency

Why do some children who are characterised by BI in infancy grow out of this temperamental style and do not develop anxiety later in the development? According to Degnan and Fox (2007) to understand the association between BI and anxiety, BI should be studied along with moderating or mediating factors rather than as a risk factor for anxiety problems in childhood and adolescence. One of the factors that influence the association between higher levels of BI and anxiety is the regulatory domain of temperament (e.g., Gulley, Hankin, & Young, 2016; White, McDermott, Degnan, Henderson, & Fox, 2011). According to Rothbart, Ellis, Rueda, and Posner (2003), self-regulation (EC) has an important role in monitoring, modulating and organising emotional reactivity (BI), which subsequently protects those showing higher BI from developing anxiety symptoms later in childhood (White et al., 2011). With the development of the EC capacity, it may help children to disengage from threatening situations and engage in more calming stimuli, thus adjusting dysregulated emotional responses and may lead lower levels of BI (Rothbart et al., 2003; Rothbart, Sheese, Rueda, & Posner, 2011). Hence, increased EC capacity may reduce the likelihood of BI dispositions producing anxiety problems.
Another factor that may affect the relationship between BI and anxiety is parental psychopathology. There is evidence on the association between parental trait level anxiety, child BI and anxiety. For example, Rosenbaum et al. (1991) showed that 21 months old offspring of parents with any anxiety disorders were likely to have higher BI scores. Also, follow-up investigations at the age of 7 suggested that these infants were more likely to develop childhood anxiety disorders compared to uninhibited and control groups. Similarly, Biederman et al. (1993) showed that 21 months to 7 years old offsprings of individuals with anxiety problems were more likely to develop childhood anxiety problems compared to uninhibited peers in 3-year intervals. Moreover, Muris, van Brakel, Arntz, and Schouten (2011) investigated the longitudinal effect of parental trait level anxiety, toddlers’ BI; their association with children’s social anxiety and total anxiety scores over 3 years. The result suggested that maternal anxiety traits and children’s BI scores were associated with the children’s overall anxiety scores. A study by Aktar et al. (2014) extended these investigations by examining the longitudinal effect of both parents’ anxiety traits and observed parental behaviours during the social reference paradigm on children’s BI. The results showed that 12 months old infants’ maternal social anxiety and other comorbid anxiety disorders were predictive of their BI scores at 30 months old. Also parental observed overprotective behaviours, the interaction between their lifetime anxiety problems and observed overprotective behaviours at 30 months were associated with children’s BI. So, as parental anxiety problems and parental behaviour in social context may have an important role of children’s temperamental disposition and shaping their internalising psychopathology.

Several mechanisms can explain the underlying pattern of this association. For example, parental anxiety problems may increase their sensitivity to offspring’s emotionality, which could also impact different styles of behaviours, such as
overprotection (Degnan et al., 2015; Rubin et al., 2002). Parents who make anxious approaches during interaction with an inhibited child may be a role model and the child may imitate the behavioural style by reacting to novelty with heightened shyness, fearfulness or wariness (Natsuaki et al., 2013). Also, anxious parents may reinforce their children’s inhibited responses by overprotective behaviours that are intended to protect the child from negative experiences (Rubin et al., 2002). As parental overprotective behaviours influence children's’ BI dispositions, children’s extreme sensitivity towards novelty also influence more parental overprotective behaviours (Brooker et al., 2015). So there may be a bidirectional association. Alternatively, the association between parental and child anxiety may posit heritability. However, it is hard to distinguish whether the influence of parental anxiety and overprotective behaviours on child anxiety is due to heritability or due to environmental risk. But within the context of the current investigation, exploration of parental variables extends the investigations of the root of anxiety and ASD.

1.4.5. BI and other factors in ASD

Turning to ASD, even though there has been a considerable amount of investigation on broader temperament traits, which have indicated a higher negative affect and lower EC in school-age children with ASD (Brock et al., 2012; De Pauw et al., 2011), there has been much less research on the associations between BI and co-occurring anxiety within ASD. These investigations commonly assessed group level (e.g., ASD vs control) differences in BI components fear and shyness. For example, Konstantareas and Stewart (2006) showed that school-age children with ASD presented greater shyness than a control group and Burrows et al. (2016) found that, even though there were no overall group mean differences in fear and shyness, the ASD group did present with greater variability in scores. De Pauw et al. (2011) demonstrated that
children who had higher ASD symptoms also rated themselves as presenting greater shyness in novel contexts. A study by Scherr, Hogan, Hatton, and Roberts (2017) utilised the Stranger Approach observational task (Fox et al., 2001) to assess the early behavioural risk markers for anxious children with ASD. The results showed that the ASD group displayed more facial fear than a control group, but observed facial fear, escape and gaze aversion was not significantly related to anxiety and ASD traits in the ASD group. So, the evidence is scarce how temperamental traits play a role in underpinning the anxiety in ASD.

The EC regulatory dimension of temperament has been proposed to be a protective factor for ASD related adaptive functioning by Johnson (2012). Indeed, parent-reported successful regulatory capacities have been associated with less social communication difficulties and overall ASD severity (Brock et al., 2012; De Pauw et al., 2011; Faja & Dawson, 2013). Uljarevic, Richdale, Evans, Cai, and Leekam (2017) also found that effective EC was associated with less co-occurring anxiety symptoms in children with ASD. So, in addition to BI, EC may provide a foundation for comprehension as to whether co-occurring anxiety problems are due to ASD related EC difficulties or EC difficulties influence ASD and anxiety problems separately. That is, when exploring anxiety, EC may provide more insight into the overlap between these disorders. However, these investigations need replication and disposition of BI in infancy and its relation to early emerging anxiety as well as ASD symptoms should be researched. The HR for ASD sibling design may provide methodological advantages for exploring early risk factors for anxiety. In the next section, I will summarise this design and the current findings as well as describe how it presents methodological benefits.
1.5. Longitudinal investigations of ASD

1.5.1. Retrospective longitudinal studies

Until the last decade, investigations into early markers of ASD in infancy and toddlerhood have involved retrospective investigations of parent reports (on initial concerns about their child) and home videos (Yirmiya & Charman, 2010). These studies have consistently shown that children with ASD have impairments in skills in the first and the second year of life (e.g., Barbaro & Dissanayake, 2009; Bernabei, Camaigni, & Levi, 1998; Osterling, Dawson, & Munson, 2002). For example, reduced frequency of eye contact, motor atypicality, lack of response to name when being called, limited use of gestures, reduced facial expressions, were commonly noted problems. In the second year of life, difficulties around joint social activities, symbolic imitative play as well as sensory sensitivities emerge (Losche, 1990).

Although the retrospective investigation has provided important information about early development in ASD, it is prone to methodological difficulties that influence the reliability of the findings (Zwaigenbaum et al., 2007). Parent reports might be limited in capturing subtle behaviours and observations may not be as comprehensive as a professional view. Recall bias may also influence parental judgement when asking questions that are related to the early developmental period of the child. This may lead to distorted information due to false memory about the past or parents tending to report only the best behaviours of their child. Analysis of video recordings with systematic coding procedures may overcome these problems. However, the purposes of the video recordings would not be systematic across families and they could vary in setting, length, and quality. In addition to these limitations, this method may limit the number of questions that can be asked by the investigators due to the lack
of control on choosing retrospective predictors. Thus, retrospective longitudinal investigations may limit the assessment on the timing and underlying mechanisms of ASD. In order to eliminate such drawbacks, the prospective longitudinal familial HR risk design has been introduced more recently (e.g., Chawarska et al., 2014; Jones, Gliga, Bedford, Charman, & Johnson, 2014; Messinger et al., 2013; Szatmari et al., 2016; Varcin & Jeste, 2017)

1.5.2. Prospective longitudinal studies

As stated earlier, heritability is a hallmark in ASD (e.g., Losh et al., 2017). Twin studies have examined to what extent genetic and environmental factors involved in the aetiology of ASD by comparing monozygotic and dizygotic twins. The results have indicated that ASD has considerable genetic influences whereas environmental factors are much less salient (e.g., Colvert et al., 2015; Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016), which suggests there is a genetic risk for siblings of individuals with ASD. Population studies have explored the recurrence rates of ASD in at least one or more full siblings, half-siblings and cousins of proband children (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Yip et al., 2017). ASD reoccurs mostly in full-siblings, moderately in half-siblings and the least in cousins (Yip et al., 2017). Among full biological siblings, the reoccurrence rate is almost 10%, and approximately 20% of unaffected siblings represent the sub-clinical threshold of ASD traits (Constantino et al., 2010). Moreover, prospective familial risk studies have shown that the recurrence rate of ASD among 3-year-old siblings is almost 20% and a further 20% of younger siblings develop other social and communication difficulties (Charman, Young, et al., 2017; Messinger et al., 2013; Ozonoff et al., 2011). Hence, there is substantial evidence of genetic liability for ASD and subsequent HR of developing it among siblings of
probands with ASD. Consequently, the recurrence of ASD in siblings of probands to identify early signs within a prospective longitudinal design would be a beneficial avenue for research.

The prospective HR design has been shown to be feasible for following up infant siblings of ASD probands from the first year of life until the 3 years of age when a reliable and stable ASD diagnosis can be made (Jones et al., 2014; Szatmari et al., 2016). The longitudinal nature of the HR design allows for testing younger siblings at various time points to understand developmental trajectories, the onset of the emerging difficulties and the effect of early abnormalities to later clinically-elevated ASD symptoms. Moreover, this design facilitates comparison of HR siblings (due to having (an) older sibling(s) with ASD) and a LR control group (due to having (an) older sibling(s) without ASD or having no history of ASD in first degree relatives), which is essential for controlling possible confounders, such as the effect of being a later-born child and to elicit whether or not the emerging problems are group-specific. In contrast to the retrospective method, HR studies provide a platform for scientists to manipulate the research by controlling predictor variables, administering standardised measures, collecting data in a standardised testing environment, and utilising multi-method approaches.

Studies that have followed the HR infant siblings have indicated potential early markers in infancy that are related to an ASD outcome at the age of 3. For example, limited flexibility in visual attention at 14 months of age (Elsabbagh et al., 2013), reduced activation in the brain regions that are related to visual and auditory processing in response to social stimuli at 4 and 6 months (Lloyd-Fox et al., 2017), reduce gesture production (Talbott, Nelson, & Tager-Flusberg, 2015) and reduced expressive language
by 14 months (Hudry et al., 2014) have all been associated with a later ASD outcome. The HR sibling design also allows for exploration of the profiles of HR siblings who do not meet the criteria for ASD diagnosis but do demonstrate sub-threshold ASD traits or developmental problems, which is referred to as the broader autism phenotype (BAP; Bolton et al., 1994). Compared to the LR controls, these groups score higher in ASD severity and present a moderately delayed profile, while also having a lower level of adaptive functions (Charman, Young, et al., 2017; Messinger et al., 2013).

In addition to explorations of the early markers of ASD, HR sibling design can provide a basis for exploring the roots of co-occurring anxiety traits. Indeed, Milosavljevic, Shephard, Happé, Johnson, and Charman (2017); Shephard et al. (2018) used this design to investigate anxiety in ASD in a cohort of HR and LR children, who were followed up to 7 years old. Milosavljevic, Shephard, Happé, Johnson, Charman, et al. (2017) explored the attention bias to threat and its association with anxiety traits cross-sectionally. Findings of this study suggested that threat bias was not related to anxiety traits in HR children with ASD. Moreover, Shephard et al. (2018) explored the predictive value of parent-reported BI components of fear (7, 14 and 24 months) and shyness (24 months) on anxiety traits (7 years). The results showed that increased fearfulness in infancy and toddlerhood was correlated with anxiety and ASD symptoms in mid-childhood. However, despite the novelty in these investigations, anxiety traits were measured in the mid-childhood when the symptoms of both conditions fully unfold and can be altered by genetic or environmental influences (or interaction between these factors). So, there is still a need for an investigation of underpinnings of anxiety traits in infancy and toddlerhood. In the general population, BI is a robust predictor of early emerging anxiety traits. So investigation of the association between
BI and early emerging anxiety traits in the HR design will shed light on the research gap.

1.5.3. The present study

In summary, the evidence suggests prevalent anxiety disorders and sub-threshold anxiety traits among individuals with ASD as well as their parents and siblings. However, why anxiety highly co-occurs with ASD has not yet been fully understood. Especially, it is not clear anxiety constitute monomorbid anxiety or ASD specific anxiety problems. To untangle this coexistence, studies investigated the directional association between both disorders in mid-childhood, but the results were mixed. The inconsistent patterns of the results between prior investigations could be the result of the change in anxiety disorders by age as well as the symptom presentation of ASD. By mid-childhood and later in the development, environmental factors, such as negative experiences in social environments, other genetic risks or epigenetic modifications may confound the phenotypic presentation. So, investigating underpinnings of anxiety in ASD in mid-childhood or later in the development may make it difficult to disentangle the overlap. Hence, it may be more informative to explore the roots of anxiety in ASD before the clinical presentation of ASD symptoms unfolds by HR for ASD design.

The BI construct in infancy and toddlerhood is involved in the aetiology of later childhood anxiety problems in the general population. Self-regulation (EC), parental anxiety and parental overprotective behaviours influence the association between BI and anxiety. There is scarce evidence on how BI (as an overall construct and social and non-social BI) and relative factors (e.g., parental variables) relates to anxiety in the ASD as well as HR for ASD. Kerns and Kendall (2012) have argued that investigating shared and distinct markers of ASD could help in understanding the nature of the overlap. So,
BI may be a useful construct to explore in an HR for ASD sibling design, which will enhance the current understanding of the nature of anxiety in ASD.

In this thesis, I will first explore the association between parent-reported BI and EC and subsequent anxiety and ASD outcomes (Chapter 2). Then, I will investigate BI in infancy by applying a novel coding scheme, examining this concurrently with ASD traits and longitudinal associations with anxiety and ASD symptoms (Chapter 3). In Chapter 4, I will analyse BI through the observational Stranger Approach and Unpredictable Toy tasks, global ratings and parent reports and investigate whether BI scores yielded in the social and non-social contexts have a similar or distinct association with anxiety and ASD scores. Chapter 5 addresses the familial aggregation of maternal and paternal anxiety and ASD traits on toddlers. Lastly, in Chapter 6, I will position the current findings based on the existing BI literature regarding the general population, discuss the limitations and put forward suggestions for future work.

Overall, the aim of this thesis is to explore whether temperamental BI is a shared or distinct pathway for early emerging anxiety traits and ASD symptoms in an HR ASD sibling design that involves employing a multi-method approach. I will address the broader aims of the current thesis through consideration of the following questions:

1. Is BI associated with later anxiety traits in infants at familial risk for ASD, as it is in infants and toddlers in the general population? If so, is BI a distinct or shared predictor of early emerging anxiety and ASD traits?
2. Do BI and EC relate to each other and how do they relate to subsequent anxiety and ASD traits?
3. Is parental report of temperament traits related to observed traits?
4. Is BI a unitary construct or do social and non-social BI constitute subdimensions? If so, do social BI and non-social BI relate similarly or differently to anxiety and ASD traits?

5. Do parental anxiety and ASD traits relate to child anxiety and ASD traits?
Chapter 2. Developmental Paths to Anxiety in an Autism-Enriched Infant Cohort:
The Role of Reactivity and Regulation

2.1. Introduction

As mentioned in Chapter 1, anxiety is the most highly co-occurring condition and between 40% and 70% of individuals with ASD present with at least one clinically elevated anxiety problem (Salazar et al., 2015; Simonoff et al., 2008; van Steensel et al., 2011). This rate is substantially higher than the prevalence estimate of 27% for anxiety problems in the general child population (Costello et al., 2005). At the trait level, children with ASD tend to score higher on anxiety measures compared to their typically developing (TD) peers (Bellini, 2004) and children with attention deficit hyperactivity disorder (Guttmann-Steinmetz et al., 2010; van Steensel & Heeman, 2017). Co-occurring anxiety and ASD symptoms may exacerbate each other and amplify the difficulties that children with ASD experience. For example, difficulties in social interaction may increase, subsequently contributing to functional impairment (Chang et al., 2012) and reduced quality of life (van Steensel, Bögels, & Dirksen, 2012). Identifying the roots of anxiety in ASD may provide a basis to understand the nature of their co-occurrence, provide clear targets for early interventions, and improve long-term prognosis.

The mechanisms underlying heightened levels of anxiety in children and adolescents with ASD are poorly understood. Anxiety problems may co-occur with ASD due to common genetic (Hallett, Ronald, et al., 2013; Tick, Colvert, et al., 2016) or environmental risk factors (Mazefsky, Conner, & Oswald, 2010; van Steensel & Heeman, 2017). Twin studies have demonstrated a tendency for increased anxiety traits in unaffected co-twins of individuals with ASD, suggesting there may be genetically-
mediated links between the two disorders (Hallett, Ronald, et al., 2013). Although heritability has not been directly examined, family studies have shown associations between increased parental anxiety and increased anxiety symptoms in children with ASD (Duvekot et al., 2018; Mazefsky et al., 2010). Environmental factors that might be more commonly experienced by children with ASD such as being a victim of bullying (van Schalkwyk, Smith, Silverman, & Volkmar, 2018), or experiencing financial challenges in the family due to the additional expense of healthcare could be important influences on childhood anxiety (Lebowitz, Leckman, Silverman, & Feldman, 2016). Furthermore, it remains unclear whether co-occurring anxiety and ASD represent distinct disorders with independent causal pathways that happen to co-occur; whether both disorders share some common causal pathways that result in phenotypic overlap; or whether anxiety might arise as a consequence of ASD symptoms (or vice versa), an example of phenotypic causality (e.g., Mayes, Calhoun, Murray, & Zahid, 2011; Wood & Gadow, 2010).

In an attempt to disentangle this link, longitudinal studies have examined the bidirectional associations of anxiety and ASD in children who have received an ASD diagnosis. Duvekot et al. (2018) have shown that higher levels of social anxiety symptoms predicted more social communication impairment over time in children with ASD aged between 2 and 10 years old. However, Pickard et al. (2017) have shown that social communication difficulties predicted higher social anxiety symptoms in children with ASD diagnosis aged between 7 and 13 years old. The findings from these studies indicate that there are likely complex bidirectional relationships between symptoms of the two conditions once they have emerged. However, studies of children with an existing diagnosis do not provide insight into whether there are shared or distinct pathways that lead to the initial emergence of ASD and anxiety symptoms. Therefore,
investigating risk factors in infancy and toddlerhood prior to the emergence of both disorders is crucial to understand the possible pathways for co-occurring anxiety in ASD. One promising domain of investigation is infant temperament traits since individual differences in temperament have been related to later symptoms of childhood psychopathology in the general population (Costello et al., 2005; Muris et al., 2011; Nigg, 2006). Thus, temperamental dispositions in infancy and toddlerhood may provide an insight into the roots of anxiety in ASD prior to the emergence of both disorders.

2.1.1. Temperament and anxiety in the general population

Rothbart and Derryberry (1981) defined temperament as constitutionally based individual differences in emotional reactivity and regulation, which themselves span the domains of affect, activity and attention. Emotional reactivity refers to the intensity, duration, and latency of emotional and motor responses to internal and/or external stimuli whereas regulation refers to the system that voluntarily monitors, modulates, inhibits or boosts emotional reactions. Both reactivity and regulatory dimensions of temperament play a crucial role in emotional adjustment in children (Gulley et al., 2016).

Within the domain of reactivity, behavioural inhibition (BI) in infancy and toddlerhood is a robust predictor of childhood anxiety problems (e.g., Bufferd et al., 2016; Buss, 2011). BI is characterised by heightened fearfulness, shyness and wariness towards novel stimuli or situations (Fox et al., 2001; Kagan et al., 1984). Children who consistently present a high degree of BI at different developmental stages are more likely to develop clinically-relevant anxiety problems in childhood and adolescence (e.g., Biederman et al., 1993; Scheper et al., 2017). Infants with BI may have a lower threshold and show more fearful responses to sudden changes in stimulation or inhibited
approach towards novelty (Kagan, 1997; Kagan et al., 1999). During toddlerhood, these children are more likely to avoid interactions with novel objects and unfamiliar people. As toddlers avoided novel situations, they may become less socially competent, less assertive, and subsequently be at elevated risk for anxiety disorders (e.g., Buss, 2011; Walker et al., 2014).

The other major dimension of temperament that has been related to later anxiety in the general population is self-regulation or effortful control (EC) which emerges at the end of the first year of life and becomes more pronounced during the preschool period (Posner & Rothbart, 2000). EC refers to the child’s ability to organise reactions to external stimuli and regulate their emotions appropriately and thus influences the severity of negative emotionality and subsequent anxiety problems (e.g., Rothbart et al., 2003). EC is not independent of the reactive domain of temperament and inversely related to negative reactivity (Gulley et al., 2016). Specifically, EC allows children to regulate their emotional or behavioural responses through flexibly employed cognitive abilities such as attention shifting and inhibitory control. Adequately executed EC may allow children to successfully engage in social interactions and prevent negative experiences that may lead to emotional maladjustment (Rothbart et al., 2011).

Higher levels of BI predispose infants and toddlers to later anxiety problems, but it may be due to the negative association between EC and negative reactivity. The association between BI and anxiety may be buffered by reduced EC capacity. For example, Buss et al. (2013) found that infants who failed to regulate their emotional responses showed more BI in unfamiliar situations and more anxiety symptoms in kindergarten. In unfamiliar situations, cognitive processes (attention shifting and inhibitory control) may help children to shift their attention from threatening stimuli and
inhibit extreme negative reactions such as sadness, fearfulness or withdrawal. Indeed, White et al. (2011) found that high levels of attention shifting reduced the risk of anxiety problems in children who had high levels of BI. Thus, successfully employed EC may reduce the likelihood of heightened BI trait that may produce subsequent anxiety problems, acting as a protective factor.

Thus far, evidence suggests that the combination of poor regulation and increased reactivity could represent risk factors for later anxiety symptoms in the general population. Examining whether these early temperamental capacities similarly relate to the development of anxiety in the context of a cohort at familial risk for ASD gives us an important way of testing the nature of anxiety within ASD. If anxiety is purely a downstream effect of ASD symptoms (phenotypic causality), one would not expect an identical pattern of infant temperamental predictors of anxiety to be present before behavioural symptoms of ASD have consolidated.

2.1.2. Temperament and anxiety in ASD

Most research on temperament in ASD has been conducted with children who have an existing diagnosis. Broadly, children with ASD often present with lower levels of EC and more negative reactivity than children with developmental delay, Fragile X and TD across toddlerhood and childhood (e.g., Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Brock et al., 2012; Burrows et al., 2016; De Pauw et al., 2011; Macari et al., 2017). These temperament traits could be related to broader internalising problems (e.g., anxiety) among individuals with ASD. Indeed, higher negative affect and lower EC have been associated with elevated internalising problems in ASD (Burrows et al., 2016; De Pauw et al., 2011). However, disentangling whether these differences in temperament emerge downstream or upstream from ASD or anxiety symptoms is
challenging in cross-sectional studies. Therefore, there is a need for prospective longitudinal studies that track individuals from infancy through to early childhood when ASD and anxiety symptoms emerge.

One fruitful research design is the study of infants with an older sibling with ASD, who have a 20% chance of developing ASD themselves (Ozonoff et al., 2011). In such studies, temperament patterns in infant siblings appear consistent with those seen in later development (e.g., Clifford, Hudry, Elsabbagh, Charman, & Johnson, 2013; Garon et al., 2016). For example, infants with older siblings with ASD showed higher BI than infants with an older typically developing sibling at 12 and 24 months (Garon et al., 2016) with effects largest in infants subsequently diagnosed with ASD (Clifford et al., 2013; Garon et al., 2016). In the regulation domain, infants with later ASD showed poorer EC than other infants at 14 and 24 months (Clifford et al., 2013; Zwaigenbaum et al., 2005). Garon et al. (2016) showed further that lower negative affect at 12 months was associated with higher EC at 24 months, which in turn was associated with lower levels of ASD symptoms at 36 months in the children at familial risk of ASD. These early studies provide some suggestive evidence of similar alterations in temperamental profile in infants at familial risk for ASD and infants with later anxiety.

Figure 2.1 shows conceptual models for developmental pathways for co-occurring anxiety and ASD, inspired by the framework outlined in Johnson et al. (2015). Model A proposes that elevated BI/reduced EC in infants with an older sibling with ASD (infant siblings) may resemble temperamental alterations seen in anxiety (in the general population) but actually represent separate developmental paths; in this case, anxiety may arise mainly as a consequence of ASD symptoms in these cohorts. Alternatively, as in Model B, elevated BI/lower EC in infant siblings could relate to
later anxiety symptoms rather than ASD symptoms; this would represent the same risk
pathway to anxiety as that seem in the general population but may be more common in
infants with familial risk for ASD because of associations at the genetic level. A further
possibility, as shown in Model C, is that elevated BI and lower EC in infant siblings
could indicate that there are some shared developmental pathways between ASD and
anxiety in infant siblings of probands such that genetic/environmental risk factors for
ASD lead to temperamental alterations that also put a child at risk for anxiety. Model D
suggest that BI and EC may also represent different types of pathways and may interact
with each other over time.

One previous study has provided an important initial step towards exploring
what could infant sibling data tell us about the reason that anxiety and ASD often co-
occur (Shephard et al., 2018). In a small group of infant siblings (N = 104) followed to
age 7, increased fearfulness at 14 and 24 months was correlated with both anxiety and
ASD at age 7. This is most consistent with the proposed conceptual model in Figure 2.1.
Model C and may suggest that high BI represents a common path to anxiety and ASD.
However, in path analysis of the same data, a pooled estimate of infant fear from 7 to 24
months was associated with anxiety but not ASD symptoms when they were entered as
correlated outcomes; covarying for ASD symptoms rendered the association with
anxiety only marginally significant. This may be more consistent with Figure 2.1.
Model B that actually higher fear is more closely related to anxiety than ASD. It is
possible that there is an important distinction between the component of infant fear that
is stable across infancy and developmental changes in fearful behaviour that occur over
the first two years of life. One critical factor may be the interaction between infant
fearfulness and EC over time (e.g., Figure 2.1 Model D) if infant fearfulness at 15 and
24 months is additionally influenced by EC over infancy and EC in infancy is related to
ASD, there may be additional associations with ASD when running simple correlations at those time points that are not there when considering the component of fear that is stable from 7 months.

Figure 2.1 Conceptual models for co-occurring anxiety and ASD inspired by the framework outlined in Johnson et al. (2015). Key: RM = Risk Marker; A = ASD; X = Anxiety; GE = Genetic and/or Environmental Risk Factors; BI = Behavioural Inhibition; EC = Effortful Control.

### 2.1.3. The present study

Structural equation modelling (SEM) was used to examine the longitudinal relationship between BI and EC and subsequent anxiety and ASD symptoms over the first two years of life in a large group of infants with and without older siblings with ASD. By including cross-lagged pathways (e.g., from a prior assessment of BI to current assessment of EC) I was able to examine (a) how different temperament traits
influence each other over developmental time and (b) how they relate to anxiety and ASD outcomes. These questions were tackled in four systematic steps for parsimony and clarity of interpretation: (1) whether higher levels of BI predicts anxiety in infants at risk for ASD (Model 2.1) to replicate previous work (Shephard et al., 2018), and examine specificity to BI (vs other components of negative emotionality, Model 2.1b). (2) how BI and EC interrelate over the first two years of life and how this interrelation associates with later anxiety (Model 2.2). (3) how BI and EC interrelate over the first two years of life and how this interrelation associates with later anxiety and ASD (Model 2.3). (4) I used mediation analysis to probe the relationship between BI, EC, and later anxiety/ASD over developmental time. This analysis allowed me to ask whether the relationship between an infant predictor and a developmental outcome can be fully accounted for by another variable, for example, whether any relations between infant BI/EC and later anxiety can actually be accounted for by anxiety symptoms. Taken together, I interpret the pattern of results in line with the theoretical frameworks presented in Figure 2.1.

2.2. Method

2.2.1. Participants

Participants in this study were 116 high-risk (HR) (52 female; 64 male) and 27 low-risk (LR) (13 female; 14 male) children who took part in the second phase of the longitudinal British Autism Study of Infant Siblings (BASIS; www.basisnetwork.org). All HR participants had at least one older sibling with a community clinical diagnosis of ASD (herein, proband). Diagnosis of probands was confirmed by expert clinicians (TC, PB) based on parent-reported Development and Wellbeing Assessment (DAWBA; Goodman, Ford, Richards, Gatward, & Meltzer, 2000), the Social Communication
Questionnaire (SCQ; Rutter, Bailey, et al., 2003), or parent-confirmed community clinical ASD diagnosis. Seventy-seven probands met the criteria on both the DAWBA and SCQ, eight probands fell below the SCQ cut-off (≥15) but were included due to meeting the threshold on the DAWBA. For 19 probands, confirmation of ASD diagnosis was only available for the SCQ while for five, neither measure was available, but they were included based on parent confirmed clinical ASD diagnosis.

The LR group had at least one TD older sibling and no first-degree family members with an ASD diagnosis (confirmed through family medical history screening). LR infants were recruited from a volunteer database at the Birkbeck Centre for Brain and Cognitive Development. Possible ASD symptoms were screened in the older siblings of the LR infants with the SCQ and none of the older siblings scored above the ASD cut-off score of 15. Further inclusion criteria for both groups were having at least one parent speaking English at home.

Exclusion criteria for both groups, based on parent report, included significant prematurity (gestational age ≤ 32 weeks), marked medical conditions such as epilepsy, heart conditions, vision and hearing impairments, cerebral palsy, or genetic conditions such as Down syndrome or Fragile X, parents with evidence of learning difficulties or unable to give informed consent. No infants had any known medical or developmental condition at the time of enrolment.

Families enrolled in this study when their babies were eight months of age or younger and attended four lab visits when children were 9, 15, 24 and 36 months of age. A battery of questionnaires was posted to parents prior to each visit and parents were asked to bring them to the lab visit.
Ethical approval for this study was given by the National Health Service National Research Ethics Service (NHS NRES London REC 08/H0718/76). At each time point, written informed consent was obtained from parents for their children to participate in the study.

### 2.2.2. Measures used in modelling

Fear, shyness, sadness and EC were measured using the Infant Behaviour Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003) and the Early Childhood Behaviour Questionnaire (ECBQ; Putnam et al., 2006). These are parent-report questionnaires which allow caregivers to rate the frequency of particular behaviours during the previous two weeks. Studies that investigated the factorial structure of both questionnaires yielded three higher-order factors: (i) Surgency and (ii) Negative Affect, which constitute the reactivity domain, and (iii) EC, which constitutes the self-regulatory domain. In the IBQ-R, fear refers to infant distress or inhibited approach to novel objects, social stimuli or novelty. In both IBQ-R and ECBQ, shyness refers to discomfort, slow or inhibited approach to novelty and uncertainty in social situations, sadness refers to general low mood and activity related to personal suffering, physical state, object loss, or inability to perform a desired action and EC characterised by ability of shifting attention, duration of attentional focusing, inhibitory control and low-intensity pleasure. The IBQ-R was completed at 9 and 15 months; the ECBQ at 24 months of age.

BI was not directly addressed in these questionnaires. Therefore, BI was measured using the IBQ-R Fear subscale (at 9 and 15 months), and the ECBQ Shyness subscale (at 24 months). Multi-method approach studies have used IBQ-R Fear (Crockenberg & Leerkes, 2006; Gensthaler et al., 2013) and ECBQ Shyness subscales
as a proxy of parent-reported BI to complement the observed BI (e.g., Geng et al., 2011). In the current sample, the cross-sectional correlation between Fear and Shyness subscales at 24 months was $r = .59, p < .001$ and the longitudinal correlation between Fear at 9 months and Shyness at 24 months was $r = .30, p = .001$; and Fear at 15 months and Shyness at 24 months was $r = .52, p < .001$. This suggests that Fear and Shyness subscales have a moderate concurrent and longitudinal relationship (only between 15 and 25 months) and this allowed us to use Shyness subscales at 24 months in longitudinal analysis. This approach is also supported by past studies, which showed significant longitudinal and cross-sectional associations between these two subscales in infancy and toddlerhood (Eggum et al., 2009; Wolfe et al., 2014). Some of the research measures (e.g., IBQ, ECBQ and CBCL) were developed in the general population and to measure the overall internal reliability of the research measures for the current sample, Cronbach’s alpha was calculated throughout this thesis. Calculation of Cronbach’s alpha is equivalent to split-half reliability, but it combines two splits of data in every possible way (Cronbach, 1951). Values between .70 and .80 indicate good, .80 and above indicates excellent internal reliability. For the current sample, Cronbach’s alpha ranged between .80 and .93 for all the IBQ and the ECBQ subscales, indicating excellent internal reliability (Kline, 2000).

Anxiety traits were measured with the raw scores of DSM-Oriented Anxiety Problems subscale of Child Behaviour Checklist 1.5/5 at 36 months (CBCL; Achenbach & Rescorla, 2000). The CBCL is a standardised parent-report questionnaire measuring emotional, behavioural and social problems. The DSM-Oriented Anxiety Problems subscale assesses for symptoms of separation anxiety disorder, specific phobia, and generalised anxiety disorder. Parents endorse one of the item responses 0 ‘Not true,’ 1 ‘Somewhat or Sometimes True,’ or 2 ‘Very True or Often True’ to specify the
frequency of problems that the child has experienced in the past two months. The CBCL has been widely used to measure emotional and behavioural symptoms in ASD (e.g., Hartley & Sikora, 2009; Havdahl, von Tetzchner, Huerta, Lord, & Bishop, 2016; Magyar & Pandolfi, 2017). Cronbach alpha for the anxiety problems subscale was .79, indicating good internal consistency for the current sample.

ASD symptomatology at 36 months was measured with the Social Communication Questionnaire (SCQ; Rutter, Bailey, et al., 2003). The SCQ is a 40-item parent-reported questionnaire which allows parents to endorse ASD related behaviours that they have observed in their children over the past three months. Total SCQ scores vary between 0 and 39 and higher scores reflect higher symptoms. Cronbach’s alpha for the SCQ was .91, showing excellent internal reliability.

2.2.3. Data analysis

Pearson correlation coefficients were calculated to assess the relationships between predictors (temperament traits measured at 9, 15, and 24 months) and outcomes (anxiety and ASD measured at 36 months). Due to the high number of comparisons made, the reported significance level was set to $p < .001$. Mean group differences, between LR and HR participants, were computed for all predictors and outcome variables using SPSS 23.

Cross-Lagged and mediation models were estimated in an SEM framework using Mplus 7.13 (Muthén & Muthén, 1998-2015). Full Information Maximum Likelihood (FIML) was used to account for missing data and Maximum Likelihood Robust (MLR) estimation was used to provide robust standard errors to account for the non-normal distributions and skewness in the anxiety outcome measure. Model fit was assessed by the Root Means Square Error of Approximation (RMSEA), the
Comparative Fit Index (CFI) and the Standardised Root Mean Square Residual (SRMR). An acceptable fit was indicated by the RMSEA of 0.05-0.08, the CFI of 0.90-0.95, the SRMR of 0.05-0.08. A good fit was indicated by the RMSEA of 0.01-0.05, the CFI of 1-0.95, and the SRMR of < 0.05 (Hu & Bentler, 1999; Kline, 2016). All model coefficients were standardised for the predictor and outcome (i.e. STDYX in Mplus; Muthén & Muthén, 1998-2015). These criteria for the fit indices were also used in Chapter 4 and 5 for checking the model fit.

I fit models sequentially for parsimony and clarity of interpretation. All models were estimated using observed (i.e. non-latent) variables only and were estimated using the combined LR and HR sample (N =143). The risk groups (LR = 0; HR = 1) was treated as a covariate in all models to control for effect and regressed on each predictor and outcome variables.

2.2.3.1. Model 2.1: Longitudinal associations between BI and anxiety.

As a baseline model, I tested the relationship between infant BI (9, 15, 24 months) and anxiety in toddlerhood (36 months), illustrated in Figure 2. This model included autoregressive pathways between three measures of BI at 9, 15, and 24 months. It further included direct pathways between each BI measure and anxiety traits at 36 months to assess the developmental timing of the association (Figure 2.2).
I used a cross-lagged model to examine the direction of longitudinal relationships between EC, BI and later anxiety. Direct pathways between each temperament traits and anxiety traits were incorporated in this model (Figure 2.3).

Figure 2.2 Model 2.1, the proposed model for the first-order autoregressive model of BI and anxiety.

2.2.3.2. Model 2.2: Longitudinal associations between BI, EC and anxiety

Figure 2.3 Model 2.2, the proposed cross-lagged association between BI, EC and anxiety
2.2.3.3. Model 2.3: Longitudinal association between BI, EC, anxiety and ASD

In the third model, I extended Model 2.2 by including ASD scores as a secondary outcome variable to assess whether the findings in the Model 2.2 are unique to anxiety or also shared with ASD (Figure 2.4).

![Diagram](image)

Figure 2.4 Model 2.3, the proposed cross-lagged association between BI, EC, anxiety and ASD

2.2.4. Exploratory mediation analysis

To explore whether the relationships between BI, EC (24 months) and anxiety (36 months) were mediated by ASD (36 months), or conversely, whether the association between BI, EC (24 months) and ASD (36 months) were mediated by anxiety (36 months), two separate mediation models were estimated. For clarity, these models included the exposure, mediator, and outcomes only and did not incorporate the cross-lagged panel structure used above. In both mediation models, indirect effects were estimated using Maximum Likelihood estimation with bootstrapped confidence intervals (1000 iterations).
2.2.5. Post hoc power analysis

A post hoc power analysis was calculated after Model 2.3 since it is the most complicated model and included the pathways in Model 2.1 & 2.2 to eliminate the replication.

The power analysis is a retrospective calculation of an observed effect driven from the sample size and parameter estimates. In SEM power calculation is not easy because there are a large number of parameters (e.g., means, intercepts, regression coefficients, variances and covariances). Within a model, each parameter can be estimated with different degrees of precision. Also, parameters may be dependent on other parameters. However, Satorra and Saris (1985) presented a straightforward approach for testing statistical power of single df test. This approach requires to be repeated for every parameter for which an estimate of power is required (e.g., Model 2.3 BI-anxiety). The main idea behind this approach is that when a model is misspecified, the model fit test statistic follows a noncentral $\chi^2$ distribution. The model $\chi^2$ statistic of the misspecified model can be considered as noncentrality parameter of the noncentral $\chi^2$ distribution. The following steps were followed to calculate the desired power for significant pathways: 1) After obtaining the model, in a separate analysis, a desired pathway was constrained to 0; 2) the difference between $\chi^2$ of the alternative model (the actual model pathways were freely estimated) and the constrained model (the model that has pathways constrained to 0) was calculated; 3) the $\chi^2$ distribution table was checked for the 1 df (since the pathways were constrained to 0 in separate analysis) at .95 significant level (it gives the $\chi^2$ of 3.84); 4) the formula of 1-nchi2(df of difference between models, $\chi^2$ difference, value from $\chi^2$ table) was run in the STATA.
2.3. Results

2.3.1. Sample characteristics

Sample characteristics, means and standard deviations for measures and risk group comparisons (effect sizes) are shown in Table 2.1. Groups did not differ in the proportion of girls and were the same age at each visit with the exception of the 24-month timepoint where the HR group were older than the LR controls. The HR group scored significantly higher than the LR group on the BI subscale at 9 and 15 months but not at 24 months. The HR group had significantly lower EC than the LR group only at 24 months. The HR group scored higher than the LR group on the anxiety subscale and ASD total scores at 36 months.
Table 2.1 Sample Characteristics and Descriptives by Risk Group

<table>
<thead>
<tr>
<th></th>
<th>High-Risk M (SD)</th>
<th>Low-Risk M (SD)</th>
<th>Group Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visit 1 – 9 m.</strong></td>
<td></td>
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</tr>
<tr>
<td>N (girls)</td>
<td>116 (52)</td>
<td>27 (13)</td>
<td>$\chi^2 (1) = .002, p = .960$</td>
</tr>
<tr>
<td>Age in days</td>
<td>274.18 (24.81)</td>
<td>283.42 (25.36)</td>
<td>$t (138) = 1.71, p = .090, d = .37$</td>
</tr>
<tr>
<td>IBQ BI</td>
<td>3.03 (1.13)</td>
<td>2.60 (.76)</td>
<td>$t (52.99) = -2.37, p = .021, d = .45$</td>
</tr>
<tr>
<td>IBQ Sadness</td>
<td>3.86 (.95)</td>
<td>3.69 (.97)</td>
<td>$t (138) = -0.83, p = .407, d = .18$</td>
</tr>
<tr>
<td>IBQ EC</td>
<td>4.68 (.65)</td>
<td>4.75 (.69)</td>
<td>$t (138) = .456, p = .649, d = .10$</td>
</tr>
<tr>
<td><strong>Visit 2 – 15 m.</strong></td>
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<td></td>
</tr>
<tr>
<td>N (girls)</td>
<td>113 (51)</td>
<td>27 (13)</td>
<td>$\chi^2 (1) = .102, p = .749$</td>
</tr>
<tr>
<td>Age in days</td>
<td>465.90 (30.36)</td>
<td>473.63 (27.82)</td>
<td>$t (139) = .83, p = .409, d = .27$</td>
</tr>
<tr>
<td>IBQ BI</td>
<td>3.47 (1.05)</td>
<td>2.94 (.88)</td>
<td>$t (135) = -2.41, p = .017, d = .55$</td>
</tr>
<tr>
<td>IBQ Sadness</td>
<td>3.99 (.97)</td>
<td>3.76 (.83)</td>
<td>$t (136) = -1.17, p = .244, d = .25$</td>
</tr>
<tr>
<td>IBQ EC</td>
<td>4.57 (.63)</td>
<td>4.83 (.60)</td>
<td>$t (136) = 1.98, p = .050, d = .42$</td>
</tr>
<tr>
<td><strong>Visit 3 – 24.</strong></td>
<td></td>
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<tr>
<td>N (girls)</td>
<td>102 (46)</td>
<td>27 (13)</td>
<td>$\chi^2 (1) = .01, p = .916$</td>
</tr>
<tr>
<td>Age in months</td>
<td>26.26 (2.23)</td>
<td>24.71 (1.20)</td>
<td>$t (120) = -3.28, p = .001, d = .87$</td>
</tr>
<tr>
<td>ECBQ BI</td>
<td>3.25 (1.07)</td>
<td>3.06 (.88)</td>
<td>$t (120) = -0.82, p = .417, d = .19$</td>
</tr>
<tr>
<td>ECBQ Sadness</td>
<td>3.11 (1.00)</td>
<td>2.55 (.60)</td>
<td>$t (58.69) = -3.53, p = .001, d = .68$</td>
</tr>
<tr>
<td>ECBQ EC</td>
<td>4.45 (.72)</td>
<td>4.72 (.41)</td>
<td>$t (61.79) = 2.45, p = .017, d = .46)$</td>
</tr>
<tr>
<td><strong>Visit 4 – 36 m.</strong></td>
<td></td>
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<tr>
<td>N (girls)</td>
<td>111 (49)</td>
<td>25 (11)</td>
<td>$\chi^2 (1) = .001, p = .983$</td>
</tr>
<tr>
<td>Age in months</td>
<td>38.88 (1.96)</td>
<td>38.72 (1.46)</td>
<td>$t (127) = -.37, p = .712, d = .09$</td>
</tr>
<tr>
<td>MSEL ELC</td>
<td>102.46 (25.10)</td>
<td>119.48 (15.26)</td>
<td>$t (57.49) = 4.40, p &lt; .001, d = .82$</td>
</tr>
<tr>
<td>Anxiety</td>
<td>3.22 (3.42)</td>
<td>1.92 (1.53)</td>
<td>$t (82.19) = -2.75, p = .008, d = .49$</td>
</tr>
<tr>
<td>ASD</td>
<td>6.26 (6.84)</td>
<td>2.64 (2.29)</td>
<td>$t (115.23) = -4.52, p &lt; .001, d = .71$</td>
</tr>
</tbody>
</table>
2.3.2. Correlations between predictor and outcome variables

Table 2.2 shows the pattern of correlations for the variables used in the modelling. Taking an alpha level of 0.001, the relationship between BI and EC was not significant. Predictors were significantly related to outcome variables: higher anxiety scores were associated with higher BI at 15 and 24 months \( (r = .35, p < .001; r = .44, p < .001; \text{respectively}) \); and lower levels of EC at 15 and 24 months \( (r = -.33, p < .001; r = -.41, p < .001; \text{respectively}) \). Higher SCQ scores were associated with lower levels of EC at 15 and 24 months \( (r = -.31, p < .001; r = -.57, p < .001; \text{respectively}) \). Anxiety and ASD scores were also highly correlated to each other \( (r = .57, p < .001) \). These patterns of associations indicate adequate associations between the predictors and the outcome variables for further modelling.
Table 2.2 Pearson Correlation Coefficients for Associations between Temperament Scales, CBCL Anxiety Scores and SCQ Scores in the HR and LR Groups Combined.

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<tbody>
<tr>
<td>1. IBQ BI 9m</td>
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<tr>
<td>2. IBQ BI 15m</td>
<td>.553*</td>
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<tr>
<td>3. ECBQ BI 24m</td>
<td>.300</td>
<td>.520*</td>
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<tr>
<td>4. IBQ Sadness 9m</td>
<td>.548*</td>
<td>.287</td>
<td>.184</td>
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<tr>
<td>5. IBQ Sadness 15m</td>
<td>.382*</td>
<td>.392*</td>
<td>.267</td>
<td>.560*</td>
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<tr>
<td>6. ECBQ Sadness 24m</td>
<td>.389*</td>
<td>.474*</td>
<td>.417*</td>
<td>.451*</td>
<td>.440*</td>
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<tr>
<td>7. IBQ Effortful Control 9m</td>
<td>-.111</td>
<td>-.067</td>
<td>-.153</td>
<td>-.097</td>
<td>-.072</td>
<td>-.101</td>
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<tr>
<td>8. IBQ Effortful Control 15m</td>
<td>-.052</td>
<td>-.126</td>
<td>-.246</td>
<td>-.003</td>
<td>-.126</td>
<td>-.134</td>
<td>.555*</td>
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<tr>
<td>9. ECBQ Effortful Control 24m</td>
<td>-.105</td>
<td>-.209</td>
<td>-.237</td>
<td>-.209</td>
<td>-.126</td>
<td>-.247</td>
<td>.432*</td>
<td>.533*</td>
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<tr>
<td>10. CBCL Anxiety Problems 36m</td>
<td>.313*</td>
<td>.369*</td>
<td>.442*</td>
<td>.253</td>
<td>.220*</td>
<td>.396*</td>
<td>-.140</td>
<td>-.345*</td>
<td>-.424*</td>
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<tr>
<td>11. SCQ 36m</td>
<td>.118</td>
<td>.147</td>
<td>.312</td>
<td>.128</td>
<td>.095</td>
<td>.286</td>
<td>-.041</td>
<td>-.330*</td>
<td>-.586*</td>
<td>.583*</td>
<td>----</td>
</tr>
</tbody>
</table>

* p < .001; IBQ: Infant Behaviour Questionnaire, ECBQ: Early Childhood Behaviour Questionnaire, CBCL: Child Behaviour Checklist, SCQ: Social Communication Questionnaire
2.3.3. Model 2.1: Longitudinal association between BI and anxiety.

I first tested the hypothesised relationship between infant BI and toddler anxiety (Figure 2.2). Overall, there was an association between BI (24 months) and anxiety traits (36 months). The fit indices of the autoregressive model indicated a good fit to the data ($\chi^2 (1) = .27, p = .605; \text{CFI} = 1.00, \text{RMSEA} = .000, \text{and SRMR} = .008$). The association between the risk group (HR vs LR) and BI at 9 and 15 months was significant ($\beta = .15, p = .018; \beta = .13, p = .043$, respectively), indicating higher BI in the HR group at these time points. All coefficients for the autoregressive pathways of BI were significant; BI at 9 months was related to subsequent BI at 15 months ($\beta = .53, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .52, p < .001$), with the similarity in standardised beta values indicating stability in magnitudes of associations between time points. Higher levels of BI at 24 months were associated with higher levels of anxiety at 36 months ($\beta = .34, p < .001$; Figure 2). Model 2.1b (see Appendix 1) indicates that this relation does not extend to other components of negative emotionality (infant sadness). This confirmed that infant BI specifically related to toddler anxiety.
Figure 2.5 The result of Model 2.1, the first-order autoregressive model of BI and anxiety. Bolds indicate a significant association, dotted lines indicate non-significant results. Standardised beta and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$.

2.3.4. Model 2.2: Longitudinal association between BI, EC and anxiety

I then tested how infant EC interrelated with infant BI in the prediction of later anxiety (Figure 2.3). The cross-lagged autoregressive model provides a good fit to the data ($\chi^2 (4) = 5.36, p = .252$; CFI = .994, RMSEA = .049, and SRMR = .024). The risk group was significantly related to 9- and 15-months BI and 15 months EC scores ($\beta = .15, p = .017$; $\beta = .13, p = .045$; $\beta = -.16, p = .010$; respectively). The autoregressive pathways indicate that BI (9-15 months: $\beta = .53, p < .001$; 15-24 months: $\beta = .50, p < .001$) and EC (9-15 months: $\beta = .56, p < .001$; 15-24 months: $\beta = .53, p < .001$) had a stable magnitude of relationship between different timepoints. Concurrent residual correlations between BI and EC were not significant ($p \geq .192$), which means that BI and EC were not interrelated within a timepoint. As for the cross-lagged paths, higher levels of EC at 15 months related to decreased BI at 24 months ($\beta = -.20, p = .009$). Both higher levels of BI and lower levels of EC at 24 months were significantly associated with higher levels of anxiety symptoms ($\beta = .24, p = .008$; $\beta = -.29, p = .014$;
respectively; Figure 2.3). Thus, better EC at 15 months appeared to be associated with lower anxiety through decreased BI in toddlerhood.

Figure 2.6 The results of Model 2.2, the cross-lagged association between BI, EC and anxiety. Bold lines indicate a significant association, dotted lines indicate non-significant results. Standardised beta and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$.

2.3.5. Model 2.3: Longitudinal association between BI, EC, anxiety and ASD

I then tested how ASD traits interrelate with infant temperament and later anxiety (Figure 4). The model fit indices suggested a good fit to the data ($\chi^2 (4) = 4.20$, $p = .380$; CFI = .999, RMSEA = .018, and SRMR = .021). Again, the risk group was significantly related to BI at 9 and 15 months, EC at 15 months, and ASD at 36 months ($\beta = .15$, $p = .016$; $\beta = .13$, $p = .043$; $\beta = -.16$, $p = .010$; $\beta = .13$, $p = .016$; respectively). The magnitude of the autoregressive pathways for BI and EC were stable from infancy
to toddlerhood (BI: 9-15 months: $\beta = .53, p < .001$; 15-24 months: $\beta = .50, p < .001$; EC: 15 months: $\beta = .56, p < .001$; 15-24 months: $\beta = .52, p < .001$). Concurrent correlations between BI and EC were not significant at all three timepoints ($p \geq .192$).

As in the previous model, the cross-lagged paths indicated that higher EC at 15 months related to decreased BI at 24 months ($\beta = -.20, p = .010$). Both higher levels of BI and lower levels of EC at 24 months were significantly associated with heightened levels of anxiety symptoms ($\beta = .24, p = .007$; $\beta = -.28, p = .013$; respectively) and ASD symptoms ($\beta = -.61, p < .001$; $\beta = .20, p = .003$; respectively). Unlike for anxiety, higher levels of EC at 9 months were related to increased ASD symptoms ($\beta = .28, p < .001$). There was also a concurrent positive relationship between anxiety and ASD symptoms at 36 months ($\beta = .43, p < .001$) (Figure 2.7). Thus, this model indicates that both BI and EC are associated with anxiety and ASD symptoms in toddlerhood, but there may be additional specific relations between EC in early infancy and later ASD.

The test of no direct effects between BI at 24 months and anxiety gave a $\chi^2$ difference of 11.01 for 5 df with 143 participants and the strength of generating the significant pathways was 74%. The power of detecting the association between BI (24 months) and ASD was 73%, EC (24 months) and anxiety was 85%, EC (24 months) and ASD was 99%, EC (15 months) and BI (24 months) was 73%. These results indicate strong power for detecting the significant associations.
Figure 2.7 The results of Model 2.3, cross-lagged association between BI, EC, anxiety and ASD. Standardised beta and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$.

2.3.6. Exploratory mediation analysis

I examined whether toddler BI and EC were equally related to anxiety and ASD symptoms. There was evidence of an indirect effect of BI (24 months) on anxiety (36 months) via ASD (36 months; $\beta = .14$, $p = .004$, 95% CI [.06, .22]). The direct ($\beta = .28$, SE = .08, $p = .001$), and the total effects ($\beta = .42$, SE = .10, $p < .001$) were significant, suggesting a partial mediation. Specifically, 33% of the total effect of BI on anxiety was operating through ASD. There was also a significant indirect effect of BI on ASD through anxiety ($\beta = .22$, $p < .001$, 95% CI [.12, .32]). The direct effect of BI on ASD was not significant ($\beta = .06$, SE = .09, $p = .472$), and the total effect was significant ($\beta = .28$, SE = .10, $p = .004$), anxiety mediated (complete mediation) the relationship
between BI and ASD and accounted for 79% of the total effect. Thus, these results suggest that BI is more strongly related to anxiety rather than ASD traits.

The indirect effect of EC (24 months) on anxiety (36 months) via ASD (36 months) was significant ($\beta = -.31, p < .001, 95\% \text{ CI} [-.41, -.21]$). The direct effect was not significant ($\beta = -.09, SE = .09, p = .331$), and the total effect was significant ($\beta = -.39, SE = .08, p < .001$), suggesting a complete mediation. Seventy-nine per cent of the total effect of EC on anxiety was operating through ASD. There was also a significant indirect effect of EC on ASD through anxiety ($\beta = -.16, p = .001, 95\% \text{ CI} [-.25, -.08]$). The direct effect ($\beta = -.43, SE = .09, p < .001$), and the total effect were both significant ($\beta = -.59, SE = .07, p < .01$). Anxiety partially mediated the association between EC and ASD, accounting for the 27% of the total effect (Table 2.3). Thus, the pattern for EC was the opposite as for BI, suggesting that EC at 24 months is more strongly related to ASD than anxiety.

2.3.7. Sensitivity analyses

All analyses were re-estimated to control for the effect of possible confounders sex and cognitive ability (MSEL). The effect of missing data was addressed by re-estimating the models with listwise deletion. The results of the models after adjustment for sex and with listwise deletion did not change. However, when controlling for cognitive ability at 36 months, the relationship between BI at 9 months and anxiety at 36 months become significant; all other patterns of findings remain the same. Further results for Models 2.1 to 2.3 are summarised in Appendix 1.
Table 2.3 Summary of Exploratory Mediation Analyses

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Mediator</th>
<th>Outcome</th>
<th>Total Effect</th>
<th>Direct Effect</th>
<th>Indirect Effect</th>
<th>Percentage of Total Effect Mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>(24 months)</td>
<td>(36 months)</td>
<td>(36 months)</td>
<td>Total Effect</td>
<td>Direct Effect</td>
<td>Indirect Effect</td>
<td>(95% CI Bootstrap)</td>
</tr>
<tr>
<td>Behavioural Inhibition</td>
<td>ASD</td>
<td>Anxiety</td>
<td>.42 (.10) ***</td>
<td>.28 (.08) **</td>
<td>.14 (.06, .22) **</td>
<td>33 %</td>
</tr>
<tr>
<td>Effortful Control</td>
<td>ASD</td>
<td>Anxiety</td>
<td>-.39 (.08) ***</td>
<td>-.09 (.09)</td>
<td>-.31 (-.41, -.21) ***</td>
<td>79 %</td>
</tr>
<tr>
<td>Behavioural Inhibition</td>
<td>Anxiety</td>
<td>ASD</td>
<td>.28 (.10) **</td>
<td>.06 (.09)</td>
<td>.22 (.12, .32) ***</td>
<td>79 %</td>
</tr>
<tr>
<td>Effortful Control</td>
<td>Anxiety</td>
<td>ASD</td>
<td>-.59 (.07) ***</td>
<td>-.43 (.09) ***</td>
<td>-.16 (-.25, -.08) **</td>
<td>27 %</td>
</tr>
</tbody>
</table>

*** p < .001, ** p < .01, * p < .05
2.4. Discussion

The primary aim of this prospective study was to examine the developmental pathways that contribute to the co-occurrence between anxiety and ASD traits. I investigated how infant reactivity and regulation related to later anxiety in a cohort enriched for later ASD symptoms. The models showed that 1) higher levels of BI at 24 months were associated with anxiety traits at 36 months; while 2) more BI at 24 months was associated with more anxiety traits at 36 months, although this was reduced by having better EC at 15 months. 3) The associations between reduced EC and higher BI and anxiety was also similar for the ASD outcome; 4) mediation analyses showed that BI was related to ASD traits through emerging anxiety traits whereas EC was related to anxiety through ASD traits.

2.4.1. Infant BI and anxiety

Model 2.1 confirmed that heightened reactivity in infancy, without the effect of regulation, was associated with higher levels of anxiety in the current cohort, consistent with the existing literature in the general population (Bufferd et al., 2016; Buss, 2011). Especially, heightened BI at 24 months was related to heightened levels of anxiety scores at 36 months in the current cohort. This is also in line with a previous study showing that shyness in toddlerhood was significantly correlated with anxiety symptoms in a cohort of HR and LR infant siblings followed up to age 7 (Shephard et al., 2018).

Prior evidence has suggested that both children with ASD and infants with older siblings with ASD had increased negative affect, sadness, shyness and fear (e.g., Clifford et al., 2013; Garon et al., 2016) but few studies have examined associations between these temperament traits and emerging internalising problems (Burrows et al.,
Model 2.1b (Appendix 1) assessed whether the observed associations between BI and anxiety were specific, or whether they may be extended to broader aspects of negative affect (specifically sadness). Planalp et al. (2017) argued that BI and sadness share similar behavioural or emotional reactions such as withdrawal, but the context that elicits them differs. Specifically, BI is characterised by reactions to novelty or over-arousing stimuli whereas sadness is characterised by broader reactions to goal blockage or loss without an approach/withdrawal orientation. The results showed that there was a significant concurrent association between BI and sadness at each time point and higher BI in infancy was related to increased sadness in toddlerhood (Model 2.1b). However, infant/toddler sadness was not related to anxiety outcome. Thus, the relationship between infant BI and later anxiety is relatively specific. Following Planap et al. (2017), it may be that the specificity of BI in predicting anxiety is due to infants’ intolerance of uncertainty in a novel context. Intolerance to uncertainty is the perception that an uncertain, unpredictable context is stressful and threatening, and this perception results in avoidance (Anderson et al., 2012). As in the general population, intolerance to uncertainty appears as a mechanism that explains the variation between anxiety and ASD in children and young adults with ASD (Boulter et al., 2014). Thus, elevated worry in ambiguous or over-arousing situations may be an early form of intolerance to uncertainty resulting in maladaptive emotionality in infant siblings.

2.4.2. Longitudinal association between BI, EC and anxiety

Model 2.2 shows how EC interrelates with BI in the prediction of later anxiety. EC has been proposed to be a protective factor against a wide range of conditions, including both ASD (Johnson, 2012) and anxiety (White et al., 2011). Thus, EC would
be expected to interrelate with other risk factors in shaping later psychopathology. The cross-lagged models that were employed allow us to directly test this possibility. Specifically, I explored the longitudinal directionality of the association between BI and EC over the time window in which behavioural symptoms of ASD and anxiety emerge. As expected, better EC at 15 months was associated with less BI at 24 months. Furthermore, greater BI in toddlerhood was associated with more anxiety symptoms; but later anxiety symptoms were reduced by having higher EC in infancy. In other words, successfully employed EC capacities in infancy reduced dysregulated emotional reactivity in toddlerhood, and reduced emotional reactivity related to reduced anxiety traits at 36 months. This is consistent with the proposal that EC and related constructs might be broad protective factors against later psychopathology (Johnson, 2012).

2.4.3. Longitudinal association between BI, EC, anxiety and ASD

Since symptoms of anxiety and ASD intertwine, it is also crucial to further investigate whether infant predictors related similarly or differently to ASD and anxiety outcomes in our cohort. Model 2.3 showed that for some paths, BI and EC related similarly to later anxiety and ASD symptoms. Specifically, successful EC capacity at 15 months related to lower levels of BI at 24 months; and lower levels of BI at 24 months related to both fewer anxiety and ASD traits (36 months). This could suggest shared developmental pathways to both anxiety and ASD traits (Figure 2.1. Model C). Alternatively, infant temperamental features may have apparently similar relations to both ASD and anxiety because phenotypic causality drives stronger associations between ASD and anxiety symptoms once they have emerged (Figure 2.1. Model B or D). To dissect this possibility, I used mediation analyses to explore whether BI and EC at 24 months relate to ASD traits through co-occurring anxiety symptoms, or whether
BI and EC relate to anxiety symptoms through ASD traits. This could tell us, for example, whether more fearful infants develop anxiety symptoms that then exacerbate their ASD symptoms, or whether infants who have poorer EC develop ASD symptoms that then lead to anxiety symptoms.

The results suggest differences between EC and BI in terms of their relationship with ASD and anxiety traits. Early BI predicted later anxiety symptoms, and this was only partially mediated by ASD symptoms. In contrast, the relation between early BI and later ASD symptoms were fully mediated by anxiety symptoms. This may mean that the degree to which early reactivity predicts later ASD is likely attributable to more anxious children tending to have higher levels of ASD symptoms in our cohort (similar to Figure 2.1, Model B). This association could be due to phenotypic causality such that anxiety exacerbates ASD related social difficulties (Duvekot et al., 2018). In other words, BI disposition increases the likelihood of children at HR of ASD for anxiety and increased anxiety problems may lead to higher ASD symptoms. So, co-occurring anxiety problems may exacerbate social difficulties such as finding it hard to join peer groups. The Model A suggested the lower levels of EC and heightened BI may explain the anxiety problems in the HR for ASD design as if it does in the general population. However, Model 2.3 and mediation analysis showed that even if the co-occurring anxiety and ASD were controlled, there were still variances between variables. So the analyses did not support Model A. Overall, these results suggest that apparent infant predictors of later ASD in sibling studies are actually related to risk for co-occurring conditions like anxiety or ADHD.

The results diverge from those observed in a smaller cohort in which Shephard et al. (2018) showed that shyness at 24 months was related to 7-year ASD but not
anxiety symptoms. Further, the current statistical approach examined the longitudinal bidirectional effects between BI and EC (see also, Eggum et al., 2009; Wolfe et al., 2014) whereas Shephard et al. (2018) specifically tested the relation between shyness at 24 months and 7 year anxiety scores. Incorporating longitudinal data and taking into account the bidirectional relation between EC and BI may reveal patterns of association that are not detectable when focusing on simple associations. Alternatively, there may be stronger associations between toddler BI and early-emerging anxiety traits at age 3 than in later development (childhood and adolescence), where other cascading effects and phenotypic interactions further complicate the relationship. Some studies suggest that anxiety symptoms increase from childhood to young adulthood (Gotham et al., 2015; van Steensel et al., 2011), less is known about continuity or change from infancy to mid-childhood. There may be a change in symptom presentation throughout development and there may not be a gold-standard age to examine anxiety so, to better understand the general presentation of anxiety, assessments should be carried out at different time points.

In contrast, strong early regulation (higher EC) was related to later ASD symptoms and this was only partially mediated by anxiety. Further, the relation between early regulation and later anxiety symptoms was fully mediated by ASD symptoms. This means that the degree to which early regulation predicts later anxiety is likely accounted for by the fact that children on the path to developing ASD often subsequently develop more symptoms of anxiety. Further, in Model 2.3, higher EC at 24 months was associated with both reduced anxiety symptoms and ASD traits at 36 months. However, the standardised beta coefficients were over twice as large for the relation between EC and ASD (Figure 2.4). Again, this suggests that lower EC is more strongly associated with later ASD than anxiety. One way to interpret this finding is that
infants within the current cohort with more genetic/early risk factors for ASD have lower levels of EC. This means infants are less likely to compensate if they also happen to be high in BI (due to genetic or other risk factors), leading to a raised risk of subsequently developing anxiety symptoms. So, anxiety may commonly co-occur with ASD because generally lower levels of EC leave children vulnerable to the effects of other risk factors that may vary in the general population.

Model 2.3 showed a positive association between better EC at 9 months and more ASD symptoms at 36 months. Many of the items that comprise the EC construct at this age ask about how long the child can pay attention to things in their environment. Thus, this significant association may reflect prolonged visual fixations in infants (sticky attention; Elsabbagh, Volein, Csibra, et al., 2009). This result also corroborates the findings from eye-tracking studies that indicated difficulties in switching attentional focus from peripheral stimuli and longer duration of attention that consistent with processing speed of the exposed stimuli in infants who diagnosed with ASD (Elsabbagh, Volein, Holmboe, et al., 2009; Richard & Lajiness-O’Neill, 2015). From 15 months and on, this association was not significant in Model 2.3. Further, by 24 months there was an association between lower EC at 24 months, and more symptoms of anxiety and ASD at 36 months. By age 2, the EC construct is less heavily influenced by questions about visual attention engagement and contains more items asking about inhibitory control, sustained attention. This change in emphasis of the construct may explain this pattern.

Alternatively, it may be that atypically strong EC early in development indicates an unusually paced developmental trajectory, which may be a sign of emerging vulnerability. One way to address this may be investigations of brain development
during the development. Especially, the prefrontal cortex is crucial to regulate thought, behaviour and facilitate learning (Thompson-Schill, Ramscar, & Chrysikou, 2009). The maturation of prefrontal cortex is essential in infancy and toddlerhood and trajectories of maturation in prefrontal cortex differ in typically developing children and children with ASD (Carper, Moses, Tigue, & Courchesne, 2002). Children with ASD show early maturation of prefrontal cortex than typically developing children and this can be linked to cognitive abilities. The positive association between EC at 9 months and ASD traits at 36 months may be the evidence of early maturation in the brain development and subsequently reflect the unusual development of cognitive skills (e.g., duration of orientation). Indeed, some have argued that the limited skills of infants are developmentally beneficial because they facilitate learning (Bjorklund, 1997; Elman, 1993) and so developing strong EC too early may be a risk factor.

2.4.4. Limitations and future directions

One limitation of the present study was the small sample size. All analyses were run in the overall sample and the post hoc power analysis for the Model 2.3 indicated strong association. But the sample size, particularly within the LR group is low. Due to this, I was unable to examine the multi-group models, which would inform us whether such associations are consistent for both HR and the LR groups. Second, the exploratory mediation analyses should be interpreted with caution since some of the mediator and outcome variables (anxiety and ASD) were measured cross-sectionally. Maxwell, Cole, and Mitchell (2011) stressed that a cross-sectional investigation of mediation might lead to substantial bias. A third limitation arises due to relying on parent-reported data, which may increase the risk of informant bias. Having an older child with an ASD diagnosis might affect the parental judgement for the younger child. Moreover, parental
anxiety problems have been found to mediates the relationship between temperament and anxiety problems (Gartstein et al., 2010). This might raise attributional bias and future research should take into account the possible effects of parental psychopathology on childhood anxiety problems. Fourth, employing a single method approach may increase the possibility of yielding results that are specific to a single method or due to shared method variance between predictors and outcome. However, the results were specific to BI and not sadness (extracted from the same questionnaire) mitigates this possibility. Further research should employ a multi-method approach encompassing both parent report and observational measurements to boost ecological validity.

2.4.5. Conclusion

The current study has three key findings. First, early BI predicts later anxiety in infants with older siblings with ASD as it does in the general population. Second, regulatory control can act as a protective factor on the path to anxiety symptoms. Finally, the current findings suggest that anxiety may co-occur with ASD in part because reduced EC in ASD reduces the ability to compensate for other background risk factors that are more likely to lead to psychopathology. Interventions designed to strengthen self-control in late infancy (but perhaps not earlier) could thus be a promising avenue for early intervention approaches.
Chapter 3. Observed Behavioural Inhibition in Infancy Predicts Parent-Reported Anxiety Traits in Toddlerhood

3.1. Introduction

Temperament has received a great deal of research interest in an attempt to understand the heterogeneity in Autism Spectrum Disorder (ASD) (Mundy et al., 2007), early developmental or behavioural markers of ASD (Garon et al., 2009) and roots of co-occurring psychiatric conditions (Shephard et al., 2018). In Chapter 2, early temperamental dispositions such as heightened reactivity (specifically behavioural inhibition; BI) and lower levels of regulation (effortful control; EC) were related to both anxiety and ASD symptoms in infants at high- and low-risk for ASD. Like most of the ASD literature, this investigation relied on parent-reported predictors and outcome variables (e.g., Clifford et al., 2013). While most previous research has investigated the reactive and regulatory domains of temperament using parent-report tools, relying on a single method approach to measure both predictor and outcome variables may increase systematic error and reduce the internal and external validity of the findings. In addition, few studies have used multimethod measurements of infant temperament traits in children at high- and low-familial risk of ASD. Therefore, to increase the ecological validity of the measurement, in this chapter, I used a multimethod approach (used observation and parent-report data) to investigate temperament traits and their association with later anxiety and ASD traits within the same cohort of children in Chapter 2.

Thus, in the current chapter, I aim (1) to develop a coding scheme to identify temperament traits (more specifically BI and EC) observed during a play session; (2) to examine how temperament varies in high and low-risk infants; (3) to investigate
whether observed temperament traits (at 15 months) relate to measured ASD traits (at 15 months); (4) to investigate the agreement between observed and parent-reported temperament traits; and (5) lastly, to explore the predictive value of temperament traits (both parent report and observed) for later anxiety and ASD traits.

3.1.1. Measurement of temperament

3.1.1.1. Parent-report temperament

Developmental scientists have designed several questionnaires based on different theoretical conceptualisations of temperament (e.g., Carey & McDevitt, 1995; Goldsmith, 1996; Rothbart, Ahadi, Hershey, & Fisher, 2001). These questionnaires are often implemented in research due to good psychometric properties and practicality of the administration (e.g., cost and time efficiency; Clifford et al., 2013; Wolfe et al., 2014). Parents are rich sources of information as they are the primary caregivers and have the most contact with their child. Therefore, they have the most opportunity to see temperamental tendencies in different contexts and gather generalised perspectives on trait level temperamental dispositions (Rothbart & Hwang, 2002).

However, parental judgements may also be affected by rater bias for several reasons. Firstly, parents who have a single child may have a lack of calibration with average child behaviour to evaluate the child’s temperamental traits (Goldsmith & Hewitt, 2003). Secondly, in families with multiple children, parental judgement may be affected by contrasting sibling behaviour. The results of twin studies have highlighted this tendency (e.g., Saudino, 2003). Given that temperament traits are constitutionally based individual tendencies (Rothbart & Derryberry, 1981) and co-twins share genetic variations, similarities in temperament traits are expected. Saudino (2003) argued that low correlations in parent-rated temperament of dizygotic twins and moderate
correlations in monozygotic twins might partly be explained by that parents exaggerate differences (contrasting effect). On the contrary, high correlations between co-twins may be the result of magnified similarities (assimilation effect). Thirdly, parental characteristics such as personality traits and psychopathology (e.g., anxiety and depression) influence parental perceptions of the child’s temperament (Aktar, Majdandzic, de Vente, & Bogels, 2013; De Los Reyes & Kazdin, 2005). For example, Kerstis, Engstrom, Edlund, and Aarts (2013) found that parents with depression perceived their child’s temperament to be more difficult than parents without depression. Parental intrusiveness, stress (Mäntymaa, Puura, Luoma, Salmelin, & Tamminen, 2006) and their own childhood experiences, such as being exposed to overprotective parenting, also affects their perception about their children’s negative temperament (Manczak et al., 2016). Finally and importantly, using parent-report measures of predictors (e.g., temperament traits) and outcome (e.g., anxiety) may be biased due to the factors listed above. Gulley et al. (2016) found that parents who perceived their child as having a difficult temperament also endorsed higher emotional or behavioural difficulties than parents who rated their child as having a less difficult temperament. While parents are a valuable source of information about their child’s behaviour, using parent-report alone may raise information bias that may reduce the internal validity of the investigations and subsequently may reduce the generalisability of the findings.

### 3.1.1.2. Observed Temperament

Observational measures have also been fundamental to the assessment of childhood temperament traits (e.g., Buss, 2011; Kiel & Buss, 2011). Observations of a child’s behaviour in the home or laboratory environment with standardised tasks increase the probability of capturing spontaneous responses (Gagne et al., 2011). This
method increases external validity because they are generally ecologically valid (Lo, Vroman, & Durbin, 2015). Observational methods, which incorporate standardised coding schemes, have generally been used to measure temperamental traits in infants and toddlers to avoid potential parental response bias (Planalp et al., 2017). Coders get trained on coding schemes to capture and code operationally defined behaviours of interest reliably and this may help to overcome recall bias that can rise in parent reports.

The observation-based techniques are not entirely free from possible errors. One of the possible flaws is related to the setting of the observation (e.g., home or laboratory), which may not be relevant to observe target behaviours. The observational technique also highly depends on the state of the child at the time of the task and might not be representative of the general child reactions. Furthermore, training coders to rate desired behaviours may be time consuming and expensive (Rothbart & Goldsmith, 1985). Although standardised coding schemes provide a framework to observe reactions, independent coders may not agree on the capture of the specific behaviour or emotional expression, which can result in poor inter-rater reliability. In particular, ambiguous or less frequent reactions may be omitted or coded less reliably. Low agreement between coders may result in bias in the generation of reliable and valid constructs of temperament through observations.

Understanding how BI scores obtained through laboratory procedures influence the strength of the association between parent reports of different traits and examining how parent-report BI relates to observed BI may extend the assessment of temperament. Several studies have examined the agreement between parent report and behavioural observations (Kiel & Hummel, 2017; Olino, Durbin, Klein, Hayden, & Dyson, 2013; Seifer, Sameroff, Barrett, & Krafcuk, 1994; White et al., 2017). For example, Seifer et
al. (1994) observed infant temperament traits in the home environment. The agreement between experimenter rating and parent-report was poor for approach (the child willingness to engage; $r = .02, p > .05$) but good for activity level ($r = .29, p < .05$). Kiel and Hummel (2017) investigated contextual influences on the agreement between two methods of rating infant temperament. There were moderate associations ($r = .35, p < .001$) between BI scores elicited in the social context through the Stranger Approach task (designed to observe child’s reactivity to a novel person) in the laboratory and parent-reported social BI whereas the total BI scores elicited in the non-social Unpredictable Toy task and parent-reported non-social BI were not significantly associated ($r = .10, p = .284$). White et al. (2017) compared a total BI score elicited through observed social and non-social BI laboratory tasks and a composite of parent-reported social and non-social BI scores. The results suggested moderate agreement between parent-report and observed BI ($r = .44, p < .001$). In contrast, Bufferd et al. (2016) compared a composite observed BI score and parent-report social BI score and found lower levels of convergence between the two assessment methods ($r = .28, p < .05$). Among these investigations, Bufferd et al. (2016) examined whether parent-reported and observed BI scores as predictors (at age 3) similarly predicted anxiety problems on a parent interview assessment in 7-year-old children who had anxiety problems. The BI scores generated from both assessment techniques significantly predicted anxiety problems at the age of 7. Taken together, these results suggest low to moderate convergence between parent-report and behavioural observation measures of temperament, but that both methods may capture important early temperamental predictors of child anxiety.
3.1.2. Multi-method approaches

Given the evidence, neither parent-report nor observational measures of temperament are sufficient alone to understand childhood temperament traits. Although the evidence suggests that the convergence between methods varies substantially between studies, depending solely on a single method may lead to a reduction in generalisability of findings, and increase the possibility of obtaining method-specific results. Further, using one methodology on its own increases the chance that shared method variance drives associations between predictors and outcome; that is, the association between the parent-reported child temperament and outcome measures (e.g., parent report child psychopathology) are merely the result of eliciting responses from a single method approach (Podsakoff, MacKenzie, Lee, & Podsakoff, 2003; Seifer et al., 1994). Besides, exploring associations between temperament and psychopathology through single method may be biased by measurement error. For example, in the context of BI, items in the widely used temperament questionnaires such as Infant Behaviour Questionnaire (e.g., Putnam et al., 2014) and anxiety measures (e.g., Child Behaviour Check List; Achenbach & Rescorla, 2000) may tap the same construct and elicit findings due to similarity in the items (e.g., “seem frightened for no apparent reason?” and “shows panic for no good reason”). In order to limit the scope of the bias, to provide a comprehensive picture of childhood temperament and increase external validity, in this Chapter, I employ a multimethod approach to studying infant temperament and its relation to later anxiety that combines both parent-report and observational techniques.
3.1.3. Measurement of ASD in infancy

Observational measures of temperament can either involve the creation of novel tasks designed to probe for specific temperamental reactions (see Chapter 4) or the creation of novel coding schemes to apply behavioural data captured for another purpose. In this chapter, I chose to create a novel coding scheme to apply to behavioural data captured through the Autism Observational Scale for Infants (AOSI). First, this enabled me to apply my coding scheme to existing longitudinal data, facilitating a link between early temperament to the later outcome. Second, the AOSI is designed to capture behaviours related to ASD and thus provides us with the opportunity to examine the intersection between early temperament and the expression of early behavioural signs of ASD.

Increased interest in identifying early signs of ASD in infancy through familial high-risk (HR) for ASD longitudinal studies (Bedford et al., 2014; Jones et al., 2014; Messinger et al., 2013; Ozonoff et al., 2011; Zwaigenbaum et al., 2005) has increased the demand for clinical measures that reliably detect behavioural traits of ASD in the first two years of life. Although HR studies have identified possible early behavioural markers of ASD, such as differences in social communication (eye contact), repetitive behaviours, and attentional problems around 12 and 18 months, factors such as different onset pattern of symptoms (early vs. late-emerging and plateau) (Ozonoff et al., 2018), phenotypic overlap between co-occurring neurodevelopmental disorders (Grzadzinski, Dick, Lord, & Bishop, 2016), broader autism phenotype (Ozonoff et al., 2014), gender and temperament (Mundy et al., 2007) result in heterogeneity and complicate the reliable capture of the ASD through screening measures within the early stages of development.
The Autism Observational Scale for Infants (AOSI; Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008) is one of the most widely used research instruments for the detection of behaviours related to ASD in infancy. The AOSI is a semi-structured experimenter-led behavioural assessment that was designed to observe early markers of ASD in infants aged between 6 and 18 months. The AOSI has moderate predictive value; longitudinal investigations have shown that the AOSI total score at 14 months but not 7 months was associated with scores from the Autism Diagnostic and Observational Schedule (ADOS) at both 24 and 36 months (Gammer et al., 2015), and predicted HR-ASD groups at 7 years old (Bedford et al., 2017).

However, the AOSI is not entirely predictive, with around 14% of the variance in later ASD symptoms at 36 months remaining unexplained. One of the factors that influence the sensitivity and specificity of the AOSI may be temperamental traits.

Infant temperament may be essential to study when seeking to improve our ability to predict later ASD from early behaviour for three reasons. First, some early signs of ASD may be apparent in domains of infant temperament like irritability or reactivity. Thus, measuring aspects of temperament in addition to other social communication behaviours may add predictive power. Indeed, Brian et al. (2008) argued that, in addition to the importance of core ASD symptoms, it is also crucial to consider the temperamental disposition of the child during the AOSI assessments. In the same study, the results of a discriminant function analysis showed that reactivity at 18 months was a significant predictor of ASD diagnosis and was thus included in AOSI items (Brian et al., 2008). The scoring of this item is based on the behavioural responses based on under- or over-reactivity to activities or toys. However, this definition is not entirely in line with the conceptualisation of temperament by Rothbart (1986) and it is not clear how specific temperament traits contribute to total AOSI scoring.
Alternatively, early disruptions in temperament associated with ASD that may emerge at a similar developmental period as early behavioural signs of the condition (Rothbart, Ahadi, & Evans, 2000) may actually confound AOSI scores. Infants who are later diagnosed with ASD diagnosis may exhibit temperamental dispositions that eventually make it challenging to detect ASD symptoms using clinical instruments reliably. For example, in Chapter 2, the results showed that disruptions in regulation (EC) were strongly linked to ASD outcome. Reduced EC may make it difficult for infants to pay attention and engage with the experimenter and toys through the duration of the AOSI assessment, affecting their score.

Finally, an infant’s temperament during the AOSI may affect their scoring on items like social interest and shared affect, social smile in a way that inflates their score but is unrelated to the presence or absence of ASD. For example, the infant might not smile or respond as expected because of BI rather than core ASD traits. In Chapter 2, temperamental reactivity (specifically BI) was more strongly related to anxiety than ASD symptoms. Components of BI, such as fearfulness and shyness, may appear crudely similar to social interaction difficulties in ASD, for example, in encountering a stranger. Elevated BI associated with later anxiety could thus confound associations between early behavioural scores on the AOSI and later ASD outcome. Exploring observed temperament traits with more detailed coding schemes may help us to tease apart these conceptually overlapping behaviours.

In summary, coding temperamental traits during the administration of the AOSI may allow us to understand whether temperamental traits affect the judgement of the experimenter that result in inflated or reduced overall AOSI scores. This may also allow us to explore the conceptual overlap between temperament and behaviours considered
to be characteristics of ASD. Thus, in this Chapter, I created an observational coding scheme designed to capture temperament traits during subtests of the AOSI which enabled me to investigate the intersection between temperament and early behavioural signs of ASD. Developing a coding scheme that is related to the AOSI may increase likely uptake by the field, as the AOSI is a commonly used measure for which many investigators have historical data captured on video that could be recoded for temperamental traits.

3.1.4. The theoretical and conceptual background of temperament coding

As the aim of the current study is to replicate the findings of Chapter 2 partially, the focus of the coding scheme will be on BI and EC. As mentioned in previous chapters, BI is an early childhood temperament trait that is characterised by fearful responses to novelty, and avoidance of interactions in novel social contexts (Fox et al., 2001; Kagan, Reznick, & Snidman, 1987). Infants can perceive novel situations as involving threat of harm or uncertainty, and behavioural or emotional responses can be consistent with fear that can be conveyed through facial expressions, distress vocalisations (e.g., crying, screaming), bodily reactions (e.g., decreased activity level, tensions on whole or part of body) or avoidant behaviours (e.g., Brooker et al., 2013; Buss, 2011). In infancy and toddlerhood, BI was generally measured by observational techniques via standardised structured observational laboratory tasks such as the Stranger Approach, the Unpredictable Toy, the Scary Mask, the Risk Room tasks that involved novelty and that were designed to elicit over-aroused fearful reactions and withdrawal (e.g., Buss, 2011; Morales, Perez-Edgar, & Buss, 2015; Walker et al., 2014).
There have been two distinct coding schemes that were widely used to measure BI. One of these coding schemes developed by Kagan et al. (1987) and Calkins et al. (1996) is based on mainly approach/withdrawal reactions. In the laboratory tasks (e.g., the Stranger Approach), latency to vocalise, latency to approach/touch the stranger/stimuli, and the duration of time spent in proximity to parent during each event was calculated to create BI scores. After determining these durations and latencies, a composite BI score was created by summing up the coded behaviours (Fox et al., 2001) or averaging these behaviours and parent-reported BI to create more accurate scores (Lahat et al., 2014; Walker et al., 2014; White et al., 2017; White et al., 2011). Lahat et al. (2014) confirmed this approach (combining two distinct measurements) through principal component analysis which suggested a single component score of BI (eigenvalue = 2.04; loadings = .62 to .81). This coding scheme omitted the facial and bodily reactions of the fearful responses. This may be due to the overlapping concept of BI, fearfulness and shyness. BI is generally referred as a broader term that has components of fearfulness (more related to responses to the non-social stimuli) and shyness and eliciting behaviours based solely on approach/withdrawal behaviours may have less accurate BI presentation.

The second commonly used BI coding system is from the Laboratory Temperament Assessment Battery (Lab-TAB; Planalp et al., 2017). This coding scheme allows researchers to ascertain the intensity (scored between 0 and 3, higher scores indicating more intensity) of the following behaviours: facial fear, facial sadness, bodily fear, and negative vocalisation. Buss (2011) utilised this coding scheme as well as latencies to freeze, cry and approach, duration of bodily sadness and withdrawal. A PCA with varimax rotation elicited a fear component (latency to freeze, duration of facial fear, duration of bodily fear, duration of freezing, and duration of proximity to
mother) that explained between 22.42 and 29.57% variance across tasks, an engagement component (latency to approach, duration of withdrawal, duration of approach, and proximity to the stimulus) that accounted for 14.03–16.57% of the variance and a sadness component (latency to cry, duration of facial sadness, duration of crying, and duration of bodily sadness) that explained 10.28–13.10% of the variance across episodes. The fear component demonstrated good internal consistency (.61–.73) whereas the engagement (.48–.54) and the sadness components (.32–.48) presented acceptable to low Cronbach’s alpha.

My new coding scheme combined these two different approaches to identifying BI to ensure a broad capture of relevant behaviours. Facial expression, bodily fearfulness, latency to approach, vocalisations, duration of interaction with the experimenter and toy were coded as aspects of reactivity. The nature of the AOSI involves social interaction through playing with the researcher. In addition, given the nature of the AOSI, I also included positive behaviours such as smiles and positive or neutral vocalisations in the reactivity coding. Some infants may find these interactions quite enjoyable and may produce more positive reactions. For the children with ASD, previous research on facial expressions has suggested that children with ASD used less complex mechanisms to generate facial expressions and produced lower levels of more complex facial expressions, e.g., fear and sadness, than the TD children (Guha et al., 2015). However, there was no difference for less complex facial expressions like smiles. Given the evidence, in addition to coding negative reactivity, the current coding scheme will include positive reactions. If we conceptualise these reactions as negative and positive on a spectrum, lower frequency of positive facial expression may indicate the presence of negative reactivity in case of difficulties in the presentation of complex facial expressions.
Self-regulatory behaviours were also of interest to explore the observed association between regulatory and reactive dimensions of temperament. In previous studies, regardless of the context of the observational setting, self-soothing strategies such as thumb sucking, touching the face, hair twirling or putting a hand into hair and any evidence of physical avoidance, such as twisting, turning away, or sinking into a chair were considered as observed regulatory strategies (Gulsrud, Jahromi, & Kasari, 2010; Jahromi, Meek, & Ober-Reynolds, 2012). In addition to these behaviours, visual orientation, which is consistent with attentional control, was considered a regulatory strategy (Jahromi et al., 2012). Orienting is involved in distraction from arousing, threatening stimuli. This may result in a shorter duration of gaze to a presented toy or experimenter and longer duration of gaze directed away or directed to the parent (Hill-Soderlund & Braungart-Rieker, 2008; Planalp et al., 2017). In other words, infants can disengage from a stressful situation or stimulus via orienting to something that is neutral and calming and so object orientation may serve as a form of regulatory behaviour in infancy.

3.1.5. The present study

Taken together, the first aim of the current study was to explore the temperament profile of a cohort of children at familial HR for ASD and low-risk (LR) controls. The HR group were further subdivided by whether they met the diagnostic criteria for ASD (HR-ASD), had a sub-clinical presentation of ASD but did not receive an ASD diagnosis (HR-ATY), and they had typical development (HR-TD). I coded temperament traits from video recordings of the AOSI, using a novel observation scheme. I then analysed the psychometric properties of the coding scheme.
The second aim was to assess whether observed temperament traits related to total AOSI score. Although the AOSI scoring involves an item for scoring overall temperamental reactivity by determining whether the child is under- or over-reactive, less is known about which subdimension of reactive temperament mainly relates to overall AOSI score and whether observed regulatory strategies relate to AOSI score.

The third aim was to explore the convergence between parent-reported and observed temperament traits. As mentioned earlier, parental judgements can be affected by various variables. Similar to investigations in the general population, in HR families who have an older child with an ASD diagnosis, parents’ perception about the later-born sibling may be affected by contrast effects such as endorsing more positive traits and fewer emotional and behavioural problems relative to the proband’s behaviour. On the contrary, parents may focus on the similarities between the proband and the younger child and may magnify concerns relating to the younger sibling and may over-report negative temperament traits. Parental characteristics may also influence parental reports. For example, parents who have children with ASD report higher internalising problems (Crea et al., 2016) and this may confound their perception about the temperament traits and outcome (anxiety and ASD).

Lastly, Chapter 2 showed that the reactive (BI) and the regulatory domains (EC) of temperament were related to anxiety and ASD traits in both HR and LR children. However, this investigation was carried out using parent-reported temperament traits. The final aim was to explore the group differences in predictors (parent-reported and observed temperament traits) and outcome (anxiety and ASD), and to explore whether observed and parent-reported temperament traits at 15 months similarly predict anxiety and ASD traits at 36 months.
3.2. Method

3.2.1. Participants

A total of one hundred infants who participated in the second phase (the same cohort employed in Chapter 2) of the British Autism Study of Infant Siblings (BASIS; www.basisnetwork.org) project were drawn for the current research. Of one hundred infants, 75 were HR (due to having an older sibling with ASD; 28 female, 47 male) and 25 LR (due to no familial history of ASD in the first and second-degree relatives; 12 female, 13 male) for ASD. In the previous chapter, group differences in predictors and outcome measures were assessed based on the risk group comparison (HR vs LR) and in the current chapter, the aim is to compare the sample based on outcome group categorisation to see whether the effect of the association is actually due to the 15 children who were diagnosed with ASD. So, participants were equally stratified into four outcome group categorisations (see 3.2.2. for details of outcome categorisation) to have enough power to examine the group differences. Due to this and the AOSI was missing for some infants, the participant number is slightly reduced in this chapter.

The infants participated in the second visit of the BASIS project after they turned 15 months old. Along with several other measures (e.g., eye-tracking, EEG and behavioural tasks), they attended a play-based research assessment that was designed to observe ASD symptoms (AOSI, see 3.2.3.) towards the end of the assessment day.

3.2.2. Clinical measures and outcome decision

A battery of clinical measures was used for the outcome group categorisation at 36 months and all participants completed the following measures: The Autism Diagnostic Observation Schedule – Second Edition (ADOS-2; Lord et al., 2012) is a
standardised, semi-structured, observational measure that assesses symptoms related to ASD, including communication, social interaction and restricted, repetitive behaviours. The ADOS was not completed for 2 LR children at 36-months, but these participants were included in the analysis. The Calibrated Severity Scores (CSS) for Social Affect and Restricted and Repetitive Behaviours subscales were reported (Gotham, Pickles, & Lord, 2009). The Autism Diagnostic Interview-Revised (ADI-R; Rutter, LeCouteur, et al., 2003) is a structured parent interview consisting of questions about developmental history, and retrospective symptoms and behaviours related to ASD. The ADI-R was administered at 36 months and standard algorithm scores which aggregate current and historical symptom information were computed for Reciprocal Social Interaction, Communication, and Restricted, Repetitive and Stereotypic Behaviours and Interests subdomains. These assessments were carried out by researchers who were not blind to risk group status or under the close supervision of clinical researchers (i.e., psychologists, speech therapists). Children completed the Mullen Scales of Early Learning (MSEL; Mullen, 1995) at each visit and the early learning composite score was used as a measure of overall developmental level.

After the 36-month visit, experienced researchers (TC, GP, CC) reviewed the ADOS-2 and ADI-R for ASD symptomatology and the MSEL for the developmental level of all the HR and LR children to ascertain diagnostic outcome based on DSM-5 criteria for ASD. Subsequently, of 76 HR infants, 29 were typically developing (hereafter, HR-TD), 29 were considered as having neither ASD nor typical development but other atypicalities due to i) scoring above ADI-R cut-off and/or scoring above ADOS-2 cut off for ASD, or ii) scoring 1.5 standard deviation below the general population mean on the MSEL composite or the MSEL expressive or Receptive Language scales (hereafter, HR-ATY), 17 met criteria for ASD diagnosis (hereafter,
HR-ASD). All 27 LR children presented typical development and were not given a research or community ASD diagnosis (Table 3.1).

Table 3.1 Sample Characteristics of Risk and Outcome Groups

<table>
<thead>
<tr>
<th></th>
<th>Low-Risk M (SD)</th>
<th>High-Risk M (SD)</th>
<th>HR diagnostic outcome groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical</td>
<td>Atypical</td>
<td>ASD</td>
</tr>
<tr>
<td>Age in day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (girls)</td>
<td>25 (12)</td>
<td>75 (28)</td>
<td>29 (15)</td>
</tr>
<tr>
<td>MSEL ELC</td>
<td>100.82 (2.83)a</td>
<td>93.30 (1.79)</td>
<td>98.46 (2.35)a</td>
</tr>
<tr>
<td>36 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (girls)</td>
<td>25 (12)</td>
<td>75 (28)</td>
<td>29 (15)</td>
</tr>
<tr>
<td>Age in months</td>
<td>38.74 (.30)</td>
<td>38.67 (.22)</td>
<td>38.70 (.31)</td>
</tr>
<tr>
<td>MSEL ELC</td>
<td>118.13 (3.24)a</td>
<td>97.35 (3.23)</td>
<td>114.46 (3.11)a</td>
</tr>
<tr>
<td>ADOS SA CSS</td>
<td>2.70 (.41)a</td>
<td>3.42 (.31)</td>
<td>1.64 (.16)a</td>
</tr>
<tr>
<td>ADOS RRB CSS</td>
<td>3.26 (.47)a</td>
<td>4.97 (.30)</td>
<td>3.75 (.44)a</td>
</tr>
<tr>
<td>ADI Social</td>
<td>1.00 (.32)a</td>
<td>4.51 (.66)</td>
<td>1.54 (.42)a</td>
</tr>
<tr>
<td>ADI Com</td>
<td>.52 (.23)a</td>
<td>5.01 (.62)</td>
<td>2.04 (.49)a</td>
</tr>
<tr>
<td>ADI RRB</td>
<td>.09 (.06)a</td>
<td>1.94 (.33)</td>
<td>.43 (.18)a</td>
</tr>
</tbody>
</table>

Bolds indicate a significant group difference between LR and overall HR group.

Superscript letters indicate a significant difference between the LR and HR outcome groups at $p < .05$ level. MSEL ELC= Mullen Scales of Early Learning Early Learning Composite Scores, ADOS SA CSS= Autism Diagnostic Observational Schedule Social Affect Calibrated Severity Scores, ADOS RRB CSS= Autism Diagnostic Observational Schedule Restricted Repetitive Behaviours Calibrated Severity Scores, ADI Social=
Autism Diagnostic Interview Social Subscale, ADI Com= Autism Diagnostic Interview Communication, ADI RRB= Autism Diagnostic Interview Restricted Repetitive Behaviour subscale.
3.2.3. Measures used to elicit observed temperament traits

The Autism Observation Scales for Infants (AOSI; Bryson et al., 2008) is a semi-structured observational assessment designed to capture early emerging ASD phenotypes (e.g., eye contact, repetitive interests, sensory difficulties, ease of switch between tasks) in infants aged between 6 and 18 months. The assessment consists of a standard set of seven interactive activities that are administered by trained experimenters to elicit desired behaviours. During the AOSI assessment, the infant sits on a parent’s lap at a table opposite and facing the experimenter to enable verbal and non-verbal reciprocal social interaction. The first 6 tasks examine the infant’s reactions to specific presses by the examiner (visual tracking, disengagement of attention, orienting to name, differential response to facial emotion, anticipatory responses, imitation of actions) and the rest of the items (social babbling, eye contact, reciprocal social smile, coordination of eye gaze and action, reactivity, shared affect, transitions, motor control, atypical motor and sensory behaviours) were coded depending on the overall judgement of the experimenter throughout the AOSI administration. Three additional items allow experimenters to judge the level of child’s engagement of attention, insistence on having or playing with particular objects or specific activities, and sharing interest, however, these items do not contribute to scoring. In the current study, the 16-item version of the AOSI was used to measure risk markers of ASD in infancy at 15 months, and the administration was videotaped. Scores for each item (which range from 0 to 2 or 3) were generated after the session based on the consensus coding of the examiner and a second trained rater following the AOSI manual (Bryson et al., 2008). The AOSI yields two types of summary scores. Total scores are based on the sum of all codes (maximum score is 38) with higher scores indexing both a greater number and/or greater severity of ASD traits. Second, the Number of Markers was calculated based on the amount of the
items that were endorsed for the child (maximum score is 14) and thus represented the range of ASD-related behaviours the child shows.

### 3.2.3.1 Task description

Five activities of the AOSI were selected to code temperament traits. Before finalising the coding scheme, twenty randomly selected AOSI videos were examined to decide on the tasks that were most likely to elicit relevant temperament traits. Among seven AOSI activities, Visual Tracking, Disengagement of Attention, Free Play (reading a book period), Social Anticipation, Differential Response to Facial Emotion were found to be relatively likely to capture desired behaviours. The other activities such as response to name or imitation were excluded from coding because these tasks did not provoke emotional reactivity or not relevant to observe regulatory behaviours. The following section includes descriptions of these tasks.

*The Visual Tracking* is consistently administered as the first activity in the AOSI assessment and was chosen as it is enriched with novelty. The experimenter shakes a rattle at the midline at the eye level for the infants to visually engage with the presented stimuli and moves it from the midline to each side. This task was chosen to elicit initial responses in the novel interaction and to see how quickly the infants warm up and start interacting with the experimenter. Coding for this activity starts when the experimenter presents the toy in front of the child and ends when the experimenter starts taking the toy away from the eye level.

*The Disengagement of Attention* is the second task which is consistently administered right after the *Visual Tracking*. In this task, a rattle is shaken to one side of an infant and once the infant engages with it, a second rattle is presented at the opposite side to measure the how quickly the child switches attention between stimuli. This
activity was designed to measure the ability of an infant to disengage and switch attention between the two stimuli. This task was chosen to code temperament traits since it is one of the first two tasks and thus may be enriched for eliciting initial reactions to the novel interaction. The coding of this task starts when the experimenter puts one of the rattles up and ends once the experimenter starts withdrawing the toys.

The third activity was *Reading a Book* episode embedded in the *Free Play*. The task involves the experimenter reading a book and mimicking the animal sounds in the book to give a chance to the child to engage and produce social babbling. This episode was chosen to measure the level of engagement with the experimenter. The coding starts when the experimenter puts the book on to the table in front of the child and ends when the book was taken away.

*Social Anticipation* involves a peek-a-boo game. This task was chosen because it is likely to elicit infants’ positive affect and level of enjoyment (which may be inversely related to BI). The task starts when the experimenter covers his/her face and ends when the experimenter put the cloth down.

*In the Differential Response to Facial Emotion task*, after establishing eye contact with the child, the experimenter changes her/his facial expression from smiling to neutral to assess the child’s head or other motor movements. This task was chosen since it resembles the ‘still face’ paradigm often used to examine the affective response to another’s emotions. The coding starts when the experimenter entirely changes her/his facial expression to neutral (corner of the mouth relaxed, and cheeks released down) and ends when experimenter raises the corner of her/his mouth.
3.2.3.2. Coding scheme

The AOSI administration for each infant was videotaped, and emotional and behavioural variables (described below) were coded later using the Mangold Interact (Mangold, (2017); [www.mangold-international.com](http://www.mangold-international.com)). Infants’ behaviours were coded based on one parameter, the duration of each emotional or behavioural reaction.

Before starting to code, as a first step, starting and ending points of each task were identified. As a general administration rule, the AOSI instructions can be repeated if the child was distracted or disengaged. Coding was carried out considering the first administration of a repeated task to avoid child’s familiarity, but if the first administration was not fully completed due to the child’s disengagement, the coding was carried out based on the second administration where the child is more easily engaged.

After determining the duration of each AOSI activity, the following behaviours were coded by a bachelor and a PhD student (SH and ME).

3.2.3.3. Reactivity Codes

*Facial expressions* of fear, sadness and smile were coded based on the AFFEX systems (Izard, Hembree, Dougherty, & Spizzirri, 1983). The AFFEX coding enables the researcher to determine distinct facial expressions based on three facial regions: eyes, brows and mouth area. Facial fear was defined by brows being straight or normal, slightly raised or drawn together, eyelids raised and tense, mouth open, corners straight back. Sadness was defined by inner corners of brows raised, outer corners lowered, eyes being narrowed or squinted, cheeks being raised, and the corners of the mouth pulled down and out, the mouth could be open or closed, and upper lip often protrudes at the centre. The smile was indicated by eyes being neutral, mouth smiling, or eyes squinted.
or narrowed, cheeks raised from smiling. *Bodily expression of fear* was also coded. Fearfulness expressed through bodily reaction was classified by a marked and sudden decrease in the activity level, freezing (no physical movement more than 2 seconds) or muscle tensing on limbs, especially on legs, arms and shoulders (e.g., firmly extended legs, arm or raising shoulders towards ears). *Latency to touch* that is characterised by the time that infants’ take to approach and touch the presented toy within the first task (*Visual Tracking*) of the AOSI. Duration of a child’s *engagement* (playing/interacting) with presented toys was coded continuously to understand whether the child comfortably interacts with the stimuli or stays withdrawn. *Negative vocalisations* include any intensity of cry, scream, whining or whimpering whereas *positive vocalisations* include any intensity of vocal expressions that reflect enjoyment and joy, such as mimicking or bubbling.

### 3.2.3.4. Regulation Codes

Duration of *visual orientation* to parent (gaze is directed to the parent’s face), to experimenter (gaze is directed to the experimenter’s face), to toy (visual engagement with the presented toy by the experimenter) and away (visual disengagement from experimenter, target toy or parent) were coded in separate classes. Any physical *escape* behaviours that are characterised by quitting or stopping interactions such as turning away, sinking into the chair, leaning back were coded. *Self-soothing behaviours* such as thumb sucking, touching own face or body, touching ears, hair twisting were also included in this class.

### 3.2.4. Parent report measures

Parent-reported temperament was measured at 15 months using the Infant Behaviour Questionnaire-Revised version (IBQ-R; Gartstein & Rothbart, 2003).
Cronbach’s alpha for the subscales ranged between .84 and for the current sample, indicating excellent internal reliability. The Child Behaviour Checklist 1.5/5 DSM-Oriented Anxiety Problems raw scores that measured at 36 months (CBCL; Achenbach & Rescorla, 2000) was used to measure anxiety traits. Cronbach’s alpha for the anxiety problems subscale was .79 for the current sample demonstrated good internal consistency. The Social Communication Questionnaire (SCQ; Rutter, Bailey, et al., 2003) was used to measure ASD symptoms at 36 months. In the current study, total scores of the SCQ was used in the analyses. The Cronbach’s alpha for the SCQ was .91, indicating excellent internal consistency for this measure in the current sample. All measures were summarised in section 2.2.2.

3.2.5. Data analysis

3.2.5.1. Psychometric properties of temperament coding

A bachelor and a PhD student (SH and ME) coded 16 videotaped AOSI administrations, to establish inter-rater reliability. Inter-rater agreement was calculated for the total duration (based on seconds) of each behaviour and emotional reactions across five different AOSI activities to assess the variations in scores that were provided by the coders. Intraclass correlation coefficients (ICC) were calculated using the two-way random effect model (due to the randomly chosen sample for ICC) with absolute agreement type. ICC scores between 1 and .75 were accepted as excellent, .74 and .60 were accepted as good and scores less than .60 were regarded as a poor agreement (Cicchetti, 1994). All analyses in this chapter were conducted using SPSS 25.

After determination of the variables that showed good inter-rater agreement, to reduce the number of the variables for parsimony and ease in the further analyses, to understand the latent structure of observed temperament constructs Principle
Component Analysis (PCA) was run on 13 coded research variables. Firstly, Pearson correlation coefficients were calculated to see the pattern of association between variables before data reduction. All correlation coefficients were checked to avoid multicollinearity ($r > .80$) and to avoid insufficient variability in variables ($r < .10$). Secondly, PCA with Varimax Rotation was run based on Eigenvalues. The Varimax Rotation was chosen because it maximises the loadings of variables on the underlying components and minimises the loadings of variables on all other components so, it is an appropriate method when the expected underlying components are unrelated. Eigenvalues ($\geq 1$) and scree plots were examined to determine the number of emerging components. Variables that loaded on components with a magnitude of $0.60$ and above were accepted as adequate loadings. Composite scores were created based on summing up behaviours that loaded on to each component. After this step, the internal consistencies of each component were calculated and components that had a lack of internal consistency (Cronbach’s Alphas $< .40$) were discarded.

### 3.2.5.2. Association between temperament and the AOSI scores

Prior to regression analyses, Pearson correlation coefficients were carried out to establish variation between variables. The association between AOSI scores and observed temperament traits were calculated by using hierarchical linear regression analysis. Before running analyses, the relevant assumptions for regression analyses were checked. Correlation coefficients revealed that predictors were not correlated with each other, suggesting singularity of predictors. Residual and scatter plots indicated normality, linearity and homoscedasticity were all met. The first stage of the hierarchical regression included observed temperament variables and the second stage included risk group (HR vs LR) variable.
3.2.5.3. Convergence between parent-reported and observed temperament

The convergence between parent-reported and observed temperament traits was examined by calculating Pearson correlation coefficients between the following conceptually matched subscales: IBQ-R Fear subscale and BI component, IBQ-R Sadness subscale and sadness component, IBQ-R High-intensity Pleasure subscales and positive affect component; and lastly, IBQ-R Duration of Orientation subscale and engagement component. In addition to correlation analysis, to visually inspect the agreement between methods, scatter plots and Bland-Altman plots (BA; Bland & Altman, 1995) were generated to examine the agreement between two distinct measurement methods.

van Stralen, Jager, Zoccali, and Dekker (2008) argued that correlation analysis is a useful method to assess the variability between variables, but it does not provide the size and the amount of the differences. Alternatively, the BA method is a special scatter graph that plots the difference between two measures (A-B; y-axis) against the mean ([A+B]/2; x-axis) of the same measures (Bland & Altman, 1995; Giavarina, 2015). This method does not indicate whether there is a good agreement between methods by statistical tests, but the general recommendation for good agreement is that 95% of the data points should lie between limits of the agreement which is 1.96 standard deviation above and below the mean of the differences between two measurements (A-B; y-axis). Scatters that lies close to y = 0 (within the confidence interval (CI) of mean differences) indicates perfect agreement (Giavarina, 2015). BA plots previously used to assess the agreement between different measures (e.g., Bennetts, Mensah, Westrupp, Hackworth, & Reilly, 2016).
Before the plotting, Z-scores were derived for each parent-report and observed temperament traits to enable cross-measure comparison on the same scale. The only assumption for the BA plot is that the differences (A-B) have a normal distribution. Before plotting, this was checked by running Kolmogorov-Smirnov test and none of the differences violated the assumption (all $p \geq .200$). Standard scatterplots will also be presented for each pair of temperament traits to investigate the pattern of the association.

### 3.2.5.4. Risk group and outcome group differences in research variables

Initially, a one-way MANOVA was conducted for observed temperament traits across the LR and the HR groups. Then another one-way MANOVA was undertaken to assess the effect of dimensional outcome groups (LR, HR-TD, HR-ATY-HR-ASD) on observed temperament traits. After both MANOVA analyses, post hoc univariate ANOVAs were run with Bonferroni post hoc analysis to assess where outcome group differences lay in all the observed temperament dimensions. Same steps were followed for the parent-reported temperament traits and anxiety and ASD scores in a separate analysis.

Assumptions of normality and homoscedasticity were checked. Sadness and BI components were positively skewed, but for both analyses, groups had equal variances; Levene Statistics were non-significant for risk groups (all $p \geq .117$) and outcome groups (all $p \geq .093$).

### 3.2.5.5. Association between temperament traits, anxiety and ASD scores

Before regression analysis, Pearson correlation coefficients were calculated to present associations between predictor and outcome variables. Three two-stage hierarchical linear regression analyses were run to investigate whether observed or
parent-reported temperament traits measured at 15 months predicted anxiety and ASD scores at 36 months.

Assumptions of multicollinearity among independent variables, linearity, homoscedasticity were examined. The first regression analysis examined the association between observed temperament traits and anxiety outcome and the second analysis included parent-reported temperament traits and anxiety scores. Since there was no significant correlation between observed temperament traits and ASD scores, further regression analysis was not run. Instead, the parent-report temperament traits were used to explore its relationship with ASD outcome. In the first stage of all regression analysis, observed temperament traits were included and in the second stage, risk group status was covaried to control the effect of the group.

After regression analysis, post hoc power was calculated using G*Power (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007), to test the population value of $R^2$ is large enough to detect the results.

3.2.5.6, *Exploratory analyses*

All analyses were run on the total sample (HR and LR combined) in the current chapter. This was mainly to eliminate the low power that can be arisen from the unequal sample size in the risk groups ($N_{LR} = 27$). Despite the small sample size in both groups, to assess whether the significant associations were driven by the HR or LR groups, Pearson correlation coefficients were calculated for each risk groups. This analysis will extend the previous analyses by indicating the source of the group effect.
3.3. Results

3.3.1. Psychometric properties of the temperament coding

3.3.1.1. IRR between coders

The results of ICC analyses showed that visual orientation to toy and facial fear had a good agreement; latency to touch, engagement, escape, visual orientation to the experimenter, visual orientation to parent, facial sadness, smile, negative and positive vocalisations, self-soothing and bodily fear had an excellent agreement between coders. Visual orientation away from the target points did not reach an acceptable ICC (.59) level and was excluded from the further analysis (Table 3.2).

Table 3.2 ICC for Inter-Rater Agreement of Observed Behaviours

<table>
<thead>
<tr>
<th>Total Duration of Behaviour</th>
<th>ICC</th>
<th>CI, 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to Touch</td>
<td>.94</td>
<td>.83, .98</td>
</tr>
<tr>
<td>Engagement</td>
<td>.97</td>
<td>.92, .99</td>
</tr>
<tr>
<td>Escape</td>
<td>.80</td>
<td>.52, .92</td>
</tr>
<tr>
<td>Looking Toy</td>
<td>.61</td>
<td>.20, .84</td>
</tr>
<tr>
<td>Looking Experimenter</td>
<td>.92</td>
<td>.78, .97</td>
</tr>
<tr>
<td>Looking Away</td>
<td>.59</td>
<td>.18, .83</td>
</tr>
<tr>
<td>Looking Parent</td>
<td>.83</td>
<td>.57, .94</td>
</tr>
<tr>
<td>Facial Fear</td>
<td>.71</td>
<td>.35, .89</td>
</tr>
<tr>
<td>Facial Sadness</td>
<td>.95</td>
<td>.88, .98</td>
</tr>
<tr>
<td>Smile</td>
<td>.93</td>
<td>.80, .98</td>
</tr>
<tr>
<td>Positive Vocalisation</td>
<td>.89</td>
<td>.67, .96</td>
</tr>
<tr>
<td>Negative Vocalisation</td>
<td>.79</td>
<td>.49, .92</td>
</tr>
<tr>
<td>Self-Soothing</td>
<td>.75</td>
<td>.41, .90</td>
</tr>
<tr>
<td>Bodily Fear</td>
<td>.77</td>
<td>.39, .92</td>
</tr>
</tbody>
</table>
3.3.1.2. Data reduction and dimension composites

Total duration of engagement across the tasks was associated with longer latencies to interact with the presented toy in the first task ($r = -.35, p < .001$) and longer duration of visual orientation to the presented toy ($r = .64, p < .001$). Longer positive vocalisations were significantly related to longer durations of gaze directed to the experimenter and duration of facial smile ($r = .31, p = .002$) whereas longer negative vocalisations were associated with longer facial sadness ($r = .67, p < .001$). Facial fear was associated with longer durations of gaze at the experimenter ($r = .21, p = .033$) and parent ($r = .38, p < .001$). Bodily expressed decreased activity was related to longer gaze at parent ($r = .22, p = .030$; Table 3.3). These results showed no multi-collinearity ($r > .80$) and enough variation between variables ($r > .10$) for the following PCA.
Table 3.3 Pearson Correlation Coefficients for Observed Temperament Variables

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<th>7</th>
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<th>10</th>
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<tbody>
<tr>
<td>1. Engagement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Escape</td>
<td>-.039</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3. Latency to Touch</td>
<td>-.252*</td>
<td>.059</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4. Positive Vocalisation</td>
<td></td>
<td>.043</td>
<td>-.091</td>
<td>-.188</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>5. Negative Vocalisation</td>
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<td>.126</td>
<td>.017</td>
<td>.081</td>
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</tr>
<tr>
<td>6. Smile</td>
<td>-.026</td>
<td>.099</td>
<td>.084</td>
<td>.313</td>
<td>-.102</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>7. Facial Fear</td>
<td>.024</td>
<td>.090</td>
<td>.023</td>
<td>-.060</td>
<td>.196</td>
<td>-.065</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8. Facial Sadness</td>
<td>-.120</td>
<td>.026</td>
<td>.059</td>
<td>-.068</td>
<td>.665*</td>
<td>-.162</td>
<td>.190</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>9. Bodily Fear</td>
<td>.086</td>
<td>-.092</td>
<td>.107</td>
<td>.075</td>
<td>.054</td>
<td>-.138</td>
<td>.072</td>
<td>-.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Looking at Experimenter</td>
<td>-.169</td>
<td>.007</td>
<td>.181</td>
<td>.314</td>
<td>.112</td>
<td>.458*</td>
<td>.213</td>
<td>.043</td>
<td>.182</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Looking at Toy</td>
<td>.637*</td>
<td>-.023</td>
<td>.046</td>
<td>.053</td>
<td>-.079</td>
<td>.028</td>
<td>.070</td>
<td>.025</td>
<td>.092</td>
<td>-.034</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Self-Soothing</td>
<td>-.090</td>
<td>.112</td>
<td>.080</td>
<td>.024</td>
<td>-.053</td>
<td>.142</td>
<td>.020</td>
<td>.035</td>
<td>-.063</td>
<td>.160</td>
<td>.060</td>
<td>.096</td>
<td></td>
</tr>
</tbody>
</table>

Mean 38.43 8.98 45.56 6.09 2.77 33.01 1.47 2.38 23.89 32.11 57.68 .51 5.37


* p < .001.
In order to explore the number of factors that could identify aspects of temperament traits, engagement, latency to touch, positive vocalisation, negative vocalisation, facial fear, facial sadness, smile, visual orientation to the experimenter, visual orientation to toy, visual orientation to parent behaviours were submitted to a PCA with orthogonal rotation (varimax). Five component solutions were identified. Bartlett’s test of sphericity was significant ($\chi^2 (78) = 241.42, p < .001$), indicating correlations between items were sufficient for running PCA. Based on Kaiser’s criterion (eigenvalues 1 and over), initial analyses suggested five components and these components explained 62.95% of the total variances. The scree plot demonstrated five points that would reflect five components. Due to the convergence between Kaiser’s criterion and scree plot, five components were calculated for further analyses. Table 3.4 indicates the factor loadings after varimax rotation. The items that clustered on a) the first component represented sadness (negative vocalisation, facial sadness) that accounted for the 15.80% of the variance; b) the second component represented positive affect (smile, visual orientation to the experimenter, positive vocalisation) that accounted for the 14.22% of the variance; c) the third component represented non-social engagement (Engagement, Visual orientation to toy) that accounted for the 13.07% of the variance; d) the fourth component represented BI (facial fear, latency to touch, visual orientation to parent) that accounted for the 10.56% of the variance; e) the last component represented regulation (escape, self-soothing, bodily fear (reversed)) that accounted for the 9.31% of the variance (Table 3.4).

Cronbach’s alpha of each component for the sadness and engagement showed good internal consistency ($\alpha = .76$ and $\alpha = .78$; respectively) whereas positive affect showed moderate ($\alpha = .63$) and BI showed modest ($\alpha = .42$) internal consistency. The
The regulation component showed poor internal consistency (α = .34) and was thus excluded from further investigations.

### Table 3.4 Principal Component Analysis

<table>
<thead>
<tr>
<th>Behaviours</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1 Negative Vocalisation</td>
<td>.90</td>
</tr>
<tr>
<td>2 Facial Sadness</td>
<td>.86</td>
</tr>
<tr>
<td>3 Positive Vocalisation</td>
<td>.12</td>
</tr>
<tr>
<td>4 Visual Orientation Experimenter</td>
<td>.06</td>
</tr>
<tr>
<td>5 Smile</td>
<td>-.24</td>
</tr>
<tr>
<td>6 Engagement</td>
<td>-.09</td>
</tr>
<tr>
<td>7 Visual Orientation Toy</td>
<td>-.03</td>
</tr>
<tr>
<td>8 Visual Orientation Parent</td>
<td>.11</td>
</tr>
<tr>
<td>9 Latency to Touch</td>
<td>-.14</td>
</tr>
<tr>
<td>10 Facial Fear</td>
<td>.33</td>
</tr>
<tr>
<td>11 Escape</td>
<td>.09</td>
</tr>
<tr>
<td>12 Bodily Fear</td>
<td>-.04</td>
</tr>
<tr>
<td>13 Self-soothing</td>
<td>-.06</td>
</tr>
</tbody>
</table>

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td>2.06</td>
<td>1.85</td>
<td>1.70</td>
<td>1.37</td>
<td>1.21</td>
</tr>
<tr>
<td>% of Variances Explained</td>
<td>15.85</td>
<td>14.26</td>
<td>13.06</td>
<td>10.55</td>
<td>9.29</td>
</tr>
<tr>
<td>Cronbach’s Alpha</td>
<td>.76</td>
<td>.63</td>
<td>.78</td>
<td>.42</td>
<td>.34</td>
</tr>
</tbody>
</table>

#### 3.3.2. Association between temperament and the AOSI total score

There was a significant negative correlation between the AOSI total score and the positive affect component ($r = -.38$, $p = .001$); however sadness, engagement and BI components were not significantly related to AOSI scores ($r = .20$, $p = .046$; $r = -.03$, $p = .791$; $r = -.01$, $p = .908$; respectively). There was no significant association between AOSI scores and parent report IBQ-Sadness, High-Intensity Pleasure, Duration of Orientation and Fear subscales (all $p \geq .623$).
The follow-up two-stage hierarchical multiple regression revealed that at the first stage, four observed temperament traits contributed significantly to the regression model \(F(4, 95) = 5.13, p = .001\) and explained the 17.8% variances in the AOSI total score. Only positive affect made a significant unique contribution to the model \((\beta = -0.37, p < .001)\). After adding categorical risk group variable into the second stage, the model improved by 4.4% \((F(1, 94) = 5.26, p = .024)\). Group variable had a significant contribution to the model \((\beta = .22, p = .024)\) and covarying group in the second model did not affect the pattern of significance and lower levels of positive affect were the only significant predictor of the AOSI total scores \(\beta = -.37, p < .001\) (Table 3.5).

Table 3.5 Hierarchical Regression with Observed Temperament Variables and the AOSI

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>b</th>
<th>SE</th>
<th>(\beta)</th>
<th>t</th>
<th>(R)</th>
<th>(R^2)</th>
<th>(\Delta R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>9.51</td>
<td>1.59</td>
<td>5.97***</td>
<td>.42</td>
<td>.18</td>
<td>.18**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sadness</td>
<td>.09</td>
<td>.05</td>
<td>.17</td>
<td>1.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>-.05</td>
<td>.01</td>
<td>-.37</td>
<td>-3.97***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Engagement</td>
<td>-.003</td>
<td>.01</td>
<td>-.02</td>
<td>-.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BI</td>
<td>.004</td>
<td>.06</td>
<td>.01</td>
<td>.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Constant)</td>
<td>7.12</td>
<td>1.87</td>
<td>3.80***</td>
<td>.47</td>
<td>.22</td>
<td>.04*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sadness</td>
<td>.075</td>
<td>.05</td>
<td>.15</td>
<td>1.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>-.05</td>
<td>.01</td>
<td>-.37</td>
<td>-4.04***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Engagement</td>
<td>.004</td>
<td>.01</td>
<td>.04</td>
<td>.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BI</td>
<td>-.002</td>
<td>.06</td>
<td>-.002</td>
<td>-.03</td>
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</tr>
<tr>
<td></td>
<td>Group</td>
<td>2.34</td>
<td>1.02</td>
<td>.22</td>
<td>2.29*</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*** \(p < .001\), ** \(p < .01\), * \(p < .05\).

3.3.3. Convergence between parent-reported vs observed temperament

Correlation analysis showed that there was a moderate significant association between observed BI and the IBQ-Fear subscale \((r = .22, p = .032)\). There were no
significant association between the sadness component and the IBQ-Sadness score \( (r = .03, p = .789) \); positive affect component and IBQ-High-Intensity Pleasure \( (r = .09, p = .407) \); or engagement component and the IBQ-Duration of Orientation \( (r = .002, p = .983) \). Furthermore, to visually inspect the agreement between two methods, scatter plots and BA plots were graphed. Figure 3.1 Plot A shows that there was not a linear association between measures. Plot B showed the pattern of agreement between the IBQ-High-Intensity Pleasure and observed positive affect component. The 95% limit of agreement lies between -2.7 and 2.7 standard deviations of the difference between both scales. Scatters lie within the CI of the mean differences shows the perfect agreement between the methods. The points widely spread in the mid-section (between \( x = -1 \) and \( x = 1 \)) indicating infants that were endorsed average scores. For these infants, scatters (lie between upper limits of the CI of the mean difference \( (y = 0) \) and \( y = 2.7 \)) indicate that parent reports higher scores than observed positive affect and moving towards \( y = 2.7 \) the differences increase. On the contrary, scatters (lie between lower limits of the CI of the mean difference \( (y = 0) \) to \( y = -2.7 \)) indicate that parent reports lower scores than observed positive affect and moving towards \( y = -2.7 \) the differences increased and observed positive affect is higher than the parent reports.

As Figure 3.2 Plot A shows, there is a floor effect for the sadness component indicating infants presented lower levels of sadness during the AOSI compared to their general state by parent report. Plot B indicates that the limit of agreement ranged between -2.7 and 2.7 standard deviations. Scatters lie between the upper level of CI of the mean difference \( (y = 0) \) towards \( y = 2.7 \), parents report higher sadness than the observed sadness whereas scatters lie between the lower limit of CI of the mean difference \( (y = 0) \) towards \( y = -2.7 \) for few cases parent reports less sadness than observed sadness.
For the duration of engagement, the limit of agreement ranges between -2.8 and 2.7. Scatter that lies within the CI limits of the mean difference (y = 0) shows perfect agreement between two methods and this agreement evident for children who had lower scores (x < -1), average scores (between x = -1 and x = 1) and higher scores (x > 1). Scatters lie between the upper level of CI of the mean difference (y = 0) towards y = 2.7 indicates that parents report higher orientation than the observed engagement whereas scatters lie between the lower limit of CI of the mean difference (y = 0) towards y = -2.7 shows that parent reports less orientation than observed sadness.

BI component explained the 4.84% variation in the IBQ-R Fear subscale. Figure 4 Plot A demonstrates floor effect due to lower levels of frequencies of BI. Plot B showed that compared to other components, parent-report fear and observed BI had the narrowest limit of the agreement, which was -2.4 and 2.5. There is a good agreement between parent report of fear and observed BI for children who had less BI). Scatters lie between the upper level of CI of the mean difference (y = 0) towards y = 2.5 indicates that parents report higher fear than the observed BI whereas scatters lie between the lower limit of CI of the mean difference (y = 0) towards y = -2.4 shows that parent reports less fear than observed BI.
Figure 3.1 A) Scatter plot reflects Z-scores of parent-reported high-intensity pleasure and observed positive affect. B) Bland-Altman plot of agreement between methods. The x-axis represents the mean of parent-reported high-intensity pleasure and observed positive affect. The y-axis represents the difference between Z-scores of parent-reported high-intensity pleasure and observed positive affect.
Figure 3.2 A) Scatter plot reflects Z-scores of parent-report sadness and observed sadness. B) Bland-Altman plot of agreement between methods.

The x-axis represents the mean of parent-reported sadness and observed sadness. The y-axis represents the difference between Z-scores of parent-reported sadness and observed sadness.
Figure 3.3 A) Scatter plot reflects Z-scores of parent-reported duration of orientation and observed engagement B) Bland-Altman plot of agreement between methods. The x-axis represents the mean of parent-reported duration of orientation and observed engagement. The y-axis represents the difference between Z-scores of parent-reported Duration of Orientation and observed Engagement.
Figure 3.4 A) Scatter plot reflects Z-scores of parent-reported fear and observed BI B) Bland-Altman plot of agreement between methods. The x-axis represents the mean of parent-reported fear and observed BI. The y-axis represents the difference between Z-scores of parent-reported fear and observed BI.
3.3.4. Outcome group differences in research variables

3.3.4.1. Observed temperament traits

The MANOVA assessing differences between the LR and the HR groups on the
four observed temperament traits (sadness, positive affect, engagement, BI) showed a
significant main effect of group, \(F(4, 95) = 2.54, p = .045, \eta^2 = .10\). Between-subjects
test indicated that the HR group were reported to have lower levels of engagement than
the HR group \(F(1, 100) = 9.15, p = .003, \eta^2 = .09\). There was not significant effect of
group on positive affect \(F(1, 100) = .002, p = .962, \eta^2 = .00\), sadness \(F(1, 100) = 1.63,
p = .204, \eta^2 = .02\) and BI \(F(1, 100) = .73, p = .396, \eta^2 = .01\).

A MANOVA assessing dimensional outcome group differences (LR, HR-TD, HR-ATY, HR-ASD), on observed temperament traits revealed a significant main effect of outcome group \(F(4, 95) = 3.39, p = .012, \eta^2 = .13\). Outcome group specifically had a
significant effect on the engagement component \(F(3, 96) = 3.38, p = .021, \eta^2 = .10\);
there were no significant group differences on sadness \(F(3, 96) = .76, p = .517, \eta^2 = .02\), positive affect \(F(3, 96) = .58, p = .632, \eta^2 = .02\) and BI \(F(3, 96) = 1.66, p = .181, \eta^2 = .05\) components.

A follow up univariate ANOVA indicated that only the HR-ASD group scored
lower than the LR group \((p = .041)\) and there were not any other significant differences
in rest of the outcome groups (Table 3.6). Overall the results revealed that HR-ASD
group had difficulties in engagement in social situations than the LR group.

3.3.4.2. Parent report temperament traits

The MANOVA testing the effect of risk group status and a separate MANOVA
assessing the effect of the outcome group categorisation on the parent-reported
temperament traits (sadness, high-intensity pleasure, duration of orientation, fear)
showed that both risk groups and outcome groups did not have significant effect on observed temperament components (risk group: $F(4, 90) = 1.66, p = .166, \eta^2 = .07$; Outcome group: $F(12, 233) = .68, p = .773, \eta^2 = .03$; Table 3.6).

### 3.3.4.3. Anxiety and ASD traits

A MANOVA to test group differences between LR and HR groups on anxiety and ASD scores showed a main effect of risk group, $F(2, 85) = 5.20, p = .007, \eta^2 = .11$. Group membership was observed to have a significant effect on anxiety ($F(1, 88) = 6.47, p = .013, \eta^2 = .07$) and ASD ($F(1, 88) = 9.61, p = .003, \eta^2 = .10$), with the HR group demonstrating higher anxiety and ASD traits than the LR groups.

To investigate the outcome group differences on anxiety and ASD scores, a MANOVA was calculated. The result suggested a main effect of group categorisation ($F(3, 84) = 22.12, p < .001, \eta^2 = .44$). Group membership was observed to have a significant effect on anxiety ($F(3, 88) = 4.15, p = .009, \eta^2 = .13$) and ASD ($F(3, 88) = 21.53, p < .001, \eta^2 = .44$). Follow up ANOVA analyses with Bonferroni Post hoc analysis showed that the HR-ASD group had significantly higher anxiety scores than the LR group ($p = .004$) and there were no significant group differences between rest of the group members (all $p \geq .066$). As for the ASD scores, Bonferroni post hoc test showed that the HR-ASD group had significantly higher ASD scores than the LR ($p < .001$), HR-TD ($p < .001$) and HR-ATY ($p < .001$) groups; the HR-ATY group also had higher ASD traits than the LR group ($p = .028$) (Table 3.6). Overall these findings suggest that the HR and the HR-ASD groups significantly differ on engagement component, anxiety and ASD scores.
Table 3.6 Risk and Outcome Group Differences in Observed Temperament Dimensions

<table>
<thead>
<tr>
<th></th>
<th>Low-Risk M (SD)</th>
<th>High-Risk M (SD)</th>
<th>HR diagnostic outcome groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Typical M (SD)</td>
</tr>
<tr>
<td><strong>Observed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sadness</td>
<td>3.08 (4.66)</td>
<td>5.85 (10.50)</td>
<td>6.55 (14.07)</td>
</tr>
<tr>
<td>Positive Affect</td>
<td>70.93 (22.66)</td>
<td>71.30 (36.80)</td>
<td>69.98 (26.01)</td>
</tr>
<tr>
<td>Engagement</td>
<td><strong>115.46</strong> (36.53)</td>
<td><strong>89.66</strong> (37.16)</td>
<td>88.32 (35.47)</td>
</tr>
<tr>
<td>BI</td>
<td>4.86 (8.49)</td>
<td>6.31 (6.93)</td>
<td>5.19 (6.16)</td>
</tr>
<tr>
<td><strong>Parent-Report</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sadness</td>
<td>3.77 (.85)</td>
<td>4.00 (1.05)</td>
<td>4.12 (.97)</td>
</tr>
<tr>
<td>Pleasure</td>
<td>6.15 (.60)</td>
<td>6.10 (.66)</td>
<td>6.14 (.55)</td>
</tr>
<tr>
<td>Orientation</td>
<td>3.58 (1.13)</td>
<td>3.26 (.85)</td>
<td>3.28 (.97)</td>
</tr>
<tr>
<td>Fear</td>
<td>3.02 (.85)</td>
<td>3.47 (1.09)</td>
<td>3.50 (.97)</td>
</tr>
<tr>
<td>Anxiety_36m</td>
<td><strong>1.91 (1.59)</strong></td>
<td><strong>4.03 (3.76)</strong></td>
<td>2.96 (3.60)</td>
</tr>
<tr>
<td>ASD_36m</td>
<td><strong>2.78 (2.32)</strong></td>
<td><strong>9.91 (7.67)</strong></td>
<td>4.17 (4.87)</td>
</tr>
</tbody>
</table>

Bolds indicate a significant group difference between LR and overall HR group.

Superscript letters indicate a significant difference between the LR and HR outcome groups at $p < .05$ level.

3.3.5. Association between temperament traits, anxiety and ASD scores

There was a significant association between parent-reported fear and anxiety ($r = .43, p < .001$), observed BI and anxiety score ($r = .36, p < .001$) but no other parent-reported or observed temperament traits at $p < .001$ significance level. There was also a significant association between anxiety and the SCQ total score ($r = .60, p < .001$).

Since the MSEL was not associated with any temperament traits (both parent report and
observed), anxiety and ASD scores, it was excluded from further analysis. Again, there were no significant associations between observed temperament traits and ASD scores whereas there were significant associations between parent-report temperament and ASD (Table 3.7). So, further regression analysis will be run using parent-reported temperament traits.

Table 3.7 Pearson Correlation Between Temperament Anxiety, ASD and Cognitive Functioning

<table>
<thead>
<tr>
<th></th>
<th>Anxiety</th>
<th>ASD</th>
<th>MSEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleasure_PR</td>
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<td>-.280</td>
<td>.039</td>
</tr>
<tr>
<td>Sadness_PR</td>
<td>.271</td>
<td>.123</td>
<td>.070</td>
</tr>
<tr>
<td>Orientation_PR</td>
<td>-.119</td>
<td>-.083</td>
<td>-.015</td>
</tr>
<tr>
<td>Fear_PR</td>
<td>.430*</td>
<td>.137</td>
<td>-.026</td>
</tr>
<tr>
<td>Positive Affect_O</td>
<td>.004</td>
<td>-.019</td>
<td>.122</td>
</tr>
<tr>
<td>Sadness_O</td>
<td>.050</td>
<td>.019</td>
<td>.067</td>
</tr>
<tr>
<td>Engagement_O</td>
<td>.029</td>
<td>-.202</td>
<td>.159</td>
</tr>
<tr>
<td>BI_O</td>
<td>.362*</td>
<td>.157</td>
<td>-.066</td>
</tr>
<tr>
<td>Anxiety</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD</td>
<td>.602*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MSEL</td>
<td>-.215</td>
<td>-.247</td>
<td>-</td>
</tr>
</tbody>
</table>


Further hierarchical multiple linear regression analysis was conducted to explore the association between anxiety and observed temperament traits considering the effect of group and ASD scores. The first stage of the regression analysis included only four temperament components and the model fit was significant $F(4,83) = 2.97, p = .024$. In this stage, observed BI was the only significant predictor of anxiety scores ($\beta = .36, p = .001$). The risk group status and ASD scores covaried and 41.4% variances in anxiety scores were explained by the variables in the second stage ($F(4,83) = 2.97, p = .024$).
Risk group status was not associated with anxiety scores ($\beta = .13, p = .164$) whereas ASD scores were significantly associated with anxiety ($\beta = .55, p < .001$). Among the variables, again observed BI was a significant predictor of anxiety ($\beta = .25, p = .005$) and after covarying group and ASD score engagement component become significant ($\beta = .21, p = .021$).

The second hierarchical multiple regression analysis was re-run replicating observed temperament traits by parent-reported variables. The first stage of the analysis explained 22.9% of the variances in the anxiety score $F(4, 79) = 5.87, p < .001$. Like the first regression analysis, parent-reported fearfulness was the only significant predictor of anxiety scores ($\beta = .42, p < .001$). Inserting risk group variable and ASD scores into the second stage increased the model by 29.4%, $F(2, 77) = 23.70, p < .001$. Controlling these two variables did not change the level of significance and parent-reported fearfulness was the only predictors of the anxiety scores ($\beta = .37, p < .001$; Table 3.8).

Post hoc power analysis was conducted to determine the power of the current sample for detecting a medium effect size. The analysis revealed that the present sample had the power of $(1-b) = .89$ in detecting a significant association between anxiety score and observed temperament variables and had the power of $(1-b) = .99$ in detecting a significant association between anxiety score and parent-reported temperament variables.
Table 3.8 Hierarchical Multiple Linear Regression for Association between Observed, Parent-Reported Temperament and Anxiety

### Anxiety by Observed Temperament Traits

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>b</th>
<th>SE</th>
<th>β</th>
<th>t</th>
<th>R</th>
<th>R²</th>
<th>ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>Positive Affect</td>
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<td>-.03</td>
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</tr>
<tr>
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<td>Sadness</td>
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<td>-.004</td>
<td>-.04</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Engagement</td>
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<td>.01</td>
<td>.08</td>
<td>.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BI</td>
<td>.17</td>
<td>.05</td>
<td>.36</td>
<td>3.44**</td>
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<td>(Constant)</td>
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<td>-1.14</td>
<td>.67</td>
<td>.45</td>
<td>.33***</td>
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</tr>
<tr>
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<td>-.01</td>
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<td>Sadness</td>
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<td>.03</td>
<td>.01</td>
<td>.14</td>
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</tr>
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<td>Engagement</td>
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<td>.01</td>
<td>.21</td>
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</tr>
<tr>
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<td>BI</td>
<td>.12</td>
<td>.04</td>
<td>.25</td>
<td>2.88**</td>
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<tr>
<td></td>
<td>Group</td>
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<td>.13</td>
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<tr>
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<td>ASD</td>
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<td>.04</td>
<td>.55</td>
<td>6.21***</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

### Anxiety by Parent-Report Temperament Traits

<table>
<thead>
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<th>Model</th>
<th>Variable</th>
<th>b</th>
<th>SE</th>
<th>β</th>
<th>t</th>
<th>R</th>
<th>R²</th>
<th>ΔR²</th>
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<tr>
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<td>.74</td>
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</tr>
<tr>
<td></td>
<td>High Intensity Pleasure</td>
<td>-.20</td>
<td>.55</td>
<td>-.04</td>
<td>-.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of Orientation</td>
<td>-.65</td>
<td>.38</td>
<td>-.18</td>
<td>-1.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear</td>
<td>1.37</td>
<td>.37</td>
<td>.42</td>
<td>3.68***</td>
<td></td>
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</tr>
<tr>
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<td>(Constant)</td>
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<td>.72</td>
<td>.52</td>
<td>.29***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sadness</td>
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<td>.31</td>
<td>.03</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>High Intensity Pleasure</td>
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<td>.12</td>
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</tr>
<tr>
<td></td>
<td>Duration of Orientation</td>
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<td>.31</td>
<td>-.15</td>
<td>-1.77</td>
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<tr>
<td></td>
<td>Fear</td>
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<td>.30</td>
<td>.37</td>
<td>4.00***</td>
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<tr>
<td></td>
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<td>.04</td>
<td>.58</td>
<td>6.65***</td>
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*** p < .001, ** p < .01, * p < .05.
Regarding the ASD scores, the first stage of the analysis (parent reported sadness, high intensity pleasure, duration of orientation, fear as predictors and ASD as outcome) did not significantly fit the data ($F(4, 79) = 2.36, p = .061$). Including risk group and anxiety scores into the second model improved the model fit by 37.3%, $F(2, 77) = 27.65, p < .001$. Being in the HR group was related to higher ASD scores ($\beta = 3.21, p = .029$) and higher anxiety scores was related to higher ASD scores ($\beta = .63, p < .001$). Parent-reported high-intensity pleasure and fear scores were significantly related to anxiety ($\beta = -.24, p = .007; \beta = -.23, p = .033$; respectively; Table 3.9).

Post hoc power analysis was conducted to determine the power of the current sample for detecting a medium effect size. The analysis revealed that the present sample had the power of $(1-b) = .79$ in detecting a significant association between ASD score and parent-reported temperament variables.
Table 3.9 Hierarchical Multiple Linear Regression for Association between Parent-Reported Temperament traits and ASD Scores.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>( b )</th>
<th>( SE )</th>
<th>( \beta )</th>
<th>( t )</th>
<th>( R )</th>
<th>( R^2 )</th>
<th>( \Delta R^2 )</th>
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<td>.78</td>
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<tr>
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<td>-2.53*</td>
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<tr>
<td></td>
<td>Duration of Orientation</td>
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<tr>
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<td>Fear</td>
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<td>Duration of Orientation</td>
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</tbody>
</table>

*** \( p < .001 \), ** \( p < .01 \), * \( p < .05 \).

3.3.6. Exploratory analyses

One of the aims of the current chapter was to assess the association between observed, parent-reported temperament traits and the AOSI scores. The only significant association was between the AOSI and the observed positive affect. When investigating group level associations, the negative association between observed positive affect and the AOSI total score was significant in the HR group (HR: \( r = - .41, p < .001 \); LR: \( r = - .26, p = .206 \)). Another aim was to investigate whether observed and parent report of temperament traits were related to anxiety and ASD traits measured at 36 months. Previously, I found a significant association between observed BI and anxiety scores and in the current analysis there was a significant association in the HR group (HR: \( r = - .45, p < .001 \); LR: \( r = -.14, p = .533 \)). The association between parent-reported fear and
anxiety was significant in the HR group, but the effect size in the LR group was medium (HR: $r = .41, p < .001$; LR: $r = .34, p = .114$). Like previous analysis, there was no significant association between observed temperament traits and ASD scores at $p < .001$ significance level. But the association between observed positive affect and ASD score was significant in the LR group at $p = .05$ significance level (HR: $r = -.04, p = .740$; LR: $r = .43, p = .040$). In terms of parent-reported temperament traits was negative significant association in high intensity pleasure and ASD scores only in the HR group (HR: $r = -.28, p = .022$; LR: $r = -.16, p = .464$). Parent-reported fear was significantly related to ASD scores only in the LR group at $p < .01$ significance level (HR: $r = .04, p = .766$; LR: $r = .54, p = .007$). Overall, except the relationship between parent-reported fear and ASD scores, all significant associations were likely to be driven by the HR group.
Table 3.10 Pearson Correlation Coefficients of Research Variables Split by Group, Top Left Represent the LR Group and Right Bottom Represents the HR Group

<table>
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<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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</tr>
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<td>1. Sadness_O</td>
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<td>-</td>
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<td>-</td>
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<td>-.057</td>
<td>.070</td>
<td>-</td>
<td>.135</td>
<td>-.202</td>
<td>.121</td>
<td>-.160</td>
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<td>7. Orientation_PR</td>
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<td>-.038</td>
<td>.059</td>
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<td>.060</td>
<td>-</td>
<td>.479</td>
<td>-.357</td>
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<td>8. Fear_PR</td>
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<td>.044</td>
<td>.269</td>
<td>.450*</td>
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<td>.063</td>
<td>-</td>
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<td>-.118</td>
<td>-.084</td>
<td>.154</td>
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<td>-.095</td>
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<td>-.109</td>
<td>.037</td>
<td>.107</td>
<td>.572*</td>
<td>-</td>
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* $p < .001$
3.4. Discussion

The present study, is the first to (i) employ a multi-method approach to investigate temperamental reactivity and regulation and their association with ASD symptoms in infancy (at 15 months), (ii) investigate agreement between parent-reported and observed temperament traits, (iii) integrate two distinct assessment methods (observed vs parent-report) to investigate whether both approach similarly relate to anxiety traits and ASD symptoms (at 36 months).

Analysis of behaviours coded via my novel coding scheme revealed four components (sadness, positive affect, engagement and BI). Lower levels of positive affect during the AOSI administration was related to total higher AOSI scores. Observed and parent-report BI was more closely associated with each other than for the other three domains of temperament. Observed BI and parent-report fear at 15 months both significantly associated with anxiety scores but not ASD scores at 36 months whereas only parent-report of lower levels of high-intensity pleasure was associated with higher levels ASD traits at 36 months. These results support and extend Chapter 2 by indicating associations between early BI and later anxiety but suggest that these may not confound early behavioural capture of ASD-related symptoms. However, individual differences in positive reactivity may influence AOSI scores and are related to later ASD, possibly contributing to the predictive association between the AOSI and ASD outcome.

3.4.1. Psychometric properties of the temperament coding

In order to observe temperamental reactivity, the current coding scheme included behaviours that were related to the theoretical constructs of BI. Latency to
approach in the initial activity of the AOSI, duration of engagement with the presented toy, facial expressions (fear, sadness and smile), bodily fear, negative and positive vocalisation were of interest. As for regulatory behaviours, escape, self-soothing and orientation of gaze (looking at presented toy, experimenter and parent) were incorporated in the coding scheme. The result of the PCA revealed five components: sadness, positive affect, engagement, BI and regulation. This finding is partly in line with results from previous research by Buss (2011) and (Kagan et al., 1987). Especially Buss (2011) and current research have yielded similar components (BI, sadness and engagement) but few behaviours loaded onto different components in the current findings. For example, Buss (2011) showed that bodily fear loaded on to fear component whereas in the current research bodily fear negatively loaded on to the regulation component; and latency to touch loaded on to the engagement component, whereas in the current research it was related to the BI component. Although Buss (2011) showed that BI and sadness were related constructs, the current findings differ from that investigation, probably due to the context of the observation. The AOSI tasks were not specifically designed to trigger temperamental reactions of infants and it consists of mainly social interactions with an adult experimenter. This raises the possibility that the meaning of specific behaviours changes depending on the context in which they are elicited, an important consideration for future research.

In line with Gulsrud et al. (2010) and Jahromi et al. (2012), self-soothing and escape behaviours loaded on to the regulation component in the current investigation. Contrary to expectations, bodily fear (generally regarded as the reactive domain of temperament) negatively loaded on to the regulatory domain. Possibly, diminished activity level and apparent freezing in the AOSI may reflect attentional focus towards the activities presented by the experimenter. However, the regulation component (bodily
fear, escape, self-soothing) had poor internal consistency and to increase the reliability of the finding this component was discarded from the rest of the analyses. The low internal consistency perhaps the result of input from the parent and the experimenter. Since the setting of the AOSI requires the child to sit on the parent’s lap and in close proximity to the experimenter, once the child got upset or fearful, the parent or the experimenter can scaffold the child and they presented shorter durations of regulatory behaviours. So, the observation of these behaviours can be confounded by other uncontrollable variables.

Planalp et al. (2017) argued that visual object orientation and the ability to disengage from arousing stimuli might serve as regulatory behaviours. To capture this, I integrated four gaze orientations (gaze at the parent, gaze at the experimenter, gaze at the toy, gaze away which did not present good interrater agreement) to examine the extent to which gaze behaviour reflects the regulatory type of behaviours. Unexpectedly, none of the visual orientation codes loaded onto the regulation component. However, gaze to the experimenter was related to the positive affect component, gaze at the toy was related to engagement and gaze at parent was associated with the BI component. Again, this may be the result of scaffolding from the parent or the experimenter that burdens the observation of regulatory behaviours or these gaze orientations reflects socially motivated interactions. Besides, none of the tasks in the AOSI was designed to arouse fear, withdrawal so there may not be anxiety-provoking stimuli to disengage the visual attention from.

Along with the facial fear and latency to touch, gaze at parent loaded on the reactive BI component. Hill-Soderlund and Braungart-Rieker (2008) suggested that infants can visually disengage from a stressful stimulus and engage with a neutral,
calming stimulation as a regulatory strategy. However, the current findings are in contrast with this argument and showed that longer duration of gaze at the parent, even though it is not conceptually regarded as a fearful reaction, was consistent with the reactive BI component. One possible explanation for this may be that children had a limited face to face contact with their parent since they were in a sitting position on their parent’s lap during the AOSI and had limited chances to refer to their parents in the arousing situations. Infants who seek eye contact with their parents were aroused and refer to their parents to convey their emotional state or, on the contrary, they may refer to parents to check their emotional state as for seeking social cues in relation to the situation.

The results of the PCA analysis should be interpreted with caution. PCA is a widely used method for the data reduction in observational research, but it is less powerful than factor analysis and it should be noted that conclusions of the PCA are specific to the sample that the data is collected from. The generalisation of the obtained components can be achieved if the other studies reveal a similar pattern of component loadings. So, it is essential to replicate this approach in bigger sample sizes and to observe these behaviours in different situations.

3.4.2. Association between observed temperament traits and AOSI scores

As mentioned in the introduction, Brian et al. (2008) argued that consideration of the infants’ temperamental disposition during the AOSI assessments is important and the AOSI scoring allows administrators to rate infants’ overall temperamental disposition as under- or over-reactive. The current investigation extended this by exploring four conceptually distinct temperament domains (all reactivity) during the AOSI administration. The results showed that lower levels of positive affect observed
during the AOSI assessment were related to higher AOSI total scores; none of the parent-reported temperament traits was related to the AOSI total score. Furthermore, the exploratory analysis showed that the lower levels of positive affect was related to the AOSI score only in the HR group. This finding is in line with a study by Filliter et al. (2015) who showed that HR infants who were diagnosed with ASD at a later timepoint showed reduced positive affect during the AOSI than the HR-ASD infant who did not have ASD when they were 12 months old. One of the explanations for this may be that decreased positive affect during the AOSI may be itself an early sign of ASD, and thus associated with higher scores because of both index ASD risk. Even though Filliter et al. (2015) observed only smile in their coding scheme, the results showed associations between higher levels of observed smile and AOSI items (reciprocal social smile, social interest and affect). So this result support that positive affect may be an early marker of ASD.

Different from Filliter et al. (2015), the observed positive affect component consisted of smile, positive vocalisation and visual orientation to the experimenter. The combination of these behaviours, especially smile and eye contact, is consistent with early social interaction abilities and associates with later complex social interactions (Striano & Rochat, 1999). Impairments in combining social smile and gaze or reduced levels of these behaviours have been found in children with ASD (Kasari, Sigman, Baumgartner, & Stipek, 1993) and HR-ASD infants (Filliter et al., 2015). One possible explanation for this association may be that the experimenters find it challenging to engage with the infants during the tasks that were designed to elicit positive affect and for those infants, the experimenters may tend to endorse higher AOSI total scores.
3.4.3. Convergence between parent-reported vs observed temperament

The results of the present investigation also provide some evidence of the convergence between observed temperament styles (positive affect, sadness, engagement and BI), and parental perception of temperament. Previous studies presented inconsistent results on whether there was an agreement between two methods (Kiel & Hummel, 2017; Olino et al., 2013; Seifer et al., 1994; White et al., 2017). Initial correlation analyses of the current investigation showed that the BI component and Fear subscale had a significant but low association ($r = .22$) compared to other matched components and subscales.

The possible reasons for this may be threefold. Firstly, parental characteristics such as personality and psychopathology affect the parental ratings and may thus lead to lower levels of convergence with the observational techniques. Secondly, the observation and parental questionnaires may reflect the same temperamental construct, but there may be situational differences in the way that the construct is expressed. According to Rothbart and Goldsmith (1985), parents and observers should use similar situations to rate child temperament to increase the convergence. Thirdly, the context of the observational tasks may affect the agreement. Buss (2011) argued that the level of threat in the BI-eliciting context is crucial to yielding higher levels of fear, withdrawal or wariness. Further, Kiel and Hummel (2017) suggested that the convergence between the two measurement methods could be low due to contextual threat-level, and showed that parent-reported temperament converged with observed BI in the low-level threat conditions with small to moderate effect size rather than high-threat conditions.

The context of the AOSI was perhaps thus not optimal for triggering sadness and BI that would converge with parent reports, but due to the main aim of this research, I
chose specific tasks in which I expected infants could show withdrawal or fearful reactions. That may be the reason for significant correspondence between two assessment techniques for BI but fewer associations for other components. Lastly, the agreement between methods may differ based on the temperamental construct being measured. For instance, Stifter, Willoughby, Towe-Goodman, and The Family Life Project Key (2008) showed moderate agreement between methods for positive affect whereas showed no congruence for the negative affect. So, it may be that the lower levels of agreement in some temperament trait may be the result of difficulty in capturing these behaviours.

3.4.4. Association between temperament traits, anxiety and ASD scores

Although previous research has investigated the correspondence between observed and parent-reported temperament traits (e.g., Kiel & Hummel, 2017; Olino et al., 2013; White et al., 2017), there has been less research that has directly compared these two methods in the prediction of anxiety and ASD traits. The current findings showed that both observed BI and parental perception of fear in infancy (15 months) were significant predictors of anxiety traits in toddlerhood (36 months). The relation between observed BI/parent-reported fear and later anxiety traits did not change once the ASD scores were incorporated in the analysis. This finding is consistent with results in Chapter 2 that showed a strong association between parent-reported BI and anxiety traits once controlling for the ASD scores (both measured at 36 months). But the current investigation extended the findings of Chapter 2 to an observational context in which observed BI correspond parent-reported fear. This finding is strong evidence that early BI relates to later anxiety but does not seem to confound the AOSI score and influence later (36 months) ASD scores. So, this suggests that there may be independent ways we
currently identify ASD. This gives us a way to start to dissociate early behavioural signs of ASD and anxiety; shows they may be separable and that they possibly have separable developmental roots. So, these results confirm that BI may be a risk factor that distinguishes anxiety traits from ASD.

Since the analyses were run on the total sample, to explore the source of the association more, exploratory correlation analyses were run for the HR and the LR group separately. The results suggested that in the HR group observed BI and parent-reported fear were more strongly associated with the anxiety than ASD traits. In the LR group, observed BI was not associated with both anxiety and ASD traits, but parent-reported fear was more strongly related to ASD traits than anxiety. Although these exploratory analyses may be underpowered due to small sample size in the LR group, the results suggested the effect of the significant association may be driven by the HR group.

Physiological reactions related to heightened BI may independently relate to anxiety rather than ASD. For example, BI and ASD share similarities such as withdrawal of BI is similar to the lack of social interaction in ASD. However, BI is more consistent with the initial reactions to novelty or sudden stimulation rather than the general responses to daily stimulation. BI disposition may be more in line with physiological changes, such as fight-or-flight responses. In the novel situations, heightened physiological reactions may influence subsequent initial withdrawal, dysregulated fear. In the DSM-5, the definition of anxiety consists of physiological changes whereas core ASD symptoms do not require this (American Psychiatric Association, 2013). So, BI may relate to anxiety due to initial arousability and it may be a risk factor that separates anxiety from ASD symptoms.
Moreover, when ASD scores were controlled in the regression analyses, the observed engagement component, that incorporated the duration of play with the presented toys and visual orientation to toys, become significant. However, it only explained 2% of the variance in the anxiety scores. This may reflect the notion that some infants may find it difficult to engage with the experimenters due to the level of shyness and pursued longer engagement with the presented toy.

In terms of the parent report temperament traits, lower levels of pleasure were related to higher ASD scores at 36 months. Although the parent-reported intensity of pleasure was not associated with concurrently measured AOSI total scores, observed positive affect was related to the AOSI scores. In general, positive affect relates to ASD traits but may be influenced by the measurement method. For example, parent-reported pleasure relates to parent-reported ASD scores and observed positive affect related to observed AOSI score. The longitudinal association may reflect the influence of the rater effect. Because of observed positive affect and parent-reported intensity of pleasure tap different aspects of the ASD measures. For example, observed positive affect includes smile, eye contact and positive vocalisations which are perhaps more in line with the state level positive affect when interacting with the experimenter and may not reflect trait level temperament. On the other hand, the parent-report intensity of pleasure could reflect more trait level affect. It is possible that consistent lower levels of pleasure in infancy relate to reduced sharing of facial expressions with others, a core feature of the ASD phenotype.

When anxiety scores were controlled in the analyses, fear become negatively associated with the ASD scores. This result is rather surprising, because in Chapter 2 when ASD scores were controlled, 24-month shyness and anxiety remained positively
associated. In the current research, at 15 months, the IBQ-R Fear subscale which includes reactions to social (more in line with shyness) and non-social stimuli was used (Putnam et al., 2014). This association could be generated due to the non-social aspect of the Fear subscale. However, further investigation is needed to explore the mechanisms that underlie this association.

3.4.5. Strengths, limitations and future directions

One of the strengths of the current research is its relatively large sample size and having the outcome group categorisation to investigate the effect of ASD group. Also, post hoc power analyses confirmed that high power generating the results in the current sample.

Secondly, multimethod measurement of infant temperament enhances our understanding of the association between childhood construct temperament and its association with concurrent ASD and longitudinal association with later anxiety as well as ASD traits. Furthermore, this association contributed to our understandings of the question of whether parental judgement is biased or whether we reliably observe temperament traits.

It is important to consider possible limitations when interpreting the results of the current study. Firstly, the coding scheme only relied on one parameter: duration, which may not reflect the overall emotional or behavioural reactions of an infant. Other studies have generally used intensity scores to determine child reaction. However, it is open to discussion whether duration provides a proxy measure of temperamental reactions. Thirdly, these observational measures only provide data for certain context for a limited of time. Children may exhibit high levels of reactions in a laboratory environment but no other settings, so, in future research it is important to investigate
this in longitudinal studies with latent class analyses to observe whether infants who scored higher on a specific temperament trait present similar temperamental trait at a later timepoint. Future studies should also employ context-specific observational tasks that were designed to trigger specific reactions (e.g., withdrawal, fear) and consider the role of parental behaviours in these tasks.

3.4.6. Conclusion

The aims of the current investigation were threefold: to explore whether i) temperamental BI masks or exacerbate identification of early markers of ASD, ii) observed temperament traits converge with parent-report temperament traits, iii) observed and parent reports temperament traits predicts anxiety and ASD traits at 36 months. To investigate these aims, I developed a novel coding scheme to employ a multi-method assessment of temperament. The first key finding presented in this chapter was that observed BI did not confound the identification of early markers of ASD traits, but lower levels of positive affect were more associated with the ASD scores. The second key finding was that observed BI converges with parent-reported fear. Lastly, observed BI was related to anxiety traits but not to ASD scores (at 36 months). To conclude, the results of this study indicate that observed BI may be a distinct risk factor for anxiety, which helps us to untangle the overlap between anxiety and ASD.
Chapter 4. Social and Non-Social Behavioural Inhibition and Their Associations with Anxiety and ASD

4.1. Introduction

In Chapter 2, I used parent-reported temperamental fear (measured at 9 and 15 months) and shyness (measured at 24 months) as a proxy measure of behavioural inhibition (BI), since both subscales reflect conceptually similar traits and result in inhibited approach. In Chapter 3, I adopted a novel coding scheme to observe BI in infancy (15 months) and employed a multimethod approach to tackle possible bias that could arise from parent-report measures and shared measurement variance (due to using same measurement method for predictor and outcome variables). However, the description of BI requires novelty in the context that behavioural and emotional responses are observed (Kagan et al., 1984). In Chapter 3, BI was observed during a play-based research instrument, which mainly involves lower levels of unfamiliarity compared to observational tasks and higher levels of social interaction with an adult experimenter. In the current chapter, I will expand the exploration of temperamental BI among 24-month-old toddlers by employing widely used structured observational tasks which were designed to provoke fearfulness, shyness and withdrawal tendencies (Fox et al., 2001; Kagan et al., 1984). Exploring whether BI observed in these social and non-social tasks similarly relates to anxiety and Autism Spectrum Disorder (ASD) traits may extend the investigations in previous chapters by exploring familial aggregation of anxiety and ASD traits. Moreover, in the general population maternal behaviours have been related to child BI and anxiety traits (e.g., Aktar & Bögels, 2017; Hudson & Dodd, 2012; Lewis-Morrarty et al., 2012). To date, there have not been many investigations of observed BI in relation to early emerging anxiety traits and how parental behaviours
affect the association between child BI and anxiety within the high-risk (HR) for ASD sibling design.

In the current chapter, I aimed to 1) observe behaviours that constitute BI in the semi-structured social Stranger Approach and the non-social Unpredictable Toy laboratory tasks in 24-month-old toddlers at high- and low-risk (LR) for ASD; 2) create a factor score for overall social BI using behaviours observed in the Stranger Approach task, experimenter-rated overall social inhibition and parent-reported shyness; and create a factor score for non-social BI with behaviours observed in the Unpredictable toy task, experimenter-rated overall object inhibition and parent-reported fear; and then investigate whether social and non-social BI similarly or distinctly predict cross-sectionally measured anxiety and ASD traits; 3) observe parental behaviours during the Stranger Approach and the Unpredictable Toy tasks and assess whether parental behaviours influence both child anxiety and ASD traits.

4.1.1. Social and non-social BI, their association with anxiety and ASD

As mentioned in Chapter 1, it is not clear whether BI is a homogenous construct or social and non-social BI constitute overall BI tendencies. To investigate this, developmental studies have compared emotional and behavioural responses observed in social and non-social contexts in infants and toddlers (Brooker et al., 2016; Kochanska, 1991; Majdandžić & Van Den Boom, 2007). The results of these investigations varied based on measurement methods and the age of the children. The results of several observational studies showed no significant association between social and non-social BI at the age of 2 (Brooker et al., 2016; Majdandžić & Van Den Boom, 2007; Rubin, Hastings, Stewart, Henderson, & Chen, 1997; Scarr & Salapatek, 1970), 3 and 4 (Dyson, Klein, Olino, Dougherty, & Durbin, 2011; Gagne et al., 2011). In contrast,
parent-reported comparisons suggested that social BI was moderately related to non-social BI at the age of 2 (Brooker et al., 2016; Eggum et al., 2009) but that this association become non-significant when children were 3, 4 and 5 years old (Brooker et al., 2016).

There have also been cross-method comparisons of social and non-social BI. Eggum et al. (2009) showed that the association between parent-reported social BI and observed non-social BI was low but significant ($r = .19$) at 18 months. However, this association became nonsignificant ($r = .13$) when children turned to 30 months old. In line with this finding, Brooker et al. (2016) showed that observed social BI was associated with parent-reported non-social BI at the age of 2 but observed non-social BI was not related to parent-reported social BI at age the age of 2, 3, 4 and 5. In contrast, Dyson et al. (2011) showed that observed social and non-social BI were associated with both parent-reported social and non-social BI in 3 and 4 years old. The discrepancy between investigations may be a result of bias that arises from different research methods. For example, Kertes et al. (2009) employed a multi-method investigation to address bias that may arise from different measurement techniques by running a principal component analysis on scores from the observed Stranger Approach (social BI) and the Risk Room (non-social BI) tasks, and parent-reported shyness and fear. The findings revealed two components for both social (with scores of the Stranger Approach and Shyness subscale) and non-social BI (with scores of the Risk Room and Fear subscale) that were moderately related to each other ($r = .28$) in 3 to 4 years old. Thus, some reported associations between parent report investigations may be attributable to measurement effects, and multimethod measurement of BI (e.g., creating a latent variable) may provide a more robust measure of BI.
Social and non-social BI may have distinct developmental trajectories across early developmental stages and could reflect distinct emotional processes early in life. Social BI across the preschool period puts children at increased risk for socially anxious behaviours in interacting with their peers whereas non-social BI was not associated with socially anxious behaviours in daily life (Brooker et al., 2016). Moreover, social and non-social BI were associated with different types of anxiety problems in 3- to 4-year-old children; social BI was related to social anxiety and separation anxiety problems whereas non-social BI was related to specific phobias (Dyson et al., 2011). These studies suggest that social BI relates to social adjustment and socio-emotional problems whereas non-social BI is related to non-social aspects of anxiety.

Exploring how social and non-social BI relates to both anxiety and ASD traits could thus provide additional information about the developmental roots of both conditions. The ASD literature has limited evidence of how BI, especially in the social and non-social contexts, relates to co-occurring anxiety and ASD traits. Scherr et al. (2017) studied a small number of children (N = 12) with ASD who were aged between 2 and 5 years old in an unfamiliar social context (Stranger Approach task). The behavioural coding scheme examined gaze, escape, and facial fear. Children with ASD looked at the unfamiliar person for a shorter time, directed their gaze away from the unfamiliar person for a longer duration, and produced more intense facial fear than their typically developing peers, and these behaviours were not correlated with anxiety (Scherr et al., 2017). However, in a parent-report study, children with ASD who were between 2 and 8 years old did not differ based on parent-report BI in social situations, and BI was not associated with maladaptive externalising behaviours (Adamek et al., 2011). In childhood and adolescence, children with high functioning ASD reported themselves as higher on BI in social and non-social contexts than their peers (Burrows
et al., 2016). Also, parent-reported non-social BI was strongly correlated with ASD symptoms (Kadak, Demirel, Gökalp, Erdoğan, & Demirel, 2015; Shephard et al., 2018). In path analyses, parent-reported non-social BI was associated with the anxiety score whereas there was no significant association between non-social BI and anxiety in children at risk for ASD. In the same cohort, social BI was related to ASD symptoms, but it was not related to anxiety traits at the age of 7 (Shephard et al., 2018). Findings regarding the associations between social and non-social BI, ASD and anxiety traits have been mixed, so further investigations that explore how situational differences in the organisation of BI scores relate to anxiety and ASD are warranted.

As mentioned in previous chapters, ASD and anxiety are highly likely to co-occur and the symptoms of both conditions share similar phenotypes (American Psychiatric Association, 2013). For instance, social communication difficulties are not only experienced by individuals with ASD, but also by individuals with anxiety problems (Halls et al., 2014). In Chapter 2 and 3, I treated BI as a unitary construct but given the evidence, BI appears to have social and non-social dimensions that do not generally link with each other. The social component of BI may lead to social withdrawal, which may explain the overlap with ASD (due to social communication difficulties). Social BI may be associated with ASD and anxiety whereas non-social BI may just be related to anxiety or also relate to ASD due to heightened responses to non-social situations (e.g., insistence on sameness). Contextual differences may be essential and both social and non-social BI may be related to ASD. Investigating this may extend the previous research and help us to better understand the developmental mechanisms that contribute to the overlap between the symptoms of anxiety and ASD.
4.1.2. Parental behaviours

Discussion on the role of parenting in ASD dates back to Kanner (1943). Kanner (1943) hypothesised that “refrigerator mothers,” who were cold and unemotional towards their offspring, may be the cause of autism. However, the ‘refrigerator mother’ hypothesis was soundly refuted and over time research has shifted away from the psychosocial factors that may cause ASD, to more sophisticated approaches that consider hypothesised biological, cognitive and genetic roots of the condition (e.g., Happé & Ronald, 2008; Mandy & Lai, 2016; Tick, Bolton, et al., 2016). It is now well-established that genetic and environmental factors are involved in the aetiology of ASD (Lyall et al., 2017; Matelski & Van de Water, 2016; Meek, Lemery-Chalfant, Jahromi, & Valiente, 2013). Despite this shift, it is important to recognise that children with ASD are part of a family system and parents have a vital role in managing behaviour, the needs of their children and in some cases delivering treatment (e.g., Green et al., 2017; Schrott et al., 2018). The context in which a child develops is an integral factor for their social adjustment, and for influencing the interaction between child temperament and psychopathology (Nigg, 2006). As in the general population, being exposed to a particular type of parenting behaviour may influence the degree to which temperament traits predispose a child with ASD to anxiety traits. Parents who have offspring with an ASD diagnosis experience more parenting stress than parents in the general population (e.g., Keenan, Newman, Gray, & Rinehart, 2016). However, there is less evidence as to how parental behaviours influence both temperamental dispositions and emotional adjustment in children with ASD.

The influence of parental behaviour on child temperament and anxiety symptoms in the general population has been widely explored (e.g., Eggum et al., 2009;
Before summarising findings from previous studies, it is essential to clarify the distinction between two terms: parenting *style* and parental *behaviour*. According to Wood, McLeod, Sigman, Hwang, and Chu (2003), parenting style does not refer to a certain set of acts of parenting, but it is a broader pattern of caregiving which provides contexts for parents to practice parental behaviours whereas parental behaviours are characterised by the specific way of interacting with children in specific contexts. Moreover, parenting style is harder to assess through observations, but parental behaviours can be readily identified using coding schemes.

Parental behaviour in parent-child interactions and novel situations plays a role in the maintenance of child BI from toddlerhood to childhood (Kiel & Buss, 2011), and can moderate the association between BI and anxiety traits (Kiel & Buss, 2012; Rubin et al., 2002). One relevant parenting behaviour is parental overinvolvement/control which is defined as parental regulation of child behaviours by solicitous behaviours, overprotection by emotionally comforting or physically protecting the child, or parental directedness to the child to behave in a particular manner (Hudson & Dodd, 2012; Kiel & Buss, 2011; Wood et al., 2003). Parental protective or intrusive behaviours that arise because of the parent’s sensitivity to their child’s inhibited temperament may exacerbate the child’s BI and anxiety traits over time (Kiel et al., 2016). For example, preschoolers are more likely to have anxiety problems in mid-childhood when they had BI and maternal overprotection in infancy and toddlerhood (Hudson & Dodd, 2012).

Parental behaviours may relate to child BI and anxiety for various reasons. During over-arousing situations, sensitive parents may perceive the emotional state of their child as vulnerable due to heightened fear or withdrawal. To reduce/sooth
heightened negative reactivity of the child, parents may then engage their children in an overly protective and directive manner (Degnan et al., 2015; Rubin et al., 2002; Vreeke, Muris, Mayer, Huijding, & Rapee, 2013). Parental interference or protective behaviours then limit the toddler’s engagement with the anxiety-arousing situations and reduce the child’s opportunity to develop adaptive strategies to cope in these environments (Kiel & Buss, 2010; Rubin et al., 2002). In contrast, the child may cling to parents because they seek comfort due to lack of coping or regulatory strategies in the novel threatening situations; this may evoke distress or anxious behaviours in parents (Hastings et al., 2011; Hudson & Dodd, 2012; Rapee, 2014). Nevertheless, the potential links between parental protective behaviours and anxiety in children at HR of ASD, have not been fully explored.

4.1.3. The present study

The present study sought to extend investigations in previous chapters by 1) examining contextual differences in BI scores (social vs non-social) in 24-month-old toddlers at high and low familial risk for ASD; 2) assessing associations between social and non-social BI and their relation with anxiety and ASD traits; 3) incorporating parental over-protective behaviours to assess their association with the child’s anxiety and ASD traits.

Given the evidence from the literature regarding children in the general population, the first aim of the current study was to measure BI traditionally via observational novelty tasks and to explore the factor structure of observed reactions. This exploration would enable me to identify whether BI is an underlying latent construct upon which fear, shyness and withdrawal load.
Research has shown that BI in social and non-social contexts relates to social functioning and anxiety problems in the general population (Brooker et al., 2016; Dyson et al., 2011). Toddlers may organise their responses in social and non-social context distinctly, and BI tendencies that are observed in these contexts may have different correlates. The second aim was thus to explore whether BI observed in social and non-social contexts related to anxiety and ASD traits similarly or followed different pathways among 2-year-old infants. For this exploration, social and a non-social BI factor scores were generated using observed, experimenter-rated and parent-reported temperament. This will allow the current investigation to overcome the potential biases that could arise from shared measurement variance and yield ecologically valid BI scores.

Lastly, parental behaviours have been related to child BI and anxiety problems in the general population. Parents who were sensitive to their child’s anxious behaviours were more likely to present overprotective behaviours. However, it is poorly understood whether parental overprotective behaviours constitute a risk factor for co-occurring anxiety problems in children at familial risk of ASD. Thus, the final aim of the current chapter is to observe parental overprotective behaviours during novelty tasks to assess whether the temperamental tendency of the toddlers influences overprotective parental behaviours and whether these behaviours also affect the anxiety traits among two-year-old toddlers who are at familial risk of ASD (Figure 4.1, Model 4.1).

4.2. Method

4.2.1. Participants

One hundred and fifty-four infants who were born between December 2012 and July 2016 enrolled in the third phase of the British Autism Study of Infant Siblings
(BASIS; www.basisnetwork.org) and the Studying Autism and ADHD Risks (STAARS; www.staars.org) project when they were 5- or 10-months-old, or younger. Among these infants, 36 had an older sibling or parent with an ADHD diagnosis. Due to the main scope of the current thesis of exploring roots of co-occurring anxiety traits in children who are at high- and low-risk of familial risk of ASD, toddlers with only a family risk for ADHD were excluded from further analyses.

Of the remaining one hundred and eighteen toddlers, 27 were low-risk controls and 91 were at high-risk for ASD. All infants were invited to participate in the BASIS projects when they were 5, 10, 14 and 24 months. For the current chapter, data were included from the 24-month time point. In addition to the participants of the BASIS-STAARS study, in order to increase the number of LR children at the 24 months visit, based on general recruitment strategy of the BASIS-STAARS project (see below for details), we recruited an additional eight LR participants.

Of this group, 57 HR (27 female, 30 male) and 28 LR (14 female, 14 male) toddlers completed the Stranger Approach and Unpredictable Toy tasks. Data from the Stranger Approach and Unpredictable Toy tasks were missing for 15 toddlers (1 LR, 14 HR). For 9 children (2 LR, 5 HR) there were problems with the video recordings; 2 HR children did not participate within the data coding window; and for the remaining children, the tasks were not available as they had not been piloted and incorporated into the main testing protocol when the visit occurred.

Ethics approval for the BASIS STAARS project was obtained from the National Health Service National Research Ethics Service (NHS NRES London REC 13/LO/0751) and to test the additional LR participants, ethical approval was obtained from the Psychiatry, Nursing and Midwifery Research Ethics Subcommittee (PNM)
Research Ethics Subcommittee (HR-16/17-3509). Parents provided informed consent for their children to participate in the current study and they were informed that they have the right to withdraw from the study at any time.

4.2.2. Measures

4.2.2.1. Observed BI

Toddler BI was measured via the semi-structured social Stranger Approach and non-social Unpredictable Toy tasks (Calkins et al., 1996; Fox et al., 2001; Kagan et al., 1987). After completing an eye-tracking battery and other behavioural tasks, the researchers informed parents, who were present during the procedures (only 2 fathers did the testing with the child), about the procedures for both tasks. Parents were instructed not to prompt the child, not to take the child onto their lap and to respond to the child as they would typically do when the child interacts with them. After the briefing, the examiner asked the child to stand in the middle of the room and said, “I am going to find more toys, can you wait for me here?” and left the room. After this, a female stranger knocked on the door, entered the room and says “hi” with a neutral tone and facial expression and from this point, the Stranger Ignore episode started. During this episode, the stranger sat quietly and pretended to be busy with reading, meanwhile did not interact with the child and refrained making eye contact with the child. After a minute, if the child had not approached the stranger, the Stranger Play episode started. During this episode, the stranger started to stack cups and played with the cups for a minute without any interaction or eye contact with the child. If the child had not approached or initiated any interaction with the experimenter, the Invitation to Play episode started. In this episode, the stranger invited the child to play with her saying, “Would you knock down my tower?” if the child did not approach the stranger, she
knocked the cups down and stacked the cups again and gave the child opportunity to interact by saying, “Could you help me?” (the duration of this episode is also a minute).

After the third minute, the stranger takes the stacking cups away, returns with a battery operated fluffy electronic robot that has big eyes, makes unpredictable movements and sounds and the Unpredictable Toy task starts. This toy is approximately 26 cm in height. The stranger places the toy approximately one meter away from the child and leaves it activated for 30 seconds. The stranger stands back from the stimuli and engages with the child by asking, “Have you seen my funny toy?” and, “Would you like to pet it?” If the child has not touched the toy within the first 30 seconds, the stranger brings the toy approximately 30 centimetres closer to the child and tries to get the child to touch it.

Since these two episodes were potentially anxiety-arousing in nature, to calm the child down to be able to continue the other tasks in the testing battery, a friendly toy play session was inserted after the Unpredictable Toy episode. A colourful squashy ball was chosen to play with the child. This session was not incorporated into the coding scheme. Each episode was videotaped and coded later. The sequence of these tasks was constant for each participant. The Unpredictable Toy task was always administered after the Stranger Approach task, so as not to influence the novel context.

4.2.2. Coding scheme

Emotional and behavioural responses characterised by BI were coded later using Mangold Interact (Mangold, (2017); www.mangold-international.com). Behaviours in each episode of the Stranger Approach and Unpredictable Toy tasks were coded based on a continuous coding system for the duration of each emotional or behavioural
reaction. In order to elicit the total amount of child reactivity, the duration, rather than the intensity, of the identified behaviours were the primary focus.

The AFFEX system was used to code *Facial expressions* of fear (Izard et al., 1983). The AFFEX coding enables the researcher to determine distinct facial expressions based on three facial regions: eyes, brows and mouth area. Facial fear was defined by brows being straight or normal, slightly raised or drawn together, eyelids raised and tense, mouth open, corners straight back. *Bodily expression of fear* was also coded. Fearfulness expressed through bodily reaction was classified by a marked and sudden decrease in activity level, freezing (no physical movement for more than 2 seconds) or muscle tensing on the limbs, especially the legs, arms and shoulders (e.g., firmly extended legs or arm and raising the shoulders towards the ears). *Latency to approach* was characterised by the time that infants’ take to initiate an interaction with the experimenter or the presented toy. *Negative vocalisations* include any intensity of cry, scream, whining or whimpering. *Hypervigilance* was characterised by a state of being wary, e.g., scanning the room anxiously. Two gaze behaviours were integrated into this coding scheme: *gaze to parent* and *gaze away* from the presented stimuli, experimenter or the parent. The duration of time that the child spent within one arm’s reach of their parent was defined as *proximity to parent*; the duration of the child’s interaction with the experimenter or presented toy was *duration of the interaction* — physical avoidance such as moving away from the stranger or the toy was coded as *escape* and attempt to *climb onto the parent’s lap* was also coded.

### 4.2.2.3. Global rating of social and non-social BI

Researchers who took part on the testing day rated toddlers’ *Social Inhibition* (global rating of social BI) and *Inhibition to Object/Environment* (global rating of non-
Social BI was measured using a 7-point Likert scale based on their overall observations during the testing day.

A score of 1 indicates higher BI, 4 indicates the optimal level of BI and 7 reflects over-familiarity towards the experimenters. Social Inhibition was characterised by a child’s initial shyness towards the experimenters at the beginning of the testing day, the duration of shyness throughout the testing day and the child’s reactions in the Stranger Approach task were also considered. Inhibition to Object/Environment was evaluated based on the child’s fearful responses to toys or the willingness of the child to enter the testing room for some children. The child’s reaction to the Unpredictable Toy task was also considered in the global ratings of BI. The scores were reversed for ease of interpretation of the results and higher scores indicated higher levels of inhibition. Although we did not measure the reliability of these codes, to increase the robustness of the measurement, we discussed possible behavioural or emotional reactions and the scores reflect the consensus between the experimenters who were present on the day.

4.2.2.4. Parent-reported BI

Social BI was measured using the Shyness subscale and non-social BI was assessed using the Fear subscale of the Early Child Behaviour Questionnaire short-form version (ECBQ; Putnam et al., 2006). The ECBQ is a standardised parent-report temperament measure that allows caregivers to rate the frequency of behaviours of toddlers during the previous two weeks. The Fear subscale refers to negative affect, including unease, worry, or nervousness related to anticipated pain or distress and/or potentially threatening situations (new place) or objects (loud vehicles, elevator or escalator). The Shyness subscale refers to discomfort, slow or inhibited approach to unfamiliar people or peers in social encounters. The Cronbach’s alpha for the Fear and
Shyness scales in the current sample was .80 and .82, indicating good internal consistency.

4.2.2.5. Parental Behaviours

Parental behaviours were coded during the Stranger Approach and the Unpredictable Toy tasks. The Duration of parental behaviours that could be characterised as interfering with the child’s behaviour was coded. These included emotionally loaded statements of parents directed to the child, such as soothing (e.g., holding child's hand, patting the child’s back), commanding, generally disruptive behaviours, comments of parents.

4.2.2.6. Parent Report anxiety and ASD traits at 24 months visit

Anxiety traits of toddlers were assessed using the Child Behaviour Checklist 1.5-5 DSM-Oriented Anxiety Problem subscale raw scores (CBCL; Achenbach & Rescorla, 2000). The CBCL was described in section 2.2.2. The Cronbach’s alpha for the current sample was .75, indicating good internal consistency.

ASD traits in toddlers were measured using the total score derived from the Quantitative Checklist for Autism in Toddlers (Q-CHAT; Allison et al., 2008). The Q-Chat is a parent-report questionnaire that consists of 25 items scored on a 5-point Likert scale of frequency ranging from 0 to 4. This measure was designed to assess ASD traits in infants and toddlers aged between 18 and 24 months. A total score on the Q-Chat can be generated by summing all items and scores vary within the range of 0-100. Higher total scores reflect a higher frequency of ASD traits. The Cronbach’s alpha of the Q-chat was .81 in the current study, indicating excellent reliability.
4.2.3. Data analysis

4.2.3.1. Psychometric properties of BI

4.2.3.1.1. Inter-rater agreement

Initially, intraclass correlation coefficients (ICC) for 20 randomly chosen videos were calculated using a two-way random effect model (appropriate for randomly chosen sample for ICC) with absolute agreement type to investigate the agreement between two coders. Since the focus was on eliciting social and non-social BI, ICC for the social Stranger Approach, non-social Unpredictable Toy and total durations from both tasks were run separately in SPSS 25. Based on the approach suggested by Cicchetti (1994), ICC scores between 1 and .75 were accepted as excellent, .74 and .60 were good and scores less than .60 were regarded as poor agreement. Following ICC, Pearson correlation coefficients were calculated for the total behaviours that were observed in the social and the non-social tasks to investigate the pattern of association between variables prior to data reduction. Multicollinearity (r > .80) and lower levels of variability (r < .10) between observed behaviours were assessed by Pearson correlation coefficients.

4.2.3.1.2. Data reduction

Principal Component Analysis (PCA) with Varimax Rotation was run using the total duration of observed behaviours in social and non-social tasks to understand the underlying structure of BI in the current sample. The number of components was determined with Eigenvalues (≥1) and scree plots. Variables loaded on components with a magnitude of .60 and above were accepted as adequate loadings. Component scores were created based on summing up behaviours that meaningfully loaded. Internal consistency of each component was calculated and components that had poor internal
consistency (Cronbach’s Alphas < .40) were excluded from further analyses to increase the reliability of the findings. Based on the result of the PCA, standardised scores were aggregated to elicit fear, withdrawal and strategies sores for the Stranger Approach and Unpredictable Toy tasks.

4.2.3.2. Path models with social and non-social BI factors

4.2.3.2.1. CFA for the social and non-social BI

Prior to modelling, the normality of the variables was checked. The scores for parental interference, child anxiety strategies and fear components were positively skewed, and transformation did not work on these variables. Both social and non-social withdrawal components had bimodal distributions and the cubic transformation was applied and distribution was improved. Since the data had ordinal variables (experimenter-rated inhibition), Spearman correlation coefficients were calculated to assess the association between variables used in the modelling. The CFA was run without controlling the risk group status since the group effect was not hypothesised for the CFA.

In structural equation modelling, one of the solutions to handle non-normal data distribution is to use Maximum Likelihood Robust (MLR) estimator (Satorra & Bentler, 1994). MLR calculates less biased standard errors, chi-square and works with different sample sizes. So, in the current analyses, MLR estimator was used to address non-normality and ordinal data in the CFA (global ratings of social and object inhibitions).

The following analyses were conducted with a Structural Equation Modelling (SEM) framework using Mplus 7.4 (Muthén & Muthén, 1998-2015). Initially, two confirmatory factor analyses (CFA) were conducted. CFA involved loadings from indicators measured via three different methods (observed, experimenters’ global
ratings and parent-report measures) onto a single factor. Collapsing these scores across different measurements provides a weighted average factor score which accounts for bias that can be raised from different measurement methods. Moreover, these factor scores provide ecologically valid scores because they could capture the state as well as the trait level BI.

To construct the social BI factor scores, a CFA was run with withdrawal, fear, strategies components observed in the social Stranger Approach task, the experimenters’ global rating of social inhibition and parent-reported shyness; and to construct the non-social BI factor scores, a CFA was run with withdrawal, fear, strategies components observed in the Unpredictable Toy task, the experimenters’ global rating of object inhibition and parent-reported fear.

4.2.3.2.2. Model 4.1: Associations between BI, parental behaviours, anxiety and ASD

After the CFAs, a path analysis was conducted to assess the associations between social and non-social BI factors, parental behaviours, child anxiety and ASD scores. In this model, BI scores were correlated predictors. The Social BI score was regressed on the parental over-protective behaviours that were observed in the social Stranger Approach task and the non-social score was regressed on the parental over-protective behaviours that were observed in the Unpredictable Toy task to assess whether child BI associated with overprotective parental behaviours. In addition, to assess the effects of BI factor scores and parental behaviours on child anxiety and ASD traits, these variables were regressed on anxiety and ASD scores which were treated as correlated outcomes. Figure 4.1 shows the proposed model.
4.2.3.2.3. Model 4.2: Associations between BI, parental behaviours, anxiety after controlling for ASD traits

As anxiety and ASD commonly co-occur, to understand whether the associations between predictors and anxiety were significant due to co-existing ASD traits, ASD traits were covaried in Model 4.2 (Figure 4.2). The effect of ASD traits on anxiety was controlled and ASD score was regressed on BI scores, parental behaviours and anxiety traits.
4.2.3.2.4. Model 4.3: Associations between BI, parental behaviours, ASD controlling for anxiety traits

In Model 4.3, anxiety traits were treated as a covariate and regressed on BI factors, parental behaviours and ASD traits.

Figure 4.2 Model 4.2 proposed model for the association between BI scores, anxiety after controlling for ASD traits.

Figure 4.3 Model 4.3 proposed model for the association between BI scores, ASD after controlling for anxiety traits.
All the models were estimated using the combined LR and HR sample ($N = 85$). The risk group variable (LR = 0; HR = 1) was covaried in the models to control for group differences and regressed on all predictor and outcome variables. Full Information Maximum Likelihood (FIML) was used to account for missing data. The criteria for the model fit indices that summarised in section 2.2.3 was used to assess the model fit.

After all these analyses, post hoc power analyses were calculated to detect the strength of generating significant pathways. The process of the post hoc power analysis was summarised in section 2.2.5.

4.3. Results

4.3.1. Psychometric properties of BI

4.3.1.1. IRR between coders

Table 4.1 shows the ICC values for the Stranger Approach, Unpredictable Toy task and the total duration of behaviours from these two tasks. The results for the totals suggested excellent inter-coder agreement for proximity to parent, latency to touch, duration of interaction, looking away, looking at parent, facial fear, bodily fear, hypervigilance, negative vocalisation, escape, climbing on parent’s lap and parental interference. However, overall Facial Sadness did not reach the average agreement limit and was discarded from further analysis. In the Stranger Approach task, looking at parent, bodily fear, escape and negative vocalisation showed good agreement whereas the rest of the behaviours had an excellent agreement. In the Unpredictable Toy Task, only Escape behaviours showed good agreement and the rest of the classes showed excellent agreement (Table 4.1).
<table>
<thead>
<tr>
<th>Observed Behaviours</th>
<th>Total Duration of Behaviour (SA+UT)</th>
<th>Total Duration of Behaviour in SA</th>
<th>Total Duration of Behaviour in UT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICC</td>
<td>CI, 95%</td>
<td>ICC</td>
</tr>
<tr>
<td>Proximity</td>
<td>.93</td>
<td>.85, .97</td>
<td>.93</td>
</tr>
<tr>
<td>Latency to I.</td>
<td>.72</td>
<td>.44, .88</td>
<td>.80</td>
</tr>
<tr>
<td>Duration of I.</td>
<td>.97</td>
<td>.93, .99</td>
<td>.99</td>
</tr>
<tr>
<td>Looking Away</td>
<td>.81</td>
<td>.59, .92</td>
<td>.86</td>
</tr>
<tr>
<td>Looking Parent</td>
<td>.84</td>
<td>.64, .93</td>
<td>.72</td>
</tr>
<tr>
<td>Facial Fear</td>
<td>.89</td>
<td>.76, .95</td>
<td>.96</td>
</tr>
<tr>
<td>Bodily Fear</td>
<td>.80</td>
<td>.58, .92</td>
<td>.74</td>
</tr>
<tr>
<td>Facial Sadness</td>
<td>.51</td>
<td>.10, .77</td>
<td>.23</td>
</tr>
<tr>
<td>Hypervigilance</td>
<td>.84</td>
<td>.65, .93</td>
<td>.79</td>
</tr>
<tr>
<td>Escape</td>
<td>.77</td>
<td>.54, .90</td>
<td>.72</td>
</tr>
<tr>
<td>Negative V.</td>
<td>.88</td>
<td>.73, .95</td>
<td>.72</td>
</tr>
<tr>
<td>Climbing</td>
<td>.87</td>
<td>.70, .94</td>
<td>.95</td>
</tr>
<tr>
<td>Parental Int.</td>
<td>.84</td>
<td>.60, .94</td>
<td>.96</td>
</tr>
</tbody>
</table>

SA= Stranger Approach, UT= Unpredictable Toy, I = interaction, V= Vocalisation, Climbing= Climbing on Parents Lap, Int= Interference
Table 4.2 Pearson Correlation Coefficients for Observed Behaviours

<table>
<thead>
<tr>
<th></th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
<th>8.</th>
<th>9.</th>
<th>10.</th>
<th>11.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Proximity to Parent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Latency to Touch</td>
<td></td>
<td>.770*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Duration of Interaction</td>
<td>-.674*</td>
<td>-.774*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Looking Away</td>
<td>-.011</td>
<td>.149</td>
<td>-.175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Looking Parent</td>
<td>.205</td>
<td>.234</td>
<td>-.151</td>
<td>-.131</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Facial Fear</td>
<td>-.020</td>
<td>.206</td>
<td>-.166</td>
<td>-.029</td>
<td>-.089</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Bodily Fear</td>
<td>.362</td>
<td>.410*</td>
<td>-.327</td>
<td>-.024</td>
<td>.092</td>
<td>.458*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Hypervigilance</td>
<td>.332</td>
<td>.460*</td>
<td>-.365</td>
<td>.080</td>
<td>.020</td>
<td>.416*</td>
<td>.615*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Negative Vocalisation</td>
<td>.217</td>
<td>.28</td>
<td>-.101</td>
<td>.050</td>
<td>.404*</td>
<td>.031</td>
<td>.042</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Moves Away</td>
<td>.117</td>
<td>.092</td>
<td>-.093</td>
<td>.067</td>
<td>-.160</td>
<td>.060</td>
<td>-.141</td>
<td>.101</td>
<td>-.028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Climbing on Parents Lap</td>
<td>.098</td>
<td>.320</td>
<td>-.161</td>
<td>.117</td>
<td>.344</td>
<td>-.056</td>
<td>.203</td>
<td>.182</td>
<td>.323</td>
<td>-.089</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>108.07</td>
<td>155.48</td>
<td>36.24</td>
<td>37.42</td>
<td>12.63</td>
<td>2.81</td>
<td>32.78</td>
<td>32.13</td>
<td>8.11</td>
<td>8.33</td>
<td>5.11</td>
</tr>
<tr>
<td>SD</td>
<td>76.41</td>
<td>60.51</td>
<td>29.35</td>
<td>22.66</td>
<td>14.28</td>
<td>10.56</td>
<td>49.31</td>
<td>52.23</td>
<td>15.79</td>
<td>11.80</td>
<td>12.73</td>
</tr>
</tbody>
</table>

* p < .001 level
4.3.1.2. Data reduction

Table 4.2 shows the interrelations between different coded variables. The results suggested enough variation between variables for PCA and the pattern of association indicated no multicollinearity.

The total duration of behaviours that were observed across tasks was then submitted to a PCA with orthogonal rotation (varimax). Kaiser’s criterion (eigenvalues 1 and over) and the scree plot demonstrated four points that would reflect four components (one inflexion point). These four components (which explained the total 69.26% of variance) were then retained for further analyses. Table 4.3 indicates the factor loadings after varimax rotation. The items that clustered on the first component represented withdrawal (proximity to parent, latency to approach, duration of interaction (negatively loaded) and explained 31.24% of the variance; the second component was fear (facial fear, bodily fear and hypervigilance) and accounted for 15.72% of the variance; the third component represented behavioural strategies (looking at parent, negative vocalisation, climbing on parent lap) and explained the 12.54% of the variance; and lastly, the fourth component included only looking away, which accounted for 9.76% of the variance, however, due to only a single item loading, this component was dropped from further analysis. After this step, the internal consistencies of each component were calculated. Cronbach’s Alpha of withdrawal ($\alpha = .93$), fear ($\alpha = .66$) and behavioural strategies ($\alpha = .62$) showed good internal consistency (Table 4.3).
Table 4.3 The Results of the PCA Analysis

<table>
<thead>
<tr>
<th>Behaviours</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Proximity to Parent</td>
<td>.909</td>
</tr>
<tr>
<td>Latency to Touch</td>
<td>.842</td>
</tr>
<tr>
<td>Duration of Interaction</td>
<td>-.841</td>
</tr>
<tr>
<td>Bodily Fear</td>
<td>.244</td>
</tr>
<tr>
<td>Facial Fear</td>
<td>-.026</td>
</tr>
<tr>
<td>Hypervigilance</td>
<td>.319</td>
</tr>
<tr>
<td>Looking Parent</td>
<td>.189</td>
</tr>
<tr>
<td>Climbing on Parents Lap</td>
<td>.049</td>
</tr>
<tr>
<td>Negative Vocalisation</td>
<td>.173</td>
</tr>
<tr>
<td>Moves Away</td>
<td>.316</td>
</tr>
<tr>
<td>Looking Away</td>
<td>.069</td>
</tr>
</tbody>
</table>

**Eigenvalues**          3.44 | 1.73 | 1.38 | 1.07
**% of Variances Explained** 31.24 | 15.72 | 12.54 | 9.76
**Cronbach’s Alpha**       .93  | .66  | .62  | -

**Bold**s indicate component loadings

4.3.2. Associations between BI components, parental behaviours, anxiety and ASD

Spearman correlation coefficients were calculated to assess the pattern of association between variables that were submitted to the CFA, as well as the associations between parental behaviours, ASD, anxiety and the MSEL scores. Table 4.4 displays the associations between the variables that were used in SEM modelling. The results showed some correlation between subcomponents: the social and non-social scores derived from the observational tasks, experimenters’ rating of inhibition and parent-reported BI. So, results showed a basis to run CFAs.
Table 4.4 Spearman Correlation Coefficients Between CFA Indicators and Outcome Variables

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
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<td>4.Strategies_SA</td>
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<tr>
<td>5.Social Inhibition_GR</td>
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<td>.315*</td>
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<td>-.262*</td>
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<td></td>
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<td>.031</td>
<td>.613*</td>
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<td>.316*</td>
<td>-.041</td>
<td>.153</td>
<td>.427*</td>
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<td>10.Object Inhibition_GR</td>
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<td>.317*</td>
<td>.098</td>
<td>-.185</td>
<td>.595*</td>
<td>.111</td>
<td>.418*</td>
<td>.274*</td>
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<td>.100</td>
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<td>12.Parental Behaviour_UT</td>
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<td>.258*</td>
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<td>-.095</td>
<td>-.260*</td>
<td>.112</td>
<td>.301*</td>
<td>.030</td>
<td>-.057</td>
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<td>-.033</td>
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<td>-.134</td>
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<td>.048</td>
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<td>-.147</td>
<td>.066</td>
<td>-.454*</td>
<td>-.471*</td>
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</tr>
</tbody>
</table>

* $p < .001$. Blue text highlights correlations between variables representing social BI. Orange text indicates variables representing non-social BI. Grey text indicates correlations between parental behaviours. Green texts highlight correlations between anxiety, ASD and MSEL.

PR= Parent Report, SA= Stranger Approach, GR= Experimenters’ Global Rating, UT= Unpredictable Toy, MSEL= Mullen Scale of Early Learning (Composite Scores).
4.3.3. CFA for social and non-social BI

The fit indices of the CFA for social BI indicated an acceptable model fit for the data ($\chi^2 (6) = 7.30, p = .294; \text{CFI} = .936, \text{RMSEA} = .051, \text{and SRMR} = .083$). For this factor, standardised factor loadings of parent-reported shyness, observed withdrawal (Stranger Approach), and experimenter ratings of social inhibition were significant, but the observed fear and behavioural strategies components did not load on this factor significantly (Table 4.5).

For non-social BI, the fit indices also suggested an acceptable model fit ($\chi^2 (6) = 7.16, p = .294; \text{CFI} = .968, \text{RMSEA} = .048, \text{and SRMR} = .095$). For this factor, except for parent-reported fear, the standardised factor loadings of observed withdrawal, fear, behavioural strategies components of the Unpredictable Toy task and experimenters’ global rating of object inhibition were significantly loaded on this factor structure. Table 4.5 summarises the items that significantly loaded on the social and non-social BI factors.

<table>
<thead>
<tr>
<th>Social BI</th>
<th>$\beta$</th>
<th>SE</th>
<th>P</th>
<th>Non-Social BI</th>
<th>$\beta$</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
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<td>.06</td>
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<td>Fear_PR</td>
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<td>.17</td>
<td>.374</td>
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<td>Fear_SA</td>
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<td>.12</td>
<td>.116</td>
<td>Fear_UT</td>
<td>.87</td>
<td>.10</td>
<td>&lt; .001</td>
</tr>
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<td>.15</td>
<td>.002</td>
<td>Withdrawal_UT</td>
<td>.64</td>
<td>.13</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Strategies_SA</td>
<td>-.13</td>
<td>.12</td>
<td>.278</td>
<td>Strategies_UT</td>
<td>.30</td>
<td>.11</td>
<td>.006</td>
</tr>
<tr>
<td>Social Inh_GR</td>
<td>.62</td>
<td>.16</td>
<td>&lt; .001</td>
<td>Object Inh_GR</td>
<td>.43</td>
<td>.14</td>
<td>.002</td>
</tr>
</tbody>
</table>

PR= Parent Report; SA= Stranger Approach; UT= Unpredictable Toy; GR= Experimenters’ Global Rating
4.3.4. Association between social and non-social BI anxiety and ASD traits

4.3.4.1. Model 4.1: Associations between BI, parental behaviours, anxiety and ASD

The path analysis provided a good fit to the data ($\chi^2(3) = 3.52$, $p = .318$; CFI = .995, RMSEA = .045, and SRMR = .034). As a covariate, group variable (HR vs LR) significantly related to anxiety ($\beta = .22$, $p = .002$) and ASD ($\beta = .24$, $p = .002$) scores, indicating higher anxiety and ASD traits in HR toddlers. Group was not associated with CFA-social ($\beta = .10$, $p = .223$), CFA-non-social BI scores ($\beta = .15$, $p = .059$), parental behaviour in the social ($\beta = .01$, $p = .948$) and non-social contexts ($\beta = .02$, $p = .827$). These results indicated no difference in the CFA-social and CFA-non-social BI in the both HR and LR toddlers.

Higher CFA-social BI was associated with higher anxiety and ASD scores ($\beta = .42$, $p < .001$; $\beta = .40$, $p < .001$; respectively) whereas higher CFA-non-social BI was related to lower levels of ASD ($\beta = -.30$, $p = .008$), but not anxiety ($\beta = -.18$, $p = .077$) traits. CFA-social and CFA-non-social behaviours were significantly related to each other ($\beta = .46$, $p < .001$).

CFA-social and CFA-non-social BI were not related to parental over-protective behaviours observed in the social ($\beta = .07$, $p = .512$) and non-social contexts ($\beta = .13$, $p = .091$), but the parental overprotective behaviours that were observed in the social context were related to higher child anxiety traits ($\beta = .29$, $p = .001$). This association was specific to anxiety and was not related to ASD traits ($\beta = .05$, $p = .559$). Also, higher anxiety traits were related to higher ASD traits ($\beta = .48$, $p < .001$; Figure 4.1). So, parental behaviours that were risen due to their reactions in the social context may influence the child’s co-occurring anxiety traits.
The post hoc power calculation showed a 97% of chance to detect the association between social BI and anxiety, a 94% chance of detecting the association between social BI and ASD, a 78% chance of generating the association between non-social BI and ASD, an 82% chance of detecting the association between parental behaviours and anxiety is 82% indicating higher power for detecting the significant associations.

**4.3.4.2. Model 4.2: Controlling for ASD traits**

In Model 4.2, ASD traits were included as a predictor of anxiety symptoms, rather than a correlated outcome, to assess whether controlling for ASD would affect associations between CFA-social BI, CFA- non-social BI, parental behaviours and anxiety traits. The model fit indices showed acceptable fit data ($\chi^2 (4) = 8.52, p = .074$; CFI = .953, RMSEA = .115, and SRMR = .053). The covariate group was not
significantly related with the anxiety after controlling for the ASD traits ($\beta = .14, p = .106$). Group was not associated with CFA-social ($\beta = .03, p = .748$), parental behaviour in the social ($\beta = -.01, p = .904$) and non-social contexts ($\beta = .04, p = .682$). However, group was significantly related to CFA-non-social BI scores ($\beta = .18, p = .036$), indicating higher non-social BI in the HR group.

The association between CFA-social BI and anxiety ($\beta = .24, p = .002$); parental overprotective behaviours and anxiety ($\beta = .27, p < .001$) remained significant (Figure 4.2). CFA-social and CFA-non-social scores were significantly related to each other ($\beta = .52, p < .001$).

![Diagram](image)

Figure 4.5 Model 4.2 for the association between BI scores, anxiety after controlling for ASD traits. Blue lines indicate the effect of covariate ASD traits, while black lines represent associations between predictors and outcome. Full lines indicate significant results. Dotted lines indicate non-significant results. Standardised beta and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$
The post hoc power calculation for this model showed a 97% of chance of detecting the association between ASD and anxiety, a 54% chance of generating the association between social BI and anxiety and an 84% chance of detecting the association between parental behaviours and anxiety.

4.3.4.3. Model 4.3: Controlling for anxiety traits

In Model 4.3, ASD and anxiety traits were reversed, and anxiety was treated as a predictor to control its effect on the ASD scores. This model fit the data better than the model 4.2 and indicated a good fit ($\chi^2 (4) = 6.17$, $p = .187$; CFI = .977, RMSEA = .080, and SRMR = .055). As a covariate, risk group was not significantly associated with ASD traits ($\beta = .13$, $p = .114$), CFA-social BI ($\beta = .01$, $p = .914$), CFA-non-social BI ($\beta = .16$, $p = .062$), parental behaviours in the social ($\beta = -.06$, $p = .549$) and non-social context ($\beta = .05$, $p = .640$).

Once controlling for anxiety, the association between CFA-social BI and ASD became non-significant ($\beta = .19$, $p = .057$) but the association was marginal. The association between CFA-non-social BI and ASD remained significant, indicating (contrary to expectations) that higher CFA-non-social BI was associated with lower levels of ASD traits ($\beta = -.21$, $p = .016$; Figure 4.3). Parental behaviours in the social and non-social contexts were not associated with child’s ASD traits ($\beta = -.10$, $p = .230$; $\beta = .01$, $p = .998$; respectively).

The post hoc power calculation for this model showed a 99% of chance to obtain the association between anxiety and ASD, a 38% power of detecting the pathways between non-social BI and ASD, a 40% power for generating the association between anxiety and parental behaviours.
Figure 4.6 Model 4.3 for the association between BI scores, ASD after controlling for anxiety traits. Blue lines indicate the effect of covariate anxiety traits, while black lines represent associations between predictors and outcome. Full lines indicate significant results. Dotted lines indicate non-significant results. Standardised beta and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$

The IQ level was controlled in the model and summarised in Appendix 2.

4.4. Discussion

The aim of this chapter was to examine the associations between BI measured in the social and the non-social contexts, parental protective behaviours, anxiety and child ASD traits within the familial HR for ASD sibling design. This investigation expanded previous empirical explorations in Chapter 2 and 3 by 1) considering contextual influences on child responses to novelty and differences in social and non-social BI scores, 2) generating social and non-social BI factor scores with observed, experimenter-rated and parent-reported measures to increase the generalisability of the BI measurement, 3) considering parental over-protective behaviours as an
environmental risk factor for child anxiety. The results suggested that pronounced temperamental reactivity (BI) in social (but not non-social) situations was associated with both anxiety and ASD traits. In contrast, higher levels of BI in non-social contexts were associated with lower levels of ASD traits, contrary to the predictions. The results also suggested that parental overprotective behaviours were related to child anxiety scores.

4.4.1. Factor structure of observed Stranger Approach and Unpredictable Toy tasks

In the general population, the terms BI, fear, shyness and withdrawal were used interchangeably for addressing reactions to novelty or unfamiliarity (e.g., Brooker et al., 2016; Buss, 2011; Eggum et al., 2009). The findings of the PCA suggested that BI is a broader construct that encompasses fearful responses, avoidance and other behavioural strategies. Eggum et al. (2009) argued that fear is a broader term than shyness/withdrawal due to it is generally observed in non-social situations because responses to novelty are organised by fear, withdrawal and other behavioural strategies. Similarly, Buss et al. (2013) argued that, regardless of the context of measurement, fear is a broader term that is seen as a response to novelty. The results of the current investigation contradict with these arguments because fear and withdrawal constituted two distinct constructs. So, when referring to the subcomponents of BI, there needs to be more precision in the definition of BI to prevent ambiguity.

Moreover, Dyson et al. (2011) argued that BI is a multifaceted construct that can be organised in the social and non-social contexts; further, the results showed that the observed social and non-social BI were not related constructs. The findings of the current study are not in line with this finding; rather, results of the CFA analysis
indicated that social BI and non-social BI factors (generated using parent-report, observational and experimenter-rated BI) were correlated. This may be due to differences in generating BI scores between studies. Firstly, Dyson et al. (2011) used only observational scores which could reflect state-level reactivity whereas in the current study, I generated factor scores that relied on different measurement methods (parent-report, observed, experimenters’ ratings). This approach increases the likelihood of capturing the child’s temperament traits from various sources and observations which in turn enrich the data. So, the scores may be related because they can reflect trait level BI. Secondly, while generating CFA scores, the correlation between same measurement methods (e.g., correlations between parent-reported fear and shyness; see Table 4.4) could raise the shared variances between two variables. Lastly, Buss (2011) demonstrated that the Stranger Approach and the Unpredictable Toy tasks were high-threat situations, and both provoke heightened reactions to novelty while the Risk Room and the Object Exploration tasks were low-threat situations. Low-threat situations may not be adequate to provoke children’s reactivity; owing to the different level threat in their investigations, high-reactive children in high-threat situations may not be necessarily high-reactive in low-threat level situations since they need more stimulation and have a higher threshold for reactivity. These results may highlight the importance of measuring temperament using multi-method measurement techniques since it increases the reliability of measuring desired traits.

4.4.2. Social BI and non-social BI, anxiety and ASD

Further, I explored whether social and non-social BI have similar associations with anxiety and ASD traits. The findings showed that a factor representing social BI was associated with both anxiety and ASD traits at 24 months (Model 4.1). This finding
is in line with the results of Chapter 2 Model 2.3 where the results suggested associations between BI and both anxiety and ASD traits. The social component of BI is related to ASD, perhaps due to similar characteristics of both traits. For example, social inhibition due to lack of social motivation may relate to ASD symptoms and could prevent toddlers from voluntarily initiating interactions with peers or adults. Social motivation refers to the psychological or biological mechanisms that influence individuals to voluntarily orient to the social world, taking pleasure in social interactions or maintain the social bond (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012). Withdrawal due to temperament and withdrawal due to lack of social motivation related to ASD traits may result in shared variances or due to overlapping symptoms (e.g., social difficulties) between anxiety and ASD, social BI may also be related to ASD.

To understand more whether the associations between social BI and anxiety are driven by emerging ASD traits, in a separate model, ASD was treated as a covariate (Model 4.2). After this, the association between social BI and anxiety remained significant. Further, once controlling for the effect of anxiety, the association between social BI and ASD was not significant. However, these findings support the results of mediation analysis in Chapter 2 that showed a strong relationship between BI and anxiety once treated ASD as a mediator. In Chapters 2 and 3, I treated BI as a unitary construct, but in the current research, I extended these studies by investigating the contribution of social and non-social BI domains to the overlap between anxiety and ASD. I hypothesised that the social component of BI could be related to ASD due to social communication difficulties. In contrast to the hypothesised association, social BI was not driven by ASD traits. So, co-occurring anxiety traits may be independent of ASD traits.
These findings contradict Scherr et al. (2017) who showed no relationship between Social BI and anxiety in children with ASD and Shephard et al. (2018) who showed link between social BI (shyness) and ASD but no anxiety, between non-social BI (fear) and anxiety score but no association with anxiety traits. Further, after controlling for ASD, social and non-social BI was not significantly related to anxiety scores. The comparison between Shephard et al. (2018) and the current findings is important because the results suggested that at the age of 7, co-occurring anxiety is a consequence of ASD symptoms whereas in the current analyses findings suggested distinct pathways. So, in the early developmental period anxiety and ASD may have a distinct pathway but by the mid-childhood due to emergence of both disorders or other genetic and environmental factors may complicate it to untangle the pathways of both conditions.

In the current chapter, the measurement of the anxiety traits was carried out at the age of 2, which is earlier than the previous investigations. The group effect in Model 4.1 was significant in anxiety and ASD traits. However, in Model 4.2 and 4.3, the group effect on anxiety and ASD was not significant. It may be that at age 2, the combination of anxiety and ASD are more pronounced in toddlers at the HR group (as in Model 4.1) and existing anxiety and ASD traits may result in group difference. But once controlling for the effect of a co-occurring trait (either anxiety or ASD), taking out some of the shared variances, the effect of group disappears. It may be that both groups have anxiety and ASD traits to some extent, but it is more pronounced in the HR group when both conditions are present concurrently. It may highlight that at age 2, the symptoms of anxiety and ASD is not fully manifest. Especially, this may be due to children who manifest their symptoms later in the development (late-onset ASD), may not have higher levels of anxiety and ASD traits at this period. So, it may be more informative to
measure anxiety and ASD at the age of 2 and 3 to better understand the link between temperament and both anxiety and ASD.

One unexpected outcome was the relationship between higher non-social BI and lower levels of ASD traits because it was hypothesised to be related to anxiety traits rather than ASD. I hypothesised that the non-social BI was to be associated with anxiety. This association remained significant once controlling for anxiety traits, suggesting that the association is independent of co-existing anxiety traits and more related to the difficulties associated with ASD. In Chapter 3, similar associations emerged when anxiety scores were controlled in the analyses and parent-reported fear become negatively associated with ASD scores. So, these results suggest that reactivity in non-social situations may relate to ASD symptoms independent from the anxiety traits. One possible explanation for this may be that toddlers who have higher ASD symptoms may show a lack of awareness of danger towards a non-social threatening stimulus, which may result in lower levels of reactivity. Alternatively, co-occurring ADHD symptoms such as lack of impulsivity may influence children at risk for ASD to present impulsivity towards a non-social stimulus. However, further investigation should explore the possible mechanisms that explain this association.

4.4.3. Parental behaviours and child anxiety and ASD

Another important finding of the current investigation is the relationship between parental protective behaviours and the child’s anxiety traits. In line with the studies conducted in the general population (e.g., Kiel & Maack, 2012; Rubin et al., 2002), parental overprotective behaviours observed during the Stranger Approach task were related to child’s anxiety traits. There was not such an association with ASD traits and there was no risk group difference in parental behaviours in the social contexts. It
may be that in the HR group, parental behaviours are independent of parental overprotectiveness due to having an older child with an ASD diagnosis. As Aktar and Bögels (2017) noted, parental behaviours could be present due to their oversensitivity towards child’s emotional state and constitute an environmental risk for children’s anxiety consequently, children learn anxious behaviours from their parent’s over-anxious behaviours. On the other hand, parental overprotective behaviours may be due to parental anxiety traits which may aggregate within the family members due to genetic or environmental risk factors (Brooker et al., 2015). Parental behaviours that were observed only in the social context related to child anxiety traits perhaps due to regulating their own social difficulties, but it is not known whether parental psychopathology (anxiety and ASD) influences parental overprotectiveness. There needs to be further investigation to explore whether parental behaviours relate to parental own anxiety or ASD traits.

Parental protective behaviours could also be exacerbated by the child’s heightened BI. Sensitive parents could perceive the emotional state of the child as vulnerable and may want to soothe heightened reactivity (Degnan et al., 2015; Rubin et al., 2002; Vreeke et al., 2013). This may limit the child’s engagement with the threatening situation and may reduce the opportunities for the child to develop regulatory strategies (Kiel & Buss, 2010; Rubin et al., 2002). In contrast to this view, the results of the current investigation showed that the level of child BI (both social and non-social) did not predict parental behaviours. So, parental behaviours may be influenced by their own anxiety traits, which in turn appear as overly protective behaviours and ultimately contribute to increased child anxiety. Further investigation is needed to explore the role of parental anxiety traits and their influence on child anxiety. This type of investigation may extend the research effort into understanding the role of
anxiety in ASD by examining the familial aggregation of these traits. To sum up, in addition to temperamental risk overprotective behaviours that are due to parental anxiety constitute a risk factor for co-occurring anxiety traits in children at familial risk of ASD. Even though there is less evidence in the ASD literature on how parental behaviours influence anxiety or ASD phenotype in children, this investigation is one of the first attempt to highlight the importance of parental behaviours on child’s psychopathology.

In addition to main analyses, post hoc power was calculated for each model separately. The results for Model 4.1 showed high power for detecting the significant associations. However, once controlling for the ASD traits, the strength for the association between social-BI and anxiety deteriorated despite the significant association in Model 4.2. The association between non-social BI and ASD was also dropped in Model 4.3. So, it may be that once taking out the effect of the co-occurring conditions, the power of generating effect also dropped but the pattern of significant associations remained the same. There may be more participants needed to test the covariates in future investigations.

4.4.4. Limitations

Despite the important findings from the current investigation, there are some limitations to consider. Although this was a novel investigation that explored the root of the overlap between anxiety and ASD in toddlerhood (24 months), one of the limitations is the measurement of anxiety traits at the age of two, which may be too early to detect them. As for the DSM-5 description of anxiety (American Psychiatric Association, 2013), anxious behaviours may lead to physiological changes so that future
studies can investigate physiological responses (e.g., heart rate) during the novelty tasks as a complementary measurement for anxiety.

The second limitation may be the cross-sectional nature of the current investigation. Although cross-sectional associations allowed me to explore how BI components relate to co-occurring anxiety and ASD, future studies may benefit from longitudinal investigations of the trajectories of social and non-social BI in order to reliably investigate developmental outcomes, for example, by using cross-lagged associations between traits over time. Also, longitudinal investigations may provide more conclusive information about the direction of the association between child characteristics and parental behaviours.

4.4.5. Conclusion

The current study sought to explore whether social and non-social BI had similar or distinct associations with characteristics of anxiety and ASD in children at familial risk of ASD, by employing multi-method assessment and considering the possible effects of parental overprotective behaviours. The findings indicate that 1) social BI was related to both anxiety and ASD traits, however, its association with the ASD traits appeared to be primarily due to co-occurring anxiety traits, 2) unexpectedly, heightened non-social BI was related to lower levels of ASD traits, highlighting the importance of assessing the correlates of both social and non-social BI, 3) parental overprotective behaviours were associated with child anxiety traits. Overall, these finding may have clinical implications and intervention efforts that target temperamental social BI and parental over-protective behaviours may mitigate early emerging anxiety traits and subsequently prevent the risk of anxiety problems at later stages in development.
Chapter 5. Familial Aggregation of Anxiety and ASD Traits

5.1. Introduction

In Chapter 4, the results showed that toddlers’ anxiety traits were influenced by the parental protective behaviours that were observed in a social situation. Furthermore, results suggested that parental over-protective behaviours were independent of children’s behavioural inhibition (BI). However, this investigation did not specify whether parental anxiety traits predispose parents to be overly sensitive towards their children’s heightened reactivity, which could result in over-protective behaviours and consequently leave the child vulnerable to anxiety problems. Aktar and Bögels (2017) argued that infants born to anxious parents not only inherit genetic vulnerability but are also exposed to parental anxious emotionality. Moreover, adoption studies suggested that parenting behaviours of adoptive parents and anxiety traits of birth parents were both related to child BI, which suggests that both genetic and environmental exposures may constitute a vulnerability to emotional maladjustment (Natsuaki et al., 2013). As for Autism Spectrum Disorders (ASD), studies have suggested that parents of individuals with the ASD diagnosis commonly present subclinical expressions of ASD and anxiety traits (Bolton et al., 1994; Folstein & Rutter, 1977; Jokiranta-Olkonemi, Cheslack-Postava, Sucksdorff, & et al., 2016; Jokiranta et al., 2013; Piven, Palmer, Jacobi, Childress, & Arndt, 1997).

Based on this evidence, both anxiety and ASD traits run in families, through genetic and environmental risks. Exploring familial traits may enable investigation that sought to explore whether anxiety and ASD traits in parents of (an) older sibling(s) with ASD purely relate to anxiety and ASD traits in infant siblings, which may confer that the familial risk for ASD drives the early emerging anxiety and ASD traits.
Alternatively, this exploration may inform whether there is apparent segregation such that parental anxiety relates to child’s anxiety or parental ASD relates to child’s ASD traits, which may suggest independent developmental paths influence the co-occurring anxiety and ASD. Thus, the aim of the current chapter is to explore single trait (either anxiety or ASD) and cross-trait (parental anxiety and ASD traits vs child anxiety and ASD traits) familial transmission of co-occurring anxiety and ASD traits.

5.1.1. Familial transitioning of anxiety and ASD

Consistent evidence suggests that psychiatric disorders are familial and genetic or environmental factors explain a considerable amount of the variance in the aetiology (e.g., Caron & Rutter, 1991; Kushner, 2014). The heritability of these disorders enables family studies to investigate not only how single psychiatric condition aggregate within family members but also how comorbid (or co-occurring) conditions transmit from parents to their offspring. Family studies investigate the shared aetiology of comorbid conditions by estimating their direct transmission (e.g., ASD in parents and ASD in offspring) against cross-trait transmission (e.g., ASD in parents and anxiety in offspring or vice-versa).

ASD is a complex multifactorial disorder and there is considerable evidence from family, twin, and genetic research suggesting that the aetiology of ASD moderately involves genetic factors (Colvert et al., 2015; Hallmayer et al., 2011; Tick, Bolton, et al., 2016; Tick, Colvert, et al., 2016; Yip et al., 2017). As mentioned earlier, anxiety problems co-occur with ASD at high rates (Simonoff et al., 2013; Simonoff et al., 2012). Twin studies that were conducted with co-twins that had an ASD diagnosis have suggested that the co-occurrence of anxiety in ASD can be partly explained by shared genetic effects and found moderate genetic correlations (.42; Tick, Colvert, et al.,
In the general population, the phenotypic correlation between anxiety and ASD traits was modest (.30 -.35) and this overlap was similarly explained by shared genetic factors (Hallett et al., 2010). On the contrary, a larger twin study found strong genetic correlations between anxiety and ASD symptoms (.53; Lundström et al., 2011). So, shared genetic factors may underly co-occurring anxiety and ASD traits from small to a strong magnitude and this suggests heritability for both disorders. Thus, the heritability of these conditions provides a basis to explore familial transmission, specifically in relation to how parental traits influence child ASD as well as anxiety traits.

Both mothers and fathers of ASD probands present a subclinical expression of ASD phenotypes, referred to as broader autism phenotype (BAP; Bolton et al., 1994) at a higher level than the parents of children in the general population (Lau, Gau, Chiu, & Wu, 2014; Lyall et al., 2014; Maxwell, Parish-Morris, Hsin, Bush, & Schultz, 2013; Piven & Palmer, 1999) and children with Down syndrome (Piven & Palmer, 1999). Especially, within fathers of ASD probands, BAP traits were more prevalent than maternal BAP traits (Maxwell et al., 2013). In addition to BAP, anxiety disorders were also highly prevalent among mothers and fathers of individuals with ASD than parents of typically developing children (Jokiranta et al., 2013). Also, mothers had higher anxiety traits than fathers in the ASD group (Foody, James, & Leader, 2015) and control group (Kuusikko-Gauffin et al., 2013). Both parental ASD and anxiety traits may relate to child problems. For example, parental BAP, especially when both parents had elevated BAP traits, was predictive of children’s language problems, motor delay and dysregulation (Rubenstein et al., 2018). Also, maternal internalising symptoms (anxiety or depression) was associated with child dysregulated ASD symptoms (Wiggins et al., 2018). Overall, BAP is more prevalent in fathers in the ASD families and anxiety traits are more prevalent in mothers than in the ASD families.
One of the possible explanations for familial aggregation of co-occurring anxiety and ASD is assortative mating which refers to the tendency for individuals to choose a partner who shows similar (non-random or positive) or different (negative) phenotypic characteristics (Nordsletten et al., 2016; Peyrot, Robinson, Penninx, & Wray, 2016; Plomin, Krapohl, & O’Reilly, 2016). Plomin et al. (2016) suggest that the explorations of assortative mating shed more light on the heritability of psychiatric disorders. Firstly, assortative mating increases the chance of additive genetic variance only for genes that are related to a specific trait (e.g., ASD) and increases the chance of genetic effects from affected parents to offspring. This could explain why psychiatric disorders have high heritability. Secondly, assortative mating may explain the co-occurrence of psychiatric conditions. Genome-wide estimation of genetic correlations across psychiatric disorders demonstrates that genetic effect may be shared between disorders (Lee et al., 2013). Thus, assortative mating may increase the probability of a child to develop ASD. This could occur either via higher ASD traits in both parents by increasing the risk of having additional anxiety traits that accompany ASD due to partner(s) anxiety traits or co-existing anxiety traits.

Accordingly, there have been investigations of assortative mating for ASD. A recent population study assessed assortative mating within and between eleven psychiatric conditions. It demonstrated a strong partner resemblance for ASD (for males with ASD diagnosis \( r = .48 \), for females with ASD diagnosis \( r = .45 \)), which was much higher than the other psychiatric conditions (Nordsletten et al., 2016). Similarly, De la Marche et al. (2015) showed a strong association between partner ASD traits \( (r = .53) \) and Constantino and Todd (2005), Lyall et al. (2014) and Schwichtenberg, Young, Sigman, Hutman, and Ozonoff (2010) found moderate concordance between partner ASD traits \( (r = .38, r = .25, r = .34, \text{respectively}) \). Overall, these findings support that
parental psychopathology may influence their preferences of partner and assortative mating for individuals with ASD. This may increase the genetic or environmental risks for their offspring to develop psychopathology and ASD may aggregate within the families subsequently.

As for the intergenerational transmission of ASD traits between parents and offspring, findings of previous studies have varied. Schwichtenberg et al. (2010) showed that paternal ASD traits were significantly associated with offspring who have an ASD diagnosis but not with the unaffected child whereas maternal scores were neither related to affected nor unaffected offspring ASD traits. Further, Constantino and Todd (2005) suggested that parental transmission could depend on the child’s sex by indicating that same-sex parent and child combinations had higher concordance than opposite-sex combinations: mother and daughters (ICC = .41), mother and son (ICC = .38) father and daughter (ICC = .49) and father and son (ICC = .58). However, De la Marche et al. (2015) pointed that these investigations heavily relied on parental ratings of self- and child-trait which could be influenced by the informant (rater) effect, and addressed this issue by generating a factor score for parents and the proband child with multiple informant ratings. The results of this investigation suggested that parental ASD traits were associated with the child traits, but that paternal ASD traits were more strongly associated with the child ASD traits than maternal traits. So, these investigations suggest that both maternal and paternal ASD traits may be additive risk for the child ASD traits, but paternal traits may be more predictive of the child traits than maternal traits, and these associations may differ based on the sex of the offspring.

In terms of the cross-assortative mating (the probability of having a partner with another psychiatric condition), Nordsletten et al. (2016) showed that for males with
ASD diagnosis, the partner resemblance for generalised anxiety disorder was .19, and social phobia was .27; for females with ASD, the partner resemblance for generalised anxiety disorder was .29, and social phobia was .38 which was the second strongest association for females. Duvekot, van der Ende, Constantino, Verhulst, and Greaves-Lord (2016) employed a multi-informant assessment of parental anxiety and found that paternal anxiety and ASD traits were associated (both self- and informant- report), but maternal traits were associated only in self-report measures which did not support the assortative mating.

Regarding cross-trait familial transmission, Duvekot et al. (2016) also showed that once they examined the transmission of ASD and anxiety separately, maternal and paternal self-report, as well as partner-rated ASD traits were associated with child ASD and anxiety symptoms. As for the cross-trait transmission, self-report maternal ASD and anxiety were associated with child ASD and anxiety traits; paternal ASD was not associated with child ASD traits, but paternal anxiety was related to child ASD traits. For the partner-report traits, maternal anxiety but not ASD was related to both child anxiety and ASD traits. Paternal ASD was significantly associated with only child ASD traits, and anxiety was associated with both child traits. Taken together, although there was evidence for cross-trait assortative mating in the general population, this was not supported in the ASD families. However, it should be noted that the differences may be due to statistical approaches (raw correlations vs path analysis) and different sample sizes in these investigations. Also, for familial transmission, if assortative mating increases the additive genetic risk, unlike Duvekot et al. (2016), one should include both parental traits into a model to accommodate the additive risk. Nevertheless, there is a need for further investigation of cross-trait familial transmission of ASD and anxiety to sort out the source of the aggregation.
Investigating single trait as well as cross-trait familial transmission of anxiety and ASD traits from parents to infant siblings of probands (who have an ASD diagnosis) may provide a better understanding of additive risk for co-existing anxiety and ASD traits before core ASD symptoms manifest themselves and interact with other possible risk factors. This investigation may be feasible because around 20% of infant siblings of probands develop ASD around their third birthday (Ozonoff et al., 2011) and approximately a further 20% of infant siblings present sub-clinical threshold ASD symptoms (Charman, Young, et al., 2017; Messinger et al., 2013; Ozonoff et al., 2011). Moreover, younger siblings of probands experience higher levels of anxiety problems (Jokiranta-Olkoniemi et al., 2016) as well as sub-clinical anxiety traits, especially in male siblings (Shivers, Deisenroth, & Taylor, 2013). Walton (2016) also found that siblings of individuals with ASD have an increased likelihood of anxiety problems, which was higher for males. Taken together, this suggests that anxiety and ASD may cluster in siblings of probands.

5.1.2. The present study

Overall, in Chapter 4, parental over-protective behaviours were found to be associated with the child’s anxiety traits. Parental over-protectiveness could be a result of a parent’s own anxiety trait that could confer genetic or environmental risk (Aktar & Bögels, 2017). In the ASD literature, it is well established that first-degree relatives of children with ASD presented with BAP and emotional problems (Jokiranta et al., 2013; Lau et al., 2014; Lyall et al., 2014; Maxwell et al., 2013; Piven & Palmer, 1999). So, parents in the HR group may have heightened BAP and anxious traits because they already have a child with ASD. To assess how co-occurring anxiety and ASD run within families, a few studies have investigated the assortative mating hypothesis
(Duvekot et al., 2016). Assortative mating confers an increased additive risk for child psychopathology by increasing the chances of transmission of co-occurring conditions from both parents to their offspring.

In the current study, investigating familial aggregation of co-occurring anxiety and ASD could expand previous investigations in Chapter 2, 3, and 4 to understand the nature of co-occurrence between anxiety and ASD, before the age of 3. To investigate this, the current investigation involved three aims. Both mothers and fathers of probands with ASD had higher BAP than the parents of typically developing children (Maxwell et al., 2013), and anxiety traits are more prevalent in mothers of the ASD groups (Foody et al., 2015). So, the first aim of the current study was to replicate the previous investigations and to explore whether parents (mothers and fathers separately) of HR toddlers had higher levels of anxiety and ASD traits than LR parents. Moreover, investigations indicated a higher prevalence of BAP traits in fathers once compared to mothers in the ASD sample (Maxwell et al., 2013), and anxiety traits were highly common in mothers compared to fathers in the ASD group (Foody et al., 2015). So, the further aim was to explore whether parents had higher anxiety and ASD traits than each other in the HR and LR groups. This will summarise the sample characteristics of the data. Also, it will inform us whether parents in the HR group have higher anxiety and ASD traits than the LR group due to having a proband and confers a higher risk for familial aggregation. Further, prior to the modelling, it may inform whether the aggregation originated from mothers or fathers due to higher anxiety or ASD traits.

The second aim was to explore the single traits transmission of anxiety and ASD independently. Schwichtenberg et al. (2010) showed that paternal ASD traits were associated with the child’s ASD traits whereas maternal traits were not significantly
associated with the child’s symptom. As for the anxiety traits, less is known about the
single trait transmission and the source of the parental traits. To explore this, two
different path analyses were conducted with anxiety as well as ASD traits. In Model
5.1a & b and Model 5.2a & b, to accommodate the association between parental traits,
both parents’ scores were included as correlated predictors of the child outcome. These
models would explain how parental traits were associated with the child trait once
excluding the co-occurring traits. These models can address whether parental anxiety
traits purely relate to child anxiety or parental ASD relate to child ASD traits in infant
siblings as well as the source of the association.

The last aim was to explore the cross-trait transmission of parental traits to
offspring. A path analysis was run incorporating the parental anxiety and ASD traits as
correlated predictors and child traits (anxiety and ASD) as correlated outcome variables.
This model would explain the effects of associations between parent traits on child
traits. This model would also reflect familial cross-trait transmission within family
members (Model 5.3a), which helps to understand whether parental anxiety and ASD
purely relate to the child’s anxiety and/or ASD traits in infant siblings. Also, it
addresses whether there is segregation in transmissions (parent anxiety-child anxiety or
parent ASD-child ASD).

To tackle the shared measurement variance, Model 5.1a, Model 5.2a and Model
5.3a were re-run with parental observed protective behaviours and child social BI scores
(summarised in Chapter 4) (Model 5.1b, Model 5.2b, Model 5.3b). The current study is
the first to examine the familial transmission of ASD and anxiety traits within the ASD
familial HR design. This exploration will expand our understanding of to what extent
familial anxiety and ASD traits constitute a risk factor for anxiety and ASD traits in toddlers.

5.2. Method

5.2.1. Participants

Similar to Chapter 4, the participant of the current project was drawn from the third phase of the British Autism Study of Infant Siblings (BASIS; www.basisnetwork.org) / the Studying Autism and ADHD Risks (STAARS; www.staars.org) project. The data from the same participants of Chapter 4 involved in the analysis but the sample size of the current chapter slightly higher since parental data were collected when the toddlers turned to 21 months, prior to the testing date and the parent-reported data was included from the participants who did not manage to attend the testing. Of one hundred and twenty-seven participants who were born between December 2012 and November 2016, 29 infants were low-risk controls, 97 were a high-risk of ASD. For the current chapter, data were included from the 24-month time point. One LR and one HR toddler were excluded due to having another sibling in the study (because both siblings shared the same parental data). There was one single mother in the LR group and since the analyses did not affect the results with and without this participant, their data were included in the rest of the analyses.

Four controls and 12 HR-ASD toddlers did not participate at the 24 months visit and one HR-ASD family withdrew, three (1 LR, 2 HR-ASD) children did not attend the laboratory visit, but questionnaire data were available for them. Eventually, the final data were available for one hundred and eight toddlers (25 LR, 83 HR-ASD).
5.2.2. Measures of target child anxiety and ASD traits

Anxiety traits in toddlers were assessed via raw scores of the Child Behaviour Checklist 1.5-5 DSM-Oriented Anxiety Problem subscale (CBCL; see section 2.2.2; Achenbach & Rescorla, 2000). To tackle the rater effect, the social BI factor scores were used to replace parent-reported CBCL DSM-Oriented Anxiety Problem subscale. As described in Chapter 4 (section 4.3.3) social BI factor scores were generated using parent-reported shyness, withdrawal, fear and strategies observed during the Stranger Approach task and experimenter-ratings of social inhibition scores (for more details see section 4.2.2.1.1).

ASD traits were measured using the Quantitative Checklist for Autism in Toddlers (Q-Chat; Allison et al., 2008) as described in section 4.2.26. The internal consistency of the Q-Chat in the current research was excellent (Cronbach’s alpha = .81).

To replicate parent-reported Q-Chat, an observational measure of the Autism Diagnostic Observation Schedule – 2nd Edition (ADOS-2; Lord et al., 2012) total calibrated severity scores were used. The ADOS is a standardised, semi-structured, observational measure that assesses symptoms related to ASD, including communication, social interaction and restricted, repetitive behaviours. The ADOS consists of four different modules and the administration of each module changes based on children’s developmental and verbal abilities. In this study, the Toddler Module total calibrated severity scores were used for each participant that attended the visit at 24 months.
5.2.3. Measures of parental anxiety and ASD traits

Maternal and paternal anxiety traits were measured using the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The STAI is a 40 item self-report questionnaire that allows respondents to report their current anxiety symptoms and severity, and their general tendency to be anxious. The STAI consists of two subscales: The State Anxiety Scale which reflects the presence of the current anxiety symptoms and the Trait Anxiety Scale which is related to the overall propensity of individuals to feel anxious. Respondents can rate their intensity of State Anxiety “at this moment” on a 4-Likert type rating scale: 1) not at all, 2) somewhat, 3) often, 4) almost always. The intensity of Trait Anxiety is rated through the frequency of feelings in general as 1) almost never, 2) sometimes, 3) often and 4) almost always. The range of the total scores of both subscales is 20-80, and higher scores reflect either higher state and/or trait anxiety level. The STAI had good internal consistency ranging from .86 to .95 (Spielberger et al., 1983) and in the current research, it is .93, indicating excellent internal consistency.

Both maternal and paternal ASD traits were measured using the second version of the Social Responsiveness Scale-Adult Self-Report form (SRS-2-ASR; Constantino, 2012). The SRS-2-ASR is a 65-item self-report questionnaire that assesses the severity of ASD traits in adults aged 19 and older. Items on the SRS-2-ASR can be rated on a 4-point Likert scale: ‘not true’, ‘sometimes true’, ‘often true’, and ‘almost always true’. T-scores for the total SRS-ASR varies between 30 and 90, higher scores indicating higher severity and scores between 60 to 90 reflect the mild to severe clinical range. In addition to total scores, SRS-2-ASR generates scores for five treatment subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted
Interests and Repetitive Behaviour; or generates two DSM-5 compatible subscales:
Social Communication and Interaction, Restricted Interests and Repetitive Behaviour.

Both the STAI and the SRS-2-ASR measures have been widely used in the ASD research to measure either parental anxiety (Conner, Maddox, & White, 2013) or ASD traits (Lyall et al., 2014) the overlap in the symptom presentation of both disorders in adults (e.g., South, Carr, Stephenson, Maisel, & Cox, 2017).

5.2.4. Data Analysis

Before modelling, sample characteristics (HR vs LR differences and between parent differences) were examined using SPSS version 24.0. Also, Pearson correlation coefficients were calculated without controlling the effect of the group and the child’s sex to explore the association between research variables. To address the research questions, a series of path analyses were run in a structural equation modelling (SEM) framework using Mplus 7.4 (Muthén & Muthén, 1998-2015). The SEM framework is an advantageous statistical approach to analyse the research questions in the current chapter because it allows analysing the association between parental predictors (maternal and parental traits) and the overall contribution of this association to child outcomes simultaneously.

The first specified path-model (Model 5.1a & 5.1b, Figure 5.1; Model 5.2a and Model 5.2b, Figure 5.2) explored the third aim of the current research which was about assortative mating and single trait transmission. To address the assortative mating, maternal and paternal scores were treated as correlated predictors and scores of both parents’ data were regressed on the child variable to address the overall family contribution. This model was run separately for ASD (Model 5.1a &b) and anxiety (Model 5.2a & 5.2b) traits.
Figure 5.1 Proposed model for the association between parent and child ASD traits. Model 5.1a represents parental ASD traits and parent-reported ASD traits. Model 5.1b represent the association between parental ASD and child observed ASD traits. PR = parent report, O = Observed

Figure 5.2 Proposed model for the association between parent and child anxiety traits. Model 5.2a represents parental anxiety traits and parent-reported anxiety traits. Model 5.2b represent the association between parental anxiety and child observed social BI scores. PR = parent report, O = Observed
The third path model (Model 5.3, Figure 5.3) addressed the fourth aim which was about the cross-assortative mating and cross-trait familial transmission of anxiety and ASD traits. This model incorporated 1) association of ASD and anxiety within parent traits to address co-occurring conditions, and between parents to address cross-assortative mating, and 2) overall association between parental and co-occurring anxiety and ASD traits in children to assess cross-trait familial transmission.

![Figure 5.3 Model 5.3 the proposed model for between parent traits associations with child anxiety and ASD traits.](image)

To overcome the shared method variance, Model 5.2.b and 5.3b were run replacing the child anxiety traits with observational social BI scores in the social Stranger Approach task. All analyses were run with observed variables and risk group status (HR = 0 vs LR = 1); regressed on all parent and child variables. Child sex (male = 0 vs female = 1) was treated as a covariate in all models that were run in Mplus.

As in the previous chapters, for analyses run in Mplus, Full Information Maximum Likelihood (FIML) was used to account for missing data. The non-normal distributions and skewness in the anxiety outcome measure were addressed using the
Maximum Likelihood Robust (MLR) estimation which provides robust standard errors. The overall model fit was evaluated by the criteria described in section 2.2.3. All model coefficients are standardised with respect to the predictor and outcome; standardised model estimates (STDYX) will be reported throughout (Muthén & Muthén, 1998-2015).

After testing all models, post hoc power analyses were calculated to detect the strength of the analysis of generating significant associations. The procedure of power analyses was summarised in section 2.2.5.

5.3. Results

5.3.1. Sample characteristics

In terms of parental data, mothers of HR children had significantly higher anxiety and ASD traits than mothers of LR children ($F(1, 95) = 4.75, p = .032$; $F(1, 96) = 5.80, p = .018$; respectively). Fathers of the HR and the LR children did not differ on anxiety and ASD scores ($F(1, 86) = 1.27, p = .263$; $F(1, 85) = 2.47, p = .120$; respectively). These results indicate higher maternal ASD and anxiety traits in the HR group whereas there was no group difference in the paternal traits.

Maternal and paternal age did not differ between HR and LR groups ($F(1, 88) = .022, p = .883$; $F(1, 80) = .181, p = .672$; respectively). So, will not be controlled in the further analysis.

Toddlers in the HR group had significantly higher anxiety and ASD traits than the LR group ($F(1, 99) = 9.98, p = .002$; $F(1, 103) = 8.81, p = .004$; respectively), indicating that the HR group significantly differs from the LR groups on the outcome variables.
Table 5.1 Group Level Difference in Parental and Child Variables

<table>
<thead>
<tr>
<th></th>
<th>Low-Risk</th>
<th>High-Risk</th>
<th>Test</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Mothers</td>
<td>21</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother Age</td>
<td>37.87 (3.56)</td>
<td>37.12 (4.06)</td>
<td>$F = .022, p = .883$</td>
<td>.01</td>
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<tr>
<td>Mother Trait Anxiety</td>
<td>36.80 (10.77)</td>
<td>39.74 (10.70)</td>
<td>$F = 4.75, p = .032$</td>
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<tr>
<td>Mother ASD</td>
<td>45.07 (7.43)</td>
<td>47.98 (8.85)</td>
<td>$F = 5.80, p = .018$</td>
<td>.06</td>
</tr>
<tr>
<td>N Fathers</td>
<td>20</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father Age</td>
<td>40.67 (5.12)</td>
<td>39.86 (5.21)</td>
<td>$F = .181, p = .672$</td>
<td>.01</td>
</tr>
<tr>
<td>Father Trait Anxiety</td>
<td>36.93 (8.45)</td>
<td>40.78 (13.44)</td>
<td>$F = 1.27, p = .263$</td>
<td>.02</td>
</tr>
<tr>
<td>Father ASD</td>
<td>47.53 (7.36)</td>
<td>54.44 (13.15)</td>
<td>$F = 2.47, p = .120$</td>
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<tr>
<td>N Child</td>
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<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child Age in months</td>
<td>24.48 (1.17)</td>
<td>24.84 (1.51)</td>
<td>$F = .99, p = .322$</td>
<td>.01</td>
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<tr>
<td>Child Anxiety</td>
<td>1.26 (1.10)</td>
<td>3.14 (2.78)</td>
<td>$F = 9.98, p = .002$</td>
<td>.09</td>
</tr>
<tr>
<td>Child Q-Chat</td>
<td>18.91 (4.95)</td>
<td>25.32 (13.05)</td>
<td>$F = 5.29, p = .024$</td>
<td>.05</td>
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<tr>
<td>Child ADOS Total</td>
<td>1.54 (.66)</td>
<td>2.94 (2.27)</td>
<td>$F = 8.81, p = .004$</td>
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<td>Child Social BI</td>
<td>-.25 (.64)</td>
<td>.06 (.87)</td>
<td>$F = 2.10, p = .152$</td>
<td>.03</td>
</tr>
</tbody>
</table>

Bolds indicate significant group difference between the HR and LR groups

5.3.2. Do mothers have higher traits than fathers?

Paired sample t-tests for the HR and the LR group were run separately to explore whether there were differences between partner anxiety and ASD traits. As for the anxiety traits, there were no significant differences between couples in the LR ($t (17) = -.30, p = .766$) and HR groups ($t (64) = -.73, p = .468$). For the ASD traits, fathers had higher scores than mothers in the LR group ($t (18) = -2.31, p = .033$) and the HR group ($t (63) = -3.02, p = .004$). So, partners had similar levels of anxiety traits in the HR and LR groups, but fathers had higher ASD traits than mothers in both groups.

5.3.3. Association between research variables

Pearson correlation coefficients were calculated to explore co-occurring anxiety and ASD traits within parents, associations between parent traits, and associations
between parent and child traits. Table 5.2 summarises the pattern of significant associations. For within-person associations, the results showed that maternal anxiety and maternal ASD traits were strongly correlated ($r = .58, p < .001$). This pattern of significance was also similar in fathers, but the associations were stronger ($r = .74, p < .001$) than mothers.

Second, as for the between partner associations, there was a small association for maternal and paternal anxiety ($r = .29, p = .009$) and moderate associations for maternal and paternal ASD traits ($r = .40, p < .001$). For the cross-trait associations, maternal anxiety and paternal ASD scores were strongly associated ($r = .38, p < .001$) whereas maternal ASD and paternal anxiety were not significantly associated ($r = .15, p < .162$).

Thirdly, for the parent-child associations, maternal and paternal anxiety and ASD traits were significantly associated with the child anxiety traits (Mother anxiety: $r = .26, p = .015$; Mother ASD: $r = .37, p < .001$; Father Anxiety: $r = .27, p = .012$; Father ASD: $r = .28, p = .010$). Neither parental anxiety traits (Mother: $r = .11, p = .292$; Father: $r = .08, p = .445$) nor ASD (Mother: $r = .05, p = .632$; Father: $r = .18, p = .105$) were significantly associated with the child ASD traits, even though child ASD traits were modestly associated with child anxiety traits ($r = .22, p = .037$). So, these associations adequate for these variables to run the proposed models.
Table 5.2 Pearson Correlation Coefficients for Parental and Child Traits

<table>
<thead>
<tr>
<th></th>
<th>1.</th>
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<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
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<td>2. F_Anxiety</td>
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<td></td>
</tr>
<tr>
<td>3. M_ASD</td>
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<td>.154</td>
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<td>4. F_ASD</td>
<td>.384</td>
<td>.735*</td>
<td>.398*</td>
<td>1</td>
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<td>5. C_Anxiety</td>
<td>.256</td>
<td>.272</td>
<td>.367*</td>
<td>.279*</td>
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<tr>
<td>6. C_ASD_PR</td>
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<td>.346</td>
<td>.336</td>
<td>.465*</td>
<td>.557*</td>
<td>1</td>
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<tr>
<td>7. C_ASD_O</td>
<td>.111</td>
<td>.084</td>
<td>.050</td>
<td>.179</td>
<td>.217*</td>
<td>.259</td>
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<tr>
<td>8. C_Social_BI</td>
<td>.069</td>
<td>-.028</td>
<td>-.021</td>
<td>-.049</td>
<td>.249*</td>
<td>.146</td>
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</tbody>
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* p < .001 level. M= Mother, F= Father, C= Child, PR= Parent-Report, O= Observed.

5.3.4. Single trait transmission

5.3.4.1. Model 5.1a: Parental ASD and child ASD traits (parent-report)

For the association between parental and child ASD traits, the model provided a good fit to the data ($\chi^2 (3) = 1.55, p = .671; $CFI = 1.00, RMSEA = .000, and SRMR = .032). There was a significant difference on child ASD score based on child sex ($\beta = -.07, p = .013$) indicating higher ASD scores in males. Risk group status (LR vs HR) was not significantly associated with child ASD scores ($\beta = .10, p = .084$), but significantly related to maternal ($\beta = .24, p = .001$) and paternal ($\beta = .19, p = .009$) ASD traits, indicating higher ASD scores in both mothers and fathers in the HR group. As for associations between maternal and paternal ASD traits, higher maternal ASD traits were associated with paternal ASD traits ($\beta = .35, p = .002; $Figure 5.4).

Regarding the parent-child transmission of ASD traits, paternal ASD traits were significantly related to child ASD scores ($\beta = .38, p = .001$) whereas maternal traits were not significantly related ($\beta = .19, p = .156$). These results suggested single trait transmission from fathers to children.
5.3.4.2. Model 5.1b: Parental ASD and child ASD traits (observed)

The model provided a good fit to the data ($\chi^2 (2) = 2.22, p = .568$; CFI = 1.00, RMSEA = .000, and SRMR = .035). There was no significant difference on child ADOS total CSS score based on child sex ($\beta = -.07, p = .467$). The risk group status was significantly associated with child ADOS total CSS scores ($\beta = .27, p < .001$), indicating higher ASD traits in the HR group. The risk group was also significantly related to maternal ($\beta = .24, p = .001$) and paternal ($\beta = .18, p = .010$) ASD traits indicating higher scores in the HR group. As for associations between maternal and paternal ASD traits, higher maternal ASD traits were associated with paternal traits ($\beta = .38, p = .001$). Regarding the parent-child transmission of ASD traits, maternal and paternal ASD traits were not significantly related to child ASD scores (mother-child: $\beta = -.07, p = .589$; father-child: $\beta = .15, p = .353$; Figure 5.4).

Figure 5.4 Association between parent and child ASD traits. Dotted lines indicate non-significant results. Standardised beta and standard errors are reported. M= Mother, F=Father, C=Child. *** $p < .001$, ** $p < .01$, * $p < .05$. 

![Diagram](image.png)
A post hoc power calculation for the association between paternal ASD and child ASD traits showed 96% power, indicating large power to generate significant associations.

5.3.4.3. Model 5.2a: Parental anxiety and child anxiety traits

The model fit indices indicated a good fit for the association between parental and child anxiety traits ($\chi^2(2) = 1.44, p = .486; \text{CFI} = 1.00, \text{RMSEA} = .000, \text{and SRMR} = .024$). There were no significant sex differences on child anxiety traits ($\beta = -.02, p = .839$). Risk group status was significantly associated with child anxiety scores ($\beta = .24, p < .001$), indicating higher anxiety traits in the HR group. Risk group was associated with maternal anxiety traits ($\beta = .24, p = .008$), indicating higher traits in the HR group, but not with paternal anxiety traits ($\beta = .13, p = .099$). As for between parental traits, as shown in Figure 5.5 maternal and paternal anxiety traits were significantly related ($\beta = .27, p = .015$). In terms of parent-child transmission of anxiety traits, maternal and paternal anxiety levels were not significantly associated with the child anxiety traits ($\beta = .17, \text{SE} = .13, p = .200; \beta = .20, \text{SE} = .13, p = .102$; respectively). So, these findings did not support single trait transmission for anxiety traits.
Figure 5.5 Parent and child anxiety traits and child social BI. Full lines indicate significant results. Dotted lines indicate non-significant results. Standardised beta and standard errors are reported. M= Mother, F=Father, C=Child. *** $p < .001$, ** $p < .01$, * $p < .05$

5.3.4.4. Model 5.2b: Parental anxiety and child social BI traits

Once the parent-reported child anxiety traits were replaced with the observational social BI scores, the model fit remained good ($\chi^2 (3) = .69, p = .990$; CFI = 1.00, RMSEA = .000, and SRMR = .020). Child sex was not associated with the child social BI scores ($\beta = .04, p = .701; \beta = -.03, p = .818$). Risk group was significantly related to child anxiety but was not significantly associated with the social BI scores ($\beta = .23, p = .001; \beta = .15, p = .140$; respectively). Risk group was related to maternal anxiety traits indicating higher maternal anxiety in the HR group ($\beta = .24, p = .024$) but risk group was not related to paternal anxiety ($\beta = .15, p = .107$).
Both maternal and paternal anxiety traits were not significantly related to child anxiety ($\beta = .22, p = .202; \beta = .16, p = .342$; respectively) and social BI scores ($\beta = .07, p = .522; \beta = -.06, p = .693$; respectively). Higher child anxiety was also related to higher social BI scores ($\beta = .22, p = .002$; Figure 5.5).

5.3.5. Cross-trait transmission

5.3.5.1. Model 5.3a: Associations between parental and child anxiety and ASD traits

Model 5.3a, illustrated in Figure 5.3, indicated a good fit to the data, $\chi^2 (1) = .01, p = .929; \text{CFI} = 1.00, \text{RMSEA} = .000, \text{and SRMR} = .002$. As for the covariates, there was significant sex differences in ASD ($\beta = -.21, p = .008$) but not in anxiety ($\beta = -.03, p = .718$) traits. Risk group was not associated with child ASD scores ($\beta = .10, p = .092$) but significantly related to anxiety sores ($\beta = .21, p = .008$) indicating higher anxiety traits in the HR group. Risk group also had a significant effect on maternal anxiety ($\beta = .24, p = .006$) and ASD scores ($\beta = .25, p < .001$); and paternal ASD score ($\beta = .21, p = .004$) but not anxiety score ($\beta = .13, p = .103$), suggesting higher traits in the HR group.

There was a strong association within parent traits. Mothers who had higher anxiety traits also had higher ASD traits ($\beta = .57, p < .001$). Similarly, fathers who had higher anxiety also had higher ASD traits ($\beta = .73, p < .001$). However, these associations may be strong due to shared method variance.

For the between-parent traits, maternal and paternal anxiety were moderately associated ($\beta = .28, p = .008$) whereas ASD traits were strongly associated ($\beta = .36, p = .001$). In terms of cross-trait associations between parents, maternal ASD traits were not associated with paternal anxiety traits ($\beta = .12, p = .353$) but maternal anxiety was associated with paternal ASD traits ($\beta = .40, p < .001$).
Regarding familial transmission, maternal ASD traits were associated with the child ASD ($\beta = .27$, $p = .050$) and anxiety traits ($\beta = .37$, $p = .007$). Maternal anxiety was not significantly associated with child anxiety and ASD ($\beta = .037$, $p = .835$; $\beta = -.10$, $p = .310$; respectively). Paternal ASD trait was related to child ASD trait but not with the anxiety trait ($\beta = .32$, $p = .031$; $\beta = -.20$, $p = .160$; respectively). Paternal anxiety was related to child anxiety but not with the ASD trait ($\beta = .32$, $p = .026$; $\beta = .09$, $p = .491$; respectively). Overall, the results of the Model 5.3a suggest cross-trait transmission from maternal ASD traits to child’s anxiety trait and single trait transmission for paternal ASD, anxiety traits to children’s trait.

Figure 5.6 Model 5.3a: Between parent traits associations with child anxiety and ASD traits. Full lines indicate significant results. Dotted lines indicate non-significant results. Standardised beta and standard errors are reported. M= Mother, F=Father, C=Child. *** $p$ < .001, ** $p$ < .01, * $p$ < .05.
The post hoc power calculations showed that a chance of generating the association between maternal ASD traits and child ASD traits was 50%, maternal ASD and child anxiety was 75%, paternal ASD and child ASD was 51% and paternal ASD and child anxiety was 56%. Overall except for the association between maternal ASD and the child anxiety, rest of the analyses were generated with medium effect.

5.3.5.2. Model 5.3b: Associations between parental anxiety, ASD, child social BI and ASD

The Model 3b, indicated a good fit to the data, \( \chi^2 (4) = 4.59, p = .334 \); CFI = .995, RMSEA = .043, and SRMR = .035. As for the covariates, child sex was not significantly related to child ASD \((\beta = .01, p = .929)\) and social BI scores \((\beta = -.01, p = .939)\). Risk group significantly associated with child ASD scores \((\beta = .30, p < .001)\) indicating higher traits in HR group, but not with social BI \((\beta = .18, p = .090)\). Risk group status had a significant effect on maternal anxiety \((\beta = .24, p = .024)\), indicating higher maternal anxiety in HR group, but not with maternal ASD scores \((\beta = .19, p = .059)\). The risk group was also significantly related to paternal ASD scores \((\beta = .21, p = .011)\), higher traits in the HR group, but not anxiety scores \((\beta = .15, p = .113)\).

Parent traits were strongly associated to each other. Maternal anxiety and ASD traits \((\beta = .56, p < .001)\), paternal anxiety and ASD traits \((\beta = .79, p < .001)\) were significantly associated with each other. For the between parental traits, maternal and paternal anxiety traits \((\beta = .42, p < .001)\) and ASD traits \((\beta = .46, p < .001)\) were positively associated with each other. For cross-trait associations, maternal ASD was associated with paternal anxiety traits \((\beta = .30, p = .024)\) and maternal anxiety was associated with paternal ASD traits \((\beta = .39, p < .001)\).
As for the cross-trait transmission, none of the parental variables were related to child ASD (Mother anxiety $\beta = .07, p = .602$; mother ASD $\beta = -.25, p = .055$; father anxiety $\beta = -.80, p = .702$; father ASD $\beta = .27, p = .326$) and social BI scores (Mother anxiety $\beta = .09, p = .515$; mother ASD $\beta = -.04, p = .748$; Father anxiety $\beta = .03, p = .885$; father ASD $\beta = -.14, p = .439$). Child observed social BI and ASD traits were not significantly associated with each other ($\beta = .12, p = .194$). So, these results are indicating no association between parental and children traits.

5.4. Discussion

The current investigation aimed to explore whether parental anxiety and/or ASD traits aggregate within families and consequently contribute to the mechanism of co-occurring anxiety and ASD traits in children within the context of familial risk of ASD design. In the current sample, mothers of toddlers who were at risk for ASD had higher anxiety and ASD traits than the mothers of LR children whereas fathers had similar anxiety and ASD traits. In the HR and LR group, partners presented similar levels of anxiety traits, but in both HR and LR groups, fathers had higher ASD traits than mothers. These findings supported single trait transmission of ASD from father to the child (Model 5.1a), but once cross-trait transmissions were examined by including parental co-occurring traits into the model, the results suggested cross-trait transmission of maternal ASD to child anxiety and single trait transmission for paternal anxiety to child anxiety problems.

5.4.1. Sample characteristics

Studies exploring parental BAP indicated that mothers and fathers of the probands had higher traits than the control groups (Maxwell et al., 2013). In line with these findings, the results suggested that mothers of the ASD probands had higher ASD
traits than the LR parents, but in contrast, paternal ASD traits were similar in both groups. For the anxiety, the literature indicated higher traits for both parents of ASD probands once compared to the control groups (Jokiranta et al., 2013). In line with this, the findings showed maternal traits in the ASD group was higher than the controls, but there was no difference in paternal anxiety traits. So, it may be that maternal traits may influence the transmission.

Before the modelling, to understand the source of the parental effect, parental group differences were examined. Partners in the HR and the LR groups had similar levels of anxiety traits. These findings contradict with the findings in the literature suggesting more prevalent anxiety problems in mothers in the ASD and control groups (Foody et al., 2015; Kuusikko-Gauffin et al., 2013). But in terms of ASD traits, similar to Maxwell et al. (2013) fathers had higher ASD traits than mothers in the HR and LR groups. The difference in emotions generally reported being influenced by gender differences (higher anxiety traits in women than men). However, in contrast, our results suggested that the similar anxiety traits in both parents, so the source of the effect may be equal between parents in both groups.

5.4.2. Concordance between parental traits

Previous studies evaluating partner concordance of ASD traits suggested moderate to strong partner resemblance (Nordsletten et al., 2016; Piven & Palmer, 1999; Schwichtenberg et al., 2010). The results of the current investigations corroborate the findings of Constantino and Todd (2005); Lyall et al. (2014); Schwichtenberg et al. (2010) who showed moderate partner resemblance in ASD traits. For the cross-trait associations, the present results are in accord with Nordsletten et al. (2016) who indicated strong ASD trait resemblance between partners, as well as a moderate
resemblance of male ASD and female anxiety. However, in the current investigation, there was no significant association between female ASD and male anxiety traits with and without controlling for the effect of the risk group status and the child’s gender (Model 5.3a). This may be that anxious males do not tend to choose partners who do have ASD traits or vice versa. Taken together, even though the investigations did not test the assortative mating, the concordance between parent traits may reflect the assortative mating in the current research. Subsequently, the associations between maternal and paternal traits may increase the likelihood of transmission of anxiety and ASD.

For the co-existing anxiety and ASD traits within parents; the current findings indicated a strong association for fathers’ as well as mothers’ co-occurring anxiety and ASD traits (Figure 5.6). In Model 5.3a, the association between anxiety and ASD traits in fathers was stronger than the maternal traits. Their higher ASD traits may make them more vulnerable to additional anxiety traits or vice-versa. This may be another factor that increases the risk of familial transmission of co-occurring anxiety and ASD from fathers to children. However, it may be that the associations of co-occurring anxiety and ASD traits in mothers and fathers can be arisen due to relying on self-reported ASD and anxiety traits that can be influenced by the rater effect. So similar to Duvekot et al. (2016), when measuring co-occurring condition, it may be methodologically more reliable to generate scores from other informants such as partners.

5.4.3. Parent-child transmission

As for the parent-child transmission of traits, correlation analyses (without controlling the group and the child sex) showed that maternal and paternal anxiety traits were not associated with the child’s anxiety and ASD traits. For the ASD traits, there
was only a significant association between father and child traits at \( p < .001 \) level. Once incorporated the parental traits simultaneously by controlling group status and the child’s sex, Model 5.1a only supported single trait transmission of ASD traits from father to child. This finding extended the earlier findings of the current investigation that showed higher ASD traits in fathers than mothers. Further, once parental and child co-occurring anxiety and ASD traits were included in the model to address the effect of parental co-occurring traits on child traits and the cross-trait transmission (Model 5.3a), the results suggested that there was segregation for parental traits. The association between maternal ASD and child anxiety traits suggest cross-trait aggregation whereas the association between paternal ASD and child ASD traits as well as paternal anxiety and child anxiety traits suggest single trait familial transmission. Existing sub-clinical anxiety and ASD traits within parents may indicate a shared vulnerability to child traits or reflect a cumulative risk for child traits. Alternatively, this model may reflect that maternal and paternal anxiety and ASD characteristics increase the likelihood of a child developing ASD or anxiety traits as a result of the accumulative risk of paternal and maternal traits on the child outcome.

A recent investigation of familial cross-trait aggregation of anxiety and ASD within families of children with ASD and their siblings did not show a clear pattern for the origin of the effect despite using multi-informant measurement (Duvekot et al., 2016). The results showed that self-rated maternal anxiety and ASD traits were related to child anxiety and ASD traits, but paternal anxiety was only related to child ASD traits (Duvekot et al., 2016). The findings of the current research do not support these findings. The inconsistent results may reflect that in this study, the infants did not have ASD diagnoses. Once the child’s clinically elevated-ASD traits emerge, the link between parental and child traits may be influenced by the environmental factors (e.g.,
parenting stress may lead more parental anxiety and children who were exposed to anxious parenting may also develop anxiety traits).

Constantino and Todd (2005) showed that the parent-child association of ASD traits were influenced by the child’s sex. In contrast to Constantino and Todd (2005), child sex was not significantly associated with child traits in this study. It may be that the measurement of anxiety and ASD traits at the age of 2 do not reflect the full symptom manifestation of both conditions and emotional dispositions are similar in both gender group. Furthermore, another covariate risk group status was not related to parent-reported child ASD traits in Model 5.1a and 5.3a but observed ASD traits (ADOS CSS total scores) were significantly associated with the risk group status in the Model 5.1b and 5.3b. As seen in Table 5.1, the partial eta is .07 for the observed traits and .05 for the parent-reported traits. So, it may be that once incorporated different variables into the models and due to bringing together different parameters, the power of detecting the effect may deteriorate. Also, the Q-Chat was designed to measure ASD traits in infants and this measure may not be sensitive to detect ASD traits at the age 2.

The post hoc power calculation was carried out to test the chance of generating significant associations between predictors and outcome variables. For Model 5.3a, the chance of generating the association between maternal and child ASD traits was 50%, which is less than the cross-trait transmission of maternal ASD and child anxiety (75%). Similarly, the chance of generating the associations between paternal and child ASD and anxiety traits were between 51% and 56%. Based on the smallest chance, a comparable sample of 109 would thus be expected to generate power at 95% probability level.
In addition to the main analyses to accommodate the rater effect on child anxiety outcome, Model 5.1a, 5.2a and 5.3a were re-run after replicating anxiety scores with social BI factor scores in a subsample of children who completed the Stranger Approach task ($N = 77$). Although having anxious parents is a risk for child’s BI tendencies (Kiel & Buss, 2012), in the current investigation, non-significant associations may reflect that BI may be independent of the parental traits. It is important to note that, while parent-reported data was related to parent traits, observational outcome measures (Social BI, ADOS total CSS) were not related to parental traits. This may be reflecting that for some toddlers, the presentation of ASD symptoms may not be clinically elevated yet and observation tools did not capture the early signs. It is also important to consider that the association between parent-rated self- and child- traits may be significant because parental observations reflect broader, trait level behaviours. However, as mentioned in earlier chapters, due to rater effect, there may be these significant associations. There is a need for future research to employ more robust measurements to address this issue.

To conclude, the current research extends the previous investigations on developmental pathways to anxiety and ASD by exploring the parental traits which are related to trait level anxiety and ASD traits in children. Especially, ASD traits in parents raise the likelihood of a child having higher ASD traits. Moreover, paternal anxiety and maternal ASD traits seem to influence co-occurring anxiety. As it was the main starting point for the current investigation, parental anxiety, as well as ASD traits were not associated with the child social BI scores. Overall, the findings suggest that background ASD risk raises the chances for anxiety, so that part of anxiety might be related to some of the causes of ASD. But there may also be separate contributions from more anxiety specific roots (i.e., the link between father and child anxiety traits). Also, BI is not related to parental ASD traits. So, there may be parts of anxiety variance directly
attributable to background ASD risk in the current cohort. So, co-occurring anxiety traits may be attributable to parental traits.

5.4.4. Limitations and future directions

Although the current investigation expanded previous chapters by exploring the parental traits and their contribution to a toddler’s co-occurring anxiety and ASD traits, the results should be interpreted with considering possible limitations. Firstly, the model fit indices in Model 2a & 2b (RMSEA= .000 and CFI=1.00) exceeded the given model fit limits (see section 4.2.4), but there are published studies that accepted these model indices as perfect fit (e.g., Cheung & Rensvold, 2002).

Secondly, although previous investigations have generally been carried out investigating parents who received a formal ASD diagnosis (Nordsletten et al., 2016), in the current study this was explored at the trait level (given the design of the current project was the infant siblings of HR for ASD and inclusion criteria involved having an older sibling with ASD diagnosis). This may amplify the discussion of whether the findings are a consequence of genetic risk or environmental risk (due to being exposed to subclinical parental anxiety traits). Accordingly, another limitation may be that although (cross) assortative mating approach is essential to understanding the familial risk of co-existing conditions, it does not distinguish whether the link between parental and child traits is due to genetic effects or due to shared environmental risk factors.

Previous research asked both parents to rate their children’s and partner’s anxiety and ASD traits to investigate assortative mating (Duvekot et al., 2016). Future investigations may benefit from employing a multi-informant assessment of parental traits to tackle the rater effect on self-reported co-occurring traits. Also, this approach may strengthen the investigations of assortative mating in ASD families when assessing
the overlapping anxiety and ASD. Furthermore, consideration of whether the family is simplex (one child with ASD) or multiplex (more than one child with ASD) may be more helpful for further investigations on familial aggregation of ASD. This is because multiplex families confer more risk of ASD in younger siblings of probands (Schwichtenberg et al., 2010) and these investigations may expand current understanding of familial risk of co-existing anxiety traits.

Lastly, although the post hoc power calculation is stronger for the single trait transmission, for the Model 5.3a the power is medium and there need to be 109 more participants to replicate these results to generate higher effect for the generalisability of the findings.

5.4.5. Conclusions

In summary, the present research explored partner concordance of ASD and anxiety traits and familial transmission of these traits from parents to young offspring. The findings suggest that there is similarity among partners for anxiety and ASD traits and that this tendency subsequently increases the probability of an infant sibling presenting with co-occurring anxiety traits. Notably, the results demonstrated the familial risk of maternal ASD and paternal anxiety traits on child anxiety traits. Thus, observed familial aggregation could be evidence of shared genetic susceptibility and familial environmental factors for co-occurring anxiety and ASD traits. This work may have research and clinical implications for classifying toddlers at risk for ASD and co-occurring anxiety conditions once it is replicated in a larger sample.
Chapter 6. General Discussion

6.1. Recap of background and the general aims

In the current thesis, the primary aim was to explore the roots of co-occurring anxiety traits in infants and toddlers who were at increased familial risk for ASD (due to having an older sibling with ASD). To understand why anxiety is prevalent in ASD, there has been considerable research (e.g., Bellini, 2006; Duvekot et al., 2018; White et al., 2009). However, these investigations generally focused on individuals with an existing diagnosis of ASD and carried out in the mid-childhood (Duvekot et al., 2016). It may be though more informative to explore the potential risk of co-occurring anxiety before the clinical presentation of ASD symptoms unfold.

There has been substantial research indicating that behavioural inhibition (BI) in infancy and toddlerhood was a robust predictor of childhood anxiety problems in the general population (e.g., Biederman et al., 2001; Buss, 2011). However, the role of BI in ASD has not been fully explored. Kerns and Kendall (2012) suggested that to understand the underlying mechanism of co-occurring anxiety and ASD, a risk marker should be investigated in relation to both conditions. Thus, BI may be a useful early construct for investigating the overlap between anxiety and ASD traits in infancy and toddlerhood.

Furthermore, one factor that could influence the presentation of BI in the general population is the regulatory domain of temperament, effortful control (EC) (Gulley et al., 2016; White et al., 2011). In the ASD literature, EC was proposed to be an essential factor for adaptive skills and ASD severity (Garon et al., 2016; Johnson, 2012). When investigating EC in a prospective design, it could be informative to understand whether EC influences anxiety through its effects on ASD symptoms, or whether it has a primary direct effect on later anxiety, independent of ASD symptoms.
Other studies have suggested that BI tendencies and anxiety traits in children could be influenced by their parent’s protective behaviours or anxiety problems (e.g., Kiel & Buss, 2011, 2012). Based on this, one possible approach to study the co-occurring anxiety and ASD is the investigations of the patterns of familial aggregation. The role of familial aggregation of ASD traits was previously explored within the families of ASD probands (e.g., Constantino & Todd, 2005; De la Marche et al., 2015; Lyall et al., 2014; Schwichtenberg et al., 2010) but the role of familial transmission of co-occurring anxiety as well as ASD traits has not been widely explored (e.g., Duvekot et al., 2016). In addition to infant BI, the role of familial aggregation of anxiety and ASD traits from parent to infant siblings was explored the underlying mechanism of anxiety in ASD.

In the present thesis, data used to address the research questions comes from two different cohorts of prospective longitudinal investigations of infants at increased familial risk for ASD. It has been consistently shown that individuals with ASD and their first-degree relatives had co-occurring anxiety symptoms and a sub-threshold clinical presentation of anxiety or ASD traits (e.g., Jokiranta-Olkoniemi et al., 2016; Jokiranta et al., 2013; Salazar et al., 2015; Simonoff et al., 2008). This enabled the high-risk (HR) for ASD design to investigate early markers of ASD in infant siblings of probands until the age of 3 (e.g., Bedford et al., 2012; Elsabbagh et al., 2013; Hudry et al., 2014; Jones et al., 2014). Such design would also be an ideal method to investigate the overlap between anxiety and ASD traits. Indeed there have been few investigations on co-occurring anxiety traits using HR design at the age of 7 (e.g., Milosavljevic, Shephard, Happé, Johnson, & Charman, 2017; Shephard et al., 2018). But these studies may be confounded by the complex presentation of both disorders and other mediators.
This design allowed for assessing the co-occurring anxiety and ASD traits in the 24- and 36-months old HR toddlers in the current thesis.

In the present study, to reduce possible bias that could arise from shared method variance, I employed different measurement methods (parent-report vs observational) for measuring the independent variable BI. I also used multivariate approaches to test conceptual models of the relation between infant BI and both anxiety and ASD trait-based outcomes. Notably, in Chapter, 2, 4 and 5 anxiety and ASD outcome were treated as correlated outcomes simultaneously. This allowed me to test whether anxiety and ASD share similar pathways with parsimony. In Chapters 2, 3 and 4, anxiety and ASD traits were controlled in separate models to address whether the predictors were more strongly associated with the anxiety or ASD traits. This allowed me to test whether the association between BI and anxiety was present due to ASD traits. The models could correspond with the previously proposed framework that anxiety may be the consequence of ASD symptoms or anxiety may exacerbate the ASD traits (Wood & Gadow, 2010).

To address the research aims, in this thesis I asked the following related research questions:

1. Is BI associated with later anxiety traits in infants with familial risk for ASD, as it is in infants and toddlers in the general population? If so, is BI a distinct or shared predictor of early emerging anxiety and ASD traits?

2. Do BI and EC relate to each other and how do they relate to subsequent anxiety and ASD traits?

3. Does parental report of temperament traits correspond with observed traits?
4. Is BI a unitary construct or do social and non-social BI constitute distinct subdimensions? If so, do social BI and non-social BI relate similarly or differently to anxiety and ASD traits?

5. Do parental anxiety and ASD traits relate to child anxiety and ASD traits?

In this chapter, I will first summarise the main aims and findings from the empirical chapters. I will integrate the current findings with previous studies to situate the findings of this thesis. I will then discuss the research and clinical implications of the outcomes. Finally, I will discuss possible limitations for the current investigations and summarise suggestions for future research.

6.2. Summary of the findings

Chapter 2 investigated i) the predictive value of BI for later anxiety and ASD traits, ii) the bidirectional associations between BI and EC over the first two years of life (measured at 9, 15, 24 months) and their correlates with both anxiety and ASD traits (at 36-months); iii) whether BI, EC and developmental outcomes (anxiety and ASD) can be fully mediated by another dependent variable (anxiety or ASD). To investigate the aims, longitudinal parent-reported BI and EC incorporated into the cross-lagged structural equation models (SEM).

In the ASD literature, EC has been proposed to be a protective factor for a wide range of co-occurring conditions and ASD severity (Garon et al., 2016; Johnson, 2012); in the general population it has been proposed that EC could be a protective factor against emotional difficulties (Gulley et al., 2016; White et al., 2011). So, EC could interact with other genetic or environmental risk factors for ASD in shaping the likelihood of co-occurring anxiety traits. The results of Chapter 2 showed that increased parent-reported BI at 24 months was associated with anxiety as well as ASD traits at 36 months. Also, this association was specific to BI; another related temperament trait
(sadness) was not associated with anxiety traits. So, this finding prepared a foundation for the rest of the analysis in this thesis.

Furthermore, EC interrelated with BI in the prediction of anxiety and ASD traits that higher levels of EC predicted fewer later ASD/anxiety traits. It could be that better EC capacity, such as the ability to shift attention from anxiety-provoking stimuli and engaging with calming stimuli, results in better reactivity to novelty and subsequently, better emotional adjustment and lower levels of ASD severity. The exploratory mediation analyses further suggested that the degree to which early BI predicts later ASD is likely attributable to more anxious children tending to have higher levels of ASD symptoms. On the other hand, the degree to which early EC capacity predicts later anxiety is likely to be due to ASD symptoms. Overall Chapter 2 suggested that as in the general population increased BI was a stronger predictor of anxiety problems than core ASD symptoms. So it may be that co-occurring anxiety traits have separate developmental pathways and exacerbate ASD traits in children at risk for ASD.

One limitation of the analyses in Chapter 2 was that the measurement of predictors and outcomes both relied on parent-reported data. In Chapter 3, I, therefore, employed a multi-method measurement of BI and EC to tackle the bias that could arise from reporter characteristics and shared measurement variance (due to asking one reporter to rate predictors and outcome measures). The first aim of Chapter 3 was to explore observed temperament traits in the LR group and the HR group that was further subdivided by the outcome decision. To assess temperamental traits BI and EC, I developed a novel coding scheme to apply to the widely used research measure of the Autism Observational Schedules for Infants (AOSI). This approach had two main advantages: firstly, it allowed me to apply the new coding scheme to existing data to explore the convergence or distinction between early signs of ASD and temperament
traits (second aim) as well as to assess the agreement between the parent-reported and observed temperament traits (third aim); secondly, to explore the relationships between temperament traits in infancy and later anxiety and ASD traits (final aim).

The principal component analysis of observed temperament traits yielded five different components: BI, EC, sadness, positive affect and engagement. Due to the lack of internal consistency, EC was excluded from further analyses. According to Posner and Rothbart (2000), EC emerges at the end of the first year of life and becomes more pronounced during the preschool period. Perhaps the behaviours that were related to EC were not common at this age and parent report could detect these behaviours better.

Only the parent-reported fear and the observed BI component indicated good agreement between methods. In addition to the specificity of BI, in Chapter 3, I showed that the measurement of the BI in the current thesis is reliable. The lack of agreement between methods for other temperament traits may be due to the effect of the parental characteristics on the rating of child temperament traits (Rothbart & Goldsmith, 1985) or that the context of the observation and the questionnaires can lead to a measurement of different dimensions of the same construct (Kiel & Hummel, 2017). Although the context of the AOSI was not designed to be fear-arousing, the observed behaviours evidenced the child’s reactions to novelty (latency to initiate interaction) as well as sudden stimulations which are in line with the main characteristics of BI (Fox et al., 2001; Kagan et al., 1984). The results also suggested that observed BI did not confound the identification of early markers of ASD and BI was not associated with the later ASD traits. Even if BI and ASD shares some phenotypes (e.g., withdrawal), in infancy, no significant association between BI and the AOSI scores in the HR group may be evident of separate pathways for the anxiety and ASD. Nonetheless, BI was associated with anxiety traits but not with the ASD traits within the HR group at the age of 3, consistent
with the findings of Chapter 2. This is probably due to the different mechanism, such as physiological reaction in the novelty, that underpins the association between BI and anxiety rather than ASD traits. Taken together, BI may be an early childhood characteristic that distinguishes co-occurring anxiety traits from ASD. The findings of Chapter 3 extended Chapter 2 to an observational context and consistent with it, the results indicated an independent pathway to co-occurring anxiety in infants at HR for ASD.

One limitation of Chapter 3 was that the behavioural task (the AOSI) was primarily a low-stress context. This may have limited my opportunity to observe temperamental traits like EC and could lead to reduced expression of BI. Furthermore, the context of the AOSI did not allow me to examine whether BI disposition is consistent or differently organised in social and non-social contexts, which may be relevant to its relation to later ASD vs anxiety traits. In Chapter 4, I thus extended the exploration of BI by employing structured observational tasks specifically developed to introduce novelty and designed to elicit BI dispositions: The Stranger Approach and the Unpredictable Toy tasks (Fox et al., 2001). In this chapter, the first aim was to examine contextual differences in BI (social vs non-social) by testing associations between social and non-social BI scores; their relation to parent-report and examiner-rated measures; and their cross-sectional association with early emerging anxiety and ASD traits at 24 months. Moreover, the second aim was to explore the role of parental over-protective behaviours that were observed during the situational tasks and its association with anxiety and ASD traits of toddlers. To assess the aims of this study, I created social and non-social BI scores by integrating observed BI components from the Stranger Approach (social) and the Unpredictable Toy (non-social) tasks, experimenter rating of social inhibition and inhibition to object and environment, parent-reported shyness.
(social) and fear (non-social). In contrast to Chapter 2 and 3, in Chapter 4, measurement of anxiety traits was carried out at the 24 months visit because the majority of this cohort had not yet reached the age of 3 in the ongoing programme of study.

The results of Chapter 4 suggested that among observed social BI components, only withdrawal was associated with both parent-report shyness and experimenter rated social inhibition. Observed non-social components were not significantly correlated with parent-reported fear but were correlated with experimenter-rated object inhibition. Although there were associations between BI measured via different methods (e.g., parent-reported shyness and social withdrawal and experimenter rating of social inhibition), none of the individuals’ observed BI components was correlated with the anxiety as well as ASD traits that were measured at 24 months. However, when I created factor scores for social and non-social BI, results showed that the social BI factor was related to both higher anxiety and ASD scores whereas non-social BI was negatively associated with ASD. Also, there were no significant associations between non-social BI and anxiety. This could suggest that specifically social BI may show more phenotypic overlap between anxiety and ASD at the age of 2. On the other hand, lower levels of non-social BI were associated with higher ASD traits but not with anxiety. The negative association between non-social BI and ASD is somewhat unexpected. This may be explained by the lack of awareness of danger towards the threatening non-social stimuli, but further investigations should be carried out. Moreover, parental protective behaviours appeared to be more closely related to child anxiety traits than ASD traits. Once controlling for the ASD traits, the association between social BI, parental protective behaviours and anxiety remained significant. In contrast, when controlling for anxiety traits, associations between social BI and ASD become non-significant, but the association between non-social BI and ASD remained significant. These results
highlighted the interaction between social BI and parental protective behaviours on child anxiety traits.

Chapter 4 introduced the role of parental protective behaviours. In the general population, evidence suggested that parental protective behaviours may be influenced by parental anxiety traits and ultimately may relate to both child BI and anxiety traits (e.g., Hudson, Dodd, & Bovopoulos, 2011; Muris et al., 2011). So, investigating the influence of parental anxiety may be another way of understanding the underlying mechanisms of overlapping anxiety and ASD. In the ASD literature, evidence suggests that parental anxiety and ASD traits could aggregate within family members of individuals with ASD (e.g., Duvekot et al., 2016). To investigate the role of familial aggregation of co-occurring anxiety and ASD traits, in Chapter 5 the aim was to explore the single trait and the cross-trait transmission of anxiety and ASD.

Regarding the single trait transmission, the results showed that the paternal ASD score was related to child ASD traits, but when incorporating the co-occurring conditions into the model to test the cross-trait transmission, results suggested that maternal ASD traits were associated with both child anxiety and ASD traits while paternal ASD was associated with child ASD, and paternal anxiety was associated with the child anxiety traits. This raises the possibility that there may be parent-of-origin effects in early risk for ASD and anxiety and/or that there are gender differences in the manifestation of these traits. The results may indicate that some parts of the offspring anxiety traits can be attributable the parental ASD traits (maternal ASD to child anxiety and ASD transmission), but there may be some anxiety specific roots (father to child transmission of anxiety). Also, the association between parental traits and child BI (the predictor of child anxiety traits) was not related to parental ASD, indicating the possibility for part of anxiety traits can be attributable to parental ASD risk. As shown
in Chapter 2, EC explains the variances in anxiety traits via ASD. So, it may be that parental ASD traits link to child anxiety due to their EC capacity. But some parts of the child anxiety can be separable and child anxiety traits may explain this part.

Overall, these findings extended the previous literature on co-occurring anxiety problems in ASD by studying HR for ASD infant siblings. The findings suggest that temperamental disposition of BI (especially social BI), is an early marker of early-emerging anxiety problems. Moreover, parental behaviours, parental trait level anxiety and ASD account for the co-occurring anxiety in ASD. These results may suggest that in the early developmental period, anxiety traits may have different developmental pathways but some of the associations between anxiety and ASD can be explained by the ASD-related difficulties (e.g., EC).

6.3. Integration with previous literature

6.3.1. Do anxiety and ASD have shared or distinct developmental mechanisms?

Wood and Gadow (2010) proposed a framework for conceptualising the co-occurring anxiety within the ASD. This framework suggested that monomorbid anxiety may be altered by an ASD pathogenic process and result in ASD-specific presentation, which could be considered as a unique syndrome apart from ASD. Indeed, Kerns et al. (2014) showed that children with ASD presented ASD specific anxieties. Even though in the current thesis the outcome anxiety measure was not designed to measure the ASD-specific anxieties, mediation analysis in Chapter 2 suggested that EC capacity relates to traditional anxiety due to existing ASD traits. So, the findings partly support this suggestion, but further research is needed to test whether this also related ASD-specific anxiety presentation. Previous literature that investigated a factor that could explain the overlap between anxiety and ASD focused on insistence of sameness (Factor et al., 2016; Gotham et al., 2013) and intolerance to uncertainty. (Vasa et al.,
2018; Wigham et al., 2015). These investigations commonly suggested that insistence of sameness and intolerance to uncertainty relates to anxiety problems due to ASD symptoms. Bellini (2004, 2006) similarly, suggested that social skill deficits and lower levels of assertion in ASD influenced the anxiety symptoms in children with ASD. But the findings of the current investigations contradict with this. As mentioned previously, these findings highlight the importance of exploration of overlap at early developmental stages. The results of the current thesis suggest that at earlier stages of the development, there may be distinct pathways to both anxiety and ASD but at later stages, the convergence begins between risk factors and ASD symptoms.

Moreover, the results indicate that BI is associated with ASD due to existing anxiety traits. Temperamental dispositions may increase the anxiety traits and anxiety traits may increase the likelihood of a child to have more ASD traits. It may be that some part of the association between anxiety and ASD may explain the true comorbidity. Also since anxiety does not fully mediate the association between EC and ASD (Chapter 2), this does not support the second proposed model by Wood and Gadow (2010) which is that individuals with ASD who have additional anxiety symptoms may differ from those with ASD who do not have anxiety due to either phenotypic heterogeneity or a sub-type of ASD, in other words, anxiety can mediate or moderate the severity of ASD. However, future studies should explore this in detail by comparing children with ASD who have and have not additional anxiety problems.

Wood and Gadow (2010) also suggested that ASD and anxiety may represent true comorbidity with distinct phenotypes, aetiology and causal pathways. In the clinical sample of individuals with anxiety disorders (without ASD diagnosis), presented at a increased rates of ASD traits (Kleberg et al., 2017; Puleo & Kendall, 2011), for example, social communication difficulties and restricted repetitive behaviours (Halls et
al., 2014; Rutter, Bailey, et al., 2003). Also, individuals with anxiety disorders can score above the cut-off for an ASD diagnosis even if excluding some items that share similar phenotypes (Anderson et al., 2012). The results from the current thesis partly support this model. Chapter 2, 3 and 4 showed that the predictive value of BI was specific to the anxiety. However, as mentioned previously, some part of the association between anxiety and ASD is partly explained by the EC. So, the findings of the current investigations indicated that associations between BI and co-occurring anxiety traits are in accord with the previous findings in the general population (e.g., Clauss & Blackford, 2012; Hudson, Dodd, Lyneham, et al., 2011; Kiel & Buss, 2011) and clinical samples (Dougherty et al., 2011; Dougherty et al., 2013). Especially, heightened reactions to novelty in children at HR for ASD as young as 15 months old were consistently associated with early emerging anxiety traits. This highlight that some part of the underpinnings of anxiety in the general population and in the ASD shares similar causal pathways.

One possible explanation for the factors that distinguish the association between BI and anxiety may be that since temperament includes biological dispositions when children come across to novelty, they could have physiological changes in the autonomic nervous system such as increased heart rate (Viana et al., 2017). Also, in DSM-5 symptoms of anxiety include physiological responses such as fight-or-flight responses whereas the ASD do not require physiological changes (American Psychiatric Association, 2013). So, BI may have a unique association with physiology as well as subsequent anxiety traits. Indeed, Kushki et al. (2013) showed that signals of the autonomic nervous system, heightened arousability, was an indicator of anxiety in children with ASD. However, this study measured the heartrate in a situation that did
not involve novelty, further research may be of benefit measuring physiological changes during the novel contexts.

6.3.2. Measurement of BI

In the clinical practice, and research (either in the HR for ASD design or general ASD studies), the diagnostic decision depends on multimethod assessments such as parent reports (e.g., the ADI-R and SCQ) and observational measurement (e.g., the AOSI and ADOS). This approach increases the reliability of the information gathered via different techniques. Measurement of temperament (specifically, BI) in the general population is carried out using combining parent-rated age equivalent tools (e.g., Putnam et al., 2006; Putnam et al., 2014) and observational standardised laboratory tasks that highly involve either a social novelty with an unfamiliar adult or non-social toys (Fox et al., 2001; Kagan et al., 1984; Planalp et al., 2017). This method is important in developmental research and both measurement techniques have unique advances and proneness to possible bias. Parent-reports are valuable since they reflect broader observations of childhood trait level temperamental dispositions (Rothbart & Hwang, 2002), but these perhaps could be influenced by the parental characteristics (e.g., parental psychopathology and personality; De Los Reyes & Kazdin, 2005; Kerstis et al., 2013) as well as tendency of parents to magnify similarities or differences between offspring (Saudino, 2003). Observational measures of BI increases the chance of observing ecologically valid responses that subsequently increase the generalisability of the research outcome. However, the observational method could capture the state of the child; for example, tiredness of the child could influence children’s reactions to the novelty. Nevertheless, to overcome possible bias, studies also employed multi-method approaches for generating more wide-ranging BI scores (e.g., Barker et al., 2015; Walker et al., 2014).
However, these studies were conducted in the general population, but there have been few investigations of BI using observational tasks in ASD samples (e.g., Scherr et al., 2017) and other temperament traits (e.g., Gulsrud et al., 2010; Jahromi et al., 2012). There has been considerable research that solely relied on parent-reported temperament traits in the general ASD literature (e.g., Adamek et al., 2011; Brock et al., 2012; De Pauw et al., 2011; Hepburn & Stone, 2006) and HR for ASD design (Clifford et al., 2013; del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014; Garon et al., 2009; Garon et al., 2016; Shephard et al., 2018; Zwaigenbaum et al., 2005) so the novelty of the current thesis was to address this gap by employing both parent-report and observational measures of BI.

In Chapter 3, I used a novel coding scheme to investigate the behavioural manifestations of BI and to measure agreement between observer and parent-reported evaluations BI. The results showed that the BI component that included the child’s initial latency to engage with the experimenter expressed facial fear and reference to the parent was in line with the parent-reported BI. As in line with the definition of BI, this finding suggests the importance of initial reactions to novelty. However, other observed components (sadness, pleasure, engagement) were not in agreement with the parent-reported measures. This may be because the context of observation did not provoke these behaviours because it was not a stressful situation and parent-report questionnaires reflected different facets of the traits. For example, observed sadness can resemble boredom or frustration in non-controlled observational setting whereas parent-report temperament tools could reflect the lower level mood, suffering, disappointment or object loss for capturing sadness (e.g., Putnam et al., 2006; Putnam et al., 2014). So, further research could benefit from setting similar parameters when measuring other temperament traits.
In Chapter 4, I used parent-report, observed and global ratings of child BI to create social and non-social BI factor scores via confirmatory factor analysis. For instance, Brooker et al. (2013) created factor scores through principal component analyses by submitting parent-reports and observational BI scores. This approach is more in line with Barker et al. (2015) who created a global measure of BI by averaging observed and parent-reported data. Perhaps merely averaging scores across methods is more powerful when the variables are correlated, but on the contrary, when there was less variance between variables, the association between a composite score and an outcome measure may not be significant. So, the application of the principle or confirmatory factor analysis may generate more reliable variables and thus could prevent measurement errors.

6.3.3. Parental behaviours and intergenerational effect

Parental over-protective behaviours in the novel context were associated with the anxiety symptoms in children from the general population (e.g., Johnson et al., 2016). Despite there has been lack of investigations on how parental behaviours play a role in children with ASD, in the current thesis, child BI was associated with parental behaviours in the social context (as shown in Chapter 4). This result may indicate that similar to BI, parental overprotectiveness predicts childhood anxiety. So, it may be that roots of anxiety may be similar in both populations, indicating distinct pathways to anxiety. This association could be evident due to the parent-child genetic link or due to being exposed to parental behaviours that could constitute an environmental risk.

To investigate the association between parental and child’s anxiety and ASD traits, I integrated paternal, maternal and child traits into path models. Especially, parental traits were treated as correlated predictors since the high correlations between parent traits could be attributable to assortative mating. In the ASD research,
investigation of the familial transmission of co-occurring anxiety and ASD is scarce (Duvekot et al., 2016). But there has been considerable evidence on a single trait, ASD transmission from fathers to children (e.g., Constantino & Todd, 2005; De la Marche et al., 2015; Lyall et al., 2014). To current knowledge, there are no investigations that explored familial aggregation of the co-occurring anxiety and ASD in the HR for ASD design. The results of the current thesis showed that there were cross-symptom associations (only from mothers ASD to both child anxiety and ASD) as well as single trait transmission (paternal anxiety-child anxiety; paternal ASD-child ASD) between parents and children ASD and anxiety symptoms. This result is partly in line with Duvekot et al. (2016) who showed a cross-symptom association. This association may be due to different methods used in the analysis, for example, Duvekot (2016) did not integrate paternal and maternal traits in to the model, but used multi-informant report of child traits whereas other studies that assess the transmission of only ASD traits and the current study treated parental characteristics as correlated predictors (De la Marche et al., 2015; Lyall et al., 2014). Overall, the associations between parental and children’s anxiety and ASD traits suggest that some part of the co-occurring anxiety and ASD may exist due to familial risk. Especially, maternal ASD traits may explain the overlapping structure of both conditions whereas paternal traits explain the single trait transmission. This may be due to the other factors that related to child’s anxiety (e.g., EC), parental traits may be related to other temperamental factors and in turn results in increased ASD traits. However, even though the current investigation was novel in terms of exploring the current design, there should be more investigations that address the familial transmission, child temperamental regulation and anxiety and ASD outcome.
6.4. Implications

The outlined findings of the current thesis have several key research and clinical implications.

6.4.1. Research implications

To date, infant siblings at HR for ASD design have provided insight into early markers of ASD before the age of 3 when clinically reliable ASD diagnosis can be made (e.g., Elsabbagh et al., 2013; Jones et al., 2014; Szatmari et al., 2016). There has been less research investigating co-occurring anxiety in ASD in mid-childhood using this design (Milosavljevic, Shephard, Happé, Johnson, & Charman, 2017; Shephard et al., 2018). Although these studies report important findings on correlates of co-occurring anxiety, later in the development, anxiety and ASD phenotypes could be influenced by various other risk factors, thus confounding the results. Indeed, studies attempted to investigate the overlap between anxiety and ASD in children and adolescents with ASD failed to show consistent results (e.g., Duvekot et al., 2018; Green et al., 2012; Pickard et al., 2017). The prospective HR design provided methodologic advances to investigate BI as a risk of early emerging anxiety in infants and toddlers who were increased familial risk for ASD. The findings of Chapter 2, 3 and 4 showed that once controlling for ASD traits BI, especially social BI, distinctly associated with anxiety traits. This method provided a fundamental basis for investigating pathways to anxiety and ASD, and further research would benefit this method to identify underlying mechanisms of other highly co-occurring psychiatric conditions, e.g., ADHD.

Another common methodology in developmental research is to ask a single reporter to rate a predictor and an outcome measure which leads on to yielding associations due to shared measurement invariance. In Chapter 2, the analyses were run
using parent-reported temperament traits (both BI and EC) and outcome measures (anxiety and ASD). Even though the results confirmed in Chapter 3, studies could benefit measuring parent-report traits in conjunction with observed measures and could enlarge the generalisability of findings.

Another important research implication may be related to statistical approaches I employed to understand these highly overlapping conditions. In Chapter 2, 3, 4 analyses were re-run controlling for the effect of co-occurring anxiety on ASD traits or vice versa. As anxiety and ASD could share some phenotypic expressions (e.g., lack of eye contact) and the measurement tools could have similar items that contribute to the higher variation between two constructs (Wood & Gadow, 2010). When exploring the association between BI and anxiety, controlling the effect of ASD traits helped us to understand whether the effect of BI on anxiety was generated due to ASD traits. Moreover, although SEM provided an advantage to integrate anxiety and ASD traits into the models simultaneously, the current investigations were run combining the HR and LR groups. This approach did not raise concerns since the BI pattern was the same in both groups and exploratory analyses confirmed that the source of the association was the HR group. Also, to address this group variable (HR vs LR) was controlled in all analyses. To be able to understand the pattern of the results in the HR and LR groups separately, with more LR infants, a group level SEM model may be feasible. Future research concerning complex co-occurring conditions (e.g., ADHD and ASD) may benefit from this approach.

6.4.2. Clinical implications

The findings of this thesis mentioned above have important clinical implications. The robust association between BI and early emerging anxiety traits suggests that social BI may be an identifiable risk marker of co-occurring anxiety in infant and toddlers who
also at risk for ASD. Clinicians could identify children who are possibly at risk for developing co-occurring anxiety by screening questions as well as brief observations that target social BI during their clinical practice. The screening questions could include reactions to unfamiliar peers or adults while observations could focus on children’s withdrawal and how long they take to interact with the clinicians comfortably. This screening procedure may be more useful to identify children who perhaps benefit from early interventions for better emotional adjustment.

Social BI may be a possible early preventative intervention target, especially for those infants and toddlers who are at familial risk for ASD. Further results of this thesis showed the influence of parental over-protective behaviours on anxiety traits of the child. Intervention programs that targeted children who were characterised as highly inhibited in social context have shown a preventative effect for childhood anxiety problems (Rapee, 2013; Rapee, Kennedy, Ingram, Edwards, & Sweeney, 2010). These interventions target parents and provide psychoeducation on how to approach their offspring avoidance, how to adjust their overprotective behaviours and how to manage their offspring anxious behaviours (Rapee et al., 2010). Intervention strategies that target preschool children suggested that the combination of social skills training for children and parental psychoeducation increase the effectiveness (Lau, Rapee, & Coplan, 2017). Parent training interventions should be complemented with social skill training (such as guided playgroup sessions with unfamiliar peers).

Another important finding of the current thesis was that EC appeared to be a protective factor for co-occurring anxiety traits, perhaps due to present lower levels of ASD traits. To current knowledge, there have not been interventions that target attention regulation in preschoolers with ASD, but in the general population, training that targets attentional control improved EC skills in children (Posner & Rothbart, 2007).
Interventions that target strengthening EC capacities through attentional training, as well as parent-mediated interventions in late infancy could thus result in better emotions and behaviour regulation and adaptive functioning.

6.5. Limitations

At the end of the discussion sections for each empirical study (Chapter 2, 3, 4 and 5), specific limitations about the studies were summarised. Here, I will discuss limitations that are more relevant to the broader issues.

Despite the novel findings of the current thesis, it is essential that they can be considered in the context of some limitations. Firstly, although the overall sample size of the current investigations is moderate and there may be some limitations due to unequal sample sizes in groups. Especially, the LR groups were quite small and it was not possible to run a multi-group analysis. Instead, the investigations were carried out by combining both HR and LR groups; and the effect of the risk group (e.g., group differences on variables) was controlled by treating it as a covariate. This approach had the advantage and was common in the field (e.g., Shephard et al., 2018), but it may limit the current research effort to examine whether the developmental mechanisms are same in both HR children and LR controls.

Secondly, another issue may be related to the HR of ASD design. As mentioned earlier the recurrence rate of ASD in infant siblings of probands is around 20% (Ozonoff et al., 2011). So, the generalisability of the findings may be a problem due to the chances of getting an ASD outcome is low. Indeed, in Chapter 2 and 3, the number of HR-ASD siblings who later diagnosed with ASD was 17. In Chapter 4 and 5, since the outcome ASD decision have not been made (the study is still ongoing). Nevertheless, it can be argued whether the observed association between BI and anxiety is driven due to children who actually have ASD. This may be a broad issue for the HR
for ASD designs (Zwaigenbaum et al., 2007) and large collaborative projects such as EU-AIMS Longitudinal European Autism Project (e.g., Tillmann et al., 2019) may help to address this issue by combining data that collected from different sites.

Lastly, I had limited control on the sample size of the current research given the study design used I used complex statistical models which required a larger sample size. However, to address this issue, post hoc power analyses were calculated. Especially, in Chapter 2, 4 and 5, power calculations were carried out for specific pathways. But these calculations were asymptotic and do not take into account the other aspect of model estimation (e.g., model fit indices, the estimator used in the analysis). For the future studies, application of SEM models should depend on clear hypothesis and there should be a minimum of 100 participants per analyses.

An important strength of the current thesis is the multi-method assessment of BI in infants and toddlers. In Chapter 2, 3 and 4, I addressed possible limitations of measuring BI in the early developmental period and tried to acknowledge these in the analysis. Also, the results of the current research suggested that there is a strong link between BI and anxiety traits. Nevertheless, a few limitations need to be mentioned in relation to the concept of BI and anxiety. Operationalisation of BI refers to initial responses of fearfulness, wariness and withdrawal to novelty and it differs from pathologic anxiety. Also, the description of anxiety involves fearfulness and worry. Since anxiety measured at the trait level in the current thesis, it was likely to observe sub-clinical levels of anxiety traits. Nigg (2006) suggests that BI may be a milder expression of anxiety problems and rather than a risk factor in childhood, it may be the early presentation of anxiety in infants and toddlers. However, due to the clear operationalisation of BI and anxiety, it is difficult to tear apart whether BI is an early manifestation of anxiety or temperamental disposition.
6.6. Targets for future investigations

As mentioned earlier, one possible mechanism that can explain some of the distinct relationships between BI and anxiety traits may be physiological responses. Such as changes in the autonomic nervous systems when being exposed to novel or threatening stimuli may explain the unique association between BI and anxiety. Indeed, evidence suggested that children with ASD had atypical (e.g., over-aroused) physiological changes in a non-threatening situation, which were more attributable to their anxiety symptoms (Kushki et al., 2013). In the general population, physiological changes in the parasympathetic nervous system (e.g., respiratory sinus arrhythmia or heart rate) generally measured during the observational BI tasks (e.g., Viana et al., 2017). These investigations indicated that interaction between the BI and physiological changes had a buffering effect on child anxiety problems. Future research may extend the findings of the current thesis by investigating the physiological changes during the Stranger Approach and the Unpredictable tasks.

Moreover, a longitudinal latent class analysis may help better understanding the changes of the BI over the course of development and its associations with the mid-childhood anxiety and outcomes. Evidence in the general population has shown that children who were characterised by heightened BI over time were more likely to develop anxiety symptoms in mid-childhood (Degnan et al., 2014). Latent class analysis in the HR for ASD design may help to investigate the pattern of change on BI over time, and its associates later in childhood after clinically elevated ASD symptoms unfold. For example, Shephard et al. (2018) showed no associations in their path models between BI components (shyness, fear) and both anxiety and ASD traits measured at the age of 7 whereas the current thesis showed a significant association. As argued earlier in the current chapter, the association between BI and anxiety may be more evident at the
early development, but at the later stages, with the full symptom expressions of ASD, it may be harder to distinguish the relationships.

Studies also suggest that BI in toddlerhood relates to specific anxiety problems, especially, social anxiety disorders in children and adolescents in the general population (e.g., Biederman et al., 2001; Clauss & Blackford, 2012). As social anxiety and ASD have phenotypic overlap (Bellini, 2006; van Steensel et al., 2011), investigating BI as an early marker of childhood social anxiety may extend the previous work that showed social anxiety problems predict social communication difficulties of ASD over time. (Pickard et al., 2017). Especially, in Chapter 4, social-BI was specifically related to child anxiety symptoms. It may be more fruitful to investigate whether early social BI disposition drives the association between social anxiety and ASD.

Lastly, Kerns et al. (2014) suggested that children can represent ASD-specific fears (such as fear of hoovers or hand dryers). The current investigations were not designed to address ASD-specific fears, but since BI perhaps more in line with the traditional presentation of co-occurring anxiety in ASD, investigating the association between BI and ASD-specific anxiety could expand the current understanding of the underlying mechanisms of the co-occurrence between anxiety and ASD.

6.7. Conclusion

This thesis was to first to investigate the underlying mechanisms of overlapping anxiety and ASD in the HR for ASD sibling design. The first key finding is that BI, specifically the social BI as a reactive domain of temperament was a robust predictor of early emerging anxiety traits and this association was not shared with the ASD traits. Moreover, EC, the regulatory domain of temperament was related to anxiety due to ASD traits, so, anxiety could be present as consequences of ASD symptoms. In terms of measurement of BI, parent report BI and observed BI (which was enriched with the
initial withdrawal toward novelty and fear reactivity) presented agreement. Moreover, parental over-protective behaviours that were observed in the novel social context were related to anxiety traits of toddlers and it was independent of BI tendencies of toddlers.

Lastly, maternal and paternal anxiety and ASD was associated with each other and maternal and paternal ASD traits were associated with the child ASD traits whereas paternal anxiety traits and maternal ASD was related to toddler’s anxiety traits. The findings of the current thesis suggest that the early social skill and attention training interventions that target infants and toddlers (by working through parents) in conjunction with the parent-training programmes could reduce the early reactive disposition for anxiety as well as regulatory dispositions to both anxiety and ASD.
Appendices

Appendix 1: Chapter 2 sensitivity analysis

Cross-lagged model for examining the specificity of BI in predicting anxiety.

We tested the specificity of BI by using a cross-lagged model to measure longitudinal and concurrent relationships between (i) BI and (ii) sadness (as another subdomain of negative affect), and their association with anxiety traits at 36 months. Overall model fit for the second model was acceptable (CFI = .970, RMSEA = .117, and SRMR = .034), despite significance for the model ($\chi^2$ (4) = 11.59, $p = .021$). Risk group was significantly related to BI at 9 and 15 months and sadness at 24 months ($\beta = .15$, $p = .019$; $\beta = .13$, $p = .040$; $\beta = .13$, $p = .024$; respectively). The autoregressive pathways indicated stable patterns in magnitude of associations for BI (9-15 months: $\beta = .54$, $p < .001$; 15-24 months: $\beta = .49$, $p < .001$) and decrease in the magnitude of associations in sadness between the 15 and 24 months (9-15 months: $\beta = .51$, $p < .001$; 15-24 months: $\beta = .30$, $p < .001$). BI and sadness was associated with each other concurrently at each time point (9 months: $\beta = .55$, $p < .001$; 15 months: $\beta = .28$, $p = .002$; 24 months: $\beta = .22$, $p = .015$) but the magnitude of the relationship decreased over time.

Cross-lagged paths indicated that more BI at 15 months is related to more sadness at 24 months ($\beta = .32$, $p < .001$) but there were no significant relationships between sadness and anxiety problems at any time point ($p \geq .133$). Like the Model 2.1, greater BI at 24 months was significantly related to higher anxiety scores at 36 months ($\beta = .30$, $p = .003$). Thus, the predictive relation between BI and anxiety was not shared with sadness and BI was used in the rest of the analysis as a subdimension of negative reactivity.
Post hoc indirect effects

We used a series of post hoc analyses to ask whether we could disentangle the nature of the relationship between BI, EC, anxiety and ASD in infancy. First, we tested whether the effects of high levels of toddler EC seemed to act on similar or distinct developmental paths to ASD and anxiety. To do this, we first tested whether the relation between 15 months EC and later anxiety was mediated by 24-month BI. There was evidence of indirect effect of EC (15 months) on anxiety (36 months) via BI (24 months; β = -.09, p = .031, 95% CI [-.17, -.03]). The direct effect (β = -.23, SE = .08, p = .005) and the total effect were significant (β = -.32, SE = .08, p < .001). Therefore, 28% of the effect of 15m EC on 36m anxiety was operating through 24-month BI. This suggests that some of the reason that better 15m EC relates to later anxiety is that it reduces the likelihood of infant fear turning into toddler shyness. Second, we tested whether the relation between 15 months EC and later ASD was mediated by 24-month BI. There was a significant direct effect between EC (15 months) and ASD (36 months) (β = -.24, SE=. .08, p = .002). However, the indirect effect (via BI) was not significant (β = -.06, p = .080, 95% [-.11, -.004]), suggesting BI at 24 months was not a significant mediator of the relationship between 15m EC and 36-months ASD scores. Thus, this analysis suggests that lower levels of EC at 15 months do not relate to later ASD through toddler BI but through a different path.

Re-running analysis with listwise deletion

Model 2.1: Longitudinal association between BI and anxiety.

The analysis was run for 110 observed variables. The fit indices of the autoregressive model indicated a good fit to the data (χ²(1) =.035, p = .851; CFI = 1.00, RMSEA = .000, and SRMR = .003). The association between risk group and BI at 9
months was significant ($\beta = .15, p = .024$). The association between risk group and BI at 15 months become non-significant ($\beta = .13, p = .062$). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .55, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .54, p < .001$), indicating stability between time points.

Regarding the timing, higher levels of BI at 24 months were associated with higher levels of anxiety at 36 months ($\beta = .39, p < .001$).

**Model 2.2: Longitudinal association between BI, EC and anxiety**

The fit indices of the autoregressive model indicated a good fit to the data ($\chi^2 (4) = 4.83, p = .305$; CFI = .996, RMSEA = .043, and SRMR = .022). The association between risk group and BI at 9 months was significant ($\beta = .16, p = .024$) and risk group 15 months BI become non-significant; and between risk group and effortful control was significant at 15 months ($\beta = -.19, p = .005$). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .55, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .53, p < .001$). Effortful control at 9 months related to subsequent effortful control at 15 months ($\beta = .54, p < .001$) and effortful control at 15 months associated with effortful control scores at 24 months ($\beta = .50, p < .001$).

As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months ($\beta = -.23, p = .003$). The association between BI at 15 months and effortful control at 24 months become significant ($\beta = -.17, p = .030$). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety symptoms ($\beta = .28, p = .002; \beta = -.27, p = .022$; respectively).
Model 2.3: Longitudinal association between BI, EC, anxiety and ASD

The fit indices of the autoregressive model indicated a good fit to the data ($\chi^2 (4) = 4.77, p = .311; \text{CFI} = .997, \text{RMSEA} = .042, \text{and SRMR} = .024$). The association between risk group effortful control was significant at 15 months ($\beta = -.19, p = .014$). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .55, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .53, p < .001$). Effortful control at 9 months related to subsequent effortful control at 15 months ($\beta = .54, p < .001$) and effortful control at 15 months associated with effortful control scores at 24 months ($\beta = .50, p < .001$).

As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months ($\beta = -.23, p = .004$). Effortful control at 9 months was associated with ASD at 36 months ($\beta = .27, p = .001$). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety symptoms ($\beta = .28, p = .003; \beta = -.27, p = .003$; respectively) and ASD symptoms ($\beta = .21, p = .015; \beta = -.58, p < .001$; respectively). Anxiety and ASD was significantly associated with each other at 36 months ($\beta = .45, p < .001$).

There was also a significant indirect effect of effortful control on ASD through anxiety ($\beta = -.17, p = .004, 95\% \text{ CI} [-.28, -.09]$). The direct effect was significant ($\beta = -.42, SE = .09, p < .001$), and the total effect was ($\beta = -.58, SE = .07, p < 0.01$). Anxiety partially mediated the association between effortful control and ASD accounting for the 27% of the total effect.
Re-running analysis covarying for sex

**Model 2.1: Longitudinal association between BI and anxiety.**

The fit indices of the autoregressive model indicated a good fit to the data ($\chi^2 (1) = .14, p = .71$; CFI = 1.00, RMSEA = .00, and SRMR = .005). The association between risk group and BI at 9 and 15 months was significant ($\beta = .15, p = .017; \beta = .13, p = .039$, respectively). Sex was not significantly related to any variables ($p \geq .056$). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .52, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .50, p < .001$), indicating stability between time points. Regarding the timing, higher levels of BI at 24 months were associated with higher levels of anxiety at 36 months ($\beta = .34, p < .001$).

**Model 2.2: Longitudinal association between BI, EC and anxiety**

The fit indices of the autoregressive model indicated a good fit to the data ($\chi^2 (4) = 4.73, p = .316$; CFI = .997, RMSEA = .036, and SRMR = .021). The association between risk group and BI at 9 and 15 months was significant ($\beta = .15, p = .016; \beta = .13, p = .040$, respectively); and between risk group and effortful control was significant at 15 months ($\beta = -.16, p = .012$). Sex was significantly related to effortful control at 24 months ($\beta = .23, p = .001$). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .52, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .48, p < .001$). Effortful control at 9 months related to subsequent effortful control at 15 months ($\beta = .55, p < .001$) and effortful control at 15 months associated with effortful control scores at 24 months ($\beta = .50, p < .001$).
As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months (β = -.21, p = .007). The association between BI at 15 months and effortful control at 24 months was significant (β = -.17, p = .030). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety symptoms (β = .24, p = .006; β = -.29, p = .014; respectively).

Model 2.3: Longitudinal association between BI, EC, anxiety and ASD

The fit indices of the autoregressive model indicated a good fit to the data (χ² (4) = 3.44, p = .487; CFI = 1.00, RMSEA = .000, and SRMR = .018). The association between risk group and BI at 9 and 15 months was significant (β = .15, p = .016; β = .13, p = .039, respectively); and between risk group and effortful control was significant at 15 months (β = -.16, p = .012). Sex was significantly related to effortful control at 24 months (β = .24, p < .001). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months (β = .52, p < .001) and BI at 15 months associated with BI scores at 24 months (β = .48, p < .001). Effortful control at 9 months related to subsequent effortful control at 15 months (β = .55, p < .001) and effortful control at 15 months associated with effortful control scores at 24 months (β = .49, p < .001).

As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months (β = -.21, p = .008). The association between BI at 15 months and effortful control at 24 months was significant (β = -.17, p = .020). Effortful control at 9 months was associated with ASD at 36 months (β = .29, p < .001). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety symptoms (β = .25, p = .005; β = -.29, p = .014).
.27, \( p = .016 \); respectively) and ASD symptoms \( (\beta = .20, \ p = .003; \ \beta = -.60, \ p < .001; \) respectively).

**Re-running analysis covarying for the Mullen Early Learning Composite (MSEL) score.**

The Mullen Scales of Early Learning (MSEL; Mullen, 1995) is a performance-based developmental measure and consisting of four subscales: visual reception, fine motor, receptive and expressive language that are combined to calculate an early learning composite score (Table 2.1). The composite MSEL score that was measured at 36 months visit was covaried in the analyses and regressed on only anxiety and ASD scores to control for the effect of the IQ.

**Model 2.1: Longitudinal association between BI and anxiety.**

The fit indices of the autoregressive model indicated an acceptable fit to the data \( (\chi^2 (5) = 14.17, \ p = .015; \ \text{CFI} = .930, \ \text{RMSEA} = .113, \ \text{and SRMR} = .058) \). The association between risk group and BI at 9 and 15 months was significant \( (\beta = .15, \ p = .018; \ \beta = .13, \ p = .043, \) respectively); the MSEL scores was negatively related to anxiety at 36 months \( (\beta = -.21, \ p = .014) \). All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months \( (\beta = .53, \ p < .001) \) and BI at 15 months associated with BI scores at 24 months \( (\beta = .51, \ p < .001) \), indicating stability between time points. Regarding the timing, higher levels of BI at 24 months were associated with higher levels of anxiety at 36 months \( (\beta = .34, \ p < .001) \) and the association between 9 months BI and anxiety was significant \( (\beta = .19, \ p = .038) \).

**Model 2.2: Longitudinal association between BI, EC and anxiety**
The fit indices of the autoregressive model indicated an acceptable fit to the data ($\chi^2 (11) = 33.05, p = .001; \text{CFI} = .910, \text{RMSEA} = .118, \text{and SRMR} = .059$). The association between risk group and BI at 9 and 15 months was significant ($\beta = .15, p = .017; \beta = .13, p = .045$, respectively); and between risk group and effortful control was significant at 15 months ($\beta = -.16, p = .010$). The MSEL scores was related to anxiety at 36 months ($\beta = -.15, p = .036$).

All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .53, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .50, p < .001$). Effortful control at 9 months related to subsequent effortful control at 15 months ($\beta = .56, p < .001$) and effortful control at 15 months associated with effortful control scores at 24 months ($\beta = .53, p < .001$).

As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months ($\beta = -.20, p = .010$). the BI at 9 months was significantly related to anxiety ($\beta = .18, p = .030$). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety ($\beta = .24, p = .006; \beta = -.24, p = .022$; respectively).

Model 2.3: Longitudinal association between BI, EC, anxiety and ASD

The fit indices of the autoregressive model indicated an acceptable fit to the data ($\chi^2 (11) = 31.15, p = .001; \text{CFI} = .940, \text{RMSEA} = .113, \text{and SRMR} = .058$). The association between risk group and BI at 9 and 15 months was significant ($\beta = .15, p = .017; \beta = .13, p = .043$, respectively); and between risk group and effortful control was significant at 15 months ($\beta = -.16, p = .010$). Group was related to ASD outcome ($\beta =$
The MSEL score was related to only anxiety at 36 months ($\beta = -.15, p = .036$).

All coefficients for the autoregressive pathways of BI remained significant; BI at 9 months related to subsequent BI at 15 months ($\beta = .53, p < .001$) and BI at 15 months associated with BI scores at 24 months ($\beta = .50, p < .001$). Effortful control at 9 months related to subsequent effortful control at 15 months ($\beta = .56, p < .001$) and effortful control at 15 months associated with effortful control scores at 24 months ($\beta = .52, p < .001$). As for the cross-lagged paths, higher levels of effortful control at 15 months related to decreased BI at 24 months ($\beta = -.19, p = .011$). The association between BI at 15 months and effortful control at 24 months was significant ($\beta = -.17, p = .030$).

Effortful control at 9 months was related to ASD at 36 months ($\beta = .28, p = .001$). Both higher levels of BI and lower levels of effortful control at 24 months were significantly associated with higher levels of anxiety ($\beta = .25, p = .006; \beta = -.23, p = .020$; respectively) and ASD symptoms ($\beta = .20, p = .003; \beta = -.59, p < .000$; respectively). Anxiety and ASD scores were significantly related to each other ($\beta = .42, p < .001$).

**Appendix 2: Chapter 4, re-running Model 4.1 covarying IQ**

The MSEL was controlled in the Model 4.4, as IQ associations have been widely reported in anxiety investigations in ASD research (e.g., Hallett, Lecavalier, et al., 2013; Kerns et al., 2014). In this model, Model 4.1 was re-run regressing MSEL on only anxiety and ASD traits as there was no evidence of any associations between reactivity, BI and IQ.
The Early Learning Composite Score was controlled in this model. The MSEL was not significantly related to anxiety traits ($\beta = -0.15, p = .133$) and the associations between CFA-social BI and anxiety remained significant when controlling for ASD ($\beta = 0.42, p < .001$). As expected, lower MSEL scores were related to higher ASD traits ($\beta = -0.45, p < .001$). When controlling for MSEL, the association between CFA-social BI and ASD remained, and the relationship between CFA-non-social BI and ASD remained significant ($\beta = -0.24, p = .047$). The magnitude of the relationship between anxiety and ASD also remained similar to results in Model 4.3 ($\beta = 0.47, p < .001$).

Figure A.1 Model 4.4 for the association between BI scores, parental behaviours, ASD and anxiety after controlling for MSEL traits. Full lines indicate significant results. Dotted lines indicate non-significant results. Standardised beta (Standard Error) and standard errors are reported. *** $p < .001$, ** $p < .01$, * $p < .05$
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