The psychophysiology of fearful temperament: A follow up study

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Summary

Fearfulness as a temperament dimension is already evident in infants. Children who vary in fearfulness differ in their behavioural and physiological reactivity to novel situations. Despite evidence linking the extremes of early fearful temperament to later psychopathology, information regarding its development and correlates is lacking.

The present research examined fearful temperament in infancy through assessments of behaviour and physiology, and compared this information with maternal reports. The proposed associations between fearfulness, sustained attention and of effortful control were also investigated. Temperament was examined longitudinally in 50 healthy infants in the 1st and 2nd year of life. Mothers reported on their children's fearfulness; behavioural distress during fear provocation, and resting and stress levels of skin conductance activity (SCA) and cortisol were examined. Skin conductance responses (SCRs) during an Orienting Habituation Paradigm (OHP) were also studied.

We successfully induced fear in children, as reflected in significant changes in behaviour and physiology. In line with previous findings, higher physiological stress reactivity was most prominent in children high in behavioural distress, but only in year 1. Mother-rated fearfulness was not associated with physiological reactivity. With respect to the development of fear, although baseline physiological levels decreased over time, behavioural and physiological stress reactivity increased, supporting the notion that fear in young children increases with time as a result of developing cognitive skills. Within-year (i.e., baseline vs. stress) but not between-year (year 1 vs. 2) stability in individual patterns of behaviour and physiological reactivity was observed. Mother-rated fearfulness and internalizing behaviour were related to better ability to delay gratification. Unexpectedly, sustained attention and effortful control performance were inversely related. Finally, no evidence of gender differences on any temperament parameter was observed. The implications of these findings for our understanding of early temperament development are discussed.

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Chapter 1 Introduction

1.1 The origin of temperament

The notion of temperament originates from the writings of the ancient Greek philosophers and physicians. Hippocrates (4th century B.C) conceptualised a theory of humours in order to explain states of health and illness (Strelau, 1998). Hippocrates began by initially associating an individuals' characteristic emotional predisposition with the primary elements of the universe; earth, air, fire and water. From these four elements, Hippocrates drew attention to four additional 'qualities': warmth, cold, moisture, and dryness, all linked to an individual's distinctive emotionality (Strelau, 1998). Upon these foundations, Hippocrates identified four humours thought to be manifestations of the above four qualities. The four humours comprised the following; blood, phlegm, white bile and black bile. According to Hippocrates, these fluids determined the nature of the body in terms of whether a person experienced good health (if the fluids were balanced) or illness (due to a severe imbalance of the fluids) (Strelau, 1998). Although Hippocrates advocated an interesting insight into the nature of 'emotionality' he failed to describe any relationship between the proportion of humours and behavioural characteristics (an essential component of one's temperament).

Building upon the theorising of Hippocrates, Galen developed the first typology of temperament. Galen's four main temperament types were labelled according to the humours that predominated in the body and included the following; sanguine (blood), choleric (bile), melancholic (black bile) and phlegmatic (phlegm, mucus) (Strelau, 1998). Unlike Hippocrates, Galen's description of these temperaments, although unsystematic and incomplete, retains psychological features. Nevertheless, the ancient Greeks provided a major contribution to the knowledge of temperament via their assertions that individual differences in behaviour can be potentially explained by physiological mechanisms. The range of behaviours in which individuals differ can be reduced to a small number of basic temperament categories (Strelau, 1998). However, Galen's typology could be seen as the first causal, explanatory theory in the area of temperament. In addition, Galen's theory, in which the categories of temperament are linked to the excess of particular humours in the organism, has received some support regarding recent investigations concerning the biological bases of temperament. Specifically, it has been demonstrated that some temperament characteristics, particularly those referring to emotions, are related to the activity of the endocrine system (Kagan, 1994; Netter, 1991; Zuckerman, 1991).

A final, fundamental contribution to contemporary theories of temperament to be discussed is that advocated by Jung (1923); a Swiss psychoanalyst who developed his own theory of personality based on the interplay of the conscious and unconscious mind. According to Jung (1923) individuals could be described as possessing two types of attitudes; extraverted and introverted. Jung (1923) asserted a biological basis for these two 'attitudes', reflecting the direction in which both general thought processes and libido (sexual drive) expressed themselves. The two attitudes were considered to be grounded in four principal psychic functions: thinking, sensation, feeling, and intuition.

Jung (1923) described the 'extraverted personality' as being oriented in ways of adaptation to the outside world, demonstrating outward physical activity and engagement with others. By contrast, the 'introverted personality' was typified as revealing an innate inclination to disregard objects, and instinctively withdraw when faced with unknown physical stimuli and social situations. The two attitude types, extraversion and introversion, proposed by Jung (1923) became the most popular temperamental dimensions ever researched; renowned personality theorists, including Cattell (1965) and Eysenck (1970), based much of their research on these concepts. Jung's (1923) suppositions have also been influential in the development of other lines of temperament research, for example Kagan's (1994) model of inhibited and uninhibited temperaments. However, Jung's (1923) extraverted-introverted personality 'types' were essentially based on speculative criteria, thus signalling the absence of any supporting empirical evidence. Researchers must therefore look to Jung's (1923) theoretical descendents (i.e. Kagan, 1994, Cattell, 1965, and Eysenck, 1970) in terms of investigating the reliability and validity of the assessment tools used to measure extraverted/uninhibited and introverted/inhibited temperament dimensions.

1.2 Theories of infant temperament

Interest in child temperament was principally re-established due to the pioneering work of the New York Longitudinal Study (Thomas, Chess, Birch, Hertzig & Korn, 1963). Thomas et al. (1963) viewed temperament as a behavioural style which refers to the *how* of behaviour. They based their theory on the fundamental assumption of the constant interaction between the organism and the environment. Individuals seemed to differ in their behavioural style from early infancy and this style persisted sometimes until adulthood. Based upon clinical insights, the above study revealed the emergence of nine dimensions of temperament upon which infants and children were seen to differ. These included approach-withdrawal, adaptability, quality of mood, intensity of reaction, distractibility, persistence, regularity, threshold of responsiveness, and activity (Thomas et al., 1963). Following qualitative examination of their data, Thomas et al. (1963) identified three temperamental constellations namely, difficult child, easy child, and slow to warm-up child. Thomas et al.'s (1963) nine-dimensional model of temperament became widely embraced by professionals, particularly those within the clinical domain (Smith & Hart, 2004).

Similarly, some years later, Buss and Plomin (1975) stated that temperament refers mainly to stylistic aspects of behaviour, such as *how* fast or slow the response is made, or how mild or intense. Buss and Plomin (1975) suggested that the behavioural aspects of temperament, which appear to be genetically inherited, are present from early childhood onwards, and that these remain stable throughout the life span. Diamond's conception of the nature of temperament played a main role in Buss and Plomin's theory. Diamond (1957) advocated the idea of four basic temperaments, which are shared by man and mammalians alike, namely *fearfulness, aggressiveness, affiliativeness,* and *impulsiveness* (Strelau, 1998). On the other hand, Buss and Plomin's (1975) theory distinguished three basic temperaments: emotionality, activity, and sociability (EAS).

In the beginning of the 1980s Rothbart and Derryberry (1981) postulated their theory, which can be best described as constitutional-psychobiological. Rothbart (1981) criticized "style" theories, because these imply that temperament characteristics are so pervasive that they are expressed in all behaviours and suggest that a child would respond with the same intensity to all modalities of expression (i. e. a child who shows intense distress would also show intense positive affect). Rothbart and

DerryBerry (1981) defined temperament as "constitutional individual differences in reactivity and self-regulation" (Rothbart & DerryBerry, 1981, p.40). According to them, *constitutional* refers to the individual's relatively enduring biological make-up influenced over time by heredity, maturation, and experience. *Reactivity* refers to the arousability of the physiological and behavioural systems of the organism, while *self-regulation* refers to the neural and behavioural processes working towards modulating this underlying reactivity (Rothbart, 1981). They also suggested three temperamental dimensions; extraversion/positive emotionality, negative affect/anxiety, effortful control (Bee, 1997).

In recent years the generic categorization system, developed by Thomas et al. (1963), of "easy" and "difficult" clusters of children have proved popular among researchers (Smith & Hart, 2004). "Difficult" children are described as frequently displaying negative mood, withdrawing, inadaptable, and extremely intense. Furthermore, Thomas et al. (1963) predicted a greater number of these children to experience troubled patterns of social and emotional development. However, more recent research has identified that the above dimensions do not sufficiently cluster together (Sanson & Rothbart, 1995). Additionally, a variety of researchers have chosen to use their own classification of "difficultness" thus hindering the ability to make comparisons between studies (Smith & Hart, 2004).

Of greater concern is perhaps the fact that the concept of a "difficult" child exerts value-laden connotations, and fails to recognise that any temperament characteristic can be perceived as "easy" or "difficult" depending upon the demands of the child's external environment (Sanson & Rothbart, 1995). There is also particular danger in labelling a child as having either a "difficult" or "easy" temperament because of the potential detrimental effects on their later development (Davison & Neale, 2001). This is best understood in terms of the self-fulfilling prophecy phenomenon first identified by Rosenthal and Jacobson (1968). Thus, if a child is *expected* to behave in a certain way, the likely consequence is that they will in fact begin to react to their external environment in line with this expectation (Rosenthal & Jacobson, 1968). An additional criticism of the use of such a global construct as "difficult" to define certain temperamental styles, is that it inhibits understanding of the unique roles of particular temperament dimensions for specialized aspects of development (Sanson & Rothbart, 1995).

Another type of categorization that has been introduced more recently in the temperament literature is based on the dimensions of approach and withdrawal. For example, some infants express excitement when confronted with a new toy or face, or when they experience a new encounter, while others appear to find it more difficult to cope with new stimuli (Goldsmith & Campos, 1990). These two temperamental dimensions define two categories of children called behaviourally inhibited and uninhibited (Schmidt & Fox, 1998). Behaviourally inhibited children tend to be more fearful and timid with people, toys and situations that are novel or unfamiliar, while uninhibited children tend to approach new people, toys and situations more spontaneously (Kagan, Snidman & Arcus, 1995). This temperament categorization has gained a lot of attention by well-known temperament scholars (Kagan et al., 1995; Schmidt & Fox, 1998; Pfeifer, Goldsmith, Davidson & Rickman, 2002) and will be used in this thesis to distinguish between infants who tend to be fearful in the face of novelty/uncertainty and those who are more sociable and spontaneous upon encountering the same unfamiliar events. According to Kagan (1994) these two temperamental profiles cannot be seen as existing on a continuum, since their physiological profiles are qualitatively different.

1.3 Proposed models of the development of temperament

Thomas and Chess (1977) have shown that specific temperament qualities can result in behavioural problems in children under adverse conditions. Since then researchers have been eager to assert an association between aspects of child temperament and social development (Davison & Neale, 2001; Gunnar, Leighton & Peleaux, 1984). However, how exactly is this association defined? Smith and Hart (2004) emphasise the need not only to authenticate mere links between temperament 'scores' and social development, but also to *model* and explain precisely the developmental processes by which temperament is likely to influence social development (Smith & Hart, 2004).

It has often been suggested that temperament may have a direct, linear impact on development (Bee, 1997). For example, an extreme temperamental feature may lead to a specific outcome, such as an extremely reactive temperament may predispose a child to respond aggressively to frustration (Rothbart & Bates, 1998). Indirect linear effects comprise of those involving a situation where a child's temperament affects the environment, which then influences their adjustment. Generally, children with characteristically different temperaments elicit different kinds of responses from the people with whom they interact (Rothbart & Bates, 1998).

Interactional models have been used to describe the relationship between child temperament and different developmental outcomes. The notion of particular types of temperament being "compatible" with particular types of environment is known as the "goodness of fit" model (Thomas & Chess, 1977). Thus, for example, an energetic, lively child living in a physically restrictive environment may be hindered in its development compared to the same child living in a less restrictive environment where their energy is channelled in a productive way (Smith & Hart, 2004).

A more multi-dimensional and arguably viable model is the transactional model (Cicchetti & Cohen, 1995), which speculates that development is the inevitable outcome of a continuous interaction between inherent child characteristics and elements of the environment. Thus, a child's physical wellbeing, cognitive aptitude, and temperament, in addition to their current parent and family circumstances and socio-cultural context, all require stringent consideration when explaining and predicting developmental pathways (Cicchetti & Cohen, 1995). Within this model, temperament is typically considered as a *risk* or *protective* factor (Smith & Hart, 2004).

There is clear acceptance among theorists that interactional or transactional models probably best explain the process of development. However, Rothbart and Bates (1998) also assert that most empirical support exists for linear-additive models (e.g., greater physiological reactivity facilitates the development of a 'difficult' temperament), because searching for interactions between temperament and different developmental outcomes sounds easier than it actually is in practise. Rothbart and Bates (1998) argue that this is simply a consequence of the poor methodological and analytical strategies employed by researchers, preventing the researcher's ability to identify such interactional effects. This issue could be rectified by conducting latent class and trajectory analyses to establish the validity of the transactional model (Smith & Hart, 2004).

1.4 Temperament characteristics

There is general consensus among temperament researchers about the specific characteristics that are important for the nature and development of temperament. What follows is a discussion of these characteristics, as well as their place in the current literature.

1. A temperament category refers to behavioural qualities in which individuals differ, and mainly to specific characteristics of behavioural qualities such as intensity, energy, strength, speed, fluctuation etc. Terms such as *trait, quality, attribute, disposition, type, category* etc. are the most prominent ones when speaking about individual differences in temperament, and these have been used, and are still in use, interchangeably by many researchers. Temperament is not viewed as a trait itself; rather it is seen to be a group of traits (Goldsmith, Buss, Plomin, Rothbart, Thomas, Chess, Hinde & McCall, 1987). Well known is Strelau's analogy of temperament traits to the acceleration of a car. Unlike the colour or the shape of a car, which are obvious and permanent, 'acceleratability' (a term invented by Strelau) manifests itself under specific conditions, such as the movement of the car, which is similar to temperament traits, which reveal themselves in behaviour and reactions. Cars differ from each other in acceleration. Several features such as type of engine, weight of the automobile and generally the "make up" elements of the car determine its 'acceleratability' and further establish stability and cross-situational consistency of the specific characteristic, which is similar to how the biochemical and physiological underpinnings of temperament work (Strelau, 1998).

Although pioneers on temperament (Thomas et al., 1963; Diamond, 1957) suggested several traits that are essential components of the temperament construct (e.g., activity level, irritability, fearfulness, intensity of response, etc.) these dimensions could not be regarded as bearing a unifying theme, apart from characterizing individual differences among young children. Goldsmith and Campos (1990) therefore came up with one of the first definitions of temperament based on behaviour. According to them, infants' temperament is defined as "the individual differences which are being revealed in tendencies to express emotions" (Goldsmith & Campos, 1990, p.1945). However, Goldsmith and Campos' (1982) definition does not make any reference to the biological basis of temperament. The idea of emotional differences being part of the temperament rubric is well established, but there is a general disagreement about the other contents of temperament (Goldsmith & Campos, 1990). For example, Rothbart and Derryberry (1981) go beyond the emotion domain to also include qualities such as orienting and motor activation in the temperament definition.

2. Temperament is characterized by relative stability over time and situation. Investigators either use individual or group differences to evaluate temperament. However, most theorists within the domain of temperament agree that temperament is about individual differences rather than species general characteristics (Goldsmith et al., 1987). Thus, in the individual

differences approach researchers usually evaluate and compare individuals' performance on different measures and at different ages or times. When the individual differences prove to be similar across time, one refers to stability, and, in case these differences repeatedly emerge across situations, one talks about cross-situational consistency (McCall, 1986). Longitudinal studies of infant temperament have shown that there is some consistency across age in behaviours that have been defined as temperamental. For example, Kagan (1994) asserts that the behavioural profile at 4 months of age can predict the level of fear at nine, fourteen, and twenty-one months of age. Others have reported modest to moderate stability across time, with associations ranging from .20 to .40 (Smith & Hart, 2004). Temperament develops over time and could be liable to lawful change or developmental discontinuity as a cause of maturation and experience. However, in order to be able to examine those developmental changes and the dynamic characteristics of temperament, researchers will have to establish first the developmental timelines and the degree of continuity in early temperament (Komsi, Raikkonen, Pesonen, Heinonen, Keskivaara, Jarvenpaa & Strandberg, 2006).

3. Temperament has a biological basis. Human development is shaped by dynamic transactions between genes-biology and environment. Most of the temperament definitions, starting from the ancient Greek, emphasize the fact that temperament has some biological basis (Strelau, 1998). Drawing from numerous temperament theories, and based on several assumptions and findings from the temperament literature, Strelau (1998) discusses a number of important issues regarding the biological basis of temperament: a) Any important psychological function, like temperament is, is co-ordinated by

brain activity (Gray, 1991), b) the role of genetic factors is of great importance in explaining individual differences in temperament traits, c) based on the latter, variables of biological nature, such as physiology, neurology, biochemistry, and hormones, should be involved somehow in the formation of temperament, since they are supposed to be transferred genetically across generations (Eysenck, 1990), d) the assumption of temperament traits being cross-cultural, could mean that these traits have been transferred from one generation to the next via biological processes, e) some temperament traits and individual differences in temperament observed from an early age onwards can not be accounted for by environmental factors, f) since some temperament traits are also present in other mammalian species, one can assume that these temperament traits must have played an important role in the process of our evolution, and that some shared biological mechanisms must mediate these temperament traits, and finally, g) stability in temperament also points to a biological background (Strelau, 1998). Although, some of the above statements in support of a biological basis of temperament seem to be weaker than others, they delineate the general orientation of the work in this era.

4. Temperament appears early in life. Individual differences are generally seen early in life and these include differences in reacting to new things, typical mood, rate of activity, preference for social interaction or solitude, and the relatively easy establishment of routines and daily rhythms (Bee, 1997). Temperament has been characterized as "the matrix from which later child and adult personality develops" (Ahadi & Rothbart, 1994, p.190).

1.5 Temperament differences in early infancy

The pioneers of the contemporary work on temperament, Thomas and Chess (1963) initiated their work when as clinicians they were struck by the individual differences in infants' behaviour, which were obvious from the first weeks of life. According to Goldsmith et al. (1987), temperament can be viewed as a relatively direct phenomenon only during infancy; as the child matures the interaction between temperament and behaviour becomes more complex (i.e. pure temperamental expressions become easily masked), thus rendering it a more difficult construct to be studied (Goldsmith et al., 1987). Recently, temperament researchers have increasingly focused their interest on infancy. This is mainly because researching temperament in early infancy could shed light on questions concerning processes that can account for the continuity and/or discontinuity of development, and also because an *early* focus could contribute to the identification of individuals at risk for later emotional and behavioural problems, and thus possibly the prevention of psychopathology through early intervention (Crockenberg & Leerkes, 2006).

Individual differences in the newborn period are evident and can be studied in terms of activity level, anger-frustration, orienting reactions as well as smiling and laughter, and distress (Rothbart, Derryberry, & Hershey, 2000). For example, Kagan and Snidman (1991) found that motor movement combined with distress to stimulation in 4-month-olds accounted for behavioural inhibition and fearfulness in 14-month-olds. On the other hand, Calkins, Fox and Marshall (1996) found that motor activity combined with positive affect in 4-month-olds predicted approach and more outgoing behaviour at 14 months. Manifestations of anger have been observed in infants as early as 2 months of age. Stability of anger reactions have been reported across 2.5 to 5, 5 to 7.5 and 7.5 to 22 months of age (Malatesta, Culver, Tesman, & Shepard, 1989). Furthermore, with respect to early orientation reactions, 4-month-old infants can disengage easily from one stimulus and focus on another, with previous experience revealing itself in their anticipation of future locations of events (Johnson, Posner, & Rothbart, 1991). Positive relations have also been reported between questionnaire and laboratory measures of smiling and laughter dimensions, and behavioural approach of objects. Finally, stability of individual differences in fear/distress reactions has not been reported until the age of 6 months, or even later (Rothbart, 1986). Kagan (1998) showed stability of behavioural inhibition from the 14 months of age onwards up to the school years.

In summary, several researchers have established the feasibility and shown the importance of studying temperament in early infancy. The current study aims to add to this body of research by examining how fearful temperament develops during the first 2 years of life. Data from physiological and observational measures will be combined with those from parental reports to gain a complete understanding of the correlates of fearful temperament in early infancy.

1.6 Heritability of temperament

The majority of researchers agree that differences in primary reaction propensities such as sensitivity to visual or verbal stimulation, and emotional responsiveness, appear to be already in place at birth, prior to any considerable interaction with the external environment (Seifert, Hoffnung & Hoffnung, 2000). Behavioural genetic methods can be used to test this assumption (Saudino, 2005). Behavioural genetics seek to identify and characterize genetic and environmental influences on behavioural stability and change. The strongest evidence for the genetic basis of infant temperament and later personality is the reliable finding of more similarity (i.e. more than twice the size of that among dizygotic twins) of temperamental characteristics among monozygotic as compared to dizygotic twins (Buss & Plomin, 1984). An example of this is provided by Buss and Plomin (1984), who examined 228 identical and 172 fraternal twin pairs whose temperament was ranked on the basis of three temperament dimensions (emotionality, activity, and sociability) when the twins were aged 5 years. The correlations between the identical twins for each dimension were high (e.g., $r = 0.63$ for emotionality), whereas those for the fraternal twins were close to zero (e.g., $r = 0.12$ for emotionality), thus suggesting a salient genetic influence on temperament.

Other evidence also suggests a heritable component of temperament, such as the notion that differences in functioning of a child's nervous system, hormone system and brain underlie differences in behaviour (e.g. Rothbart, 1989; Gunnar, 1990). For example, Kagan, Snidman and Arcus (1993) claim that differences in behavioural inhibition (shyness) originate from differing thresholds for arousal of the amygdala and the hypothalamus, which direct responses to uncertainty. Inhibited or shy children are believed to have a lower threshold of arousal, and are therefore more prone to become tense and alert in the presence of uncertainty (Kagan, Reznick & Snidman, 1990). Kagan, Reznick and Snidman (1990) reported correlations of approximately .60 between measures of behavioural inhibition ("shyness") in children of the ages 2 to 5 and a number of physiological measures, including muscle tension, pupil dilation, heart rate, and the chemical composition of urine and saliva. Therefore, what children inherit is not inhibition or 'shyness', but a propensity for the brain to react in particular ways (Bee, 1997). Therefore it is not surprising that heritable factors seem to explain a substantial proportion of the variance in HR (Singh, Larson, O' Donnel, Tsuji, Evans & Levy, 1999) and cortisol levels (Bartels, de Geus, Kirschbaum, Sluyter & Boomsma, 2003).

Recent evidence also points to a strong genetic influence on brain activity, which suggests a heritable component of the autonomic nervous system (ANS) activity (van Beijsterveldt, Molenaar, de Geus, & Boomsma, 1996). HR and HR variability, as well as respiratory sinus arrhythmia (RSA), which has been related to emotion regulation, have all been reported to be genetically determined. As for the stress hormone cortisol, apart from environmental factors (e.g. pre- and postnatal adverse experiences) that have been shown to influence cortisol levels in humans and non-human animals, a genetic contribution has been identified. Bartels et al. (2003) reported a genetic basis of individual differences in the cortisol awakening response in 12-year-olds twins. Since both ANS and HPA axis activity have been shown to play a prominent role in studies of individual differences in development the study of their genetic influence seems crucial.

Genes are dynamic in nature and the quality and quantity of their expression can change over time, thus contributing to the developmental change and/or continuity of temperament (Saudino, 2005). Within the framework of behavioural genetics the impact of genetic factors will not be weakened as the child matures and starts to interact with his or her environment. Interestingly, heritability might be different across two ages although the same genes are expressed at each age, or the heritability of one dimension might be similar between two ages although different genes are expressed at each age (Saudino, 2005). As with developmental research, where individual trajectories of change are more informative of underlying processes rather than cross-sectional age differences, the impact of genetic factors to the change and continuity of a temperament dimension is more interesting than whether the genetic impact is present or absent at a single age (Saudino, 2005).

Notwithstanding the important role of genetic factors in temperament, researchers must not negate the effect of environmental influences. For example, despite high correlations in temperament scores among identical twins there remains a considerable amount of variance, which is due to the effect of the child's environment (i.e,. common/shared environemt, unique/unshared environment). These factors concern those that differ between and within families (e.g., parenting style, scheduling of activities, methods of soothing, maternal sensitivity etc.) (Saudino, 2005).

Although genetic influences are clearly important in the development of temperament in general, and fearful temperament in particular, the current study was unable to include the role of genetic factors in fear development.

1.7 Current orientation in the research of temperament

Although most definitions of temperament make reference to the fact that there is a biological bases to temperament, for a long time research has been dominated by the widespread use of parental reports on children's temperament. More recently, however, several shifts have occurred that have changed the focus of temperament research into a more biological direction (Goldsmith, Lemery, Aksan & Buss, 2000).

Four lines of research can be identified with respect to the biological correlates of temperament. A number of researchers focus on brain structures involved in temperament's manifestations. Others study the physiological correlates of temperament, or concentrate on the biochemical markers of temperament traits (Strelau, 1998). Finally, more recently, people have become interested in the behaviour-genetic approach to the study of temperament (Goldsmith et al., 2000).

Although each of these biological orientations in temperament research in infants contributes in its own way to the understanding of the biological correlates of temperament and its manifestations, we have chosen to focus on the physiological correlates. The role of physiology in the study of temperament, and its development over time, has been of great importance. We will show in the next chapters how temperament is linked to the physiological systems, specifically the Autonomic Nervous System (ANS; Chapter 4) and the Hypothalamic-Pituitary-Adrenal (HPA) axis system (Chapter 5), and discuss some research which shows that a proper understanding of the temperament - physiology relationship is important in our understanding of infant temperament (Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996).

1.8 Aims of the current thesis

The main aim of our study was to investigate the development of fearful temperament during the first 2 years of life. To this end we studied fearful temperament on two occasions in a sample of 50 normally developing infants. Based on previous research that has identified different biobehavioural patterns in high and low fearful children we combined data from parents, independent observers and physiology to obtain a better insight into the correlates of fearfulness. Subsidiary goals were to explore individual, age and gender differences in fearful temperament, as well as their stability over time. Finally, based on previous research, we examined the relations between fearfulness and the later developing temperament construct of effortful control, as well as the association between effortful control and the temperament dimension of sustained attention.

Chapter 2 Temperament methods in early childhood

2.1 Existing methods, their advantages and disadvantages

In contemporary research three main methodologies have been used to assess temperament in early infancy and childhood: questionnaires, observation, and physiology. What follows is a description of the role of each of these methods in research on early childhood temperament, as well as a discussion of the advantages and disadvantages of assessing temperament in these ways.

Questionnaires: In the last, almost four decades, temperament researchers have mostly used questionnaires or interviews to assess temperament characteristics in young children. The basic merit of using questionnaires in temperament research is that it enables researchers to study temperament relatively quickly and in large samples. In youngsters, questionnaires are usually completed by the parents or other caregivers, and/or teachers. According to Strelau (1998) more than 50 questionnaires have been developed for the study of temperament in infants and children. Usually these questionnaires ask parents to identify whether specific temperament characteristics are present in their child, at home or in other familiar surroundings, and if so, whether (or how often) these characteristics/behaviours occurred in the past week, month, or year. Among the most frequently used questionnaires in young children are the Infant Behaviour Questionnaire (IBQ; Rothbart, 1981) and the Child Behaviour Checklist (CBCL; Achenbach & Rescorla 2000), which both are employed in the current study.

Despite the fact that questionnaires have been the most frequently used tool in temperament research, several researchers have criticized their validity. For example, Kagan's critique (1994) is based on 6 arguments. First, high levels of subjectivity, mainly attributed to the parents' impressions of their children's characteristics, have been identified. Questionnaire findings could reflect stability of the parents' attributions (expectations) of their children's temperament rather than actual stability of children's behaviour and characteristics (Carranza, Perez-Lopez, Salinas & Martinez-Fuentes, 2000). Also, mothers tend to reduce inconsistencies observed in their children behaviours and stick to an abstract symbolic idea about their child's temperament, which helps them to interpret what they see in a child in everyday words (Kagan, 1994). Second, parents can not always discern specific qualities in the child's everyday behaviours. For example, some parents find it difficult to discriminate among the different ways that their child smiles or cries. This could be accounted for by the parents' educational background or personality (Kagan, 1994). Third, the mother's experience affects how her child is judged. Thus a mother with more children will be using different referents in her evaluations as compared to a mother with just one child. As in the case of fraternal twins, mothers tend to magnify differences between these siblings more than independent observers do (Kagan, 1994). Fourth, since the temperament construct involves more than the verbal report of children's straightforward reactions and observed behaviour, questionnaires are limited in that they do not give researchers access to temperament related physiological qualities like muscle tension, heart rate, brain activity, etc. Therefore, whilst parents can report whether their children recover quickly from stress or are relaxed, they can not report whether these observed states are accompanied by, for example, a reduction in HR or muscle tension. Fifth, some children show reactions to specific events in the laboratory that have not been noticed before at home. To the mother these reactions are inconsistent with her child's typical behaviour. This cross-situational inconsistency in behaviour is a factor that could affect the accurateness of parental judgments of their children's temperament (Kagan, 1994). However, the same argument has been used to criticize observational measures. Thus, it has been suggested that observational measures screen children's behaviour only under controlled conditions and for a short period of time when a child's behavioural repertoire might not be explicitly revealed (Vaughn, Taraldson, Cuchton, & Egeland, 2002). In that case mothers' perceptions of their children's temperament might be more accurate, since mothers acquire a more detailed and integrative picture of their children's behaviour. Finally, parents often perceive the same behaviours in different ways. For example, not all mothers will interpret a child's shyness as fearful behaviour, but consider it sensitivity or just caution. It has been suggested that parents' reports of children's temperament inform us more about the parents' personality traits than those of their children (Seifer, 2002; Vaugh et al., 2002). For example, research has shown correlations between mothers' personality measures and their children's behaviour as measured independently in the laboratory (Matheny, Wilson, & Thoben, 1987).

Another issue regarding questionnaire's validity is related to the way some questionnaire research is conducted. Parents often receive a set of questionnaires by post and have to complete these in a limited amount of time, without necessarily being in the right state of mind to provide the best answers to the questions of interest (Goldsmith & Hewitt, 2003). Also, parents rarely have they been primed in advance regarding the kinds of behaviours to be rated. Although the validity of temperament questionnaires has been questioned (Vaughn, Taraldson, Crichton, & Egeland, 1981; Vaughn, Taraldson, Cuchton & Egeland, 2002), other evidence acknowledges their objective validity and important long-term contribution to temperament research (Rothbart & Hwang, 2002).

For all of these reasons, researchers have started to use other methods in combination with questionnaires to attain optimal validity of findings.

Observation: Another fundamental method for assessing temperament has been through observation in natural settings (i.e., at home) or in the laboratory. According to Strelau (1998), observation was the first method in the study of temperament, since both Hippocrates and Galen's theories (see Chapter 1) were developed on the basis of observations in natural settings. Observations have gained popularity because they enable us to assess dimensions of temperament which can not be measured via questionnaires or interviews (Kagan, 1994). Observational techniques have been frequently used in infants and children's research in natural setting such as the home, play groups or nursery settings.

Studies using natural observation usually do not last more than 3 hours, trained observers rate the child's behaviour and the inter-rater agreement is established. In research using observation the focus of attention is on specific behaviours and interactions where temperament characteristics are expected to be revealed, such as feeding, bathing, playing, etc. (Strelau, 1998). Due to the fact that these observations are conducted in the child's natural environment, this method has some inherent limitations. For example, one has no control over the conditions in which the child' behaviour is recorded. Also, observers often miss specific reactions that are part of a whole range of behaviours which altogether complete the puzzle of the child's temperament, and in case video recordings are made the child must be young enough not to be aware of the presence of the camera. Laboratory observations help to solve some of these problems.

In laboratory research the conditions under which the child's behaviour is observed are controlled and specific behaviours are provoked through which temperament characteristics can manifest themselves. In addition, laboratory research enables the use of video recordings from which footages are used to code children's behaviour by independent observers to eliminate the element of subjectivity. Researchers have developed laboratory tools for the measurement of young children's temperament in a systematic way. Among these is the Laboratory Temperament Assessment Battery (Lab-TAB), developed by Goldsmith and Rothbart (1999), which is employed in the current study.

Physiology: As with observation, physiological measurements can be used to reveal aspects of children's temperament that can not be obtained by questioning parents or other significant persons. It is well known that an individual's report of his/her physical or emotional arousal at a given point in time or during a specific event does not necessarily give the same result as actually measuring his/her physiological arousal (Kagan, 1994). As discussed in the previous chapter, the study of the ANS has gained an important place in temperament research. Specifically, physiological responses such as changes in SCA and HR, which are part of the ANS, have long been regarded as important physiological correlates of temperament (Strelau, 1998).

For example, higher SC and HR levels have been related to activity of the behavioural inhibition system (i.e., fearfulness; Scarpa, Venables, Raine & Mednick, 1997). Furthermore, it has long been known that biological factors, which are transmitted genetically, account for a significant proportion of the observed variation in behaviour (Goldsmith et al., 2000). According to Kagan and Snidman (1999) part of the variation in fearfulness that can be observed in young children (and therefore their proneness to develop anxiety disorders) is inherited.

Appropriate levels of physiological arousal are important to survival and adaptation. Rapid activation of the stress physiological system during exposure to stress is crucial to deal with the acute emergency, but the fast termination of the stress response, once the stressor is over, is equally important in the process of recovery. In case one of these processes is impaired, one is more vulnerable to stress and less able to face everyday adversity (Gunnar, 2006). There are clear individual differences in stress reactivity and regulation which go back to individual differences in temperament. For example, more outgoing children are less inhibited and approach novel events quite readily. On the other hand, inhibited children are more cautious and may take longer to recover from negative events.

According to Strelau (1998) researchers examining the biological correlates of temperament are confronted with the fundamental issue of what is the phenomenon we seek to explain and which are the dimensions that we need to investigate. Although inconsistencies have been reported in the relation between different temperament parameters, for example behaviour and physiology, the study of temperament physiology is crucial in order to get a complete understanding of the complex nature of this important psychological construct (Kagan, 1994).

Convergence between methods: Weak associations have been obtained in studies using both questionnaire and behavioural assessments of infant temperament. The lack of agreement between these two methods has identified them as non-equivalent instruments for the measurement of temperament (Carranza et al., 2000). We discussed already that the level of subjectivity identified in questionnaire measures, and the unreliability of both questionnaires and observational instruments are partly to blame for this. The same result holds when it comes to correlations between questionnaires and physiological measures. An explanation here is that questionnaires measure a pattern of behaviours related to a temperament characteristic across different occasions, while physiological measurements mostly tap into the reactions of an individual to a given stimulus and it is assumed that this reflects the temperament characteristic (Strelau, 1998). Similarly, the convergence of physiological measures and direct observation is low to modest. According to Calkins and Dedmon (2000) behaviour and physiology "are likely to be interdependent components of a larger self-regulatory system" (pp.116).

In conclusion, each of the above three lines of research, for different reasons, is important in the study of young children's temperament, and they should therefore, if possible, all be included in their assessment. Kagan (1994) suggested that the information we get from each one of these measures is unique, with the different methods being able to shed light on only one part of the phenomenon we want to study.

2.2 Temperament constructs in the current study

Strelau (1998) asserts that "the three basic inquiries - how to measure temperament, what must be measured, and who is the subject of temperament assessment, are inseparable" (p. 273). In the previous sections, two of these inquiries have been covered; how to measure temperament and who should be the topic (see Chapter 1). What remains is to delineate the temperamental characteristics which are the focus of the current study. These are: fearfulness, sustained attention and effortful control. In the following sections the dimesnions of interest will be described and previous attempts to study these constructs by means of questionnaires, observations and/or physiology will be presented.

2.2.1 Fearfulness

As mentioned in the previous chapter, a lot of temperament research has focused on fearful temperament, also called behavioural inhibition. Behavioural inhibition (henceforth, fearfulness) refers to the tendency to react with fear and wariness in the face of uncertainty (Schmidt & Fox, 1998). Fearful children are behaviourally inhibited; they tend to stay in close proximity to their parents, while displaying long latencies to approach novel stimuli; behaviourally uninhibited children, on the other hand, tend to be bold, sociable and spontaneous when facing new situations and events. Behavioural inhibition and fear are present in infancy and their features remain relatively stable throughout childhood (Pfeifer et al., 2002). Inherited individual differences in fearfulness have been reported in rats, dogs, and chimpanzees (Rothbart, 1981). Given the important role of fearfulness in children's development, fear assessments have been included in this study.

Fear measured by questionnaires: As mentioned before, the most common method of studying temperament has been by means of questionnaires, such as the Infant Behaviour Questionnaire (IBQ, Rothbart, 1981). Since its introduction, the IBQ has been one of the most widely used parent-report instruments for assessing early temperament. Lately, Gartstein and Rothbart (2003) revised the IBQ into the IBQ-R, by adding nine new scales, while maintaining its discriminant validity. In their study, the IBQ-R was completed by the parents of 360 infants, aged between 3-6, 6-9 and 9-12-months of age. The internal consistency was reported for 14 scales, which made up the final form of the IBQ-R. Both developmental effects and gender differences have been reported. Older infants are rated higher on the dimensions of fear and distress to limitations, and boys obtain higher scores on activity and high intensity pleasure, while girls are rated higher on fear (Gartstein & Rothbart, 2003). Because of the current study's focus on fear, the IBQ-R 'fear' dimension was used. This dimension includes 16-items which relate to the baby's startle or distress to sudden changes in stimulation or to novel physical objects or social stimuli. An example of an item is *"how often during the last weeks did the baby cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)"?* Parents rated each item on a Likert scale, ranging from 1 (never) to 7 (always). The items required the parents to rate the frequency of specific temperament-related behaviours as observed in their child over the past week. The IBQ fear scale score represents the mean score calculated over the 16 items as completed by the caregiver. Cronbach's alphas for the fear scale for the age groups of 3-6, 6-9, and 9-12 months are .90, .89, and .87, respectively (Gartstein & Rothbart, 2003). In our study the Cronbach's alpha for the fear scale for the age group of 6-12 months was .77. In the current study children were assigned to a high or low fearful group by the median split procedure (see Chapter 3).

High and low fearful (i.e., inhibited/uninhibited) infants tend to develop different phenotypical profiles later on in life. Extremely fearful infants are more likely to become introverted and anxious children, while fearless children are more outgoing and at risk for developing externalizing behavioural problems (Kagan, Snidman & Arcus, 1995). In our 2nd year follow-up study we collected information about the children's internalizing and externalizing behavioural profiles through maternal report. To this end, we used the Child Behaviour Checklist (CBCL; Achenbach & Rescorla, 2000). The internalizing score represents the sum of the scores of 4 internalizing syndromes (e.g. emotionally reactive, anxious/depressed, somatic complaints and withdrawn). The externalizing score is computed by the sum of the scores of 2 externalizing syndromes (e.g. attention problems, aggressive behaviour). For both scales borderline scores range between 65 and 69 and clinical scores between 70 and 100. According to Achenbach and Rescorla (2000) the internal consistency (Cronbach's alpha) for non-referred and matched referred children for the internalizing scale is .89 and for the externalizing scale .92. The alpha's in the current study were .89 for the internalizing and .78 for the externalizing scales. For the purposes of the current study children were assigned to a high/low internalizing/externalizing group based on the median split procedure (see Chapter 3). Blair (2002) reported that negative emotionality in infancy was related to both higher internalizing and externalizing CBCL scores at age 3. Age and gender effects on the CBCL are small but significant (Rescorla, 2005).

Fear measured by observation: Kagan (1994) noted that three kinds of events are likely to elicit fear in young children: intrusion of personal space, exposure to unfamiliar objects and actions, and exposure to unfamiliar people, especially those who behave in an unpredictable way (Kagan, 1994). Studies on infant fearfulness have used a range of different stimuli, ranging from mechanical toys (Gunnar, Leighton & Peleaux, 1984), hand puppets (Nachmias et al., 1996), people dressed in unfamiliar or frightening (bear) costumes (Kagan, 1994) to examination procedures such as inoculations (Gunnar, Brodersen, Krueger & Rigatuso, 1996). Goldsmith and Rothbart developed a reliable and frequently used laboratory-based observational battery, the Laboratory Temperament Assessment Battery (Lab-TAB; 1988). To date, the Lab-TAB includes 20 standardized episodes which target the 5 temperament dimensions of fear, pleasure, frustration, activity level, and interest. Emotional parameters such as latency to act, behavioural acts, average and peak intensities are coded within each episode, with the goal of decoding the complex organisation of early emotions (Goldsmith & Rothbart, 1999). The Lab-TAB includes 4 episodes which tap into the emotion of fear. One of these episodes, the mechanical toy episode, is employed in the current study.

Fear measured by physiological measures: As mentioned in Chapter 1, most definitions of temperament make reference to the fact that there is a biological basis to temperament. Some researchers focus on brain activity (cortical arousal), others on physiological and/or the biochemical markers of temperament, and more recently some have started to investigate the genetic correlates of temperament (Strelau, 1998). In the current study we focussed on one physiological (i.e., SCA) and one biochemical marker (i.e., cortisol) of temperament. SCA, which is mediated by the ANS, has been used as a physiological index for different temperament traits, such as fearfulness, extraversion, neuroticism, anxiety and sensation seeking (Strelau, 1998).

In children's studies researchers either measure skin conductance level (SCL), which is recorded over a relatively longer period of time and is the tonic level of arousal, or skin conductance response (SCR), which is the acute or phasic reaction to a given stimulus, usually occurring 1-3 sec after the onset of the stimulus (Dawson, Schell & Filion, 1990). SCL as an index of fearfulness has been measured in young children during the Still-Face (SF) paradigm (Ham & Tronic, 2006) and while they watch fear inducing film clips (Gilissen, Koolstra, van Ijzendoorn, Bakermans-Kranenburg & van der Veer, 2007). SCR is usually recorded following the presentation of unpredictable loud noises (Gao, Raine, Dawson, Venables & Mednick, 2007).

Cortisol, a popular hormone in temperament research, is the product of the HPA axis, which is also under the activation of the sympathetic branch of the ANS. Cortisol has been linked to fearfulness, impulsivity, sensation seeking and anxiety (Strelau, 1998). There is increasing evidence that early pre- and postnatal experiences play an important role in later outcome by influencing HPA axis development (Huizink, Buitelaar & Mulder, 2004). Cortisol can be assessed in saliva without any inconvenience to the participants. Researchers collect baseline measures of cortisol at home or at the beginning of a laboratory session, and compare these with stress cortisol levels collected approximately 20 minutes after the onset of a stressor. Stressors used in research with young children are separation from the mother (Nachmias et al., 1996), stranger approach (Buss, Malmstadt, Schumacher, Dolski, Kalin, Goldsmith & Davidson, 2003) or competition (Donzella, Gunnar, Krueger & Alwin, 2000).

2.2.2 Attention span

Two attentional temperament dimensions have been identified: distractibility and attention span or persistence (Thomas & Chess, 1977). Distractibility refers to how easily the attention of the child is drawn away from ongoing behaviour; attention span/persistence refers to the length of time a child is occupied with an activity or able to sustain attention in the face of distractors. In the current study we focussed on the attention span dimension. In young children researchers usually measure duration of orienting (Rothbart, 1981) and sustained attention (Kochanska, Murray & Harlan, 2000) with both variables being related to attention span. A novel stimulus causes initial stimulus orienting, followed by sustained attention. Stimulus orienting involves the detection of a change (stimulus) in the environment (Choudhury & Gorman, 2000). Sustained attention is associated with information processing related to the stimulus, is voluntary and controlled by the individual (Stroganova, Orekhova & Posikera, 1998). By the age of 3 months infants can usually engage in five- to ten-seconds of sustained attention. Decreases in sustained attention have been reported between 3 and 6 months of age, with a subsequent increase from 7 months onwards (Colombo, 2002). It has been suggested that sustained attention develops earlier in girls than in boys (Greenberg & Waldmant, 1993).

According to Colombo, Shaddy, Greenhoot and Maikranz (2001) sustained attention can be more reliably assessed during presentations of dynamic rather then static stimuli and studies therefore usually employ complex three-dimensional stimuli within interactive and play-based situations (Choudhury & Gorman, 2000). In the current study we measured sustained attention in the 1st and 2nd years of life through observation only. The ability to sustain attention has long been linked to the development of self-regulation, an important characteristic of temperament, which involves the ability to selectively regulate incoming information from stimuli (Rothbart & Derryberry, 1981). Orienting and sustained attention early in life are believed to become integrated into the later developing temperament construct of EC (Rothbart, Derryberry, & Hershey, 2000). In the current thesis we measured effortful control in the 2nd year of life through observation.

Orienting measured by physiology: In the current study we also measured orienting response to unpredictable loud noises via Skin Conductance Response (SCR). Measures of orienting via SCR have been shown to reflect how an individual processes novel environmental stimuli. In adult studies SCRs have been related to arousal, attention, information processing and emotion (Gao et al., 2007). However, little is known about the development of the skin conductance orienting response in young children. We collected physiological measures of the orienting response in the 1st and the 2nd year by using an orienting habituation paradigm (OHP), which consisted of the presentation of 10 unpredictable loud noises.

Sustained attention measured by observation: In the laboratory attentional measures of temperament include children's duration of looking and sustained play (Rothbart, Derryberry & Hershey, 2000). Kochanska, Murray and Harlan (2000) measured sustained attention at 9 months of age by placing infants on a blanket for 5 minutes with 3 soft blocks. Duration of looking and manipulation as well as facial intensity of attention, were scored every 10 seconds. Kochanska, Coy, Tjebkes and Husarek (1998) assessed sustained attention in the laboratory by measuring the length of time infants engaged with a set of coloured blocks. In the current study, sustained attention to two stimuli was measured. One stimulus, used in both years, was a colourful music box which contained a prince revolving around himself whilst music played. The other stimulus was an age appropriate colourful toy for the measurement of sustained play. Duration of looking and manipulation were scored every 30 seconds. Each stimulus was presented for two minutes.

Sustained attention measured by physiology: Physiological indicators of sustained attention have also been used as an index of this temperament dimension. These include mainly measures of HR variability and Respiratory Sinus Arrythmia (RSA; Choudhury & Gorman, 2000). HR deceleration and high resting RSA have been associated with sustained attention and generally good attentional ability (Calkins, Dedmon, Gill, Lomax & Johnson, 2002). Although HR was collected during the execution of these tasks, HR as a temperament index was outside the scope of the current thesis.

2.2.3 Effortful control

Effortful control (EC), the ability to suppress a dominant response in order to perform a subdominant response (Posner & Rothbart, 2000; Rothbart & Ahadi, 1994; Rothbart & Bates, 1998), is a key element of executive functioning. EC ability begins to develop in the second year of life, with rapid developmental increases between the ages of 3- and 6-years (Carlson, 2005; Carlson & Wang, 2007; Reed, Pien, & Rothbart, 1984) and has been shown to formalise as a personality trait by 45 months of age (Kochanska & Knaack, 2003). Researchers have demonstrated that the ability to execute tasks requiring effortful control is a protective factor against the development of externalizing behavioural problems. Krueger, Caspi, Moffitt, White and Stouthamer-Loeber (1996) reported that poor delay of gratification, an aspect of effortful control, was correlated with higher levels of delinquent and aggressive behaviour; whereas, successful delay of gratification was linked to adaptive behaviours.

Effortful control measured by questionnaires: Mothers' ratings of effortful control are usually assessed via the Child Behaviour Questionnaire (CBQ; Rothbart, Ahadi & Hershey, 1994) which is addressed to children aged between 3-8 years of age. As mentioned above, EC ability begins to develop in the 2^{nd} year of life. Kochanska, Murray and Harlan (2000) reported convergence between contemporaneous behavioural and parental (CBQ) measures of effortful control at 22 and 33 months of age. In the current study EC was assessed in the 2^{nd} year follow-up study through behavioural observation only.

Effortful control measured by observation: Observational measures of effortful control require the child to suppress a dominant response and perform a subdominant response, and target different components of EC such as delaying, slowing down motor activity, suppressing/initiating activity in response to a signal and lowering of voice (Kochanska & Knaack, 2003). EC has been assessed in children as young as 22 months-old (Kochanska, Coy & Murray, 2001; Kochanska & Knaack, 2003). In the current study the ability to delay gratification was targeted. To this end, two delay tasks were used: for one task the child was required to wait to hear a bell ring before s/he was allowed to retrieve a snack from a transparent cup and in the other the child had to wait for 3 minutes before opening his/her present (Kochanska, Murray & Harlan, 2000).

Effortful control measured by physiological measures: The main physiological methods used in temperament research to assess EC are electroencephalogram (EEG) and HR. Wolfe and Bell (2003) found that increased EEG activity and HR were associated with effortful behaviour in 25 4-year-olds. As mentioned earlier, HR measures have been collected in our laboratory, but are not part of this thesis.

In summary, the current study aimed to conduct a two-year longitudinal study on the physiological, observational/behavioural and questionnaire correlates of fearfulness in very young children. Because attentional and self-regulatory dimensions of temperament play an important role in fear, and emotional development more generally (with, for example, fearless children having greater problems with sustained attention and/or delay of gratification), a second goal was to study the behavioural correlates of sustained attention and effortful control in the same group of children.

Chapter 3 Observation of fearfulness; Methodology and results

3.1 Fearfulness as a behavioural characteristic in temperament research in infancy

As mentioned before, fearfulness - a child's initial withdrawal to unfamiliar or challenging events (e.g. Kagan, 1989) - has been a popular target of inquiry in the last decades. Given the above definition one can infer that measures of shyness with strangers, approach or withdrawal to unfamiliar objects or people, and timidity in fear eliciting situations would be appropriate for examining the nature and development of behavioural inhibition (Kagan & Saudino, 2001). Many attempts have been made to study fearfulness as a temperament characteristic in infancy by employing measures of observation. The following review aims to explain the role of fearfulness in temperament research, as well as to delineate the current orientation of my research in infancy and early childhood with respect to measuring fearfulness through observation.

3.1.1 Behavioural observation in infants and young children

Gunnar, Leighton and Peleaux (1984) examined the effects of temporal predictability as one of the main pathways to understanding human responses to aversive events in 1-year-old-infants. It was predicted that a fearful infant might react more strongly to novel events due to a lack of predictability (Gunnar et al., 1984). In this study two noise-making mechanical toys were used as potentially frightening stimuli: a cymbal-clapping monkey and a 'gun shooting' robot. Each infant saw only one of the toys, with the toys being counterbalanced across conditions. Behavioural scoring was undertaken by two observers seated behind a one-way mirror during the experiments.

No maternal reports were used in this study. With respect to temporal predictability, it was found that infants responded less fearfully to toys that played in the predictable as opposed to the unpredictable schedule; infants were less afraid when they were able to predict how long the toy would stay off each time it was inactive rather than how long it would be active once it started playing. Unfortunately, Gunnar et al. (1984) did not collect other measures to support their results, such as questionnaires or physiological data.

Nachmias et al. (1996) studied the behavioural inhibition system (BIS) and the moderating role of attachment security in 77 18-month-olds via maternal reports (Toddler Behaviour Assessment Questionnaire, TBAQ; Goldsmith, 1987) and stress reactivity (cortisol). Behavioural inhibition was measured by recording the toddler's reactions to several novel events, while attachment security was examined approximately one week later using the Ainsworth Strange Situation test (Ainsworth, Blehar, Waters & Wall, 1978). In an earlier study Calkins and Fox (1992) tested 14-month-old infants for inhibition and attachment during the same experimental session but Nachmias et al.'s study assessed inhibition and attachment in separate sessions to ensure independence of measurement of the two constructs. The novel, potentially threatening stimuli used in this study were: a) a live, boisterous clown who invited the child to play; b) a noisy, mechanical clown robot that moved around; and c) two talkative hand-puppets who invited the child to play (Nachmias et al., 1996). Video recordings of the session were made for scoring purposes. The authors found a moderating role of attachment security in inhibited children as reflected by the activity of their HPA axis system. Inhibited, insecurely attached toddlers were more stressed (higher cortisol) in the face of novel events than inhibited securely attached ones (Nachmias et al., 1996). Inhibition of approach was not related to attachment security, thus contradicting Kagan's claim that resistant attachment reflects high inhibition, whereas avoidant attachment reflects low inhibition (Nachmias et al., 1996). Kagan's hypothesis was confirmed in the earlier study by Calkins and Fox (1992). Nachmias et al. (1996) proposed that the difference in testing-time might account for the discrepancy in findings between the two studies. In the Calkins and Fox' s (1992) study children's behaviour in the strange situation might have been affected by their reactions to the inhibition test, which took place immediately prior to the strange situation episode.

3.1.2 Behavioural observation in longitudinal designs

Kagan (1994) and his team tested 462 healthy 4-month-old infants on a battery of visual, auditory and olfactory stimuli. Twenty percent of the sample was characterized as highly reactive and highly distressed, whereas 40% showed the opposite profile of low motor activity and low distress when confronted with the same cluster of stimuli. Later, the same children returned to the lab when they were 14- and 21-months-old, and they were again administered a battery of unfamiliar social and non-social events. These included episodes such as interacting with an unfamiliar experimenter, the placement of heart rate electrodes and a blood pressure cuff, being confronted with a stranger dressed as a clown and an unfamiliar mechanical robot (Kagan, 1994). Infants who were found to be highly reactive at 4 months were found to be significantly more fearful both at 14 and 21 months of age. The majority of the children were assessed once more in the laboratory at 4.5 years. This time they were exposed to an unfamiliar experimenter and a socially challenging context which included interaction with two other unfamiliar peers. Infants who had been classified as highly reactive were more withdrawn, both in the stranger approach and the peer interaction context (Kagan, 1994). The fact that only 4% of the sample presented a different profile at 4.5 years than the one observed at 4 months of age, contributes to the notion of temperament's stability over the first years of life (Kagan, 1994).

Gunnar, Brodersen, Krueger and Rigatuso (1996) conducted a longitudinal study of cortisol reactivity and behavioural responses in 83 healthy infants in response to inoculations. 72 Infants completed all testing at 2, 4, 6 and 15 months of age, while a comparison group of 2-, 4-, and 6-month-olds received mock exams without inoculations for examining developmental changes in response to exam procedures. Behavioural distress was scored every 30 seconds on a 4-point scale (ranging from 0 = no evidence of distress, to 4 = high levels of crying) (Gunnar et al., 1996). Results showed an increase in behavioural distress from 6 to 15 months, with girls showing more distress. Behavioural distress decreased between 2 and 6 months, but increased again at 15 months. Gunnar et al. (1996) noted that according to previous reports the increase in average distress between 6 and 15 months might reflect increased anger.

Rothbart, Derryberry and Hershey (2000) examined individual differences in temperament longitudinally from infancy (n = 59) to 7 years of age (n = 26). They measured fear through behaviour at 6.5 months of age with a mechanical monkey, a mechanical bird, and a stranger episode; at 10 months of age with a stuffed bear, a mechanical dog and a mechanical duck; and at 13.5 months of age with a mechanical duck, a jack-in-the-box, and a social episode. At 7 years fear was assessed via the CBQ questionnaire only. Mothers also completed the IBQ questionnaire at all times during infancy. No differences were found across age in latency to fear reactions or duration of fear with the exception of a decrease in fear intensity across age ($p < 0.05$). IBQ fear was correlated across age. Reliable but low fear stability was found from 6.5 to 13 months of age. Observed fear was moderately correlated with IBQ fear at 6.5 and 10 months of age. Only laboratory fear at 13.5 months (r = .46) and IBQ fear at 6.5 months (r = .61) predicted CBQ fear at 7 years. No sex differences or sex by age interactions were found. However, although the authors had successfully measured fear in infancy, they had not done so with identical episodes across ages.

3.1.3 Fearfulness measured by the Laboratory Temperament Assessment Battery (Lab-TAB)

Goldsmith and Rothbart (1988) developed a reliable and frequently used laboratory-based observational battery, the Laboratory Temperament Assessment Battery (Lab-TAB), for the study of early temperament. To date, the Lab-TAB includes 20 standardized episodes which target the 5 temperament dimensions of fear, pleasure, frustration, activity level and interest. The Lab-TAB can be used partly or as a whole to examine temperament differences in infancy and in young children. Lab-TAB's basic merits include the ability to assess behavioural patterns of responding across episodes which are designed to tap into a specific emotion and to assess early developing emotion systems (Kochanska, Coy, Tjebkes & Husarek, 1998). The presence of an emotion, its latency and peak intensity are coded within and across episodes. A description of some relevant studies follows next.

Buss and Goldsmith (1998) studied whether specific coping behaviours such as approach and interaction with the stimulus, or withdrawal and looking to the mother, are empirically rather than just conceptually associated with the regulation of emotions such as anger and fear. Four Lab-TAB episodes were selected for their ability to cause distress or fear in young infants. These were the Attractive Toy behind the Barrier (anger), Arm Restraint by Parent (anger), Remote-Controlled Spider (fear), and Unpredictable Mechanical Toy (fear). Infants in the age range of 6 to 18 months participated in this study. The mothers were present during the procedure, but were requested to remain neutral and unresponsive (Buss & Goldsmith, 1998). All the episodes were videotaped for scoring purposes. The authors found that fear was not easily regulated under stressful situations. Most coping behaviours in the fear-eliciting episodes were ineffective in reducing distress. Nevertheless, coping behaviours were found to have a regulatory effect during the anger episodes. The authors concluded that contextual differences determine the observed emotional differences. Novelty and threat elicit fear, whereas a feeling of restraint is crucial for anger. These two systems (anger and fear) are complex and not necessarily similarly regulated (Buss & Golsmith, 1998). The authors did not report any sex or age differences in their results.

Kochanska, Coy, Tjebkes and Husarek (1998) investigated individual differences in early emotionality in 8 to 10-month-old infants. Specifically, they examined fear among other emotions (i.e. joy, angry distress, and discomfort) by using the Stranger Approach, the Unpredictable Toy, the Masks and the Parasol Opening Lab-TAB episodes. The IBQ was completed by both parents. Kochanska et al. (1998) aimed to examine whether there would be coherence of responding within and across episodes (i.e. children prone to anger are also likely to be prone to distress), as well as whether there would be any convergence between behavioural measures and parental questionnaires (IBQ). Coherence in responding within and across episodes was observed for all emotions except anger, but no correlations were found between behavioural measures and IBQ. Unfortunately, the authors did not explain this finding. Although, their data supported cross episode consistency for all emotions except anger, Kochanska et al. (1998) did not employ a longitudinal design and the temporal stability of their findings could therefore not be examined. Finally, although older infants and girls have been reported to obtain higher scores on the IBQ fear dimension (Gartstein & Rothbart, 2003), Kochanska et al. (1998) did not report whether there were sex or age differences in their sample.

3.1.4 Underlying social factors of gender differences in temperament

As Hay (2007, p. 1534) states "sex is a salient social category for young children". Children learn from an early age about gender stereotypes and start to form extreme views about differences between the sexes. Within the family system, parents tend to interact differently with their sons and daughters. Specific factors such as parents' temperament, their values and attitudes regarding gender roles, the quality of the marital relationship, as well as the cultural and socioeconomic background of the family system directly influence childrens' socialization of gender roles. For example, girls are generally more encouraged to show their emotions and express vulnerability, while boys are discouraged to act accordingly. Thus fear and withdrawal are linked to feminine nature, while fearlessness and higher levels of activity and anger/aggression are linked to masculine nature (Brody, 2000). Since these qualities are to some extent imposed on children by their parents, it is not surprising that studies based on parent reports of temperament have yielded higher fear scores for girls and higher activity levels for boys (Gartstein & Rothbart, 2003). Moreover, within peer groups children conform to the display of specific sex-role stereotypic behaviours to become socially accepted and popular. For example, boys tend to suppress their emotional expressiveness but act more aggressively in peer fights than girls do.

3.1.5 Fearfulness measured by questionnaires and observation in the current study

This section describes how fearfulness, as a temperament characteristic, was measured in the current study. The chapter ends with a presentation of the results and a discussion of the findings (see sections 3.4 & 3.5).

Since development has been shown to affect how individuals perceive potential fearful events (Ollendick, Langley, Jones & Kephart, 2001), the main purpose of the present study was to examine temperament differences longitudinally in one- and two-year-old-children under novel and stressful conditions, and to explore the relations between different assessments of temperament correlates (i.e., maternal reports and observation measures). Mothers rated their children's temperament by completing the IBQ-R (Gartstein & Rothbart, 2003) in year 1, and the Child Behaviour Checklist (CBCL; Achenbach & Rescorla, 2000) in year 2. The fear eliciting stimulus that was employed in the current study was the Unpredictable Mechanical Toy episode from the Lab-TAB (Goldsmith & Rothbart, 1999).

3.2 Hypotheses

In line with the development of inhibition of approach towards novel objects, which increases with age in infants, fear increases in the first year of life (Gartstein & Rothbart, 2003). Thus we predicted (1) age effects. We predicted (a) that relatively older children tested in year 1 would obtain higher scores on the IBQ-R fear dimension and show more behavioural distress during fear exposure (Gartstein & Rothbart, 2003; Gunnar et al., 1996); (b) that relatively older children in year 2 would have higher CBCL internalizing scores and more behavioural distress during fear exposure, and (c) a significant increase in fear distress from year 1 to year 2 (Gunnar et al., 1996). (2) Research on behavioural distress often fails to report on the existence of gender differences. This might be due to the fact that the social factors that underlie gender differences are not yet apparent in these early ages (Hay, 2007). Thus we expected no gender differences on the IBQ-fear scale and no gender differences in behavioural distress during fear challenge. No gender differences were expected on the CBCL internalizing dimension (see Achenbach & Rescorla, 2000, p. 61). (3) In line with previous studies, we predicted that questionnaire and behavioural measures of fear would be positively correlated. Thus, for example, mothers who rated their children as fearful were expected to have children who showed more behavioural distress (Kagan, 1994; Kochanska, et al., 1998). (4) Finally, we predicted inter-individual consistency in measures over the time, such that more fearful children in year 1 would also be more fearful children in year 2 (Rothbart, Derryberry & Hershey, 2000; Kagan, 1994).

The results of the testing done in each year will be presented separately, after which they will be compared in the final section of this chapter. Since the methods and procedures were identical in both years, these are only described once.

3.3 Methods

3.3.1 Participants

Year 1 sample

Participants were 50 infants (mean age in months = 9.7, sd = 2.2) and their mothers; all participants had been born in Cardiff, Wales. Participants were recruited from local nurseries and leisure centres.

For analysis purposes the sample was divided into four subgroups: boys (n = 27, mean age = 9.9, sd = 2.0) and girls (n = 23, mean age = 9.5, sd = 2.5), younger (n = 27, mean age = 8.0, sd = 1.0) and older (n = 23, mean age = 11.7, sd = 1.0). Of the 27 boys 14 belonged to the younger (mean age = 8.3, sd = 0.9) and 13 belonged to the older (mean age = 11.5, sd = 1.4) group, and of the 23 girls 13 belonged to the younger (mean age = 7.7, sd = 0.9) and 10 to the older group (mean age = 11.9, sd = 1.5). A chi-square test for independence indicated no significant association between gender and age (χ^2 (1) = .109, p = .74). Boys and girls did not differ in age (t (48) = -.55, p = .59).

Year 2 sample

Approximately 12 months after the first assessment the families were contacted again by mail or telephone, and asked whether they were willing to participate in the follow-up study. Of the original sample (N = 50) 10 participants could not be located or did not respond to our letter or phone calls. Therefore, 40 children (80%) returned to the laboratory for a visit in their 2nd year of life.

Of the 40 participants, 7 were unable to complete the 2nd study[1] and could therefore not be used in the analyses. These 7 children did not differ from the other participants in mother-rated IBQ-fear (U = 126.500, p = .84) or in the composite Lab-TAB score (t (47) = 10, p = .31). So in the end 33 children (66%) completed the study (mean age in months = 22.3, sd = 3.2). This sample was again divided into the same four subgroups; boys (n = 18, mean age = 22.6, sd = 3.4) and girls (n = 15, mean age = 21.9), young (n = 18, mean age = 19.9, sd = 2.1) and old (n = 15, mean age = 25.1, sd = 1.9). Of the 18 boys 8 belonged to the young group (mean age = 19.8, sd = 2.5) and 10 to the old group (mean age = 24.9, sd = 1.8), and of the 15 girls 10 belonged to the young (mean age = 20.1, sd = 1.8) and 5 to the old group (mean age = 25.4, sd = 2.1). A chi-square test for independence indicated again no significant association between gender and age (χ^2 (1) = 1.63, p = .20). Boys and girls did not differ in age (t (31) = -.65, p = .52).

3.3.2 Procedure

Information sheets containing the purpose and procedures of the study, contact details as well as photographs of the procedure were distributed to nurseries' and leisure centres' managers (see Appendix 1). The infants' caregivers were paid £10, plus the transportation costs for travelling to and from the School of Psychology, and were also provided with an age appropriate story book for their child. Full informed consent from the parents was obtained before the start of the study. Since it was anticipated that some infants may become mildly distressed by the presentation of the novel/fear eliciting stimuli, parents were notified of this issue before agreeing for their children to take part in this study. Parents were also assured that they could withdraw their child at any stage of the study without loss of payment or

[1] Seven infants did not complete the experiment due to fussiness in response to the demands of the experimental procedure, specifically the placement of the electrodes.

complimentary gift. Finally, as underlined in the parent consent form, the study involved the use of a video camera for the purposes of the behavioural coding. Therefore, parents were also requested to provide permission for this to take place whilst being assured that the data obtained in the study would be kept strictly confidential, used only for coding and teaching purposes and would eventually be destroyed after a standard period of 5 years.

a) Baseline

The primary caregiver accompanied the child to the laboratory. Parents were briefed about the procedure of the study and the consent form was issued and signed. An informal interview was conducted with the caregiver during the warm-up period. This interview was intended to elicit the mother's predictions about how the infant would react in the upcoming episodes and to help the parent familiarise with the laboratory and experimenter. The experimenter interacted with the mother and the child for approximately 20 minutes in order for the child to adapt to the novel environment. The child was free to play with a range of (calming) toys in the room.

b) Presentation of Unpredictable Toy

Goldsmith and Rothbart (1999) induced stress by using a remote controlled toy dog which approached the infant and barked. However, this study employed a different mechanical toy as an analogous stimulus to induce fear. Specifically, a mechanical remote controlled noise producing robot was employed. Although the procedure during the presentation of the robot followed Goldsmith and Rothbart's (1999) protocol strictly, in the present study the mother was requested to leave the room and a new experimenter entered who presented the robot to the child.

First, the infant was carefully placed in a car seat and strapped in safely by the mother. Then the mother was asked to leave the room and go into an observation room, from where she could closely observe the child's reactions through a one-way mirror. An unfamiliar experimenter, wearing a white coat and large glasses, entered the experimental room and acted in a non-responsive way to the child. She placed the robot approximately 1.5 m away from the child. Caregivers were assured that they could request for the episode to be terminated at any point in time they felt the child became too distressed.

The experimenter was sitting on the floor. The robot moved forward until it stopped 15 cm in front of the child. The toy remained in front of the child, while moving its arms and legs rapidly and making a noise. If the child reached for the toy, it was moved slightly back, out of child's reach. The toy then moved backwards until it was in its original position where it remained silent and stationary for 5 seconds. After the 5 seconds pause, the entire procedure was repeated twice more for a total of three trials. At the end of the 3rd trial, the toy was activated once more whilst stationary for a final 10 sec.

After the fear episode, the mother returned to the room and was asked to interact with her child in any way she liked. After the child had calmed down, the robot was offered for the child to investigate and play with it. Calming toys were made available again. The caregiver and the child had the opportunity to stay in the room for as long as was needed for the child to recover from this experience.

Behavioural Coding

For the unpredictable toy episode the Lab-TAB's guidelines were followed (see Appendix 2). Coding took place post-testing based on video recordings. For scoring purposes it was important for both the torso and the head of the child to be clearly visible (i.e., videotaped). Special emphasis was placed on gaining clear, full frontal shots of the face. It was also necessary to include both the child and the robot in the video frame when the toy was rearing up in front of the child. A digital video camera was used to record the behavioural responses of the children on digital tapes. The contents of the tapes were then transferred to the main laboratory computer by using Adobe Premier Pro 2.0 software. There were nine epochs in the fear episode; three in each trial (robot walking forward, robot moving in front of child 1st part, robot moving in front of child 2nd part). The variables to be coded from this episode were: (1) Intensity of facial fear (0 = no facial region shows codeable fear movement; 1 = only one facial region shows codeable movement, identifying a low intensity fear, or expression is ambiguous; 2 = only 2 facial regions show codeable movement, or expression in one region is very clear; 3 = an appearance change occurs in all 3 facial regions, or coder otherwise has impression of strong facial fear); (2) Intensity of facial sadness (0 - 3, see Intensity of facial fear rating); (3) Intensity of distress vocalizations (0 = no distress, 1 = mild vocalization that may be difficult to identify as hedonically negative, 2 = definite whimpering, limited to a short (1 - 2 second) duration, 3 = longer whining, fussing, mild protest, or low-intensity cry (cry has extended or rhythmic quality), 4 = definite non-muted crying, 5 = full intensity cry/scream (child is loosing control)); (4) Intensity of bodily fear (0 = no sign of bodily fear, 1 = decreased activity: an apparent and sudden decrease in the activity level of C, 2 = tensing: visible and sustained tensing of the muscles, associated with decreased activity, 3 = freezing or trembling: tensing of the entire body with no motion, or trembling due to extreme muscular tension); (5) Intensity of escape (0 = no escape, 1 = mild or fleeting escape behaviour (e. g. turning away, sinking into chair), 2 = moderate escape behaviour resulting in significant, but not extreme attempts to get away or resist [full body movements such as arching back, twisting away, and leaning away are included as well as hitting, pushing, and/or slapping], 3 = vigorous escape behaviour, usually involving linked, intense full-body movements like those found in '2' [these movements usually last for the entire epoch]), and (6) Presence of startle response (0 = absent, 1 = present) (Goldsmith & Rothbart, 1999). In the Results section we present the data for these Lab-TAB variables separately. However, the high reliability among these variables (Cronbach's alpha = .88 for year 1 and α = .88 for year 2) enabled us to create a composite score by accumulating individual ratings for the behavioural variables across the fear episode (Buss & Goldsmith, 1998).

After the end of the robot episode the child was encouraged by the mother or the experimenter to play with the robot. On that basis another variable, also part of the Lab-TAB coding scheme, was created: the touch-not touch variable, which reflects whether the child was willing to touch the robot or not (scored as 'yes' or 'no'). We were interested to examine whether children who did not touch the robot would have higher IBQ-fear and internalizing ratings, and show higher levels of distress during fear exposure.

Four coders scored the episodes independently. Intra-correlation coefficients between coders were calculated for 12 of the 49 infants in year 1, and for 10 out of the 33 children in year 2. These coefficients ranged between .70 - .99 across variables.

c) Maternal reports

Year 1: Infant Behaviour Questionnaire-Revised (IBQ-R)

As a measure of maternal perceptions of child temperament the Infant Behaviour Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003) was completed prior to the start of testing in year 1. The IBQ has been used successfully in research on individual differences in emotionality (Goldsmith & Campos, 1990).

The IBQ-R has been designed to measure temperament in infants between the ages of 3 and 12 months, by assessing 14[2] dimensions of temperament. The IBQ-R is a 191-item questionnaire. Parents rate each item on a Likert scale, ranging from 1 (never) to 7 (always). The items require the parents to rate the frequency of specific temperament-related behaviours as observed in their child over the past week. In this study we only used the IBQ-R dimension of fear. This scale includes 16-items which relate to the baby's startle or distress to sudden changes in stimulation, novel physical objects or social stimuli. An example of an item is: *"how often during the last weeks did the baby cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)"?* Older infants and girls are rated higher on this dimension (Gartstein & Rothbart, 2003).

[2]The 14 temperamental dimensions assessed by the IBQ-R are: activity level, distress to limitations, approach, fear, duration of orienting, smiling and laughter, vocal reactivity, sadness, perceptual sensitivity, high intensity pleasure, cuddliness, soothability, and falling reactivity / rate of recovery from distress

Year 2: Child behaviour checklist and language development (CBCL/1.5-5; Achenbach & Rescorla, 2000)

In the current study the CBCL was completed by the mothers as part of the 2nd year follow up study. The parents were sent the CBCL at home by mail prior to testing and they were asked to bring it in on the day of testing. Only the composite scales of internalizing and externalizing behavioural problems were used for the purposes of the current study. Higher levels of infant negativity have been associated with higher levels of internalizing and externalizing behavioural problems in childhood (Blair, 2002). Age and gender effects on the CBCL internalizing and externalizing dimensions have not been reported in normative samples (Achenbach & Rescorla, 2000, p. 61).

3.4 Results

3.4.1 Statistical analyses

Data for one child could not be collected due to equipment failure; therefore the behavioural data of 49 children were entered for analyses. Moreover, 5 mothers failed to send back the IBQ-R questionnaire and therefore IBQ-fear data for only 45 children were entered for analysis purposes. Due to skewness of some dependent measures (IBQ-fear, CBCL dimensions, Lab-TAB escape and Lab-TAB startle response) both nonparametric and parametric statistical tests were used to analyse the data. Non parametric Mann-Whitney tests were carried out to screen for effects of age and sex on IBQ-fear / CBCL, Lab-TAB startle response and intensity of escape; independent samples t-test were used when screening for the same effects on all other Lab-TAB variables. Mann- Whitney tests were also carried out in both years when examining for the effects of touch-not touch variable on the IBQ-fear and the CBCL dimensions. Finally, in order to test for relations within and between the observational and questionnaire measures correlations were carried out in both years. When correlations were performed between normally distributed variables Pearson's coefficient was used; in all other cases Spearman's correlations were employed.

3.4.2 Results for year 1

IBQ-fear

Although there was no effect of sex on IBQ-fear ($mean_{GIRLS} = 2.7$, sd = 1.1, $mean_{BOYS} = 2.6$, sd = 0.9; U = 240.500, $p = .88$), there was an effect of age group ($mean_{YOUNG} = 2.4$, sd = 0.9, $mean_{OLD} = 3.1$, sd = 1.1; U = 152.00 $p < 0.05$), with older infants being rated by their mothers as more fearful. Table 3-1 shows the descriptive IBQ-fear information for the total sample and the different subgroups.

Table 3-1 IBQ-fear: Means and standard deviations for the total sample and the different subgroups

	fear	
	Mean	**SD**
Group (n = 45)	2.7	1.0
girls (n = 18)	2.7	1.1
boys (n = 27)	2.6	0.9
young (n = 26)	2.4	.9
old (n = 19)	3.1	1.1

Behavioural dimensions

Regarding the observed behavioural dimensions of the Lab-TAB (i.e., intensity of facial fear, intensity of facial sadness, intensity of vocal distress, intensity of bodily fear, presence of startle response, intensity of escape behaviour, and touch - not touch of the robot) data were first screened for effects of age and gender. Table 3-2 presents descriptive data for the total sample and the different subgroups with respect to these variables.

Table 3-2 Lab-TAB intensity scores: Means and standard deviations for the group and the different subgroups

	facial fear		facial sadness		vocal distress		bodily fear		startle response		escape behaviour	
	M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Group (n= 49)	2.0	0.8	1.3	1.1	1.8	1.7	1.5	0.9	0.1	0.2	0.4	0.8
girls (n= 23)	1.9	0.8	1.1	1.0	1.5	1.6	1.5	0.9	0.2	0.2	0.6	1.0
boys (n= 26)	2.1	.77	1.5	1.1	2.0	1.8	1.5	0.9	0.02	0.1	0.3	0.5
young (n= 26)	1.6	0.7	0.8	1.0	1.0	1.6	1.1	0.8	0.1	0.1	0.1	0.4
old (n= 23)	2.4	0.6	1.9	0.9	2.6	1.5	1.9	0.6	0.1	0.2	0.7	1.0

Note: the 'touch-not touch' dimension is not included in the above Table, since it is a binary variable

Age effects

A significant effect of age was found on 'intensity of facial fear' ($mean_{YOUNG}$ = 1.6, sd = 0.7, $mean_{OLD}$ = 2.4, sd = 0.6; t (47) = -4.43, p<0.01), 'intensity of facial sadness' ($mean_{YOUNG}$ = 0.8, sd = 0.9, $mean_{OLD}$ = 1.9, sd = 0.9; t (47) = -4.32, p<0.01), 'intensity of vocal distress' ($mean_{YOUNG}$ = 1.0, sd = 1.6, $mean_{OLD}$ = 2.6, sd = 1.6; t (47) = -3.53, p<0.01), 'intensity of bodily fear' ($mean_{YOUNG}$ = 1.1, sd = 0.8, $mean_{OLD}$ = 1.9, sd = 0.6; t (47) = -4.07, p<0.01) and finally on 'intensity of escape behaviour' ($mean_{YOUNG}$ = 0.1, sd = 0.4, $mean_{OLD}$ = 0.7, sd = 0.9; U = 174.500, p<0.01) with older children having higher scores on these dimensions (see Figure 3-1). Age effects were not found on 'startle response' ($mean_{YOUNG}$ = 0.1, sd = 0.1, $mean_{OLD}$ = 0.1, sd = 0.2; U = 294.500, p = .91).

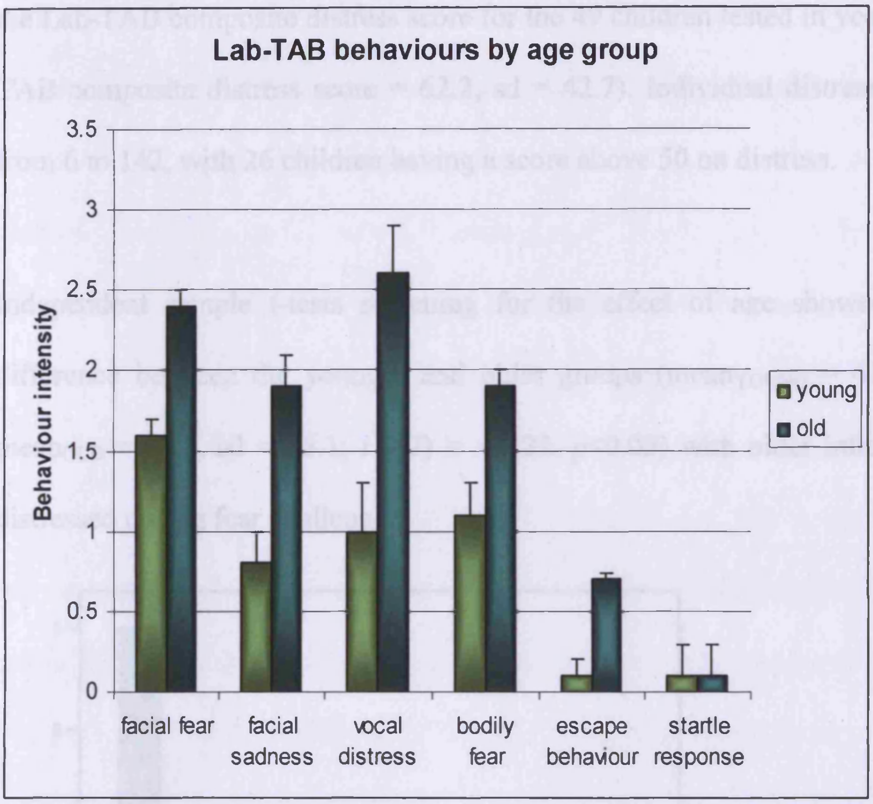

Figure 3-1 Age group effects on Lab-TAB behaviours, with error bars indicating the SE's

Regarding 'touch-not touch', participants were divided into those who were willing to touch the robot and those who were not willing to touch the robot ($n_{TOUCH} = 26$, $n_{NOT\ TOUCH} = 23$). Of the 23 infants who did not touch the robot 6 belonged to the younger and 17 to the older group, and of the 26 who touched the robot 20 belonged to the younger and 6 to the older group. Chi-square tests revealed a significant effect of age (χ^2 (1) = 12.66, p<0.01) with older children being less willing to touch the robot.

Finally, a composite score created by accumulating the scores of each infant on the behavioural variables (henceforth, Lab-TAB composite distress score) was used to further analyse the observational data. Figure 3-2 shows the frequency distribution of the Lab-TAB composite distress score for the 49 children tested in year 1 (mean Lab-TAB composite distress score = 62.2, sd = 42.7). Individual distress scores ranged from 6 to 142, with 26 children having a score above 50 on distress.

Independent sample t-tests screening for the effect of age showed a significant difference between the younger and older groups ($mean_{YOUNG}$ = 41.4, sd = 37.5, $mean_{OLD}$ = 85.7, sd = 36.1; t (47) = - 4.21, $p<0.05$) with older infants being more distressed during fear challenge.

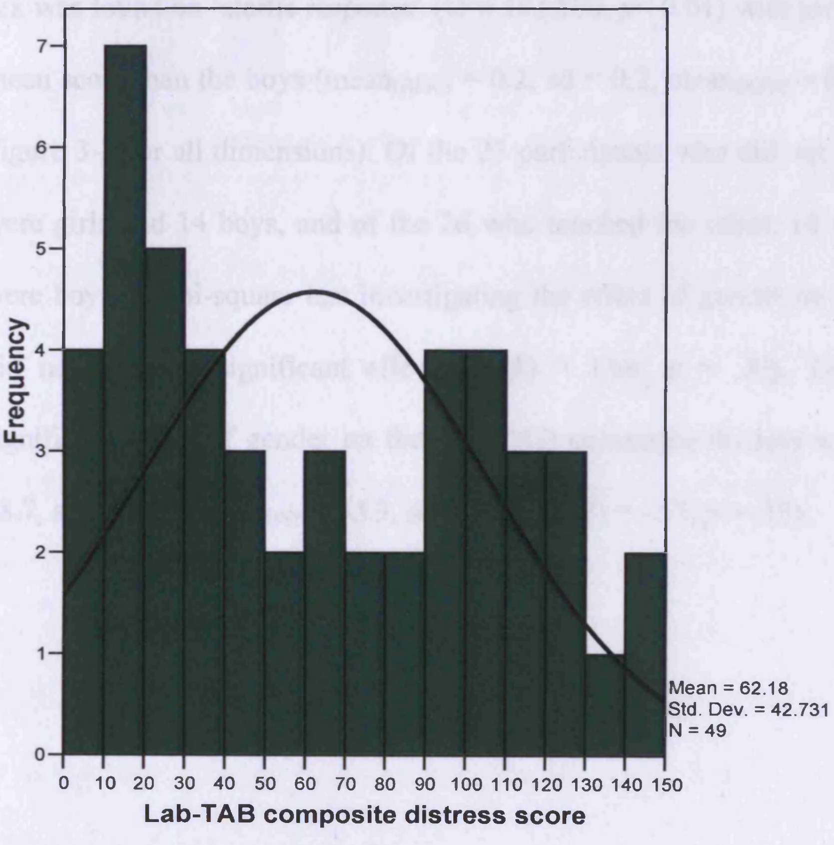

Figure 3-2 Frequency of the Lab-TAB composite distress score in year 1

Effect of gender

No significant effect of gender was found on 'intensity of facial fear' ($mean_{GIRL\ S}$= 1.9, sd = 0.8, $mean_{BOYS}$ = 2.1, sd = 0.8; *t* (47) = -.89, *p* = .37), 'intensity of facial sadness' ($mean_{GILRS}$ = 1.1, sd = 1.0, $mean_{BOYS}$ = 1.5, sd = 1.1; *t* (47) = -1.39, *p* = .17), 'intensity of vocal distress' ($mean_{GIRLS}$ = 1.5, sd = 1.6, $mean_{BOYS}$ = 2.0, sd = 1.8; *t* (47) = -.97, *p* = .34) 'intensity of bodily fear' ($mean_{GIRLS}$ = 1.5, sd = 0.9, $mean_{BOYS}$ = 1.5, sd = 0.9; *t* (47) = -.17, *p* = .86), or 'intensity of escape behaviour' ($mean_{GIRLS}$ = 0.6, sd = 1.0, $mean_{BOYS}$ = 0.3, sd = 0.5; U = 273.00, *p* = .55). A significant effect of sex was found on 'startle response' (U = 193.500, $p < 0.01$) with girls having a higher mean score than the boys ($mean_{GILRS}$ = 0.2, sd = 0.2, $mean_{BOYS}$ = 0.02, sd = 0.1; see Figure 3-3 for all dimensions). Of the 23 participants who did not touch the robot 9 were girls and 14 boys, and of the 26 who touched the robot, 14 were girls and 12 were boys. A chi-square test investigating the effect of gender on 'touch-not touch' did not reveal a significant effect (χ^2 (1) = 1.06, *p* = .30). There was also no significant effect of gender on the Lab-TAB composite distress score ($mean_{GIRLS}$ = 58.7, sd = 44.0, $mean_{BOYS}$ = 65.3, sd = 42.2; *t* (47) = -.53, *p* = .59).

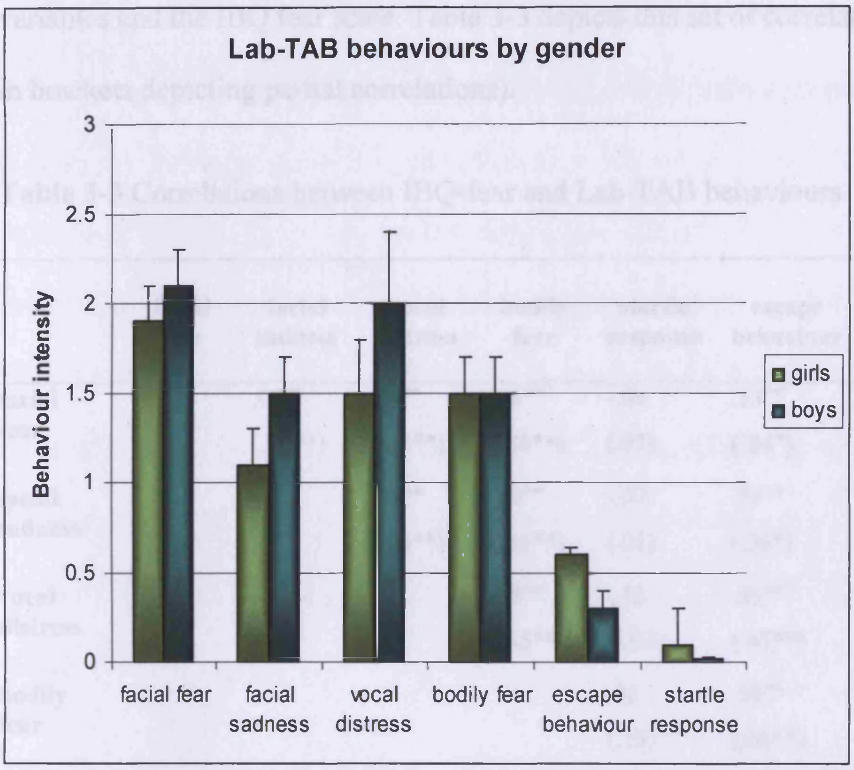

Figure 3-3. Gender effects on Lab-TAB behaviours, with error bars indicating the SE's

Finally, we examined whether the groups of children who touched and did not touch the robot differed in IBQ-fear and Lab-TAB distress. Analyses showed that infants who avoided contact with the robot had been rated as more fearful by their mothers ($mean_{TOUCH} = 2.3$, sd = 0.2, $mean_{NOTTOUCH} = 3.0$, sd = 1.0; U = 133.500, $p<0.05$) and by independent raters in the laboratory ($mean_{TOUCH} = 39.1$, sd = 36.5, $mean_{NOTOUCH} =$ 88.2, sd = 33.8, t (47) = -4.87, $p<0.01$).

Correlations between observational and questionnaire measures

Although we observed positive and significant correlations between most of the Lab-TAB variables, no significant correlations were found between the Lab-TAB variables and the IBQ fear scale. Table 3-3 depicts this set of correlations (with values in brackets depicting partial correlations).

Table 3-3 Correlations between IBQ-fear and Lab-TAB behaviours

	facial fear	facial sadness	vocal distress	bodily fear	startle response	escape behaviour	distress[a]	IBQ-fear
facial fear		.92** (.90**)	.89** (.84**)	.90** (.88**)	-.06 (.03)	.55** (.34*)	.94** (.91**)	.22 (.10)
facial sadness			.95** (.93**)	.89** (.86**)	-.07 (.01)	.54** (.36*)	.95** (.95**)	.22 (.06)
vocal distress				.88** (.85**)	-.13 (-.02)	.55** (.45**)	.95** (.96**)	.21 (.02)
bodily fear					.06 (.15)	.59** (.46**)	.95** (.93**)	.21 (.10)
startle response						.10 (.08)	-.01 (.05)	.25 (.26)
escape behaviour							.64** (.57**)	-.01 (-.05)
distress								.14 (.04)

[a] Lab-TAB composite distress score

*= Correlation is significant at the 0.05 level
**= Correlation is significant at the 0.01 level

Next, we used a median split procedure to compare high and low IBQ-fear groups (n = 44; median IBQ-fear score = 2.6, high fear group (n = 22): mean IBQ-fear score = 3.4, low fear group (n = 22): mean IBQ-fear score = 1.8) on the different Lab-TAB behavioural scores. No significant effect of fear group was found on 'intensity of facial fear' ($mean_{HIGH}$ = 2.0, sd = 0.7, $mean_{LOW}$ = 1.8, sd = 0.8; t (42) = -1.24, p = .26), 'intensity of facial sadness' ($mean_{HIGH}$ = 1.4, sd = 1.1, $mean_{LOW}$ = 1.0, sd = 1.1; t (42) = -1.25, p = .22) 'intensity of vocal distress' ($mean_{HIGH}$ = 1.9, sd = 1.8, $mean_{LOW}$

= 1.3, sd = 1.6; t (42) = -1.20, p = .25) 'intensity of bodily fear' ($mean_{HIGH}$ = 1.6, sd = 0.8, $mean_{LOW}$ = 1.2, sd = 0.9; t (42) = -1.48, p = .15) 'startle response' ($mean_{HIGH}$ = 0.1, sd = 0.2, $mean_{LOW}$ = 0.1, sd = 0.1; U = 197.00, p = .18) 'intensity of escape behaviour' ($mean_{HIGH}$ = 0.4, sd = 0.8, $mean_{LOW}$ = 0.3, sd = 0.5; U = 225.000, p = .64) or the Lab-TAB composite distress score ($mean_{HIGH}$ = 65.1, sd = 43.2, $mean_{LOW}$ = 50.0, sd = 41.0; t (42) = -1.2, p = .24). Similarly, high and low Lab-TAB distress groups (n = 44; median distress score = 57.5, high distress group (n = 23): mean distress score = 95.6, low distress group (n = 21): mean distress score = 22.7) did not differ in IBQ-fear ($mean_{HIGH}$ = 3.6, sd = 0.9, $mean_{LOW}$ = 3.5, sd = 0.8; U = 197.500, p = .30).

3.4.3 Results in year 2

One mother did not return the CBCL; therefore for this condition n = 32. Figure 3-4 and Figure 3-5 present the frequency distributions for scores on the CBCL internalizing and externalizing scales, respectively. Figure 3-4 illustrates that two children are in the clinical range on the CBCL internalizing scale with scores of 71 and 73 (borderline range: 65-69, clinical range: 70-100, for both scales). The mean of the internalizing scale was 45.3 (sd = 11.3) and the median was 43.0, with scores ranging from 29 to 73. Figure 3-5 illustrates that one child was in the clinical range on the CBCL externalizing scale with a score of 89 (this child scored 73 on the internalizing scale). The mean of the externalizing scale was 51.4 (sd = 9.6) and the median was 51.0, with scores ranging from 32 to 89.

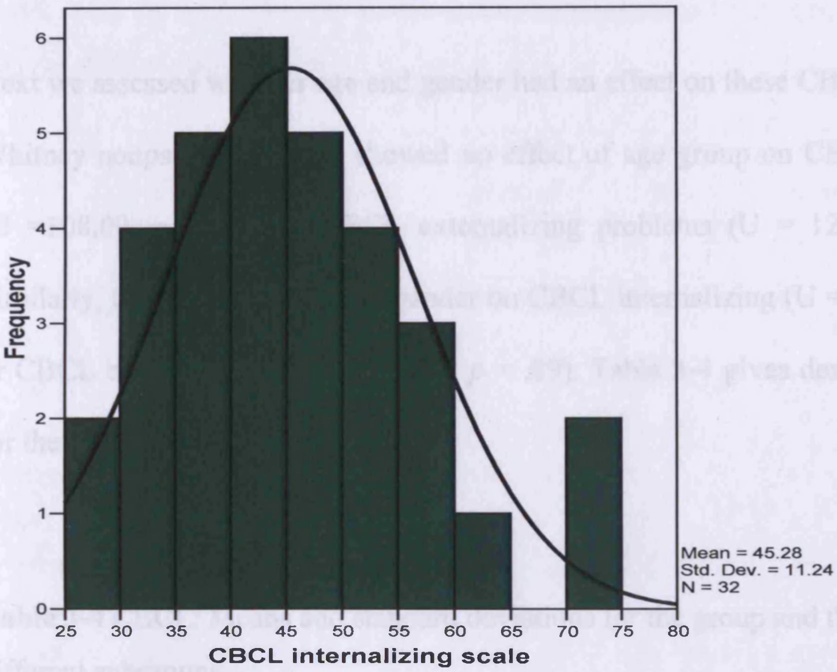

Figure 3-4 Frequency of the CBCL internalizing scale (clinical range ≥ 70)

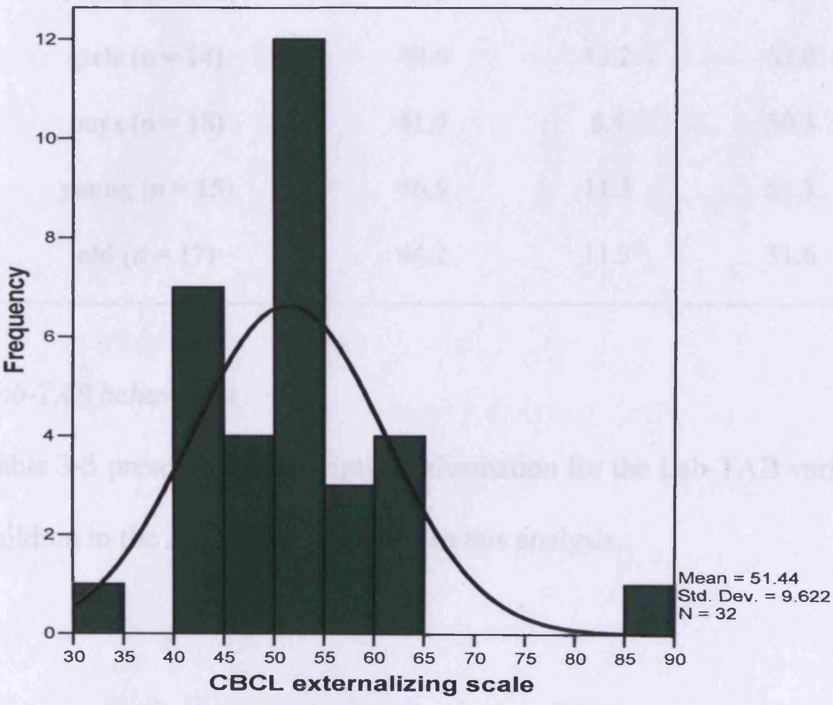

Figure 3-5 Frequency of the CBCL externalizing scale (clinical range ≥ 70)

Next we assessed whether age and gender had an effect on these CBCL scores. Mann Whitney nonparametric tests showed no effect of age group on CBCL internalizing (U =108.00, p = .49) or CBCL externalizing problems (U = 124.500, p = .91). Similarly, there was no effect of gender on CBCL internalizing (U = 84.500, p = .12) or CBCL externalizing (U = 122.500, p = .89). Table 3-4 gives descriptive statistics for the CBCL scales.

Table 3-4 CBCL: Means and standard deviations for the group and the different subgroups

	internalizing		**externalizing**	
	Mean	**SD**	**Mean**	**SD**
Group (n = 32)	45.3	11.2	51.4	9.6
girls (n = 14)	49.6	13.2	53.0	12.2
boys (n = 18)	41.9	8.4	50.3	7.2
young (n = 15)	46.5	11.5	51.3	6.7
old (n = 17)	44.2	11.3	51.6	11.8

Lab-TAB behaviours

Table 3-5 presents the descriptive information for the Lab-TAB variables. Data of 33 children in the 2nd year are included in this analysis.

Table 3-5 Lab-TAB intensity scores: Means and standard deviations for the group and the different subgroups

	facial fear		facial sadness		vocal distress		bodily fear		startle response		escape behaviour	
	M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Group (n= 33)	2.4	0.9	1.9	1.2	2.8	1.9	2.0	0.8	0.02	0.04	1.1	1.1
girls (n= 15)	2.5	1.1	1.8	1.1	2.5	1.7	1.9	0.8	0.01	0.1	1.2	1.1
boys (n= 18)	2.3	.82	2.0	1.3	2.8	2.0	2.0	.89	.02	0.04	1.0	1.2
young (n= 16)	2.7	0.9	2.2	1.0	3.1	1.6	2.2	0.7	0.01	0.04	1.1	1.2
old (n= 17)	2.1	0.9	1.7	1.3	2.3	2.1	1.8	0.9	0.02	0.1	1.0	1.2

Note: the 'touch-not touch' dimension is not included in the above table, since it is a binary variable.

Age effects

A significant effect of age was found on 'intensity of facial fear' ($mean_{YOUNG} = 2.7$, sd = 0.9; $mean_{OLD} = 2.1$, sd = 0.9; t (31) = 1.77, $p<0.05$, one-tailed), with younger children having higher scores. No effect of age group was found on 'intensity of facial sadness' ($mean_{YOUNG} = 2.2$, sd = 1.0, $mean_{OLD} = 1.7$, sd = 1.3; t (31) = 1.17, p = 2.5), 'intensity of vocal distress' ($mean_{YOUNG} = 3.1$, sd = 1.6, $mean_{YOUNG} = 2.3$, sd = 2.1; t (31) = 1.32, p = .20), 'intensity of bodily fear' ($mean_{YOUNG} = 2.2$, sd = 0.7, $mean_{OLD} = 1.8$, sd = 0.9; t (31) = 1.34, p = .19), 'intensity of escape behaviour' ($mean_{YOUNG} = 1.1$, sd = 1.2, $mean_{OLD} = 1.0$, sd = 1.2; U = 136.00, p = 1.0), or 'startle response' ($mean_{YOUNG} = 0.01$, sd = 1.2, $mean_{OLD} = 0.02$, sd = 0.05; U = 135.500, p = .99). Regarding the 'touch-not touch' variable ($n_{TOUCH} = 14$, $n_{NOTOUCH} = 19$) of the 19 children who did not touch the robot 9 belonged to the younger and 10 to the older group, and of the 14 who touched the robot 7 belonged to the younger and 7 to the older group. Chi-square tests did not reveal a significant effect of age (χ^2 (1) = .02, p = .88).

Figure 3-6 shows the frequency distribution of the Lab-TAB composite distress score for the 33 children tested in year 2 (mean Lab-TAB composite distress score = 89.2, sd = 48.4). Individual scores ranged from 8 to 153, with 24 out of the 33 children having a score above 50 on distress.

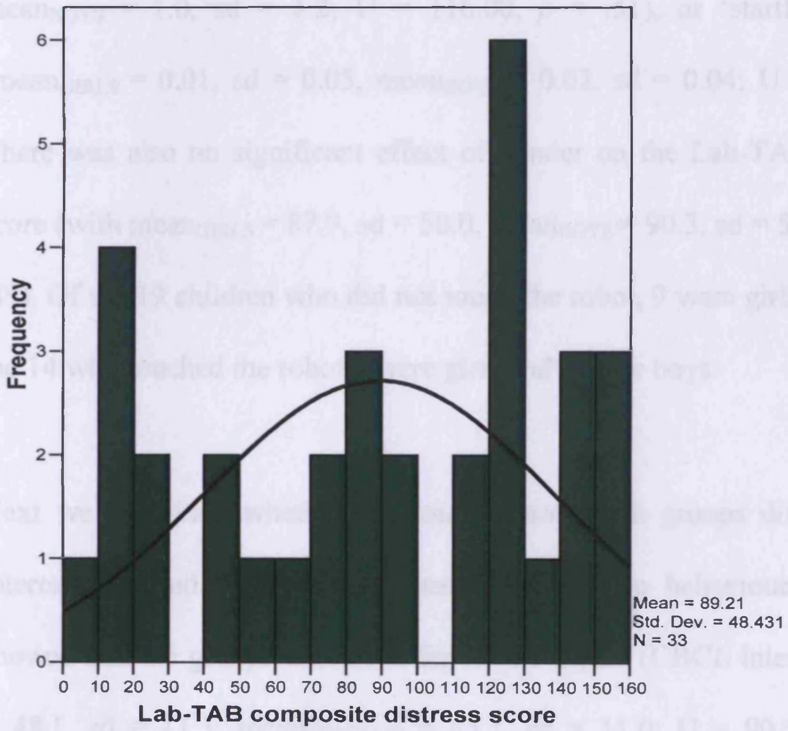

Figure 3-6 Frequency of the Lab-TAB composite distress score in year 2

An independent samples t-test showed no effect of age group on the composite Lab-TAB distress score ($mean_{YOUNG}$ = 100.7, sd = 39.5, $mean_{OLD}$ = 78.4, sd = 54.5; t (31) = 1.34, p = .19).

Gender effects

No significant effect of gender was found on 'intensity of facial fear' ($man_{GIRLS} = 2.5$, $sd = 1.1$, $mean_{BOYS} = 2.3$, $sd = 0.8$; $t\ (31) = -.35, p = .73$), 'intensity of facial sadness' ($mean_{GIRLS} = 1.8$ $sd = 1.1$, $mean_{BOYS} = 2.0$, $sd = 1.2$; $t\ (31) = .38, p = .71$) 'intensity of vocal distress' ($mean_{GIRLS} = 2.5$, $sd = 1.7$, $mean_{BOYS} = 2.8$, $sd = 2.0$; $t\ (31) = .50, p = .62$) 'intensity of bodily fear' ($mean_{GIRLS} = 1.9$, $sd = 0.8$, $mean_{BOYS} = 2.0$, $sd = 0.9$; $t\ (31) = .19, p = .85$), 'intensity of escape behaviour' ($mean_{GIRLS} = 1.2$, $sd = 1.1$, $mean_{BOYS} = 1.0$, $sd = 1.2$; $U = 116.00, p = .51$), or 'startle response' variable ($mean_{GIRLS} = 0.01$, $sd = 0.05$, $mean_{BOYS} = 0.02$. $sd = 0.04$; $U = 123.000, p = .68$). There was also no significant effect of gender on the Lab-TAB composite distress score (with $mean_{GIRLS} = 87.9$, $sd = 50.0$, $mean_{BOYS} = 90.3$, $sd = 51.7$; $t\ (31) = -.14, p = .89$). Of the 19 children who did not touch the robot, 9 were girls and 10 boys, and of the 14 who touched the robot 6 were girls and 8 were boys.

Next we examined whether the touch - not touch groups differed on the CBCL internalizing and externalizing dimensions, and in behavioural distress. Analysis showed that the groups did not differ on the CBCL (CBCL internalizing: $mean_{TOUCH} = 48.1$, $sd = 11.3$, $mean_{NOTOUCH} = 43.1$, $sd = 11.0$; $U = 90.500, p = .18$; CBCL externalizing: $mean_{TOUCH} = 51.1$, $sd = 8.8$, $mean_{NOTOUCH} = 51.7$, $sd = 10.1$; $U = 115.500, p = .69$) but they did in Lab-TAB distress score ($mean_{TOUCH} = 70.5$, $sd = 56.3$, $mean_{NOTOUCH} = 103.0$, $sd = 37.6$; $t\ (31) = -1.9, p<0.05$, one-tailed).

Correlations between observational and questionnaire measures

Although we observed highly positive correlations between the questionnaire variables and between most of the observational variables (see Table 3-6) no significant correlations were found between the observational and questionnaire measures. For example, the CBCL internalizing scale did not correlate with the Lab-TAB 'intensity of facial fear' variable (rho = .20, ns) or the Lab-TAB composite distress score (rho = .19, ns). The same applies for correlations between the CBCL externalizing scores and the Lab-TAB variables (see Table 3-6 for details).

Table 3-6 Correlations between questionnaire (CBCL) and behavioural (Lab-TAB) ratings

	facial fear	facial sadness	vocal distress	bodily fear	startle response	escape behaviour	distress[a]	CBCL-Int[b]	CBCL-Ext[c]
facial fear		.91**	.87**	.89**	-.11	.62**	.92**	.20	.10
facial sadness			.94**	.90**	-.09	.64**	.93**	.12	.01
vocal distress				.95**	-.09	.59**	.95**	.19	.02
bodily fear					-.16	.67**	.97**	.16	-.03
startle response						-.35*	-.13	-.01	-.05
escape							.75**	.35	.14
distress								.19	.03
CBCL-Int									.52**

[a] Lab-TAB composite distress score

[b]CBCL internalizing dimension [c] CBCL externalizing dimension

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

Next we assigned children to high or low internalizing groups based on their median CBCL internalizing score (n =32, median CBCL internalizing score = 43.0) and compared these subgroups (high CBCL internalizing group: n = 15, mean CBCL internalizing score = 54.7, low CBCL internalizing group: n = 17, mean CBCL internalizing score = 37.0) on their Lab-TAB intensity scores. The groups did not differ on 'intensity of facial fear' ($mean_{HIGH}$ = 2.6, sd = 1.1, $mean_{LOW}$= 2.2, sd = 0.8; t (30) = -.98, *p* = .33), 'intensity of facial sadness' ($mean_{HIGH}$ = 2.1, sd = 1.2, $mean_{LOW}$ = 1.7, sd = 1.2; *t* (30) = -.80, *p* = .43), 'intensity of vocal distress' ($mean_{HIGH}$ =3.0, sd = 1.9, $mean_{LOW}$ = 2.4, sd = 2.0; *t* (30) = -.76, *p* = .45), 'intensity of bodily fear' ($mean_{HIGH}$ = 2.0, sd = 0.9, $mean_{LOW}$ = 1.9, sd = 0.8; t (30) = -.27, *p* = .79), 'startle response' ($mean_{HIGH}$ = 0.01, sd = 0.03, $mean_{LOW}$ = 0.02, sd = 0.06; U = 113.500, *p* = .60) or the Lab-TAB composite distress score ($mean_{HIGH}$ = 93.1, sd = 51.9, $mean_{LOW}$ = 82.5, sd = 45.7; *t* (30) = -.62, *p* = .54). The groups only differed in 'intensity of escape behaviour' ($mean_{HIGH}$ = 1.5, sd = 1.2, $mean_{LOW}$ = 0.7, sd = 1.0; U = 74.000, $p < 0.05$) with children in the high internalizing group showing more escape behaviour.

The same analysis was performed with respect to high and low externalizing CBCL subgroups (n =32; median CBCL externalizing score = 51.0; high CBCL externalizing group: n = 17, mean CBCL externalizing score = 57.2, low CBCL externalizing group: n = 15, mean CBCL externalizing score = 44.9) but no differences were found on any of the Lab-TAB variables ('intensity of facial fear' [$mean_{HIGH}$ = 2.5, sd = 1.1, $mean_{LOW}$ = 2.3, sd = 0.8; *t* (30) = -.51, *p* = .62], 'intensity of facial sadness' [$mean_{HIGH}$ = 1.9, sd = 1.2, $mean_{LOW}$ = 1.9, sd = 1.2; *t* (30) = -.06, *p* = .95], 'intensity of vocal distress' [$mean_{HIGH}$ = 2.6, sd = 1.9, $mean_{LOW}$ = 2.8, sd = 1.9; *t* (30) = .25, *p* = .80], 'intensity of bodily fear' [$mean_{HIGH}$ = 1.9, sd = 0.9, $mean_{LOW}$ = 2.1, sd = 0.8; *t* (30) =

.82, p = .50], 'startle response' [$mean_{HIGH}$ = 0.01, sd = 0.03, $mean_{LOW}$ = 0.03, sd = 0.06; U = 109.500, p = .50], 'intensity of escape behaviour' [$mean_{HIGH}$ = 1.2, sd = 1.1, $mean_{LOW}$ = 0.9, sd = 1.2; U = 115.000, p = .66], and the Lab-TAB composite distress score [$mean_{HIGH}$ = 78.1, sd = 54.0, $mean_{LOW}$ = 87.9, sd = 42.5; t (30) = .05, p = .96]). Finally, again using the median split procedure we compared high (n = 16, mean distress score = 128.9) and low (n = 17, mean distress score = 46.1) Lab-TAB distress groups (median distress score = 91.5) on the CBCL scales but no effect of distress group on CBCL internalizing ($mean_{HIGH}$ = 46.1, sd = 10.8, $mean_{LOW}$ = 44.5, sd = 11.9; U = 108.000, p = .47) or CBCL externalizing behaviour were found ($mean_{HIGH}$ = 52.1, sd = 6.0, $mean_{LOW}$ = 50.8, sd = 12.4; U = 101.500, p = .32).

3.4.4 Results of year 1 - year 2 comparisons: Developmental effects

Behavioural dimensions

Table 3-7 presents the descriptive information for the composite distress score in year 1 and 2. We employed paired sample t-tests to examine the effects of time on the Lab-TAB composite distress score. A McNemar test was performed when exploring time effects on the 'touch-not touch' dichotomous variable. Finally, Pearson and Spearman correlations were employed to test for relations between behavioural and questionnaire measures collected in both years.

Table 3-7 Means and standard deviations for the cosposite score in year 1 and 2

Year 1		***Year 2***	
Mean	**SD**	**Mean**	**SD**
55.5	38.3	87.5	48.2

Note: The touch-no touch score is not included in the above table because it is a yes-no variable

Gender was not used in these analyses since previous results did not show a significant effect of gender. A paired samples t-test revealed an effect of time on the Lab-TAB composite distress score (t (31) = -2.86, p<0.01). A McNemar test did not indicate a significant effect of time on the 'touch-not touch' variable (n = 32, exact p = .27). Of the 32 children 13 did not want to touch the robot when they were 1 year old and 18 children did not want to touch the robot when they were 2 year old. A chi-square test showed that there was no relationship between how the children acted towards the robot in year 1 and year 2 (χ^2 (1) = 1.50, p = .22).

Finally, Table 3-8 presents the Spearman and Pearson's correlations between the behavioural and questionnaire measures collected in the two years. Generally, none of the between year behavioural correlations was significant with values ranging from -.30 to .19, and this result remained when controlling for within year group age differences (with values in brackets depicting partial correlations). Figure 3-7 shows the relation between the Lab-TAB composite distress scores collected in children (n = 32) across two years.

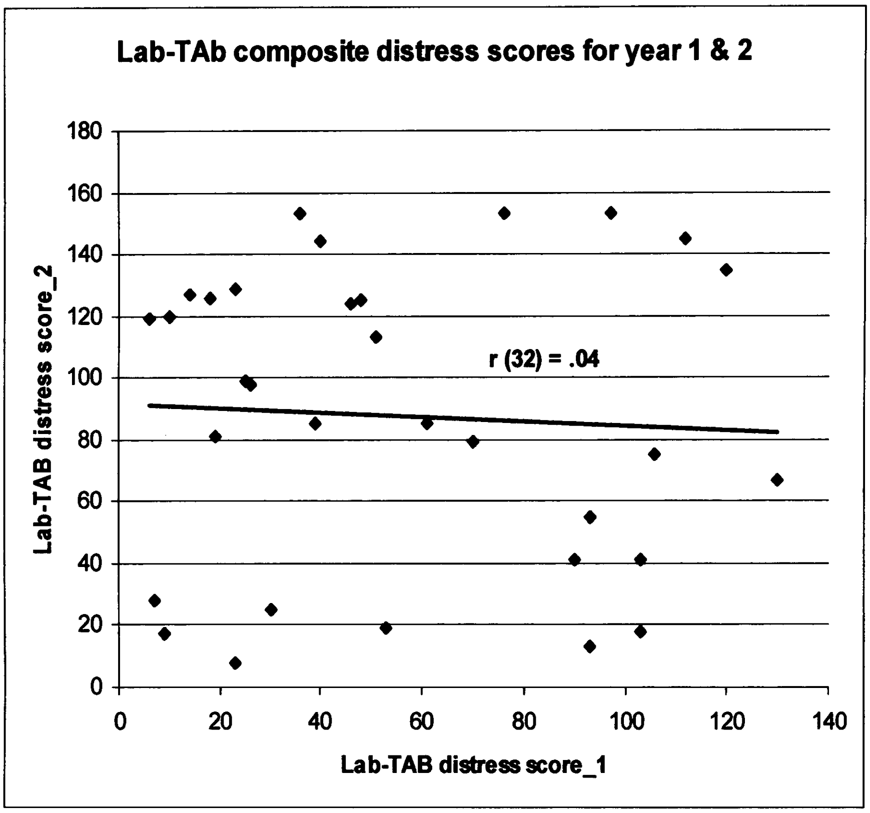

Figure 3-7 Relationship between the Lab-TAB distress scores collected in years

Table 3-8 Correlations between behavioural and questionnaire measures collected in year 1 and 2. The values in brackets indicate the correlations when controlling for age

	distress_1[a]	**IBQ-fear**
distress_2[b]	.04 (.17)	-.33 (-.29)
CBCL-Int[c]	.13	-.02
CBCL-Ext[d]	-.10	-.14

[a] Lab-TAB composite distress score_1 [b] Lab-TAB composite distress score_2

[c] CBCL internalizing dimension [d] CBCL externalizing dimension

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

3.5 Discussion

Since development has been shown to affect how individuals perceive potentially fearful situations (Ollendick et al., 2001), the main goals of the present study were (i) to examine individual differences in fearful temperament longitudinally in one- and two-year-old-children under novel and fearful conditions, and (ii) to explore the relationships between different types of temperament assessments (i.e., maternal reports and observational measures). Subsidiary goals were to examine age and gender effects within these year groups, and whether early fearful temperament would be associated with later internalizing behaviour, as has been previously reported in more extreme-group-based temperament studies (Kagan, 1994). Identifying stability in temperament characteristics as antecedents of later personality development has been an important challenge in developmental theory and research (Carranza et al., 2005). To our knowledge, not many studies have examined the development of fearfulness as a temperament characteristic in the first two years of life. This section will be structured following our initial hypotheses.

We predicted that relatively older children tested in year 1 would obtain higher scores on the IBQ-R fear dimension and have higher levels of behavioural distress during fear exposure; higher scores on the CBCL internalizing dimension and higher levels of behavioural distress during fear exposure were also expected in relatively older children tested in year 2, and a significant increase in fear distress was expected from year 1 to year 2. Consistent with previous research and in line with our hypothesis we found a significant effect of age on IBQ-fear ($p<0.05$), with older infants in year 1 (mean age 11 months) being rated as more fearful than younger infants (mean age 8 months). According to Gartstein and Rothbart (2003) higher levels of fear towards the end of the first year of life is consistent with the development of inhibition of approach towards novel objects. In year 1 relatively older children were also found to be more distressed during the robot exposure across all dimensions (see Table 3-2), and year 2 children, as a group, were again more distressed than year 1 children (see Table 3-5). Gunnar et al. (1996) reported an increase in behavioural distress from 6 to 15 months of age. The effect of age on fear could be explained by developmental factors, particularly the increasing cognitive abilities that emerge in the 1st and 2nd year of life. Development has been shown to have an effect on how individuals perceive fear eliciting situations (Ollendick et al., 2001). For instance, a young infant might be unaffected by events that will frighten him/her at a later age in life. Although the specific underlying mechanisms responsible for these changes are not yet fully identified, it is likely that these are due to the developing cognitive abilities in the older child, specifically the ability to recognize and appraise unfamiliar events as entailing potential danger (Ollendick et al., 2001). However, contrary to our expectation, relatively older children in year 2 were not significantly more distressed or rated higher on the CBCL internalizing dimension when compared to their younger counterparts. The limited age range of the 2nd year sample might account for these findings with the younger group being 19.7 months old and the older group being 24.8 months old. We also did not use the same questionnaire for the measurement of temperament in both years. Had we done this, the expected developmental effect with respect to internalizing behaviour might have been detected. Still, Achenbach and Rescorla (2000, p.61) did not find an age effect on the internalizing dimension in a normative sample of 18 - 44 and 45 - 71 months olds.

To summarize, we found clear age effects within the first year of life and also when comparing year 1 and year 2 data, with both patterns reflecting increased fearfulness in older children. It will be interesting to find out whether fear development continues in year 3 or whether around the age of 24 months the highest level of fear has been achieved. The fact that we found no differences in fear intensity between 20 month olds and 25 month olds suggests that the former explanation is a more likely one.

Very few studies in young children have been interested in gender differences in temperament. We did not expect any gender differences on the IBQ-fear or the internalizing scales nor on the behavioural distress during fear exposure. Indeed, we found no gender differences on the IBQ-fear (see Table 3-1) or CBCL internalizing scale (see Table 3-4), nor did girls differ from boys in observed behavioural distress in year 1 or year 2 (see Table 3-2 & Table 3-5 respectively). Girls only differed from boys in the startle response in year 1. Feminine nature has been linked to stronger withdrawal tendencies in culturally diverse samples. Gartstein and Rothbart's study (2003) is, however, one of the few studies in young children, which found a significant gender difference on IBQ-fear (mean girls = 2.62; mean boys = 2.39). Their study can be criticised in that their findings were based on a large sample (N = 360 participants; males: n = 179, females: n = 181), the actual difference in IBQ-fear scores was small, and the effect size was weak (d = .23). Social factors are clearly important in the development of gender differences and it might therefore be not surprising that gender differences are not yet pronounced in one- and two-year-olds (Hay, 2007).

A third prediction was that different types of temperament assessments (i.e., questionnaires, observational measures) would be correlated. Thus, for example, we expected that infants higher in mother-rated fearfulness and/or internalizing behaviour would also show more behavioural distress. There was no support for this hypothesis. We can think of several explanations for this result. First, our sample was not only relatively small but also normal in composition; we did not select infants who were extremely fearful based on mothers' ratings. It is possible that with more extreme scoring children and/or a larger sample we would have observed stronger relationships between these different temperament parameters. On the other hand, other studies also found only low positive or non-significant relations between questionnaire and observational measures of infant temperament (Kochanska et al. 1998; Crockenberg & Leerkes, 2006). We already discussed in the Introduction to this Chapter that one important criticism regarding questionnaires' validity has to do with the parents' inclination to give socially desirable answers (Kagan, 1994). For example, a mother who prefers her child to be outgoing and relatively fearless would ignore or deny her child's fearful characteristics when completing a questionnaire. Therefore, in these instances parents' reports cannot reliably predict actual fear, a proposition supported by the finding that 85% of the mothers predicted that her child would not be afraid of the robot prior to exposure. Moreover, 70% of the mothers whose children cried in year 1 predicted that her child would not be afraid of the robot in year 2 ("because s/he is older now"), which is contrary to the general picture we obtained, with 2-year-olds being more fearful than in year 1. And finally, no significant correlations were found between maternal temperament ratings and behavioural distress measures.

As mentioned in Chapter 2, some children show reactions to specific events in the laboratory that have not been noticed before at home. To the mother these reactions are inconsistent with her child's typical behaviour. This cross-situational inconsistency in behaviour is a factor that could affect the accurateness of parental judgements of their children's temperament in novel situations (Kagan, 1994). Kochanska et al. (1998) also found no correlations between the IBQ and Lab-TAB fear behaviour (i.e., the mechanical toy dog), as did Crockenberg and Leerkes (2006) between IBQ-distress to novelty and withdrawal [r (63) = .09, ns]. To summarize, weak associations between maternal reports and observational measures of children's temperament have generally been found, and these have been attributed to the subjective nature of the questionnaire measures and/or the differences between home vs. laboratory assessments (see Chapter 2). We also found no significant correlations between laboratory measures of fearfulness and parental reports and therefore it can be concluded that these two methodologies are non-equivalent tools for the measurement of temperament (see also Carnicero et al., 2000).

Finally, we predicted consistency in levels of fear distress between year 1 and year 2. Contrary to that expectation, no correlations were found between the behavioural ratings collected in year 1 and year 2 and one has to conclude therefore that there was no stability in fear behaviour over time. Previous research also observed a lack of stability over time and this has been mainly attributed to developmental changes in the expression of temperament (Carranza et al., 2005). Two-year-old children can express themselves more clearly than one-year-olds: they can call their mother for support, modulate the intensity of their crying to express their fear more clearly, and use their motor skills to protest and make clear what they (don't) want. This can result in higher fearfulness scores in the older children. We observed that in the 2nd year the children were more difficult to approach, they refused more often to comply with procedures and were more distressed during fear exposure. As mentioned in the Methods section of the current chapter, 7 two-year-olds could not be tested for these reasons. Gunnar et al. (1996) also found no cross age stability between 2 and 15 months of age in relation to inoculation distress. Rothbart, Derryberry and Hershey (2000) found moderate cross age stability in observed fear between 6.5 and 10 months ($r = .31$), between 6.5 and 13.5 months ($r = .22$), and between 10 and 13.5 months of age ($r = .27$). Observed fear at 13.5 months and IBQ-fear at 6.5 months (but only these two variables!) predicted CBQ fear at age 7 years. Stability of temperament over time has mainly been reported in extreme-group studies. For example, Calkins, Fox and Marshall (1996) reported that 88% of extremely inhibited 14-month-old children had been classified as high in activity and crying behaviour at age 4 months. Kagan, Snidman and Arcus (1995) reported that about one-third of extremely inhibited one- to two-year-old children preserve these characteristics through late childhood and adolescence, and that the stability correlations over a period of 5 to 6 years range from 0.3 to 0.5. Some temperament characteristics might stay stable for a period and then change, or stay stable for one individual and change for another (Chess & Thomas, 1990). Temperament features can be influenced by changes in maturation of neurological organization and/or exogenous factors such as parenting styles, and other social and educational parameters. One challenge for temperament research is to detect those temperament features that are consistently displayed across situations and time and that stay salient throughout maturation (Rothbart & Derryberry, 1981).

We also found no relations between maternal fear ratings in year 1 (IBQ-R) and year 2 (CBCL). As the child gets older temperament develops and manifests itself through phenotypically distinct channels (Carnicero et al., 2000). Therefore, it is possible that mothers' impressions about their children's behaviour follow these different temperamental manifestations, and thus would not be expected to remain stable over time. Also, the absence of convergence between the questionnaires in the 1st and the 2nd year might be accounted by the fact that we used different questionnaires in each year and were not able to test for temporal consistency across the years.

The CBCL is a standardized instrument that is used most often in research on *maladaptive* functioning in the preschool years. We employed the CBCL to examine normal, adaptive internalizing development in 2-year-olds and to find out whether fearful temperament in year 1 would be related to more general internalizing behaviour in year 2. As mentioned in the Results section, and not unexpectedly given the nature of our sample, the range of CBCL scores was restricted (see Figure 3-4-& Figure 3-5) with only one child scoring in the clinical range on both the externalizing and the internalizing scales, and only one other child scoring in the clinical range on the internalizing scale only; none of the children fell in the borderline range. This might explain the absence of any relations between early fearful temperament (Lab-TAB/IBQ-fear) and later internalizing scores. Additionally, the significant correlation found between CBCL internalizing and externalizing scales (rho = .52, see Table 3-6) might have confounded the expected results. Although, both behavioural profiles are associated with negative emotionality in general, they are expected to differ in terms of emotion types and regulation (Eisenberg, et al., 2001). For example, internalizing behaviour has been associated with emotions like anxiety, fear, depression, psychosomatic reactions, and withdrawal, while externalizing behaviour has been associated with anger, aggressive behaviour and delinquency (Eisenberg et al., 2001). Finally, although we found that children who did not want to touch the robot in year 1 were higher on mother-rated IBQ-fear, the same was not true when comparing the touch - not touch groups on the CBCL internalizing behaviour in year 2. The latter result could again be due to the different questionnaire used, or because parents change their impressions of their children's temperament over time.

To conclude, we successfully measured the behavioural changes in one- and two-year-old children in response to fear. Although quite a few studies on fear in young children have been conducted, questions with respect to the development of fear/behavioural inhibition over time, and how this contributes to the development of coping in everyday life remain unanswered. To our knowledge, not many studies have examined the development of fearfulness as a temperament characteristic over the first two years of life.

Does fear increase with age?

We found a clear effect of time on fear, with older children showing more intense fear. We explained this by referring to the increasing cognitive abilities which emerge by the end of the 1st and in the 2nd year of life.

Are there gender differences in fearful temperament?

Although the literature on infant temperament lacks information on gender differences in fear, feminine nature in general has been linked to a greater tendency toward fearfulness and withdrawal. However, our results did not indicate that girls and boys differed in fear.

Are there associations between different assessments of fearful temperament?

The current study sought to examine relations between different assessments of fearful temperament. Like in other studies, no significant association was observed between parent-rated and observed fear (Kochanska et al. 1998; Crockenberg & Leerkes, 2006)). This was attributed to the fact that parents change their impressions of their children's temperament over the course of development for personal (e.g., social desirability) or situational reasons.

Stability of temperament

We observed a lack of stability in fear distress across time, which was attributed to developmental changes in the expression of temperament and the fact that we did not adopt an extreme-group based approach (Carranza et al., 2005).

The current analysis was based on multiple comparison tests for examining our hypotheses. This should be noted as a limitation to the current set of results, since the probability of revealing a significant difference over the course of analysis when there is not one (Type I error) might have increased.

Continuity and change in temperament research should not be viewed as mutually exclusive. Rather than studying continuity and change it might be more relevant to focus on the specific mechanisms and processes that lead a specific temperament characteristic to change or stay stable over time (Carranza et al., 2005). Recently, with the advent of more user-friendly experimental methods, physiological assessments have been added to research on temperament in young children. In the following chapters we will combine parental reports and observational data with physiological measures and find out whether physiological assessments of fear can shed further light on our main questions about young children's fearful temperament.

Chapter 4 Skin conductance and fearfulness: Methodology and results

4.1 Skin conductance as a physiological index of temperament

Activity of the Autonomic Nervous System (ANS) has been an important focus in research on temperament. Measures of Skin Conductance Activity (SCA) and Heart Rate (HR) have been mostly used in research on infant temperament. Skin Conductance Activity (SCA), also described as Electrodermal Activity (EDA), is a non-invasive and semi-temperature independent method of assessing the level of an individual's stress response (Hernes, Mokrid, Fremming, Odegarden, Martinsen & Storm, 2002). For the purposes of this study the term SCA was adopted. Despite the fact that HR is a frequently used and well-recognised measure of temperament, the current study focussed on SCA specifically.

SCA measures the psychogalvanic reflex response or emotional sweating by reflecting changes in the sympathetic nervous system (SNS) through supplying a small amount of electrical current across two electrodes placed on the surface of the skin (Storm, Myre, rostrup, Lien & Raeder, 2002). The SNS is activated under stressful events by increasing HR and oxygen flow to prepare the organism in advance for anticipating and facing negative emotions and situations (Dawson et al., 1990). Unlike heart rate and blood pressure, which are influenced by both the sympathetic and the parasympathetic nervous system, SCA is only influenced by changes in the SNS and does not seem to be affected by circulatory changes in relaxed subjects (Storm et al., 2002). Since Fere in 1888 showed that presenting an external stimulus (visual, auditory, gustatory, olfactory) could heighten the electrical conductivity of the skin, SCA has been proven to be a useful psychophysiological method with wide applicability: 'the lie detector' or the so-called guilty knowledge test (GKT) is one of the best known examples of its use (Dawson et al., 1990). From the beginning, this response system has been closely related to psychological concepts of emotion, arousal, and attention (Dawson, Schell & Filion, 2000).

Glands of the skin are divided into apocrine and eccrine (Venables & Christie, 1980). While the function of the apocrine glands is not well understood, more is known about the function of eccrine sweat glands. Eccrine glands develop from the epidermis and cover most of the body. Due to greater density of eccrine glands on soles and hands emotionally evoked sweating is mostly studied in these areas (Dawson et al., 1990). Gladman and Chiswick (1990) stated that the functional onset time for the sweat glands is the 36^{th} week of gestation, while Jorgenson, Salinas, Dowben and John (1988) proposed that the emotional sweat glands are mature after the second week of life. The SNS controls the palmar and plantar eccrine sweat glands by secreting acetylcholine in the postganglionic synapses (Dawson et al., 1990). Each time the SNS responds to the individual's emotional state the sweat glands are filled, decreasing the resistance and increasing the conductance of the skin. Lykken and Venables (1971) suggested to measure skin conductance directly with a constant-voltage system instead of skin resistance. This was because skin conductance had been found to be linearly related to the number of active sweat glands and their rate of secretion (Dawson et al., 2000).

Clear individual differences in SCA have been reported. SCA has been shown to be related to the activation of the behavioural inhibition system (BIS), which takes place during "responding to punishment, passive avoidance, or frustrative-nonreward" and generally in anxious situations in which active avoidance is not possible (Dawson et al., 2000). Given that SCA is thought to be the most responsive system under anxious situations, higher SCA is supposed to reflect a more anxious nature, whereas low SCA is supposed to reflect a more fearless nature (Dawson et al., 2000). Moreover, in the cognitive domain SCA has been suggested to reflect individual differences in higher cognitive processes such as those involved in attention and the processing of information (Dawson et al., 2000). Social stimulation, as well as the anticipation and performance of a task, have been shown to produce increases in SCA, bringing forward the notion that effortful allocation of attentional resources during any task causes autonomic activation (Dawson et al., 1990). However, importantly, SCA studies have shown that approximately 5-10% of the normal population does not show a SCA response (being SCA non-responders; Tranel & Damasio, 1994). There does not seem to be a gender difference in this respect, just a notion of a different sweating activation pattern (Jorgenson et al., 1988).

In different physiological studies researchers choose to measure either Skin Conductance Level (SCL) or Skin Conductance Response (SCR) as an index of SCA. Dawson et al. (2000) stated that "the tonic level of skin resistance or its reciprocal skin conductance is the absolute level of resistance or conductance at a given moment in the absence of a measurable phasic response, and this is referred to as SRL (skin resistance level) or SCL (skin conductance level). Superimposed on the tonic level are phasic decreases in resistance (or increases in conductance) referred to as SRRs (skin resistance responses) or SCRs (skin conductance responses) (Dawson et al., 2000, p. 201). For estimating SCA researchers analyse the frequency and amplitude of the waves, as well as the baseline recordings (Hellerud & Storm, 2002). SCL's typical values range between 2-20 μSiemens (μS), whereas SCR's typical amplitude and latency values range between 0.2-1.0 μS and 1-3 sec, respectively (Dawson et al., 2000). Figure 4-1 shows the basic components of SCA. Changes in SCL reflect increases or decreases in tonic arousal and are often measured during the presentation of a chronic, continuous stimulus and on an ongoing basis over a longer period of time (Dawson et al., 2000). On the other hand, SCR which is thought to be a reliable component of the orienting response is elicited by any novel, unexpected, significant, or aversive stimulus and is often used in so-called "orienting response habituation" paradigms. This paradigm consists of the repetitive presentation of a discrete stimulus, such as a loud tone, with a clear inter-stimulus interval and gives recordings of the initial elicitation and subsequent habituation of the SCR (Dawson et al., 2000). The specific use of SCL and/or SCR measures related to different types of stimuli and in different populations is explained in the following studies.

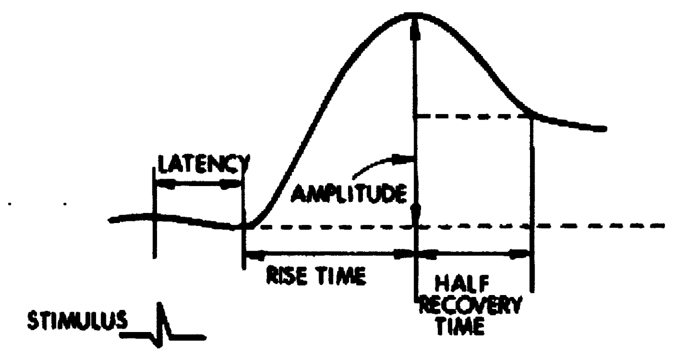

Figure 4-1.Basic components of SCA (Dawson et al., 2000)

SCL has been used in physiological studies as an index of SCA under the presentation of a continuous stimulus. Munro, Dawson, Schell and Sakai (1987) investigated SCL in college students during a continuous and demanding task. The task consisted of a series of digits presented visually every 1 sec for a duration of 48 msec. Students had to press a button whenever the digit “0” was presented. SCL increased sharply from baseline suggesting a heightening of autonomic activity during tasks that require effortful allocation of attentional resources (Munro et al., 1987).

SCR is thought to be a reliable component of the SC orienting response (Dawson et al., 2000). Although several studies have been conducted using SCR and its components as the main dependent variables, most studies combine the measures of SCR and SCL. Tranel, Fowles, and Damasio (1985) used a discrete stimulus paradigm in order to study the effects of facial stimuli on SCR. 55 slides were presented, 42 involved the presentation of unfamiliar faces, 8 involved the presentation of famous faces and 5 slides were used as initial buffer items. It was found that SCRs were significantly higher in response to famous faces (Tranel et al., 1985).

A different line of research has used this response system to assess temperament differences between individuals. Ham and Tronick (2006) examined resilience in respect to emotion regulation in 12 5-month-old infants and their mothers during the Still-Face (SF) paradigm, which is designed to examine infants’ reactivity to perturbations. SCL and HR, as well as respiratory sinus arrhythmia (RSA), were the physiological measures employed in this study (Ham & Tronick, 2006). The dyads were first categorized into four groups based on whether or not the infants protested during the SF episode for more than 25% of the time and whether or not the infant recovered from their initial protest. SCA was used for the first time in this type of study. Increases in SCL were found in all groups of infants, apart from the group that recovered after the SF, which showed the lowest mean SCL across all episodes. The mothers of more easily-recovered infants were the ones who had themselves lower SCL's. According to the authors the "best resilient response might be to not become distressed at all" (p. 301). Ham and Tronick (2006) pointed out the feasibility of measuring SCL in this type of research, which will add to the physiological data in infants' research.

Gilissen, Koolstra, van Ijzendoorn, Bakermans-Kranenburg and van der Veer (2007) investigated the physiological reactions of young children, aged 3 and 4 years old, to fear inducing films clips in relation to the child's temperament and the quality of the parent-child relationship. Seventy-eight children watched two film clips, one with the parent present and one alone. Each session began with a baseline clip, which was followed by either a neutral or a fear-eliciting clip (Gilissen et al., 2007). SCL increased during the fear-eliciting films, regardless of whether the parent was present or not. Temperamentally fearful children, who had a non-secure relationship with their parents, showed higher SC reactivity to the fear-eliciting films as compared to fearful children who were in secure relationships with their parents, and to less fearful children (Gilissen et al., 2007).

In addition to the individual differences in normal populations, specific patterns of electrodermal response have been associated with emotional and behavioural difficulties (El-Skeikh, 2005) and with diagnosable psychopathology (Dawson et al., 2000). Children high in externalizing and antisocial behaviour have often been reported to show lower levels of SCA (e.g., Snoek, van Goozen, Matthys, Buitelaar &

van Engeland, 2004). Herpertz, Mueller, Qunaibi, Lichterfeld, Konrad and Herpertz-Dahlmann (2005) reported lower levels of SCR in 8-13-year-old children with conduct disorder (CD) with and without comorbid attention deficit hyperactivity disorder (ADHD). In this study Herpertz et al. (2005) used 18 slides from the International Affective Picture System (IAPS), which have been proven to evoke emotional responses. CD boys with and without comorbid ADHD had lower SCRs to all categories of slides independent of slide valence, as compared to healthy controls and ADHD children (Herpertz et al., 2005). Similarly, Van Goozen, Matthys, Cohen-Kettenis, Buitelaar and van Engeland, (2000), found lower baseline SCLs in young children with disruptive behaviour disorders (DBD) as compared to normal control children.

Moreover, several studies in schizophrenic groups have reported SCR hyporesponding or non-responding in these patients (Dawson et al., 2000). Bernstein, Frith, Gruzelier, Patterson, Straube, Venables and Zahn (1982) reported that 50% of the schizophrenics who took part were non-responders compared to only 5%-10% of controls. Raine (1997) asserts that underarousal is particularly associated with antisocial behaviour, either because antisocial individuals are characterised by fearlessness, which makes them less sensitive to the aversive consequences of behaviour in general and the experience of receiving punishment in particular, or because a higher threshold of arousal leads antisocial individuals to seek out stimulation and take risks in order for their arousal levels to reach a more optimal level. Raine, Venables, Mednick and Pollock (1999) investigated SCA in 134 male criminals with schizotypal personality disorder. They found that their SCR's were relatively low during the presentation of 15 orienting tones of 75-dB (with 25ms rise time and 1 sec duration). However, although schizotypal criminals seemed to have lower SCR's the effect was not significant. If it is true that a SCR reflects the allocation of attentional resources within information processing conditions (Dawson, Filion & Scell, 1989), antisocial and criminal personalities may be characterised by a deficit in the attentional system (Raine et al., 1999). Finally, SCA abnormalities have been reported in depressed patients. Depressed patients have been shown to have low SCLs and produce small SCRs to different types of stimuli (Dawson, Schell, & Catania, 1977).

4.2 SCA measures in infants and young children

Storm (2000) measured SCA and behaviour in 20 preterm infants who were 29 weeks old, 3 minutes before, during and after the heel stick procedure. The number of waves, the amplitude of the waves, and the level of the behavioural state all increased ($p<0.001$) during the heel stick, and gradually decreased back to baseline levels. Although the group consisted of 13 girls and 7 boys, the authors failed to report whether there were any sex differences. Hellerud and Storm (2002) investigated SCL and behavioural state in four groups of infants (i.e., newborn preterm, more than 1-week-old preterm, newborn terms and 3-month-old terms) under two conditions, heel prick or immunization (nociceptive), and routine nursery handling (tactile). The preterm infants showed significant increases in the number of waves per second, in amplitude and in baseline, except for the wave amplitude of the preterm newborns during heel prick. The term infants displayed a more varied pattern, but the number and amplitude of the waves increased significantly during both procedures, while in the 3-month-old group a significant increase was only found in the amplitude of waves during immunization (Hellerud & Storm, 2002). Tactile stimulation of preterm newborn infants produced higher increases in SCA compared with nociceptive stimulation, although the behavioural arousal was higher during nociceptive stimulation (Hellerud & Storm, 2002). The authors concluded that non-painful (tactile) stimulation produces higher levels of physiological stress than painful stimuli in preterm and term neonatal infants (Hellerud & Storm, 2002). Harrison, Boyce, Loughnan, Dargaville, Storm and Johnston (2006) studied SCL in 21 infants under three different environmental temperature conditions (open cot, incubator and overhead radiant heater) as well as prior to, during and following completion of routine procedures (i.e., nappy change, oral feeds, and heel lance procedure). Postnatal age at the time of the study ranged from 1 to 123 days. The authors found that the mean SCL, the amplitude of the waves, and the number of waves per second did not differ significantly between painful and non-painful conditions. However, the number of waves per second failed to return to baseline following completion of the heel lance procedure while this was not the case following completion of the non-painful procedures (Harrison et al., 2006). The authors concluded that recovery to baseline seems to be harder after painful stimulation.

Scarpa et al. (1997) combined SC and HR measures to compare physiological arousal patterns in 3-year-old inhibited and uninhibited children (N =1.795). Both SCL and SCR were recorded while children listened to a standardised audiotape containing 6 stimuli selected to elicit orienting responses (three 75-dB tones of 1 sec duration at 1000 Hz and three 75-dB tones of 1 sec duration at 1311 Hz). Higher SCLs and SCR latencies but not SCR magnitudes were found in the inhibited group ($p<0.0001$ and $p<0.013$ respectively). Laboratory tasks are usually challenging events and behaviourally inhibited children have long been characterised as shy with strangers and timid in unfamiliar situations (Kagan, 1994). Like others, Scarpa and colleagues (1997) supported the notion that increased reactivity in inhibited children may reflect a lower threshold of arousal in limbic areas such as the amygdala and hypothalamus. Temperament and biological factors seem to render some children more vulnerable to fear and anxiety (Kagan & Snidman, 1999). However, the question remains whether physiological measures can predict stable temperamental features over a longer period of time.

4.3 SCA in longitudinal designs

Hernes et al. (2002), in a longitudinal study, examined plantar SC changes in 39 full-born, healthy infants during the 1st and 3rd days of life, at 3 and 10 week of life, and at 6 and 12 months of life. The infants were exposed to ten auditory stimuli of 2 sec duration, with an effect of approximately 75 dB, which were produced by a loudspeaker 30 cm away from the infant's ear. Habituation was defined as three consecutive non-responses after presentation of the auditory stimulus. Mean SCL, the number of waves per second and wave amplitude increased during the first 10 weeks of life (Hernes et al., 2002). SCL increased from 2.12 on the first day of life to 2.76 at the 10th week of life and started to decrease at the 6th month of life to 2.54 and finally stabilize at one year to 2.30. The mean SCL was significantly lower on the 1st, and the 3rd day of measurement as well as on the 3rd week compared to the results from measurements on the 10th week of life, the 6th month of life and the 1st year of life. During auditory stimulation the number of infants who responded and the amplitude of their SCR increased during the first 10 weeks of life, and then stabilised during the

1st year of life. Eight percent responded on the 1st day of life and 62% responded at the 6th and 12th months of life (p<0.002). Although a few infants showed signs of habituation this did not reach significance. Neither recovery time, nor mean latency time changed significantly during the 1st year (Hernes et al., 2002). The above findings led the authors to conclude that this part of the SNS is mature in the first 10 weeks of life (Hernes et al., 2002). The authors failed to report whether there were any gender differences in their sample.

Fowles, Kochanska, and Murray (2000) examined SCA in 92 4-year olds on a battery involving tests of fearfulness and effortful control, such as breaths, noises (expected and unexpected), a pointing game, a gift wrap task, slides, and a cupcake mischief task (Fowles et al., 2000). During stimuli with psychological significance clear SCRs were elicited. Overall, induction of psychological conflict and exposure to emotional film clips did not elicit increases in SCL (Fowles et al., 2000). The same group of children had also been behaviourally assessed 6 months earlier and when they were 2 years old, but at that time no physiological examination was carried out. Modestly significant correlations between SCL and the temperament dimensions of fear and effortful control were found at age 4 only; no significant correlations between temperament and SCR were found. With respect to gender, Fowles et al. (2000) found that fearfulness was more strongly related to SCL in girls, while the same result was not found for effortful control (Fowles et al., 2000).

Gao et al. (2007), examined the development of skin conductance orienting response (SCOR), habituation and reorienting[3] in childhood. The sample consisted of 200 Mauritian children tested longitudinally at 3, 4, 5, 6 and 8 years of life. SCR's were recorded while children listened to a standard auditory tape consisting of 6 tones through headphones; three 75-dB tones of 1 sec duration at 1000 Hz, followed by three 75-dB tones of 1 sec at 1311 Hz frequency (Gao et al., 2007). Results showed that both initial SCOR and habituation increased with age, and that SC reorienting is absent from ages 3 to 8. No gender differences were observed in the development of SCOR. Orienting and habituation seemed to mature continuously and the authors suggested that this could be related to the children's cognitive development during these ages (Gao, et al., 2007).

4.4 SCA as an index of fearfulness in the current study

The current study measured SCA as an index of fear/stress in a group of healthy infants in response to a fear-eliciting episode and in response to the presentation of loud noises. The methodology, results and discussion of this set of data will be presented in the next sections of this chapter (see sections 4.6, 4.7 & 4.8).

Trying to overcome limitations such as measuring SCA only in relation to one task (El-Sheikh, 2005) our goal was to measure SCA during three different experimental phases. Starting with a baseline assessment, we next used an orienting response habituation paradigm (OHP), which was followed by a fear-eliciting event. While SCA as a physiological tool has been proven successful in recording stress in humans

[3] Reorienting results from any change in stimulus presentation, indicating sensitivity in the orienting response system (Gao et al., 2007).

(Hernes et al. 2002; Scarpa et al., 1997), until now very few studies have been conducted in young children between the first and the second year of life.

Our orienting habituation paradigm (OHP) consisted of the presentation of 10 auditory stimuli (i.e., loud noises; Dawson et al., 2000) to elicit SCRs in a way similar to Hernes et al. (2002). Furthermore, we adopted Goldsmith and Rothbart's (1999) "unpredictable mechanical toy" episode from their standardised instrument for the laboratory assessment of early temperament, the Lab-TAB. The elements of novelty and intrusiveness in this episode are expected to elicit fear.

4.5 Hypotheses

The present study examined changes in SCL and SCR in one-year-olds as a result of different manipulations, and we repeated these experimental procedures once more one year later when the children were 2 years old, to examine the stability of the SC patterns over time. Specifically, we predicted (1) that SCL's would increase from baseline to fear, and (2) that children would show habituation to the loud noises (as reflected by reductions in SCR's during the OHP paradigm), and (3) that there would be individual differences in SCL and SCR which were related to differences in fearful temperament as rated by the mothers and as observed by independent raters during the unpredictable toy episode. Similarly, and in line with previous work in older children (e.g., Herpertz et al., 2005; van Goozen et al., 2000) we predicted that 2-year-old children relatively high in internalizing behaviour and those relatively high in externalizing behaviour, as rated by their mothers on the CBCL (Rescorla, 2005), would have different physiological profiles during stress and non-stress conditions.

(4) We predicted positive correlations within the different physiological measures and between these across time; and we predicted that the individual differences in physiological reactivity related to different temperament profiles would be stable over time. (5) Because earlier studies on SCL and SCR in infants failed to report on the existence of sex differences (Hellerud & Storm, 2002; Hernes et al., 2002; Storm, 2000), an additional purpose of this study was to find out whether boys and girls responded differently or similar in this respect. However, since behavioural analysis in Chapter 3 did not show any gender effects in fearfulness, we hypothesized that there would be no significant SCA differences between boys and girls. (6) We tested for age differences within each year. Hernes et al. (2002) reported a stabilization pattern between the ages of 6 and 12 months, with higher SCA in 6 month olds. Unlike heart rate, as far as we are aware there is no evidence of a decrease in SCA over time. We therefore predicted no effect of age on SC resting levels. However, because we found an increase in fearful distress with age (see section 3.4.4), and because we predict SCA to be a reflection of fearfulness and stress, we predicted that SC stress levels would be higher in year 2 as compared to year 1.

The results for each year will be presented separately and compared in the final section of this chapter. Since the methods and procedures were identical for both years, these are only described once.

4.6 Methods

4.6.1 Participants

Year 1 Sample

Participants were 50 infants (mean age in months = 9.7, sd = 2.2) and their mothers; all participants had been born in Cardiff, Wales. For analysis purposes the sample was divided into four subgroups: boys (n = 27, mean age = 9.9, sd = 2) and girls (n = 23, mean age = 9.5, sd = 2.5), younger (n = 27, mean age = 8, sd = 1.0) and older (n = 23, mean age = 11.7, sd = 1.0). Of the 27 boys 14 belonged to the younger (mean age = 8.3, sd = 0.9) and 13 belonged to the older (mean age = 11.5, sd = 1.4) group, and of the 23 girls 13 belonged to the younger (mean age = 7.7, sd = 0.9) and 10 to the older group (mean age = 11.9, sd = 1.5). A chi-square test for independence indicated no significant association between gender and age (χ^2 (1) = .109, p = .74). Boys and girls did not differ in age (t (48) = -.55, p = .59).

Year 2 sample

Approximately 12 months after the first assessment the families were contacted again by mail and telephone and asked whether they were willing to participate in a follow-up study. Of the original sample (N = 50) 10 participants could not be located or did not respond to our letter or phone calls. Therefore, 40 children (80%) returned to the laboratory for a visit in their 2nd year of life.

Of the 40 participants, 7 were unable to complete the 2nd study[4] and could therefore not be used in the SCA analyses. These 7 children did not differ from the other participants on any of the physiological variables measured in year 1 (i.e., SCL

[4] Seven infants did not complete the experiment due to fussiness in response to the demands of the experimental procedure, specifically the placement of the electrodes.

baseline, SCL stress, mean SCR, frequency of SCRs, SCL reactivity, with all *p*'s ranging between .19 and .71), nor did these 7 children differ from the participating children in mother-rated IBQ-fear (U = 126.500, $p = .84$) or on the composite Lab-TAB distress score (t (47) = 10, $p = .31$). So in the end 33 participants (66%) completed the study (mean age in months = 22.3, sd = 3.2). This sample was again divided into the same four subgroups; boys (n = 18, mean age = 22.6, sd = 3.4) and girls (n = 15, mean age = 21.9), young (n = 18, mean age = 19.9, sd = 2.1) and old (n = 15, mean age = 25.1, sd = 1.9). Of the 18 boys 8 belonged to the young group (mean age = 19.8, sd = 2.5) and 10 to the old group (mean age = 24.9, sd = 1.8), and of the 15 girls 10 belonged to the young (mean age = 20.1, sd = 1.8) and 5 to the old group (mean age = 25.4, sd = 2.1). A chi-square for independence indicated again no significant association between gender and age (χ^2 (1) = 1.63, $p = .20$). Boys and girls did not differ in age (t (31) = -.65, $p = .52$).

4.6.2 Procedure

a) Baseline

As described in section 3.3.2, the experimenter interacted with the mother and the infant for about 20 minutes prior to testing in order for the dyad to get used to the laboratory environment. On completion of the warm-up period, the electrodes were attached to the infant's foot. Due to an increase in cognitive development (see Chapter 3) two-year-olds turned out to be a lot more difficult to test than the one-year-olds. During equipment attachment, the parent stayed next to the child to minimize distress. Mothers often assisted the experimenter by trying to distract the child of the electrodes, or by presenting the electrodes' attachment as a "cool" thing to be happening.

The infant was carefully placed in a car seat and fastened safely by the mother. After the electrodes were attached, baseline SCL was recorded for a period of 2-3 minutes, while the infant was still quietly interacting with the parent.

b) Orienting Habituation Paradigm (OHP)

The child remained seated in the baby seat. The parent was asked to sit in a couch directly behind the infant and to remain minimally responsive to the child during this procedure. The couch was placed close to the baby's seat, so the infant could easily reach for, touch or look for the parent. Auditory stimuli, consisting of 1 second bursts of broad spectrum white noise at 75 dB were presented for approximately 3 minutes (approximately 100 cm away from the infant's ears). Specifically, auditory stimuli were presented after 15, 30, 45, 70, 85, 115, 160, 180, 195 and 250 sec after the procedure started (n = 10). At this moment the recording of SCR's stopped. This procedure was an adapted version of the one used by Hernes et al. (2002). It was made clear to the parent that she could modify the loudness of the white noise if she wished at any time, but none of the parents requested to do so. Parents were also informed that the procedure would be discontinued immediately if they thought their child became too upset. After the completion of this part of the procedure, the mother had the opportunity to comfort her child before the next phase of the study started.

c) Presentation of the unpredictable toy

This episode and its coding procedure have already been described in previous chapters (see section 3.3.2). For the purposes of the current analyses the Lab-TAB composite distress scores created by accumulating infant's ratings on the behavioural variables across the fear episode, was used (see section 3.3.2).

d) Maternal reports

Year 1: Infant Behaviour Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003)

As mentioned previously in section 3.3.2 the IBQ-R subscale assessing fearfulness was used in the current analyses. An example of a fearfulness item is: *"how often during the last weeks did the baby cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)?".*

Year 2: Child behaviour checklist and language development (CBCL/1.5-5; Achenbach & Rescorla, 2000)

Only the composite scales of internalizing and externalizing behavioural problems were used for the purposes of the current study (see section 3.3.2).

4.6.3 Skin conductance measures

For all episodes of the study SCA was measured. During the baseline and the unpredictable toy phase SCL was measured as an indication of arousal, whilst during the OHP SCRs were recorded. An applied voltage of 50 mV rms (root-mean-square) in line with the two-electrode system was utilised. The two-electrode system comprises a measuring electrode (M), and a counter current electrode (C). The C electrode is placed on the medial side of the non-dominant foot over the abductor hallucis muscle adjacent to the plantar surface (inside "arch" of foot), and the M electrode is placed midway between the phalanges and a point directly beneath (the 'mid-point' of the heel) (Edelberg, 1967).

Software program

Skin conductance was sampled with a frequency of 50 Hz, with a 16-bit resolution. The skin conductance recording and analysis software program were custom made using the PSYLAB program (PSYLAB, 2005). The amplitude threshold was adjustable and set at 0.02 μS (Hernes et al., 2002). This method produces artefacts when an electrode is disconnected from the skin, but is not sensitive to movement or changes in the normal temperature range (Hernes et al, 2002). During measures of SCL the software program recorded and analysed the mean SCL amplitude set at 30 second intervals. The valleys and the peaks were established when the derivative of the waves was zero. The amplitude of the wave was calculated from the bottom of the valley prior to the peak, to the height of the peak.

Additionally, during the recording of SCRs elicited by auditory stimuli the software program used coherent averaging to identify whether or not the infant responded to the sound given. This method superimposes all the responses and eliminates the spontaneous waves, allowing it to calculate the mean amplitude of the reaction to the stimulus (Hernes et al., 2002). A SCR was defined as an increase in conductivity occurring within a latency window of 1-3 seconds post-stimulus (Dawson et al., 2000). Amplitude exceeding 0.01 μS was considered to indicate an elicited response (Raine et al., 1999), although this value can vary (Herpertz et al., 2005; Harrison et al., 2006).

PSYLAB accessories

In addition to the PSYLAB software, accompanying equipment was required for the proper measurement of SCA. This included: a PSYLAB Stand Alone Monitor

(SAM), EL 1 electrodes and a wide bandwidth bioamplifier to fasten the electrodes ready for recording; and wipes for preparing the skin before attaching the electrodes. The electrodes were fixed to the skin by disks of double-sided adhesive collars. Additionally, strong adhesive tape was used in order to control the electrodes' stability. To improve the electrodes' conductance, conductivity paste was applied to the electrodes using buds (Hernes et al., 2002). One important reason for using double-sided adhesive collars is to control the size of the skin area that comes into contact with the electrode paste (Dawson et al., 2000). Whenever needed a sock was used in order to distract the child's attention from the electrodes and the leads.

4.7 Results

4.7.1 Statistical analyses

Due to the difference in nature of the two components of SCA (SCL and SCR), each component was analysed separately. With respect to SCL an outlier was defined as an individual value more than 2.5 SDs above or below the mean value of the group (Snoek et al., 2004). Two outliers were detected and excluded from the analysis. SCL was recorded in 30 sec. blocks. In total, we measured SCL for 210 sec. (i.e., 7 blocks) during baseline and for 210 sec. during stress (also 7 blocks). For the purposes of analyses two overall mean composite scores over these 210 sets of seconds were calculated; mean SCL baseline and mean SCL stress. The mean SCL of the baseline and stress conditions had to be square root transformed to fulfil the criteria of normal distribution. However, mean SCL values in μS rather than their transformed values are presented for ease of interpretation. Two separate repeated measures analysis of variance (ANOVA) with condition (baseline vs. stress) as the within subjects' factor and age (younger vs. older) for the 1st analysis, and gender (boys vs. girls) for the 2nd as between subjects' factors were employed to test for main effects of condition and the between subjects effects on SCL. Repeated measures ANOVAs were also employed to examine for SCL differences between more and less fearful and distressed infants based on maternal ratings and observational scores (IBQ-R/CBCL and Lab-TAB).

With respect to the SCR analysis in the OHP we were interested in (a) the individual mean SCR amplitude calculated over 10 noises, (b) the individual's SCR frequency (with an SCR defined as a response exceeding 0.01 μS; SCR frequency therefore ranges between 0-10), (c) and the effects on SCR amplitude and SCR frequency of age, gender and temperament based on IBQ-fear/CBCL and Lab-TAB scores. SCR amplitude was found to be positively skewed and even the most successful data transformation resulted in a non-normal distribution. Therefore we decided to use non-parametric tests to meaningfully analyze the data, since recent evidence (Wilcox, 2002) has shown that even with large and equal cell sizes, standard parametric procedures might not be as robust as initially thought (Fung, Raine, Lynam, Venables, Loeber, Steinhauer & Stouthamer-Loeber, 2005). Mann-Whitney non-parametric tests were employed to test for the effects of age, gender and temperament on SCR amplitude. SCR frequency was normally distributed and independent sample t-tests were used to test for the effects of age, gender and temperament.

Finally, Pearson's correlations were employed to examine the associations within the SCL variables (SCL baseline, SCL stress, SCL change, and SCL %increase), since these were transformed to normal distributions. Pearson's correlations were also employed when testing for relations between SCL and SCR frequency. However, because SCR amplitude was not normally distributed Spearman's correlations were calculated between SCL and SCR amplitude, and between SCR amplitude and SCR frequency. IBQ-fear was skewed and therefore Spearman's coefficients were calculated between SCA and temperament.

4.7.2 SCL and SCR in 1-year-old children

SCL

Table 4-1 presents descriptive data for the main SCL variables per condition and for the different subgroups. Data of 48 children were included in this analysis. Table 4-1 indicates that children in year 1 showed higher SCLs during stress.

Table 4-1 Means and standard deviations (in µS) for each subgroup for the baseline and stress conditions.

	baseline		stress	
	Mean	**SD**	**Mean**	**SD**
Group (n = 48)	13.5	9.4	17.5	11.4
girls (n =22)	14.0	10.0	18.5	11.5
boys (n =26)	13.0	9.0	16.6	11.4
young (n =25)	13.7	9.8	16.7	11.8
old (n =23)	13.2	9.0	18.4	11.1

Figure 4-2 shows the mean SCL for the baseline and stress conditions. Repeated measures ANOVAs revealed a significant main effect of condition with an increase in SCL from baseline to stress ($F\,(1, 47) = 16.39, p < 0.01$).

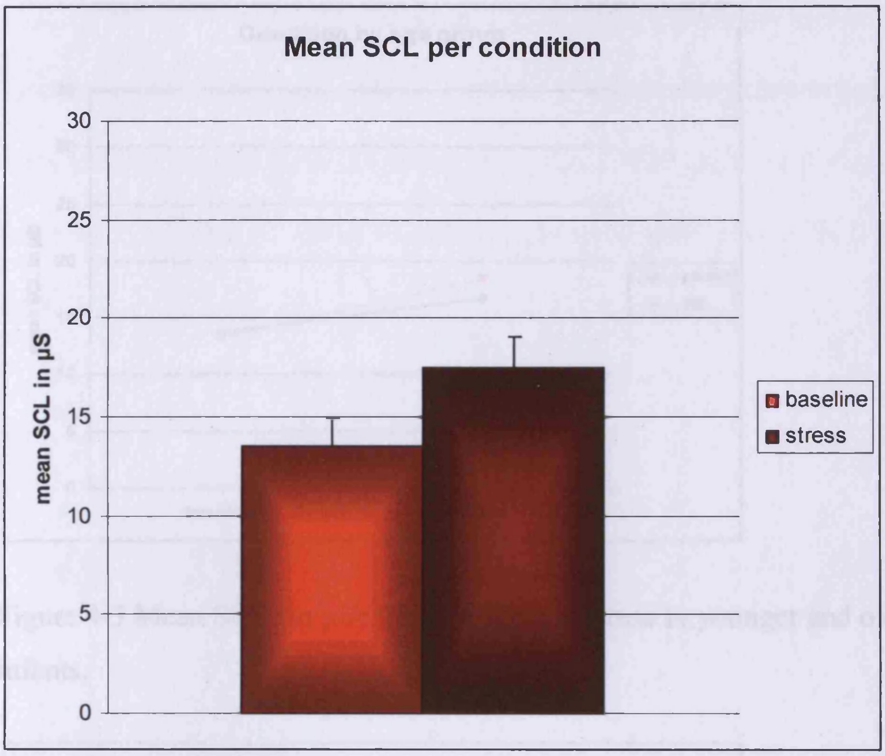

Figure 4-2 Mean SCL (in µS) for baseline and stress with standard error bars indicating SE's.

There was no main effect of age group (F (1, 46) = .24, p = .63) or sex (F (1, 46) = .17, p = .69), nor were there significant interactions between condition and age group (F (1, 46) = 1.48, p = .23; see Figure 4-3) or condition and sex (F (1, 46) = .34, p = .56; see Figure 4-4).

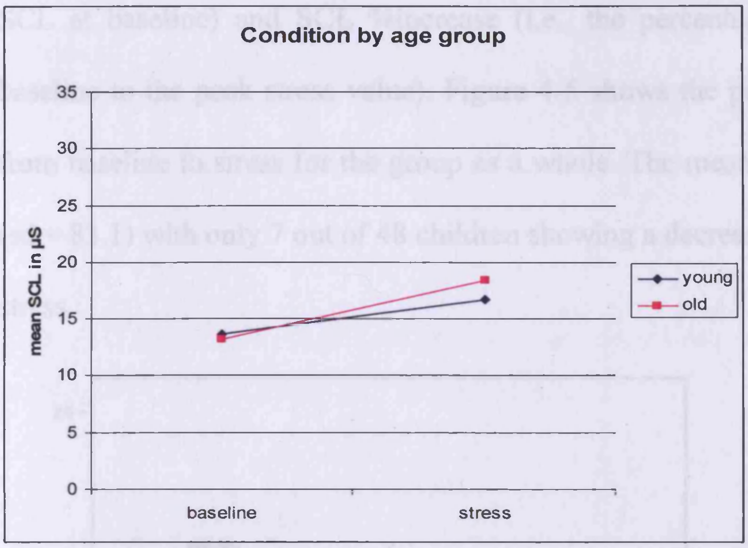

Figure 4-3 Mean SCL (in µS) for baseline and stress in younger and older infants.

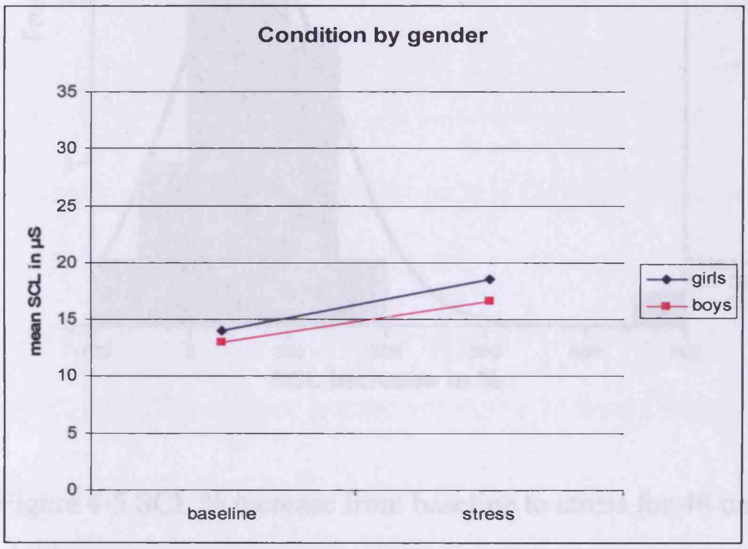

Figure 4-4 Mean SCL in µS for baseline and stress in boys and girls

SCL reactivity

We next examined more specifically the effects of SCL change or reactivity as a result of stress, by calculating two new variables: SCLΔ (i.e., the highest mean SCL recorded during the stress phase out of any of the 7 30-sec blocks minus the mean

SCL at baseline) and SCL %increase (i.e., the percentage increase in SCL from baseline to the peak stress value). Figure 4-5 shows the percentage increase in SCL from baseline to stress for the group as a whole. The mean SCL %increase was 63.8 (sd = 83.1) with only 7 out of 48 children showing a decrease in SCL from baseline to stress.

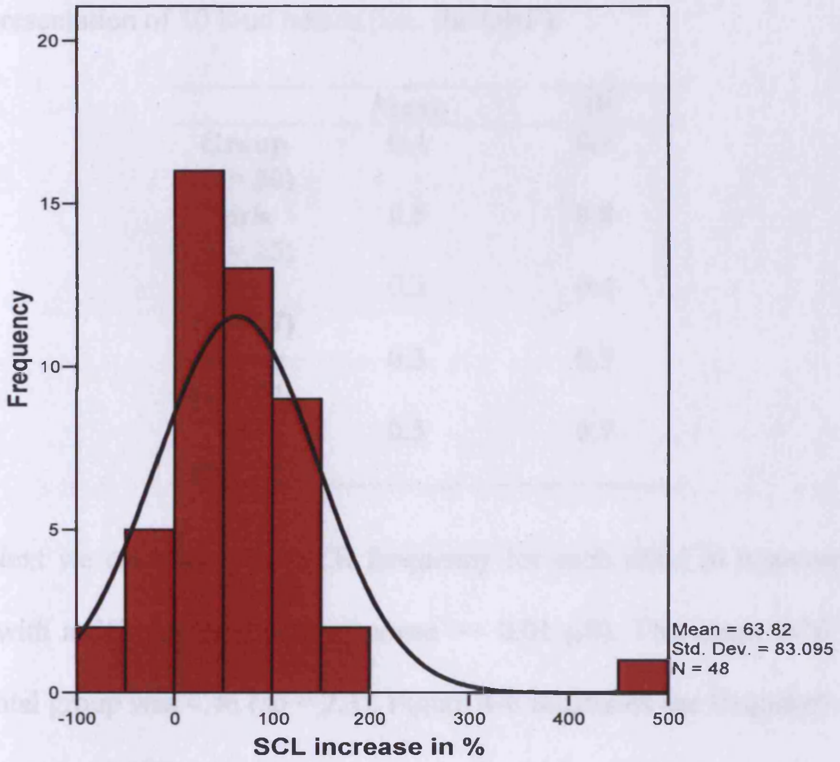

Figure 4-5 SCL % increase from baseline to stress for 48 one-year-old children

Two separate independent samples t-tests were conducted to examine the effects of age group and sex on SCLΔ and SCL %increase, respectively. No main effect of age group on SCLΔ (t (46) = -1.23, p = .23) or SCL %increase (t (46) = -.59, p = .56) was found. Similarly, no effect of sex on SCLΔ (t (46) = .17, p = .86) or SCL %increase (t (46) = .56, p = .58) was observed.

SCR

Descriptive information for SCR amplitude in 50 one-year-olds is presented in Table 4-2.

Table 4-2 Mean SCR amplitudes and standard deviations during the presentation of 10 loud noises (i.e., the OHP).

	Mean	SD
Group (n = 50)	0.4	0.6
girls (n = 23)	0.5	0.8
boys (n = 27)	0.3	0.4
young (n = 27)	0.3	0.5
old (n = 23)	0.5	0.7

Next we calculated the SCR frequency for each child in response to the 10 noises (with a SCR defined as a response >= 0.01 μS). The mean SCR frequency for the total group was 4.46 (sd = 2.3). Figure 4-6 illustrates the frequency distribution of the number of SCRs shown in response to the 10 loud noises.

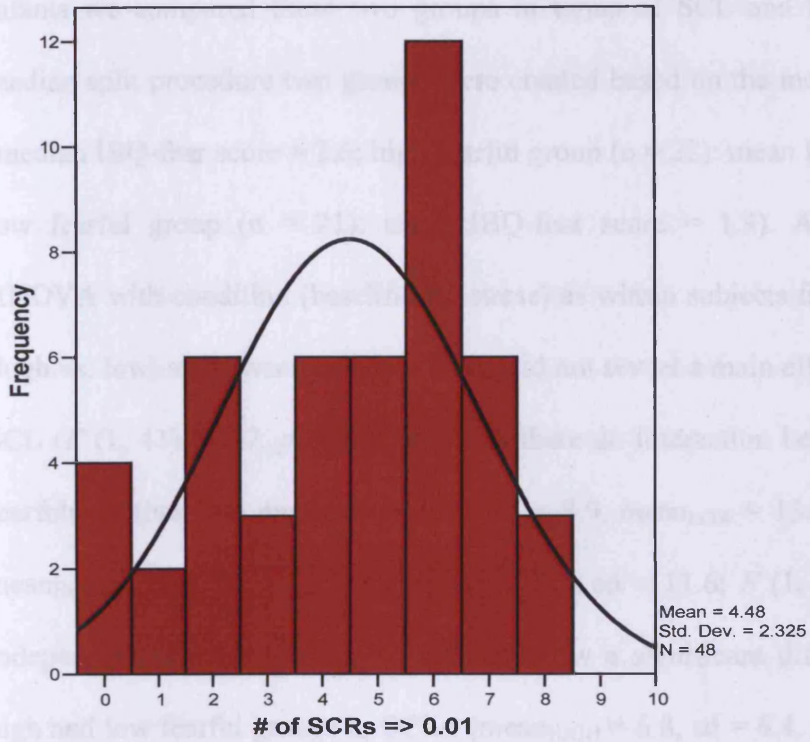

Figure 4-6 Frequency distribution of number of children showing a SCR (defined as a SCR >= 0.01 μS; score range 0-10) in 50 infants.

With respect to SCR amplitude, nonparametric Mann-Whitney tests indicated no significant effect of age group ($mean_{YOUNG} = 0.3$, sd = 0.5, $mean_{OLD} = 0.5$, sd = 0.7; U = 234.500, $p = .14$) or gender ($mean_{GIRLS} = 0.5$, sd = 0.8, $mean_{BOYS} = 0.3$, sd = 0.4; U = 286.500, $p = .64$). However, independent samples t-test showed a significant effect of age group with older infants showing more SCR's ($mean_{YOUNG} = 3.8$, sd = 2.3, $mean_{OLD} = 5.3$, sd = 2.1; t (48) = -2.36, $p < 0.05$), but there was no effect of gender ($mean_{GIRLS} = 4.7$, sd = 2.2, $mean_{OLD} = 4.3$, sd = 2.4; t (48) = .66, $p = .51$).

Effect of temperament on SCL and SCR

In order to examine whether infants with a more fearful temperament, as rated by their mothers on the IBQ-fear scale, have higher SCLs and SCR levels than less fearful infants we compared these two groups in terms of SCL and SCR. Following the median split procedure two groups were created based on the median IBQ-fear score (median IBQ-fear score = 2.6; high fearful group (n = 22): mean IBQ-fear score = 3.4, low fearful group (n = 21): mean IBQ-fear score = 1.9). A repeated measures ANOVA with condition (baseline vs. stress) as within subjects factor and fearfulness (high vs. low) as between subjects factor did not reveal a main effect of fearfulness on SCL (F (1, 41) = .27, p = .60), nor was there an interaction between condition and fearfulness (baseline $mean_{HIGH}$ = 13.5, sd = 8.9, $mean_{LOW}$ = 13.8, SD = 10.8; stress $mean_{HIGH}$ = 18.5, sd = 11.9, $mean_{LOW}$ = 15.7, sd = 11.6; F (1, 41) = 2.1, p = .16). Independent samples t-tests also did not show a significant difference between the high and low fearful groups in SCLΔ ($mean_{HIGH}$ = 6.8, sd = 6.4, $mean_{LOW}$ = 3.8, sd = 6.8; t (42) = -1.48, p = .15) or SCL %increase ($mean_{HIGH}$ = 74.6, sd = 105.8, $mean_{LOW}$ = 46.6, sd = 59.7; t (42) = -.68, p = .50).

Next we used Mann-Whitney nonparametrics and t-tests to test for an effect of fearfulness on SCR amplitude and frequency. Two fearful groups were created based on the IBQ-fear median score (n = 45, median IBQ-fear score = 2.6; high fearful group (n = 23): mean IBQ-fear score = 3.4, low fearful group (n = 22): mean IBQ-fear score = 1.9). No effect of fearfulness was observed on SCR amplitude ($mean_{HIGH}$ = .56, sd = .36, $mean_{LOW}$ = .28, sd = .36; U= 206.500, p = .29) or SCR frequency ($mean_{HIGH}$ = 4.2, sd = 2.6, $mean_{LOW}$ = 4.5, sd = 2.1; t (43) = .45, p = .65).

Finally, we assigned the infants to a high or low distress subgroup based on their Lab-TAB composite distress score (n = 47, median distress score = 61.0; high distress group (n = 24): mean distress score = 100.8, low distress group (n = 23): mean distress score = 23.8). A repeated measures ANOVA did not reveal a significant main effect of distress group on SCL (F (1, 45) = 1.31, p = .26), but there was a significant interaction between condition and distress group (baseline: $mean_{HIGH}$ = 13.5, sd = 8.6 $mean_{LOW}$ = 13.6, sd = 10.4, stress: $mean_{HIGH}$= 19.9, sd = 11.1, $mean_{LOW}$= 14.9, sd = 11.6; F (1, 45) = 8.24, p< 0.01; see Figure 4-7). Further independent samples t-tests showed that the interaction was attributable to a significant difference between the high and low distress groups during stress but not during baseline (for means and SDs see above; t (45) = -1.77, p< 0.05, one-tailed).

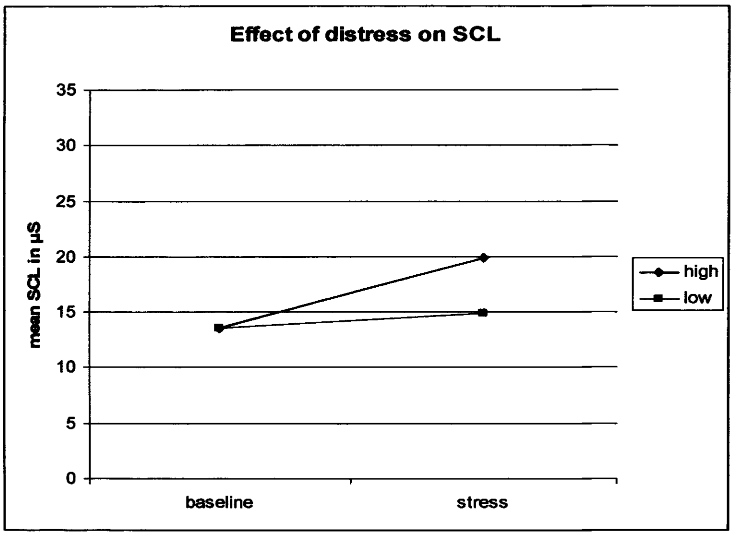

Figure 4-7 Mean SCL (in µS) for baseline and stress in high and low distress groups

There was also a significant difference between the distress groups in SCLΔ with high distress infants showing a stronger increase in SCL from baseline to stress ($mean_{HIGH}$ = 8.2, sd = 5.7 $mean_{LOW}$ = 3.2, sd = 6.9; t (45) = -2.72, p < 0.01). However, the same result was not found for the SCL %increase ($mean_{HIGH}$ = 72.9, sd = 46.6, $mean_{LOW}$ = 52.61, sd = 110.4; t (46) = -.43, p = .67).

Finally, the two distress groups were compared in terms of SCR (n = 49, median distress score = 53; high distress group (n = 24): mean distress score = 100.8, low distress group (n = 25): mean distress score = 25.2). Interestingly, because the behavioural distress and SCR data were collected in different paradigms, a significant difference between the two distress groups was observed in terms of SCR amplitude ($mean_{HIGH}$ = .39, sd = .36, $mean_{LOW}$ = .42, sd = .78; U = 213.500, $p > 0.05$; one-tailed) and SCR frequency ($mean_{HIGH}$ = 5.0, sd = 2.4; $mean_{LOW}$ = 3.8, sd = 2.1; t (47) = -1.93, $p < 0.05$; one-tailed) with more distressed infants showing a lower SCR amplitude but a higher SCR frequency during the OHP.

Correlations

SCL baseline was highly correlated with SCL stress (r = .83, $p < 0.01$) and negatively correlated with SCL %increase (r = -.40, $p < 0.01$). SCL stress was correlated with SCLΔ (r = .52, $p < 0.01$), and SCL %increase was highly correlated with SCLΔ (r =.57, $p < 0.01$). SCLΔ was significantly correlated with observed distress (r= .32, $p < 0.05$). SCR amplitude was significantly correlated with SCR frequency (rho = .75, $p < 0.01$), with SCL baseline (rho = .51, $p < 0.01$), SCL stress (rho = .69, $p < 0.01$) and SCLΔ (rho = .58, $p < 0.01$). The SCR frequency was correlated with SCL baseline (r = .50, $p < 0.01$), SCL stress (r = .64, $p < 0.01$), and SCLΔ (r = .34, $p < 0.05$). Table 4-3 presents this set of correlations. Since age appeared to have an effect on Lab-TAB distress and IBQ-fear (see section 3.4.2), partial correlations were calculated between these two variables and the SCL variables, and the values are presented in brackets.

Table 4-3 SCL and SCR correlations with temperament

	SCLbase[a]	SCLstress[b]	SCLΔ[c]	SCL%[d]	SCRamp[e]	SCRfreq[f]	fear[g]	distress[h]
SCLbase		.83**	-.02	-.40**	.51**	.50**	.07 (-.05)	.04 (-.01)
SCLstress			.52**	.12	.69**	.64**	.18 (.002)	.22 (.19)
SCLΔ				.57**	.58**	.34*	.27 (.11)	.32* (.32*)
SCL%					.21	.05	.11 (.15)	.07 (.20)
SCRamp						.75**	.21 (.19)	.23 (-.20)
SCRfreq							-.03 (-.27)	.24 (.13)
fear								.21

[a] mean SCL during baseline [b] mean SCL during stress [c] SCL change score
[d] SCL increase in % [e] mean SCR amplitude [f] mean frequency of SCRs
[h] Lab-TAB composite distress score

*.Correlation is significant at the 0.05 level (two-tailed)
**. Correlation is significant at the 0.01 level (two-tailed)

4.7.3 Statistical analyses in year 2

As for year 1, year 2 results were analysed with repeated measures ANOVAs to test for main effects of condition (baseline vs. stress) as within subjects' factor, and age group (younger vs. older), gender (boys vs. girls) and high and low internalizing/externalizing (CBCL) and distressed (Lab-TAB) temperament groups, as between subjects' factors.

SCR amplitude was positively skewed and therefore nonparametric tests were used. Mann-Whitney tests were employed to test for the age and gender effects, and for the effects of temperament on SCR amplitude. Parametric independent samples t-tests were used to test for between subjects' factors and temperament effects on SCR frequency.

SCR amplitude was positively skewed and Spearman's correlations were therefore calculated between SCR and the other physiological and temperament variables. Moreover, since both CBCL dimensions and IBQ-R fear have previously been found to be skewed (see section 3.4.1) Spearman's correlations were also employed here when exploring the relations between temperament and SCA. In all other cases Pearson's correlations were used.

4.7.4 SCL and SCR in 2-year-old children

SCL

Table 4-4 presents descriptive data for the main SCL variables per condition and for different subgroups. Data of 33 children are included in this analysis. Table 4-4 shows higher SCLs during stress in all groups.

Table 4-4 Mean SCL and standard deviations (in μS) for each subgroup for baseline and stress conditions

	baseline		stress	
	Mean	**SD**	**Mean**	**SD**
Group (n = 33)	6.9	4.8	13.5	8.6
girls (n =15)	7.5	4.5	14.0	8.5
boys (n =18)	6.5	5.1	13.1	8.9
young (n =18)	8.1	5.6	15.0	10.9
old (n =15)	5.6	3.3	11.6	4.2

Figure 4-8 shows the mean SCL during baseline and stress. Repeated measures ANOVAs revealed a significant main effect of condition, with an increase in SCL from baseline to stress (F (1, 32) = 90.54, $p < 0.01$).

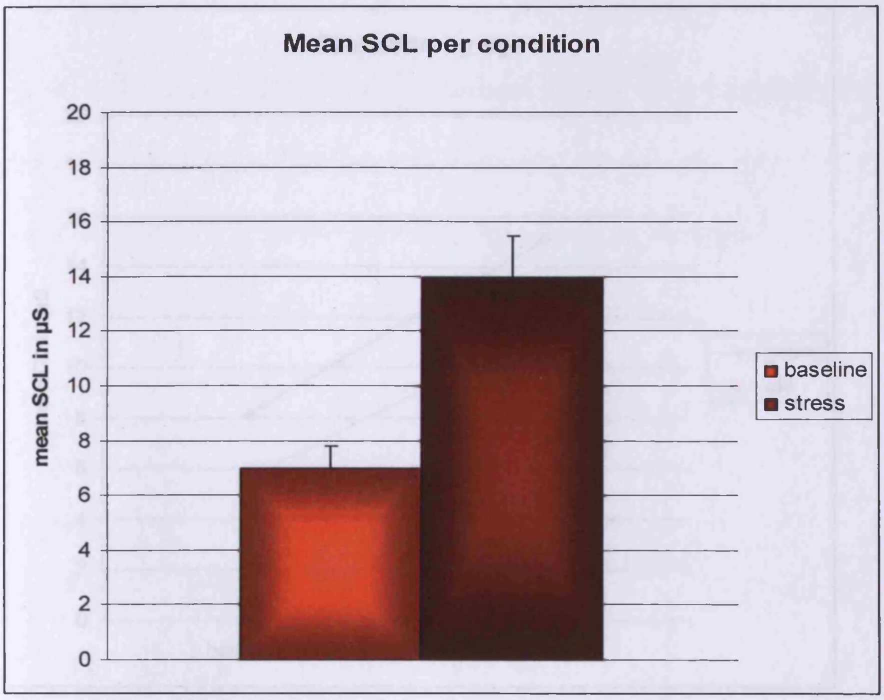

Figure 4-8 Mean SCL (in µS) for baseline and stress with error bars indicating the SE's.

Analysis did not show a main effect of age group (F (1, 31) = 1.36, p = .25) or sex (F (1, 31) = .19, p = .66), nor were there significant interactions between condition and age group (F (1, 31) = .30, p = .59; see Figure 4-9) or condition and sex (F (1, 31) = .07, p = .79; see Figure 4-10).

Figure 4-9 Mean SCL (in µS) during baseline and stress in younger and older groups

Figure 4-10 Mean SCL (in µS) during baseline and stress in boys and girls

SCL reactivity

Figure 4-11 shows the percentage increase in SCL from baseline to stress for the total group of 2-year-olds. The mean SCL %increase was 201.33 (sd = 250.48) with only one child showing a decrease in SCL from baseline to stress.

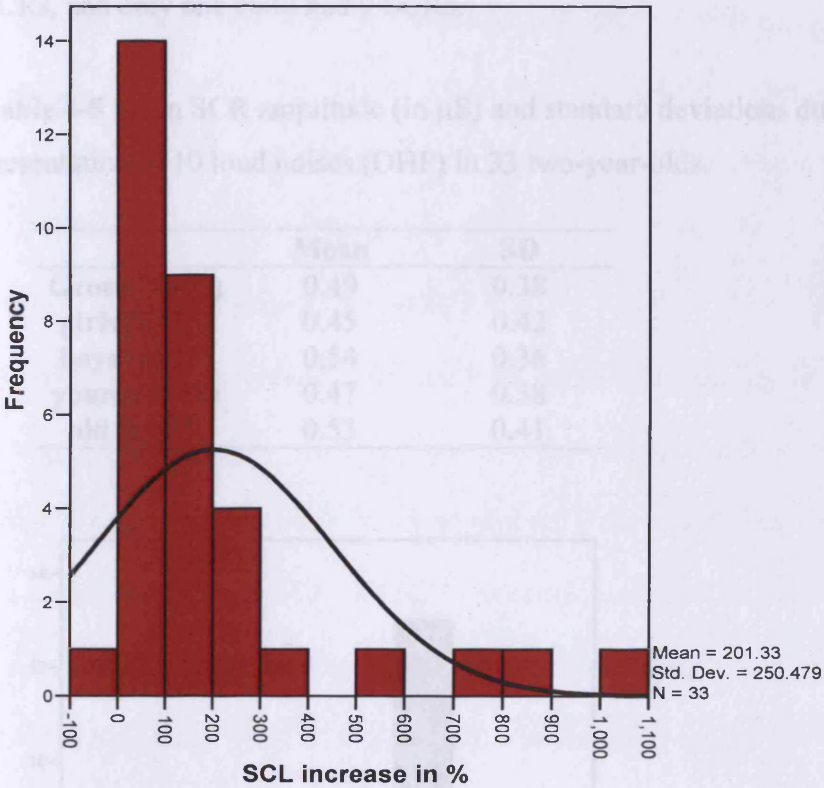

Figure 4-11 SCL % increase from baseline to stress for 33 two-year-old children

Two separate independent samples t-tests were conducted to examine the effects of age group and sex on SCLΔ and SCL %increase, respectively. No main effects of age group on SCLΔ (t (31) = -.57, p = .58) or SCL %increase (t (31) = 1.13, p = .27) were found. Similarly, no effects of sex on SCLΔ (t (31) = .20, p = .85) or SCL %increase (t (31) = .20, p = .85) were observed.

SCR

The descriptive information for SCR amplitude is presented in Table 4-5. Figure 4-12 illustrates the frequency distribution of SCR frequency. The mean SCR frequency was 5.5 (sd = 2.0) with all the children producing at least one SCR, 13 children had 7 SCRs, and only one child had 9 SCRs.

Table 4-5 Mean SCR amplitude (in μS) and standard deviations during the presentation of 10 loud noises (OHP) in 33 two-year-olds.

	Mean	SD
Group(n=33)	0.49	0.38
girls (n=15)	0.45	0.42
boys (n=18)	0.54	0.36
young(n=18)	0.47	0.38
old (n=15)	0.53	0.41

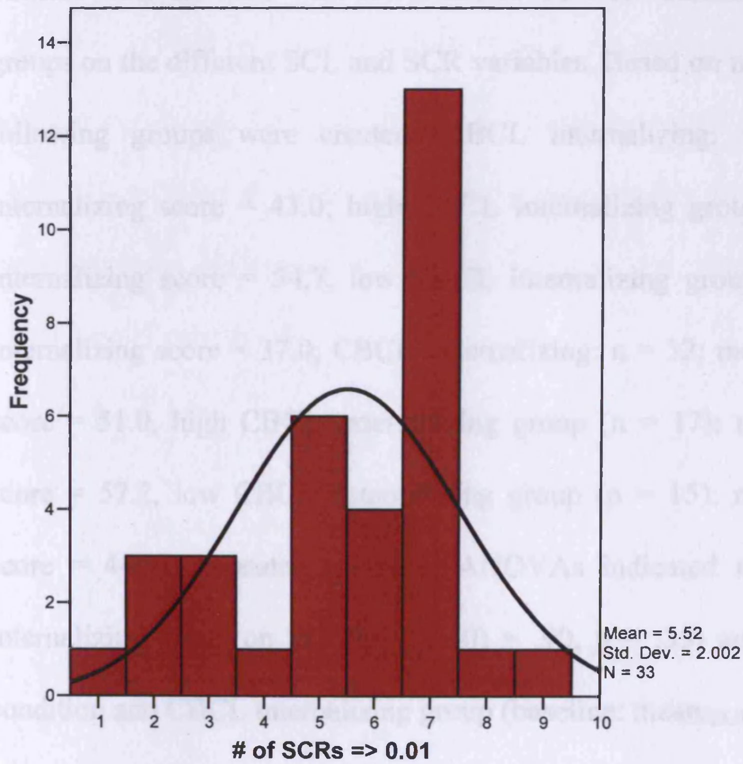

Figure 4-12 Frequency distribution of number of children showing an SCR (defined as an SCR >= 0.01 μS; score range 0-10) in 33 2-year-olds.

Non-parametric Mann-Whitney tests indicated no significant effect of age group ($mean_{YOUNG}$ = .53, sd = .41, $mean_{OLD}$ = .47, sd = .38; U = 122.00, p = .66) or gender ($mean_{GIRLS}$ = .45, sd = .42, $mean_{BOYS}$ = .54, sd = 36; U = 106.00, p = .31) on SCR amplitude. There was also no effect of age group ($mean_{YOUNG}$ = 5.6, sd = 2.2, $mean_{OLD}$ = 5.4, sd = 1.9 t (31) = -.29, p = .77) or gender ($mean_{GIRLS}$ = 5.1, sd = 2.4, $mean_{BOYS}$ = 5.9, sd = 1.6 t (31) = -1.18, p = -.82) on SCR frequency.

Effect of temperament on SCL and SCR

In order to examine whether infants with more internalizing or more externalizing behaviour, as rated by their mothers on the CBCL, have different physiological patterns than the ones with lower scores on these dimensions, we compared these groups on the different SCL and SCR variables. Based on median split procedures the following groups were created (CBCL internalizing: n = 32, median CBCL internalizing score = 43.0; high CBCL internalizing group (n = 15): mean CBCL internalizing score = 54.7, low CBCL internalizing group (n = 17): mean CBCL internalizing score = 37.0; CBCL externalizing: n = 32; median CBCL externalizing score = 51.0, high CBCL externalizing group (n = 17): mean CBCL externalizing score = 57.2, low CBCL externalizing group (n = 15): mean CBCL externalizing score = 44.9). Repeated measures ANOVAs indicated no main effect of CBCL internalizing group on SCL (F (1, 30) = .90, p = .35) and no interaction between condition and CBCL internalizing group (baseline: $mean_{HIGH}$ = 2.6, sd = 1.0, $mean_{LOW}$ = 2.4, sd = .80; stress: $mean_{HIGH}$ = 3.7, sd = 1.2, $mean_{LOW}$ = 3.3, sd = .90; F (1, 30) = .69, p = .41). Also, no effects of CBCL internalizing group were found on SCLΔ or SCL %increase (SCLΔ: $mean_{HIGH}$ = 9.6, sd = 6.8, $mean_{LOW}$ = 6.5, sd = 4.5; t (30) = -

1.51, p = .14; SCL %increase: $mean_{HIGH}$ = 227.25, sd = 288.22, $mean_{LOW}$ = 182.49, sd = 226.78; t (30) = - .61, p = .55). A Mann-Whitney nonparametric test found no effect of CBCL internalizing group on SCR amplitude ($mean_{HIGH}$ = .58, sd = .45, $mean_{LOW}$ = .43, sd = .34; U = 103.000, p = .37) or SCR frequency ($mean_{HIGH}$ = 5.3, sd = 1.7, $mean_{LOW}$ = 5.6, sd = 2.3; t (30) = .53, p = .60).

The same analysis was repeated for the CBCL externalizing groups. No effect of CBCL externalizing group was found on SCL (F (1, 30) = .19, p = .66) but there was a significant interaction between condition and CBCL externalizing group (baseline: $mean_{HIGH}$ = 5.9, sd = 3.9, $mean_{LOW}$ = 7.9, sd = 5.8; stress: $mean_{HIGH}$ = 13.1, sd = 5.8, $mean_{LOW}$ = 13.3, sd = 11.2; F (1, 30) = 5.37, $p < 0.05$) with the high externalizing group having a lower SCL baseline. Independent sample t-tests (baseline: t (30) = 1.22, p = .23) and (stress: t (30) = -1.11, p = .28) showed non-significant differences between the groups for each condition. Therefore, whilst the pattern of progression from SCL baseline to stress is similar for both groups, the interaction highlights the initial apparent difference in baseline between the groups (see Figure 4-13 for illustration of this effect).

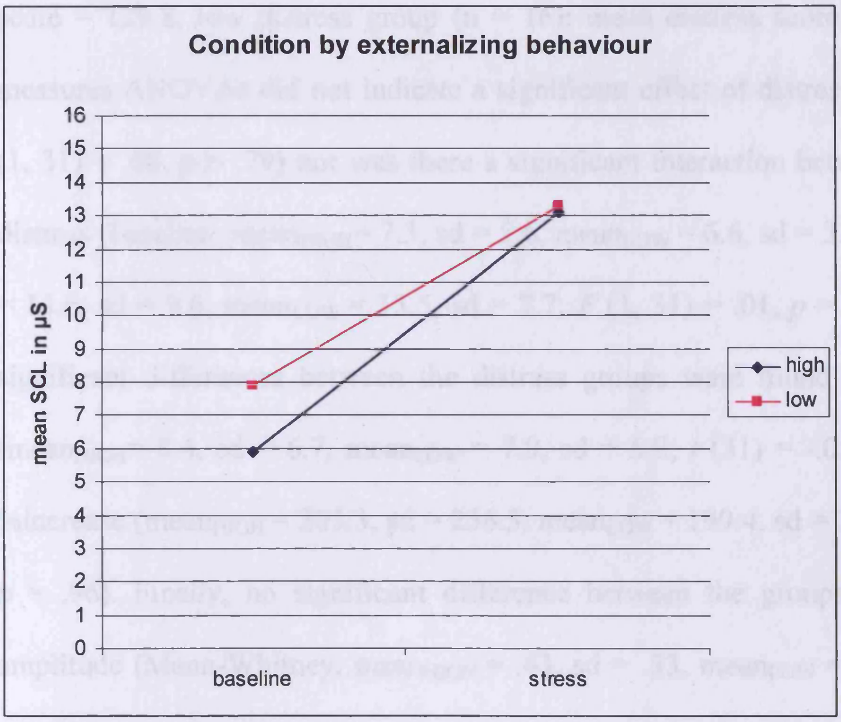

Figure 4-13 Mean SCL (in µS) during baseline and stress in high and low externalizing groups

Although the CBCL externalizing groups did not differ on SCLΔ ($mean_{HIGH}$ = 8.8, sd = 4.6, $mean_{LOW}$ = 7.0, sd = 6.9; *t* (30) = -1.28, *p* = .21) they differed on SCL %increase ($mean_{HIGH}$ = 293. 9, sd = 315.6, $mean_{LOW}$ = 101.0, sd = 89.3, *t* (30) = -2.55, $p < 0.05$) with children relatively higher in externalizing behaviour showing a stronger increase in SCL from baseline to stress. Mann-Whitney nonparametric tests indicated no differences between the CBCL externalizing groups on SCR amplitude ($mean_{HIGH}$= .56, sd = .41, $mean_{LOW}$ = .42, sd = .37; U = 94.500, *p* = .22) or SCR frequency ($mean_{HIGH}$ = 5.8, sd = 1.4, $mean_{LOW}$ = 5.1, sd = 2.6; *t* (30) = -.88, *p* = .39)

Finally, we assigned our children to a high or low distress group based on their Lab-TAB composite distress scores, and compared these groups in terms of SCL and SCR (n = 33, median distress score = 98.0; high distress group (n = 17): mean distress score = 129.8, low distress group (n = 16): mean distress score = 46.1). Repeated measures ANOVAs did not indicate a significant effect of distress group on SCL (F (1, 31) = .08, p = .79) nor was there a significant interaction between condition and distress (baseline: $mean_{HIGH}$= 7.3, sd = 5.6, $mean_{LOW}$ = 6.6, sd = 3.9; stress: $mean_{HIGH}$ = 13.6, sd = 9.6, $mean_{LOW}$ = 13.5, sd = 7.7; F (1, 31) = .01, p = .93). Moreover, no significant differences between the distress groups were found in terms of SCLΔ ($mean_{HIGH}$= 8.4, sd = 6.7, $mean_{LOW}$ = 7.9, sd = 5.0; t (31) = -.05, p = .96) or SCL %increase ($mean_{HIGH}$ = 203.3, sd = 256.5, $mean_{LOW}$ = 199.4, sd = 252.2; t (31) = -.04, p = .96). Finally, no significant difference between the groups in terms of SCR amplitude (Mann-Whitney: $mean_{HIGH}$ = .43, sd = .33, $mean_{LOW}$ = .57, sd = .44; U = 113.000, p = .42) or SCR frequency ($mean_{HIGH}$ = 5.1, sd = 2.1, $mean_{LOW}$ = 5.9, sd = 1.8; t (31) = 1.18, p = .25) was found.

Correlations

SCL baseline was highly positively correlated with SCL stress (r = .80, $p< 0.01$), SCLΔ (r = .45, $p< 0.01$) and significantly negatively correlated with SCL %increase (r = -.67, $p< 0.01$). SCL stress was correlated with SCLΔ (r = .86, $p<0.01$). Finally, SCR amplitude was significantly correlated with SCR frequency (rho = .48, $p< 0.01$) and SCL stress (rho= .42, $p< 0.05$). Table 4-6 presents these correlations.

Table 4-6 SCL, SCR and temperament correlations

	SCLbase[a]	SCLstress[b]	SCLΔ[c]	SCL%[d]	SCRamp[e]	SCRfreq[f]	CBCL-Int[g]	CBCL-Ext[h]	distress[i]
SCLbase		.80**	.45**	-.67**	.24	-.11	.05	-.04	.09
SCLstress			.86**	-.18	.42*	.06	.09	.07	.06
SCLΔ				.29	.22	.21	.09	.13	.06
SCL%					-.03	.16	.23	.21	-.01
SCRamp						.48**	.16	.32	-.04
SCRfreq							-.03	.09	-.11
CBCL-Int								.52**	.20
CBCL-Ext									.03

[a] mean SCL during baseline [b]mean SCL during stress [c]SCL change score [d]SCL increase in % [e]mean SCR amplitude [f]mean frequency of SCRs
[g]CBCL internalizing dimension [h]CBCL externalizing dimension [i]Lab-TAB composite distress score

*Correlation is significant at the 0.05 level (two-tailed)

** Correlation is significant at the 0.01 level (two-tailed)

4.7.5 Results of comparisons of year 1 and year 2: Developmental effects

As compared to the one-year-olds the two-year-olds, as a group, turned out to be more difficult to test. This has been already reported in Chapter 3 where behavioural distress was found to be significantly higher in the two-year-olds than in the one-year-olds (distress_1 = 57.5, sd = 39.75, distress_2 = 88.7, sd = 47.9, t (32) = -2.84, $p<$ 0.01). Table 4-7 illustrates the descriptive information for the groups across time and conditions.

Table 4-7 Mean SCLs (in µS) and standard deviations of the group of children who were both tested in year 1 and year 2 (n=32)

	Year 1				***Year 2***			
	baseline		**stress**		**baseline**		**stress**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Group (n = 32)	12.9	8.8	17.1	10.6	6.8	4.8	13.4	8.7

To examine whether there would be an effect of time and condition on SCL a repeated measures ANOVA was conducted with condition (baseline vs. stress) and time (year 1 vs. year 2) as the within subjects factors. Gender was not used in this analysis since our previous showed no gender differences in this respect.

There was a significant main effect of time (F (1, 30) = 7.48, $p<$ 0.01), a significant effect of condition (F (1, 30) = 79.41, $p<$0.01), and a significant interaction between time and condition (F (1, 30) = 7.25, $p<$ 0.05; see Figure 4-14).

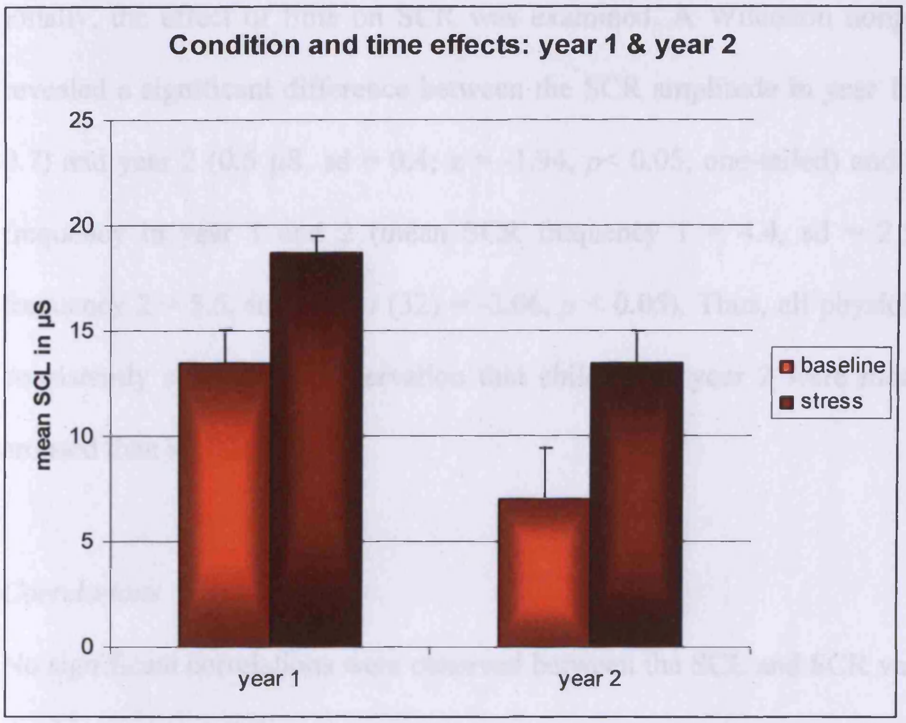

Figure 4-14 Mean SCLs in µS (with bars indicating the SE) during baseline and stress, for year 1 and year 2

Paired samples t-tests examined the significant time effects and the significant interactions between time and condition further. Paired samples t-tests showed a significant difference between year 1 and year 2 baselines (baseline_1 = 13.5, sd = 9.3, baseline_2 = 6.9, sd = 8.6; t (31) = 3.72, p< 0.01). However, there was no significant difference between year 1 and year 2 stress levels (stress_1 = 18.7, sd = 13.9, stress_2 = 13.5, sd = 8.6; t (31) = -1.27, p = .21).

Paired samples t-tests analysed the effect of time on SCLΔ and SCL %increase. SCLΔ_1 (7.0, sd = 9) was significantly lower than SCLΔ _2 (8.2, sd = 5.8; t (32) = 5.19, p< 0.01). Similarly, SCL %increase_1 (72.6, sd = 92.3) was significantly lower than SCL %increase_2 (202.0, sd = 250.3; t (32) = -2.60, p< 0.05).

Finally, the effect of time on SCR was examined. A Wilcoxon nonparametric test revealed a significant difference between the SCR amplitude in year 1 (0.4 μS, sd = 0.7) and year 2 (0.5 μS, sd = 0.4; z = -1.94, $p < 0.05$, one-tailed) and between SCR frequency in year 1 and 2 (mean SCR frequency 1 = 4.4, sd = 2.5, mean SCR frequency 2 = 5.5, sd = 1.9; $t\ (32) = -2.06$, $p < 0.05$). Thus, all physiological results consistently support the observation that children in year 2 were more fearful and aroused than in year 1.

Correlations

No significant correlations were observed between the SCL and SCR variables across the two years. Table 4-8 presents the correlations between SCL, SCR and temperament as we measured these between year 1 and year 2. Pearson and Spearman correlations were performed as appropriate.

None of the SCA variables in year 1 were found to correlate with the SCA variables in year 2. Similarly, no significant correlations were found between temperament in year 1 and SCA in year 2, or temperament in year 2 and SCA in year 1. The only significant correlation (out of the many we calculated) was between the SCL %increase in year 2 and the composite distress score in year 1; children who showed more distress in year 1 during the fear challenge showed a stronger SCL increase from baseline to stress in year 2.

Table 4-8 Correlations between SCA parameters and temperament dimensions between year 1 and year 2

	SCLbase_1[a]	SCLstress_1[b]	SCLΔ_1[c]	SCL%_1[d]	SCRamp_1[e]	SCRfreq_1[f]	fear[g]	distress_1[h]
SCLbase_2[i]	.28	.18	-.04	-.29	.13	-.02	.12 (.22)	-.25 (-.17)
SCLstress_2[j]	.32	.21	-.06	-.22	.19	.02	.06 (.15)	-.07 (-.04)
SCLΔ_2[k]	.10	.11	-.01	-.04	.08	.09	-.11 (.03)	.23 (.05)
SCL%_2[l]	-.13	-.04	.01	.25	.02	.08	-.18 (-.09)	.38* (-.04)
SCRamp_2[m]	.26	.07	-.25	-.27	.16	.24	-.13 (-.10)	-.10 (-.22)
SCRfreq_2[n]	.03	.02	-.07	-.04	-.04	-.01	-.30 (-.12)	.20 (.19)
CBCL-Int[o]	.14	.09	.03	.06	.16	.22	-.02 (.23)	.13 (.07)
CBCL-Ext[p]	.06	-.06	-.02	.11	.03	.21	-.14 (.12)	-.09 (-.31)
distress_2[q]	-.19	-.25	-.18	.01	-.03	.22	-.33 (-.30)	.04 (.09)

[a] mean SCL during baseline_1 [b] mean SCL during stress_1 [c] stress minus baseline score_1 [d] SCL increase in %_1 [e] mean SCR amplitude_1 [f] mean frequency of SCRs_1 [g] IBQ-fear [h] Lab-TAB composite distress score_1 [i] mean SCL during baseline_2 [j] mean SCL during stress_ 2 [k] stress minus baseline score_ 2 [l] SCL increase in %_2 [m] mean SCR amplitude_2 [n] mean frequency of SCRs_2 [o] CBCL internalizing dimension [p] CBCL externalizing dimension [q] Lab-TAB composite distress score_2

*.Correlation is significant at the 0.05 level (two-tailed)

**. Correlation is significant at the 0.01 level (two-tailed)

4.8 Discussion

This study assessed physiological reactivity in a group of healthy infants during the first two years of life by using SCA, a measure of the sympathetic nervous system (SNS). To our knowledge this is the first study to examine (a) a range of SCA parameters in very young children, (b) the effects of gender and temperament on these parameters, and (c) whether these parameters are relatively stable over time. We will explain and discuss the main findings by reviewing the evidence for each of the 6 hypotheses as described in the Introduction to this Chapter.

SCA has been proven to be a reliable method to detect autonomic arousal in young infants under stressful situations (Hernes et al., 2002; Storm, 2000). For example, Ham and Tronick (2006) previously reported SCL increases in 5-month-olds during a Still-Face paradigm. In line with our predictions, 41 out of 48 children (86%) in the 1st year and 32 out of 33 children (97%) in the 2nd year responded with an increase in their SCL from baseline to stress. Results of repeated measures ANOVA tests confirmed that SCLs were significantly higher during stress than during baseline in both years. These results render our experimental manipulations successful and enable us to start investigating individual differences in physiological reactivity in infancy and early childhood.

A 2nd paradigm, the OHP, was used to examine individual differences in SCR.
Clear individual differences in SCR amplitude and SCR frequency were observed in year 1, with 4 children showing no SCR response, 13 children showing 6 SCRs and 3 children showing 8 SCR's (see Figure 4-6). As for the two-year-olds, all children

showed at least one SCR, 13 showed 7 responses and 1 showed 9 SCRs (see Figure 4-12).

Other hypotheses were related to the effects of gender and age. We predicted that time would have no effect on SC resting levels, but would affect SC stress reactivity. We were uncertain about the effect of gender on SCA since previous research failed to examine the effect of gender on SCA. No effects of gender or age group were observed within the years. However, contrary to prediction, a significant effect of time was found for SCL baseline, with one-year-olds having higher SCL baselines. As predicted and in line with our observation of more fearful distress in two-year-olds, SC stress parameters (both SCL %increase and SCL reactivity) were significantly higher in year 2. Similarly, SCR amplitude and SCR frequency increased significantly with age, confirming Gao et al's (2007) results that orienting responses increase in young children over time. In Chapter 3 (see section 3.4.4) the finding of significantly more behavioural distress in two-year-olds as compared to one-year-old children was attributed to developmental factors, particularly the increasing cognitive abilities in infants which emerge with time. According to Gartstein and Rothbart (2003) the effect of age on fear is consistent with the development of inhibition of approach towards novel events, which as part of development increases towards the end of the 1st year of life and extends in the 2nd year. The current SCA results suggest that the SNS develops in similar ways, as reflected by the higher SCL reactivity and SCR values found in two-year-olds.

It is evident from previous research that individual differences in SCA are associated with temperament, and more specifically, that higher levels of SCA are associated with a more anxious and inhibited temperament. For example, Fowles et al. (2000) found modest correlations between SCL and observed fear (r = .21, $p < 0.05$) but no effect of temperament on SCR. We predicted that there would be individual differences in SCL and SCR related to differences in fearful temperament as rated by mothers and as observed by independent observers during the unpredictable toy episode. Mother-rated fearful temperament in year 1 was not correlated with any of the SCA indices, nor did high and low fearful groups differ in terms of SCA. The same results apply to year 2 when we used the CBCL internalizing scale. An important criticism regarding questionnaires' validity refers to the parents' inclination to give socially desirable answers to questionnaires in general or to specific questions in particular (see also Kagan, 1994). We discussed this issue in Chapter 3 and concluded then that parental reports cannot reliably predict fear. It is also important to note that the current between-group analyses were based on median split procedures and that the range of CBCL internalizing scores, due to the nature of our sample, was restricted (see Figure 3-4). We also discussed this limitation in Chapter 3 and concluded that had we used a more extreme group based design, the findings might have been different. In contrast to these questionnaire results, we found in year 1 that level of observed distress during fear challenge was related to stronger SCL reactivity (i.e., the observed distress - SCLΔ correlation was .32, $p < 0.05$). We also found an effect of temperament on SCL and SCR, but again only in the first year of life. Specifically, more distressed infants not only had higher SCLs during stress but also showed more SCRs during the OHP despite lower SCR amplitude. It therefore seems that one-year-olds, who were more afraid of the robot, startled more during the presentation of 10 noises, which supports the notion that both paradigms, albeit different in nature, tap into fearfulness. However, observed distress did not correlate with SCA in the 2nd year, nor were there differences between the distress groups on any SCL or SCR variables. As mentioned in the Results section of Chapter 3, there was no significant correlation between the Lab-TAB distress scores of year 1 and year 2 ($r = .04$), which was attributed to developmental changes in the expression of fearful temperament (Carranza et al., 2005).

We also predicted, in line with research in conduct disordered children (Snoek et al., 2004; Van Goozen et al., 2000), that young children with externalizing behavioural problems would have lower SCA. In the current study and based on median splits we found that children who were rated relatively high in externalizing behaviour had lower baseline SCLs but a stronger SCL increase from baseline to stress. Given that SCL stress levels did not differ between high and low externalizing groups the difference in baseline SCL stands out. However, we have to be cautious in interpreting this finding. The CBCL score ranges were small (see Figure 3-4 & Figure 3-5) and only one child scored in the clinical range on both the externalizing and the internalizing scales, and none of the children scored in the borderline range. It will be interesting to find out whether this difference is a stable one and has implications for further temperament development. Finally, no effect of externalizing on SCR amplitude or frequency was found.

Finally, we predicted positive correlations between the different SCA measures as well as inter-individual continuity in physiological response patterns across time. Unexpectedly, no stability in SCA between year 1 and year 2 was observed, nor were children high in SCL and/or SCR in year 1 also high in these parameters in year 2. The previously reported clear developmental changes in fearful temperament (i.e., more distress in year 2 than in year 1) and physiology (lower SCL baselines but higher SCL increases due to stress in year 2), could be responsible for these findings. Further follow-up research on SC reactivity in the same sample will enable us to sort out whether these patterns stabilize or continue to change over time.

Following Ham and Tronick (2006), the current study provides further evidence that SCA is a valid measure in the study of the biological basis of children's temperament. We successfully induced fear and stress in one- and two-year-olds, as reflected in the strong increase in SCL following baseline measurement.

Does fear increase with age?

Clear effects of time were revealed, with two-year-old children showing lower SC baselines but higher SC stress reactivity levels, as well as higher SCR amplitudes and SCR frequencies.

Are there gender differences in fearful temperament?

No effects of gender were observed with respect to any SC variable in year 1 or year 2.

What are the consequences of a more or less fearful temperament?

We found that more fearful children in year 1 had higher SCLs during stress, a stronger increase in SC from baseline to stress, and more SCRs during the OHP despite showing lower SCR amplitude. Moreover, more externalizing two-year-olds had lower SCL resting levels and a stronger SCL increase from baseline to stress, although there was no difference in SC stress levels between high and low externalizing groups.

Stability of SCA

Unexpectedly, there was no inter-individual stability in SCA between year 1 and year 2, nor was there intra-individual stability in SCL/SCR.

In the current study multiple comparison tests were carried out on this set of data. This should be noted as a limitation of our analysis, since the probability of committing Type I error might have increased.

Since this study is the first to examine the development of SCA early in life, further longitudinal research is needed to identify which physiological parameters are important, how physiological response patterns are related to individual differences in temperament, and whether relations between physiology and temperament continue to develop as the child gets older.

Chapter 5 Cortisol and fearfulness: Methodology and results

5.1 Cortisol as a physiological index of temperament

The other physiological measure employed in the current study is the end product of the hypothalamic-pituitary-adrenal axis (HPA), cortisol. Well documented in research on the mammalian stress response is the activation of the HPA axis system. The importance of the HPA axis in responding to and coping with stress has led to this system becoming an important focus of attention in current investigations of human and non-human stress reactivity (Gunnar, 1992).

Apart from epinephrine and norepinephrine (or adrenalin and noradrenalin) which are released in response to stress by the sympathetic nervous system (SNS), a different group of hormones, called glucocorticoids, is also involved in coping with stress. Glucocorticoids, such as cortisol, are steroid hormones which are secreted by the adrenal gland as a consequence of a cascade of events mastered by the brain (Sapolsky, 2004). When something stressful happens, even in the form of a thought, the hypothalamus releases the corticotrophin releasing hormone (CRH). CRH within seconds stimulates the pituitary to release adrenocorticotrophin hormone (ACTH). After ACTH reaches the adrenal gland, the secretion of the glucocorticoids, in the form of cortisol in primates, happens in a few minutes (Sapolsky, 2004). Cortisol can be measured in plasma and its metabolites can be assessed in urine. In addition, cortisol can be detected in saliva, which is a less stressful way of sampling than from blood, and has the added advantage that it can be collected more frequently than sampling from urine. Recent modifications in the radioimmunoassay, which allow reliable assessment of cortisol in small amounts of saliva, have led researchers to assess salivary cortisol levels in stress studies (Gunnar, 1992).

Once secreted in the bloodstream cortisol bounds to protein. However, when cortisol is assessed in saliva, only the unbound fraction of the hormone, approximately 10%, is picked up (Kirscbaum & Hellhammer, 1989). This free, non - protein bound fragment is supposed to be the more biologically active form of plasma cortisol with only 1-2 minutes difference in detection time from the plasma levels. Salivary cortisol as an index of psychological stress has been used in both ambulatory and laboratory paradigms where it has been proven to be a reliable, non-invasive method of assessing cortisol levels (Seeman, Singer, Wilkinson & McEwen, 2001).

As an endocrine system the HPA axis is characterised by a circadian rhythmicity with the highest levels around 30 min after waking up, followed by a dampening shift over the next hour or two, and then a more gradual decrease over the rest of the day (Gunnar & Vasquez, 2001). Studies disagree on the age of appearance of the circadian rhythm of the HPA axis: some suggested that it is apparent at three months of age (Spangler, 1991), while others suggest that it is not there till the 9th month of age (de Weerth & van Geert, 2002), although even then infants show large intra-individual variability in cortisol levels with no clear decline of levels during the day. In some studies the circadian rhythm was regarded as present, when evening values (2200-2400 h) are at least 2 nmol/l smaller than morning values (0600-0800 h; de Weerth, Zijl & Buitelaar, 2003). The well-known adult diurnal rhythm has been suggested to emerge by age 2 (Goldberg, Levitan, Leung, Masellis, Basile, Nemeroff & Atkinson, 2003) or even as late as 3 or 4, when children have given up their afternoon naps

(Gunnar & Donzella, 2002). The development of a cortisol circadian rhythm has been related to the establishment of a more normal sleep-wake rhythm with less naps over the day, and to physiological and/or behavioural individual differences (de Weerth, Zijl & Buitelaar, 2003). A stress hyporesponsive period (SHRP) has been reported to gradually emerge during the first year of life, when it is difficult to produce elevations in cortisol levels in response to stressors. This period has been reported to protect the infant's developing brain from the impact of elevated glucocorticoids (Gunnar, & Fisher, 2006; Sapolsky & Meaney, 1986). However, there is also a general consensus that activation of the HPA axis system to noxious or strongly arousing stimuli is apparent from the first day of life (Gunnar, 1992). Thus, cortisol reactivity to stress has been shown to decrease in infants between 2 and 6 months (Lewis & Ramsay, 1995) and between 6 and 15 months of age (Gunnar et al., 1996). A lack of cortisol response has been reported in most 15-month-old infants (Gunnar et al., 1996). It has been suggested that the SHRP is maintained under sensitive and responsive caregiving, and that any disturbance in these conditions may produce large increases in cortisol levels to stressors (Albers, Riksen-Walraven, Sweep & de Weerth, 2008). However, despite the notion of a SHRP it is possible to elicit a stress response in infants as young as 6 months (Buss et al., 2003).

Given the dynamic nature of the stress response, sizable individual differences in cortisol reactivity are expected, not only between individuals but also within an individual across different situations (Gunnar, Tout, de Haan, Pierce & Stansbury, 1997). It is believed that after the 6th month of age individual differences in stress reactivity become apparent (Ramsay & Lewis 2003).

Research on the effect of gender on cortisol reactivity remains inconclusive. Studies in adolescents and adults show either stronger cortisol reactivity in men (Kirschbaum, Wust & Hellhammer, 1992; Kirschbaum, Klauer, Filipp & Hellhammer, 1995) or no gender difference (Collins & Frankenhaeuser, 1978). With respect to stressor type, men have been reported to be more physiologically reactive to psychological stressors involving achievement, while women are more physiologically reactive in pharmacological challenges or psychological stressors involving social rejection (Stroud, Salovey & Epel, 2002; Uhart, Chong, Oswald, Lin & Wand, 2005). In early ages, Gunnar, Connors, and Isensee (1989) found that newborn boys repeatedly responded to a brief discharge examination with relatively higher cortisol levels as compared to girls, who also habituated faster. However, according to Kirschbaum and Hellhammer (1994), most reports fail to provide information about the existence of gender differences in cortisol.

Several findings point to a positive correlation between cortisol reactivity and infant distress, while basal cortisol levels have been shown to correlate with measures of temperament. While higher basal and reactivity cortisol levels have been related to inhibited and internalizing behaviour (Kagan, Reznick & Snidman, 1987; Schmidt, Fox, Rubin, Sternberg, Gold & Smith, 1997; de Haan, Gunnar, Tout, Hart & Stansbury, 1998; Smider, Essex, Kalin, Buss, Klein, Davidson & Goldsmith, 2002) the same cortisol patterns have occasionally been related to more outgoing and externalizing behaviour (Gunnar et al. 1989; Spangler & Schieche, 1998; Dettling, Gunnar & Donzella, 1999). In addition, children high in externalizing and antisocial behaviour have been reported to show weaker cortisol responses under stress as compared to normal controls (Van Goozen et al., 2005). Cortisol is necessary for survival and social adaptation. However, dysregulation as well as chronic activation of the HPA axis system has been shown to have deleterious consequences for one's health (Sapolsky, 2004). Poor regulation of cortisol has been shown to have an effect on the activity of the immune system, as well as a negative impact on brain areas involved in cognitive processes such as attention and memory, ultimately leading to loss of hippocampal neurons and decreases in dendritic branching (Bugental, Martorell & Barraza, 2003). Thus, this evidence makes it an imperative to confirm the role of the HPA axis in the physiological underpinnings of individual differences in emotional behaviour. A review of relevant studies for our research purpose will follow next. At the end of each section, a summary table with experiment details is provided.

5.1.1 Cortisol in infants and young children

Gunnar, Connors, Isensee and Wall (1988), in an attempt to establish the association between emotional distress and HPA axis functioning, conducted two stress studies in newborns selected from a normal care nursery. Their rationale was grounded in the notion that the distress-cortisol relation can best be examined in young infants who are still unable to use any psychological defences to hide negative feelings or expressions (Gunnar et al., 1988). In the 1st study the association between distress and cortisol was examined through the occurrence of several events that took place in treatment rooms adjacent to the nursery. These events included routine neonatal circumcision without anaesthesia, weighing and measuring, and the heel-stick blood sampling procedure (Gunnar et al., 1988). Cortisol was assessed in plasma. Blood sampling time was kept below 8 minutes in order not to affect the data by causing more stress to the infants. Behavioural distress during circumcision was found to be significantly higher in comparison to the other procedures. It was found that although all manipulations produced significant increases in plasma cortisol, the differences in behavioural distress were not systematically related to individual differences in plasma cortisol levels (Gunnar et al., 1988). In study 2 the authors examined whether non-nutritive sucking as a soothing method could mediate the relation between behavioural and physiological stress. Newborns were randomly assigned to a Soothing or a Non-Soothing group. Manipulations this time included the heel stick blood-sampling procedure and a discharge examination. A pacifier was used as a soothing tool in the Soothing group from the time the manipulation started until the time the post-stress blood sample was taken. Although the pacifier reduced the behavioural distress significantly, no effect was found on plasma cortisol. Plasma cortisol levels were high in both conditions. Sex differences were not found in either study. The authors concluded that distress and cortisol are dissociated in newborns, and that this relation should not be further examined in this age group as it impedes our understanding of how these behaviour - adrenocortical patterns become organized in the developing organism (Gunnar et al., 1988).

Nachmias et al. (1996) examined the moderating role of attachment in the relation between behavioural inhibition (BI) and salivary cortisol. Participants in the study were 77 18-month-olds with their mothers, whose reactions were assessed during a series of novel events. According to the authors to produce an increase in cortisol a child should not only perceive an event as potentially threatening, but also realize that s/he is unable to cope with it. The hypothesis was that behaviourally inhibited children would only produce an increase in cortisol levels if they were in an insecurely attached relationship (Nachmias et al., 1996). Participants were tested twice, with one week between the two sessions. Mothers completed the Toddler

Behaviour Assessment Questionnaire (TBAQ; Goldsmith, 1987), from which only the Fear Scale was used in the analyses. At time 1 behavioural inhibition and attachment security were measured during three novel events; a) a live, boisterous clown who encouraged the child to play with him, b) a noisy mechanical clown robot that moved around, and c) two talkative hand puppets which invited the child to play with them. The mother was instructed to get involved in her child's coping attempts, but only for half of the time each stimulus was presented; the rest of the time she remained uninvolved. This manipulation examined the mother's role as a coping recourse in response to novelty. At time 2, approximately one week later, mothers and their children took part in the Strange Situation task (Ainsworth & Wittig, 1969). Four saliva samples were collected from the child, one before and one approximately 45 minutes after the start of each testing session; sampling time was controlled and testing usually began at 9.30 am. The results showed higher post-session cortisol levels in inhibited, insecurely attached children. TBAQ fear was correlated with less approach behaviour to the clown, the robot, and the puppets. The authors also found an overall increase in post-session cortisol of 50%-70%, which surprised them given the notion of HPA axis hyporesponsivity between 6 and 18 months of age. No sex differences were observed. Finally, the authors reported that there was no significant difference in inhibition of approach between the different attachment groups (i.e., avoidant, resistant, secure) and that inhibition of approach and attachment security are thus independent factors.

Gunnar, Tout, De Haan, Pierce and Stanbury (1997) studied individual differences in temperament and social competence in preschool children, and their relation with cortisol. They examined adrenocortical activity in shy/inhibited and surgent/outgoing children in school, and during the transition of entering a new school year. Two studies were conducted. In study 1 approximately 12 samples were collected from each child during the Group Formation period, that is the first weeks of the fall quarter, and approximately 19 samples were taken during the Familiar Group period, i.e., the first 4 weeks of the winter quarter. Parents completed the Child Behaviour Questionnaire (CBQ; Ahadi, Rothbart & Ye, 1993). The CBQ generates three higher-order factors: surgency, negative affectivity and effortful control. The teacher completed the Teacher-Child Rating Scale (T-CRS; Hightower, Spinee & Lotyczewski, 1989) to collect data on social competence. Finally, during the formation group period trained observers measured the children's social behaviour on a daily basis during the initial one-hour free-play period at the beginning of each class session. Results showed that surgent children had higher cortisol levels during the early weeks of school, whereas solitary children and children with higher CBQ negative affect scores had higher cortisol levels later in the year. Additionally, low CBQ effortful control was related to higher cortisol reactivity. Lower teacher-reported effortful control was related to a higher median cortisol level, but only in the Familiar Group phase. The association between elevated cortisol and behaviour later in the school year seemed to be mediated by children's age. Only younger children had higher cortisol levels during the familiar group period, whereas the relation between behaviour and cortisol was no longer significant when age was statistically controlled for. A gender difference was found for solitary/negative behaviour, a factor drawn from the children's daily social behaviour. Boys scored lower than girls on this factor (Gunnar et al., 1997).

Next, the authors carried out a second study to examine the validity of their results in a larger sample. According to Gunnar et al. (1997) surgent children will show a decrease in cortisol over the year if they are in healthy relationships with their peers. However, if they become disliked or are rejected then a rise in cortisol can be expected. To test this, the children's socio-metric status, their negative social interactions and their socially appropriate conflicts were added as measures in study 2 (Gunnar et al., 1997). Finally, cortisol levels collected at school and at home were compared to find out whether domestic levels are higher, testing previous evidence that domestic levels are higher than levels at playgroups or day-care settings. Cortisol was collected again during the Group Formation (first 3 weeks of fall quarter) and Familiar Group (first 6 weeks of winter quarter) phases. During both periods saliva samples were collected approximately 1.5 hour after the beginning of the class, thus around 10:30 am and 2:30 pm. Parents were instructed to collect the samples at home at approximately the same times (Gunnar et al., 1997). Teachers completed a shortened version of the CBQ and rated boys as more surgent. Trained observers rated the children's social behaviour as well as their social conflicts. Higher cortisol levels at home than in school were observed, but this result only applied to the samples collected during the Familiar Group period. Teacher-reported surgency was significantly correlated with observed levels of aggressive/angry behaviour and conflict, while effortful control was negatively correlated with aggressive/angry behaviour; negative affectivity was negatively correlated with conflict. As predicted, children high in surgency and social preference, an index of how much a child was liked by its peers, showed a decrease in cortisol from Group Formation to Familiar Group periods. Children who had high median cortisol levels during the Familiar Group period showed lower levels of conflict, tended to be less liked by their peers and belonged to the group of younger children. Children with high median cortisol levels during both Group Formation and Familiar group periods were also rated as lower in effortful control. Moreover, children who changed from normal to high median cortisol later in the school year were the ones who engaged less in conflict and negotiations with peers. Children with consistently high cortisol levels were found to be the ones who were higher in negative affectivity and solitary/sad behaviour but lower in conflict. Overall, shyness was not found to be related to elevated cortisol reactivity (Gunnar et al., 1997).

Dettling, Gunnar and Donzella (1999) examined HPA axis activity in 70 39-106-month old children in childcare centres and at home. Their rationale stemmed from the results of a previous study on cortisol reactivity in the preschool years by Tout, de Haan, Cambell & Gunnar (1998). Tout et al. (1998) reported an 81% increase in cortisol from morning to afternoon in preschoolers attending full-time childcare. However, Tout et al. (1998) did not sample cortisol at home and it remained therefore unclear whether the rise in cortisol was due to the childcare context or the specific group of children. Dettling et al. (1999) collected saliva samples two days at home and two days at childcare, between 10.30-11:00 and 15:30-16:30. Parents completed the CBQ (Ahadi et al., 1993) and teachers completed a shorter version of the CBQ, the Teacher Behaviour Questionnaire (TBQ) for assessing child temperament (negative affectivity, surgency and effortful control). The results were similar to the results of Tout et al. (1998): significant increases in cortisol levels were found over the day for children attending fulltime child-care, but this was particularly true for younger children. Cortisol levels at home decreased from morning to afternoon in most children, regardless of age. Higher cortisol levels were related to shyness in boys, and to impulsivity, poor self-control and aggression in both sexes.

Generally, social skills and self-regulatory behaviours develop throughout the school years and a lack of these skills may hinder social adaptation and could produce elevations in cortisol, especially in younger children, who have not yet developed these skills. Dettling et al. (1999) suggested that the rising cortisol pattern over the day in the nursery setting may be related to the length of the childcare day. This is because a different result was found in Gunnar et al.'s study (1997), where the childcare program was shorter (i.e., 2.5 hours). Another factor that also could have contributed to this result is the quality of the centre, and specifically the quality of the naps. In conclusion, in contrast to most other studies, this study found that higher cortisol was related to externalizing aspects of behaviour (poor-self control, anger, aggression) based on teacher and parental ratings (Dettling et al., 1999).

Donzella et al. (2000) examined cortisol reactivity (and vagal tone) in 61 3-5-year-old children in response to competition. Previous research in adults in response to competition reported larger increases in cortisol in those individuals who are more emotionally engaged (Kugler, Reintjes, Tewes & Schedlowski, 1996). Donzella et al. (2000) expected to find a larger cortisol increase in children who were more surgent. Testing was carried out in schools for several months during the school year, and lasted approximately 1.5 hour during the free play period of each school day. Competition involved a familiar experimenter and one child each time. The protocol was setup such that children won the first three consecutive games (Win Period), lost the next three (Lose Period) and finally won the final games and received a prize.

Salivary samples were collected for several days for baseline measurement and testing sessions were videotaped for coding purposes. Based on these video recording four categories of affect were identified; positive, anger/frustration, tense/anxious, and sadness. Turn-taking behaviour was also scored. The short version of the CBQ was completed by the teacher. Results showed that surgent children showed higher cortisol reactivity in response to the competition than shy children (Donzella et al., 2000). Teachers rated high cortisol responders as more surgent and as lower in effortful control. Surgency was correlated with positive affect upon winning and with negative affect after loosing, which suggests that surgent children might have stronger cortisol reactivity due to greater engagement in the competition. Overall, only 15% of the children produced an increase in cortisol during the competition. Seven out of 8 children who showed a cortisol response were boys. Boys also tended to be more tense and angry (Donzella et al., 2000). Although research on stress reactivity has mostly focused on the association between shy, inhibited temperament and higher increases in cortisol reactivity, this study showed that surgent temperament is also associated with increased cortisol reactivity (Donzella et al., 2000).

Buss, Schumacher, Dolski, Kalin, Goldsmith and Davidson (2003) set out to test the association between frontal electroencephalographic (EEG) asymmetry and cortisol in 6-month-old infants. Both asymmetry in activation of the prefrontal cortex and cortisol reactivity have been linked with fearful behaviour and withdrawal, but only a few studies in nonhuman primates examined whether there is a relation between these two. For example, Kalin, Larson, Shelton and Davidson (1998) reported an association between greater right frontal EEG asymmetry and higher cortisol levels in rhesus monkeys with trait-like fear phenotypes. Based on Kalin et al.' s (1998) findings Buss et al. (2003) predicted that both resting and greater relative right frontal EEG activation would be associated with baseline and cortisol reactivity, as well as with more withdrawal-related behaviours. Participants were 85 6-month-old infants with a right-handedness family history (Buss et al., 2003). EEG was recorded during three conditions; baseline/resting, stranger approach, and a peek-a-boo game. Salivary cortisol samples were collected from the infants at the end of the three conditions and during three consecutive days at home. Following an extreme-group design procedure used previously in temperament studies, the authors divided the infants into three EEG activation groups (average, right and left) based on their EEG asymmetry during baseline and stranger approach conditions. There was no effect of gender, but infants with extreme right frontal EEG during rest had higher cortisol during stress, and infants with extreme right frontal activation during stranger approach had the highest basal cortisol. Although several reports have linked withdrawal behaviour to cortisol reactivity, this finding could not be replicated in this study.

Haley and Stansbury (2003) examined possible links between infant stress regulation and parental responsiveness in 43 5- and 6-month-old infants and their parents. Cortisol and HR were measured during a modified version of the still-face procedure (Tronick, Als, Adamson, Wise & Brazelton, 1978), during which the parent faces the child but is instructed to remain unresponsive. The modification included an additional still-face reunion sequence to make the procedure more stressful and enable better measurement of regulation and recovery during reunion (Haley & Stansbury, 2003). Two saliva samples were taken; the first was sampled 3-5 minutes before testing took place and the second one 30 minutes after the end of the first still-face episode. Behavioural scoring was done through video recordings and parents completed the IBQ as an infant temperament index. More responsive parents had children who were better in regulating negative affect (and HR). Infants of more responsive parents spent more time looking at their parents, which has been hypothesized to serve as an external source of regulation and reflect coping ability (Haley & Stansbury, 2003). Cortisol increased as a result of the procedure, but not as high as in more physically invasive procedures. This finding illustrates how different procedures can be used to systematically study infant reactivity and regulation of the HPA axis. Higher IBQ negative affectivity was found to be associated with higher baseline cortisol levels. No gender differences in cortisol were found.

The following table (Table 5-1) shows a summary of all the studies described in this section.

Table 5-1 Cortisol in infants and young children

Authors	Hypotheses	Participants	Stimuli	Physiology	Results	Sex differences
Gunnar et al. (1988). 1st study	Cortisol-distress relationship can be best examined in young infants	89 newborns	Routine neonatal circumcision, weighing, measuring, heel stick and blood sampling	Cortisol in plasma	Differences in behavioural distress were not systematically related to plasma cortisol levels	Not found
Gunnar et al. (1988). 2nd study	Could soothing mediate the relations between behavioural and physiological stress?	89 newborns	A pacifier was used as a non-nutritive soothing tool in the Soothing group.Heel stick blood sampling procedure and a discharge examination for distress assessment.	Cortisol in plasma	The pacifier reduced behavioural but not physiological distress.	Not found
Nachmias et al. (1996)	Is attachment a moderator in the relation between behavioural inhibition and corisol?	77 18-month-olds	A clown, a mechanical clown robot and 2 talkative hand puppets. Attachment: strange situation	4 saliva samples	Higher post-session levels in inhibited, insecurely attached children	Not found
Gunnar et al. (1997). 1st study	Examined individual differences in temperament and social competence in preschool children and their relation with cortisol	29 preschool children	CBQ teacher rating scale for social competence; behavioural assessment on a daily basis during one hour of free play	12 saliva samples during Group formation and 19 samples during Familiar group period.	Surgent children had higher cortisol levels during the early week of school, whereas solitary children had higher cortisol levels later in the year	Not found
Gunnar et al. (1997). 2nd study	Surgent children will show a decrease in cortisol over the year if they are in healthy relationship with their peers	46 preschool children	In addition to 1st study's assessments the children's sociometric status, negative social interaction and socially appropriate conflicts were examined	Saliva samples at home and school	During the familiar group period cortisol levels were higher at home than in school. Hypothesis confirmed	Not found
Dettling et al (1999)	Will cortisol at home and in childcare increase from morning to afternoon as shown by Tout et al. (1998)	70 39-106-month-olds	CBQ for parent and teacher	Saliva samples at home and during childcare for 2 consecutive days	Only young children showed increased levels over the day in childcare, while levels decreased over the day at home	Shy boys showed higher cortisol levels
Donzella et al. (2000)	Surgent children show larger cortisol increases in response to competition	61 3-5-year-olds	Competition involved a win and a lose period, and a final win period when the child received a price	Saliva samples for several days at school	Hypothesis confirmed	7 out of 8 cortisol responders were boys
Buss et al. (2003)	Right resting and task frontal EEG would be associated with higher cortisol	85 6-month-olds	Stranger approach, peek-a-boo game	Saliva samples at the end of the stressors and during 3 days at home. EEG during stressors	Hypothesis confirmed	Not found
Haley & Stansbury (2003)	Infants of more responsive parents show better regulation of stress	43 5-6-month-olds	Still-face procedure	1 saliva sample pre-testing and a 2nd 30 min after. HR throughout	Hypothesis confirmed	Not found

5.1.2 Cortisol in longitudinal designs

Kagan, Reznick and Snidman (1987) tested the reactions of two cohorts of children, selected in the second or third year of life to be either behaviourally inhibited or uninhibited, to unfamiliar and cognitively challenging events. The authors in particular aimed to illustrate the importance of combining behavioural and physiological measures in order to get a better understanding of child development (Kagan et al., 1987). The assignment of a child to either group required the child to consistently show withdrawal or approach behaviour to a variety of incentives. In cohort 1 children were first seen at 21 months and were on that occasion confronted with several unfamiliar events or objects, including an unfamiliar female experimenter, unfamiliar toys, a trio of acts that was difficult to recall and imitate, a talking robot, and a temporary separation from the mother. In cohort 2 children were first seen at 31 months of age and were evaluated during a session with an unfamiliar peer of similar age and sex and an unfamiliar woman. Cohort 1 was again seen when the children were 4 years old, while cohort 2 was seen at 3 ½ years of age. The procedure was again carried out when the cohort 1 children were 5 ½ years old, but unfortunately for cohort 2 the data were still incomplete when the report was published (Kagan et al., 1987). At 5 ½ years the child's evaluation included interaction with an unfamiliar peer in a laboratory setting, a school setting with classmates, a testing situation with an examiner for 90 minutes, and a room that contained unfamiliar objects involving an element of risk. Behavioural inhibition and disinhibition proved to be stable characteristics for these cohorts of children until their 6th year of life. Interestingly, uninhibited rather than inhibited behaviour was more preserved in both cohorts, and this was accounted for by the socialisation principles of the American parents (Kagan et al., 1987). Specifically, American families regard wariness and withdrawal to challenge as rather unacceptable qualities and reward outgoing behaviour and the inhibition of fear and shyness. With respect to the physiological measures, the authors examined cortisol levels amongst a whole range of biological parameters. Saliva samples were taken from children in cohort 1 at 5 ½ years before and after the laboratory session with the unfamiliar experimenter and at home for 3 consecutive days during the early morning hours. Laboratory cortisol levels more than any other physiological measure correctly predicted the initial 21-month behavioural classification at the level of 78%. Higher cortisol levels were found in inhibited as compared to uninhibited children, both at home and in the laboratory. Finally, both the laboratory and the home cortisol levels were significantly correlated with the index of inhibition at 5 ½ years. The authors claimed that they found support for the notion of lower threshold of reactivity in limbic and hypothalamic structures to unfamiliar and challenging situations in inhibited as compared to uninhibited children. Unfortunately, the study did not test for sex differences and the authors failed to address the issue why about 50% of the inhibited children had relatively low cortisol levels (Kagan et al., 1987).

Gunnar et al. (1996) examined longitudinally changes in cortisol and behaviour in 83 healthy infants in response to inoculations. 72 Infants completed all testing at 2, 4, 6 and 15 months of age, while a comparison group of 2-, 4-, and 6-month-olds received mock exams without inoculations to examine the developmental changes in response to exam procedures. Behavioural distress was scored every 30 second on a 4-point scale (0 = no evidence of distress to 4 = high cry; Gunnar et al., 1996). One saliva sample was collected upon arrival at the clinic and one more 25 minutes after the start of the inoculation procedure. Results showed a significant decrease in cortisol responses from 2 to 4 months, but not from 4 to 6 months, and a lack of cortisol response in most 15-month-old infants, despite an increase in behavioural distress from 6 to 15 months. For the comparison group, the same shift in cortisol was observed between 2 and 4 months. Behavioural distress decreased between 2 and 6 months, but increased again at 15 months. Gunnar et al. (1996) attributed the dampening in cortisol between 2 and 4 months and 6-15 months to the day-night biobehavioural organisation (i.e. marked increases in nighttime sleep are reported) in this period. As for the decrease in cortisol responses between 6-15 months, they also discussed their results in relation to the establishment of attachment relations (e.g., parental presence may decrease cortisol response) and the development of memory capacity (i.e., the reduction of novelty because of repetition). Finally, sex differences were observed only at 2 months of age with girls showing higher cortisol and behavioural reactivity but lower pretest cortisol than boys (Gunnar et al., 1996). The authors doubted that the latter result could be replicated since previous research had reported stronger cortisol reactivity in males than in females.

Lewis and Ramsay (1999) examined the effects of maternal soothing on cortisol and behavioural reactivity during infant inoculation at 2, 4 and 6 months of age. The sample consisted of 55 healthy infants. On each occasion a baseline and a 20-min post-inoculation sample were collected. No effect of maternal soothing on cortisol or behavioural responses to stress was found (Lewis & Ramsay, 1999). A 2nd sample consisted of 74 healthy infants. The procedure was identical to study 1 apart from the visit at 4 months, during which a videotape was made for deeper examination of maternal soothing. The results showed again a significant decline in cortisol between 2 and 6 months but longer distress in 2-month-olds (Lewis & Ramsay, 1999). While there was evidence of consistency in maternal soothing over time, there was no effect of maternal soothing on infant cortisol or behavioural responses to stress. Lewis and Ramsay (1999) concluded that other maternal behaviours may mediate infant distress, and that differences in adrenocortical functioning and behavioural responses during stress might be related to individual differences in temperament.

Goldberg et al. (2003) examined stability of baseline and stress cortisol levels across time and stressors. 27 Infants aged 12 to 18 months were exposed to two stressors administered 1 week apart. The first stressor was the strange situation (Ainsworth & Witting, 1969) and the second was an adaptation of a 'coping' session used by Nachmias et al. (1996; a remote controlled toy, a boisterous clown, and a puppet show involving two animal figures). One saliva sample was collected upon arrival and the other two 20 and 40 minutes after the end of stressor. Time-matched home samples were collected to control for circadian rhythm and to examine baseline stability. Stability was found in cortisol baseline, peak percent change and area under the curve (AUC, cortisol concentration *x* time) across time and stressors. Individual differences were observed in time of peak response after stressor, with half of the infants showing a peak response 20 minutes after the stressor and the other half at 40 minutes post-stress. Less than half of the infants (44%) showed stability in time of peak stress response across stressors, and no consistency in time of peak stress response was observed in the rest of them. While, large individual differences in cortisol reactivity are often reported, the optimal collection time for detecting the peak stress response has net yet been established.

De Weerth and van Geert (2002) examined cortisol in 20 infants (aged between 5-8 months) and their mothers for 13 consecutive weeks. The study's purposes were to examine intra-individual variability, diurnal rhythm and developmental trends in the infant cortisol levels. Results showed a decrease in basal cortisol between 5 and 8 months, and a negative relation between cortisol production and sleep. Although, infants showed a circadian rhythm in cortisol this was not as marked as in adults. Thus, in line with Gunnar and Donzella (2002), the authors concluded that a significant cortisol decline from morning to mid-afternoon is probably not established until the 4th year of life. Moreover, while the mothers showed inter-individual variability but high intra-individual stability across assessments, infants showed high intra-individual variability across assessments with relative stability across individuals (de Weerth & van Geert, 2002).

De Weerth, Zijl and Buitelaar (2003) also examined the development of the cortisol circadian rhythm in infancy. To that end, they measured cortisol levels in 14 infants at home between 2 and 5 months, sampling saliva 5 times per day. Factors related to napping, feeding, sleep quality were recorded. Although a cortisol circadian rhythm was apparent at a group level as early as the 2nd month of age, individual differences in persistence and stability of the rhythm were also observed. Evidence of day-to-day variability in cortisol rhythm, as well as the probable natural spread in the exact age of the rhythm's appearance, as reported in this but also in other studies, must be taken into account when conducting studies on the development of the cortisol circadian rhythm.

To summarise, young children are not as able as older ones in regulating their emotions in response to challenge, probably due to the fact that they still lack some of the essential cognitive and social skills to cope with their environment. Although higher cortisol levels have been reported in inhibited and shy children, occasionally a similar pattern has been observed in more outgoing and surgent children (Donzella et al., 2000). Although studies increasingly combine different methodologies (i.e., physiology, parental report, behavioural observation) to study temperament and its correlates, it is worth noting that correlations between different physiological measures, but also between physiology and behaviour, are at best moderate. Finally, different kinds of stressors have been used to examine the effect of temperament on stress responses in young children, with the strange situation, circumcision, and maternal absence or separation as the most prominent ones. More recently, the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1999) has been used in the study of cortisol.

The following table (Table 5-2) shows a summary of all the studies described in this section.

Table 5-2 Cortisol in longitudinal designs

Authors	Hypotheses	Participants	Stimuli	Physiology	Results	Sex differences
Kagan et al. (1987)	Examined the importance of combining behaviour and physiological measures	2 cohorts of children at 21 and 31 months, which were again seen at 4 and 3 ½ years respectively. Cohort 1 was seen once more at 5 ½ years	Unfamiliar toys and people, a talking robot, separation from mother, unfamiliar peer and unfamiliar experimenter	Saliva samples at 5 ½ years before and after the laboratory session and at home for 3 consecutive days	Cortisol correctly predicted the initial 21 month behaviour classification	Not reported
Gunnar et al. (1996)	Examined changes in cortisol longitudinally in healthy infants	72 infants at 2, 4, 6 and 15 months	Mock exams with inoculations	One baseline and 1 saliva sample 30 minutes after the onset of the stressor	Cortisol responses significantly decreased from 2 to 4 months only. No response after 6 months	At 2 months girls showed greater cortisol reactivity
Lewis & Ramsay (1999)	Examined the effects of maternal soothing on cortisol and behaviour reactivity during inoculations	55 healthy infants at 2, 4 and 6 month	Inoculations	One baseline and one 20-min postressor saliva sample	No effect of maternal soothing on cortisol or behavioural distress	Not reported
Goldberg et al. (2003)	Examined stability of baseline and stress cortisol levels across time and stressors	27 infants at 12 and 18 months	Strange situation, mechanical toy, a clown and a puppets show	One saliva sample at baseline, a 2nd at 20 and a 3rd at 40 minutes at the end of stressors	Stability in cortisol baseline, peak percentage change and AUC across time and stressors.	Not reported
de Weerth, Zijl & Buitelaar (2003)	Examined the development of cortisol circadian rhythm in infancy	14 healthy infants	No stress exposure. Saliva sampling at home	5 saliva samples per day at home for 4 months (at 2, 3, 4 and 5 month).	Individual differences in the emergence of the cortisol rhythm, and in the persistence and stability of the rhythm	Not found
de Weerth & van Geert (2002)	Examined individual differences in the development of the diurnal rhythm	20 infants aged between 5-8 months and their mothers	No stress exposure. Saliva sampling at home	Saliva sampling at home for 13 days	Decrease in basal cortisol from 5 to 8 months; clear intra-individual variability	Not found

5.1.3 HPA axis activity and the Lab-TAB

Buss, Davidson, Kalin and Goldsmith (2004) observed fear-related behaviour in 80 24-month-old children in response to 4 mildly threatening contexts. Behavioural observations were combined with different psychophysiological measures, including basal and reactive cortisol levels, baseline heart rate, respiratory sinus arrhythmia and pre-ejection period (PEP). Taking part in the study required the participants to visit the laboratory twice, with 1 week interval between visits. Physiological measurements were only done during the 2nd visit. Several episodes from the Preschool Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith, Reilly, Lemery, Longley & Prescott, 1994) were used. Children underwent three stranger approaches, two during the first visit and one during the second visit, involving physiology. One stranger approach gave children the chance to escape the stressor and the other one was designed such that the child was unable to escape. The latter version was replicated in the 2nd physiology visit. It was hypothesized that children who in less threatening conditions (i.e., stranger approach with ability of escape) would react with more intense fear would find it more difficult to regulate their fear responses in general (Buss et al., 2004). The study also used a risk room task and inhibition behaviour was observed. Two saliva samples were collected during each visit; one upon arrival and the 2nd at the end, and parents collected 4 saliva samples daily for three consecutive days in the week between the two visits. The times that parents had to sample cortisol from the children were: 8-9 a.m., 11-12 p.m., 3-4 p.m., and 8-9 p.m. (Buss et al., 2004). Buss et al. (2004) found that freezing during the stranger free episode (i.e., the less threatening context) was related to higher basal cortisol levels. In contrast to earlier temperament findings (Nachmias et al., 1996; Kagan et al., 1987) inhibition, facial fear, crying and escape behaviour were not related to higher levels of cortisol.

In fact, inhibition was related to lower basal cortisol and not to cortisol reactivity. Schmidt et al., (1997) found that higher morning cortisol levels were associated with more inhibition and that higher laboratory cortisol levels were related to less inhibition. According to Buss et al. (2004) an important function of the HPA axis is to help the organism prepare for coping with challenge, and this explains why cortisol increases have been reported in both surgent and shy children,. Fifteen percent of the children consistently coped by freezing which seemed to be the less appropriate coping strategy for reducing distress (Buss et al., 2004). Although small, this number (15 %) is similar to the percentages reported in research on behaviourally inhibited children (Kagan, Reznick, Clarke, Snidman & Carcial-Coll, 1984). Buss et al. (2004) did not report any gender differences.

5.1.4 Cortisol as an index of fearfulness in the current study

The studies described above indicate that cortisol is a reliable parameter in studies of child temperament, especially in studies of stress reactivity and stress regulation in infants and children. As mentioned earlier, dysregulation as well as chronic activation of the HPA axis system have been shown to have deleterious consequences for one's health (Sapolsky, 2004). Poor regulation of cortisol has been shown to have an effect on the activity of the immune system, as well as a negative impact on brain areas involved in cognitive processes such as attention and memory, ultimately leading to loss of hippocampal neurons and a decrease in dendritic branching (Bugental, Martorell, & Barraza, 2003). Moreover, recent evidence from non-human animal and human studies has linked perinatal stress to a general susceptibility to psychopathology (Huizink, Mulder, & Buitelaar, 2004). If exposure to stress affects individuals differently, with some being more resilient than others, and if being more sensitive to stress initially affects later coping with stress (Gunnar & Quevedo, 2007), then it is clearly imperative to examine the HPA axis and its role in explaining individual differences in emotional behaviour from the very beginning of life.

There has been an increase in the number of studies that assesses cortisol in infants, which is due to recent advances in the methodology of the radioimmunoassay procedures that enable us to measure cortisol from extremely small amounts of saliva. While the number of studies measuring cortisol over the course of development and in relation to temperament has increased, the number of questions to be answered has as well, since findings are inconsistent. For example, although higher cortisol levels have been reported in inhibited children and children high in internalizing behaviour, a similar pattern has been observed in more outgoing and surgent children (Donzella et al., 2000; Gunnar et al., 1997; de Haan et al., 1998). Thus a clear relation between cortisol and different temperament profiles has not been established. Moreover, inconsistencies have been reported with respect to the role of gender in cortisol; sometimes differences are not observed (Lewis & Ramsay, 1999), or when they are present they go in different directions (Gunnar et al, 1996; Kirschbaum & Hellhammer, 1994). Finally, although a proper HPA axis functioning is highly important for a person's normal adaptation and wellbeing, the development of the HPA-axis in the first years of life has rarely been studied (Gunnar et al., 1996, Ramsay & Lewis, 1999).

The present study assessed individual differences in baseline cortisol and cortisol change in response to a fear challenge longitudinally in a group of healthy infants, and their relationships with fearful temperament. The results and the discussion on this set of data will be included in subsequent sections (5.4 & 5.6).

5.2 Hypotheses

The goal of the study was to examine adrenocortical reactivity in one-year-old infants in response to a psychological stressor to examine relations between different cortisol parameters and explore the associations between these stress hormonal parameters and behavioural distress and mother-reported temperament. The study was repeated once more when children were 24 months old to examine stability and/or change in these parameters and relationships over time. Subsidiary goals were to find out whether there were age (in months) and sex differences in cortisol levels. Specifically, we predicted that (1) cortisol would increase from baseline as a result of fear/stress and (2) there would be individual differences in cortisol reactivity with children higher in fearfulness/internalizing behaviour (as rated by the mothers and/or observed by independent coders) showing greater cortisol reactivity. Moreover, since previous research in older children has shown lower cortisol levels in aggressive or conduct disordered children (Dettling et al., 1999; van Goozen et al., 2000) we expected (3) less cortisol reactivity in more externalizing children. Additionally, because of the existing inconsistent evidence with respect to gender differences in HPA axis activity we were interested (4) in finding out whether boys and girls would differ in cortisol, even though our behavioural analyses in Chapter 3 did not reveal gender differences in observed distress or maternal rated temperament. Moreover, in line with earlier reports of a decrease in cortisol baseline over the first two years of life (Gunnar et al., 1996) we expected (5) lower cortisol baseline in relatively older children (i.e., within year-group comparisons) and (6) lower cortisol baseline in year

2 as compared to year 1. In contrast to findings by Gunnar et al. (1996), and based on our previous reported findings with respect to higher levels of behavioural distress and SCL, we expected stronger cortisol reactivity in year 2 as compared to year 1. Finally, (7) we predicted positive correlations between the physiological measures across time.

5.3 Methods

5.3.1 Participants

Year 1 sample

Participants were 50 infants (mean age in months = 9.7, sd = 2.2) and their mothers; all participants had been born in Cardiff, Wales. Participants were excluded because of extremely high cortisol values (n = 5) or insufficient saliva (n = 1), leaving a sample of 44 infants (mean age in months = 9.5, sd = 2.2). For analysis purposes the sample was divided into four subgroups: boys (n = 23, mean age = 9.5, sd = 1.8) and girls (n = 21, mean age = 9.4, sd = 2.6), younger (n= 26, mean age = 7.9, sd = 1.0) and older (n =18, mean age = 11.7, sd = 1.4). Of the 23 boys 13 belonged to the younger (mean age = 8.3, sd = 1.0) and 10 to the older group (mean age = 11.2, sd = 1.2), and of the 21 girls 13 belonged to the younger (mean age = 7.6, sd = 1.0) and 8 to the older group (mean age = 12.3, sd = 1.4). A chi-square test for independence indicated no significant association between gender and age (χ^2 (1) = .132, p = .72), and there was no significant difference in age between boys and girls (t (42) = .21, p = .84).

Year 2 sample

Approximately 12 months after the first assessment the families were contacted again by mail and telephone, and asked whether they were willing to participate in a follow-up study. Of the original sample (n = 50) 10 participants could not be traced or did not respond to our letter or phone calls. Therefore, 40 children (80%) returned to the laboratory for a visit in their 2nd year of life.

Of the 40 participants, 7 were unable to complete the study (see section 3.3.1) and could therefore not be used in the analyses. These 7 children did not differ from the other participants on any of the cortisol indices measured in year 1 (i. e., cortisol baseline, cortisol stress and cortisol reactivity; with all *p*'s ranging between .35 and .69), nor did these 7 children differ from the participating children in mother-rated IBQ-R (fear: U = 126.500, $p = .84$) or on the composite Lab-TAB score (t (47) = .10, $p = .31$). So in the end 33 participants (66%) could be used in the cortisol study (mean age in months = 22.3, sd = 3.2). Of these 33 children one child was excluded because of extremely high cortisol values and 2 children gave an insufficient amount of saliva. The group was divided into the same four subgroups; boys (n = 17, mean age: 22.6, sd = 3.4) and girls (n = 13, mean age: 22.0, sd = 3.5), young (n = 15, mean age: 19.6, sd = 1.7) and old (n = 15, mean age: 25.1, sd = 1.9). Of the 17 boys 8 belonged to the younger group (mean age: 19.6, sd = 1.7) and 9 to the older group (mean age: 24.9, sd = 1.8), and of the 13 girls 7 belonged to the younger (mean age: 19.6, sd = 1.8) and 6 to the older group (mean age: 25.4, sd = 2.1). A chi-square test for independence indicated no significant association between gender and age (χ^2 (1) = .83, $p = .36$), and boys and girls did not differ in age (t (28) = -.59, $p = .56$).

5.3.2 Procedure

a) Baseline

As described in section 3.3.2, the experimenter interacted with the mother and the infant for about 20 minutes prior to testing in order for the dyad to get used to the laboratory environment.

b) Orienting Habituation Paradigm (OHP)

This procedure has been described in section 4.6.2.

c) Presentation of Unpredictable Toy

This episode and its coding procedure have already been described in previous chapters (see section 3.3.2). For the purposes of the current analyses the Lab-TAB composite distress scores created by accumulating infant's ratings on the behavioural variables across the fear episode, was used (see section 3.3.2).

d) Maternal reports

Year 1: Infant Behaviour Questionnaire-Revised (IBQ-R)

As mentioned previously in Chapter 3 the IBQ-R subscale assessing fearfulness was used in the current analyses. An example of a fearfulness item is: *"how often during the last weeks did the baby cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)?"*.

Year 2: Child behaviour checklist and language development (CBCL/1.5-5; Achenbach & Rescorla, 2000)

Only the composite scales of internalizing and externalizing behavioural problems were used for the purposes of the current study (see section 3.3.2). As we reviewed before, externalizing behaviour has often been linked to reduced ANS activity (Snoek et al., 2004).

5.3.3 Salivary Cortisol

Four salivary cortisol samples were collected from each participant; two baseline and two stress samples. The 1st saliva sample was collected shortly after the infant and mother arrived at the laboratory; the 2nd one was collected 15 minutes after the first. The 1st stress sample was taken immediately after the termination of fear challenge and the 4th 25 minutes after the 3rd sample.

All samples were collected between 9 and 11 am. Each sample collection took no longer than 2 minutes. Sorbettes and cryovials (Salimetrics, State college, USA) were used for collecting the saliva from the infant's mouth. Oral stimulants to increase saliva flow were not used to avoid contamination (Schwartz, Granger, Susman, Gunnar & Laird, 1998). In the light of evidence that milk in saliva can interfere with the cortisol assay (Magano, Diamond & Gardner, 1989) the mothers were informed that their children could not be fed during the experimental procedure. Due to the ages of the children and the likelihood of teething, caution was taken to report any indications of blood in the mouth or on the sorbettes, which could result in elevated cortisol levels.

Samples were immediately stored and frozen at -20°C until they were shipped in dry ice for analysis. All samples were assayed with Elisa cortisol assays. Saliva samples were spun at 1500rpm for 15mins at 4^0C and assayed in duplicate. Data were transferred to a computer using the assay software KC4, which creates a standard curve. The concentration of cortisol present in each sample was calculated from the standard curve. A standard curve was generated for every plate of samples assayed. The average intra- and interassay coefficients of variation were 4.33 % and 9.25 %, respectively.

For each individual cortisol reactivity was calculated in two ways: CortΔ (i.e., the cortisol peak stress value minus the mean cortisol value of the two baseline values), and the percentage increase (%increase) between the peak cortisol value and mean baseline level, with higher CortΔ and %increase values indicating greater cortisol reactivity (Ramsay & Lewis, 2003).

5.4 Results

5.4.1 Statistical analyses

Cortisol data were examined for outliers. An outlier was defined as an individual value more than 2 SDs above or below the group means (Schmidt-Reinwald, Pruessner, Hellhammer, Federenko, Rohleder, Schurmeyer & Kirschbaum, 1999). Two outliers were detected and excluded from the analysis at time-1 (n = 42) and 2 at time-2 (n = 28). To normalize the cortisol distributions, the convention of $\log_{10}$ transformation was employed. However, mean cortisol values in ng/ml rather than their transformed values are presented for ease of interpretation. Initial analyses were conducted to determine whether any associations would be observed between children's medication usage and their cortisol levels. Two composite scores were used for the analyses; cortisol baseline (mean of the two baseline samples) and cortisol stress (mean of the two stress samples). Two repeated measures analysis of variance (ANOVA) with condition (baseline vs. stress) as the within subjects' factor and age (younger vs. older; for the 1st analysis) and gender (boys vs. girls, for the 2nd analysis) as between subjects' factors were employed to test for main effects of condition, age and gender on cortisol. Repeated measures ANOVAs were also employed to examine cortisol differences between more and less fearful infants based on maternal ratings (IBQ-R/CBCL) and observed behaviour (Lab-TAB).

In order to examine whether individual variability in children's baseline levels accounted for variation in cortisol reactivity, temperament and/or age (in months), analyses of covariance (ANCOVAs) were performed with baseline cortisol entered as covariate. According to Bugental, Martorell and Barraza (2003) ANCOVA is the most appropriate measure to control for individual differences in initial values.

Finally, Pearson's correlations were employed to examine associations between the cortisol variables, since these were transformed to fulfil the criteria of normal distribution. With respect to the associations between cortisol, temperament and observational measures, Spearman's correlations were used in case of not-normally distributed variables (IBQ-fear, CBCL-externalizing and internalizing scales), while Pearson's correlations were employed in all other cases.

Influence of medication use on salivary cortisol

According to a recent study (Hibel, Granger, Kivligham & Blair, 2006), the use of specific kinds of medications might affect individual differences in salivary cortisol. In infants (6 months of age), acetaminophen, specifically Tylenol, has been shown to flatten cortisol levels in response to stress. Use of other common medications such as teething gels, antibiotics and cold-related remedies has not been shown to affect cortisol levels (Hibel et al., 2006). None of the children in our sample had taken acetaminophen, as reported by mothers, during their visits in both years. Nine infants had taken medication (i.e. teething gels, antibiotics) in year 1 and 5 in year 2, but none had taken any medication in the weeks leading up to testing. Pearson's correlations were conducted to screen for positive associations between medication usage and cortisol production but none of these reached acceptable significance level, with all r's ranging between .09 and .25 in year 1 and -.01 and .09 in year 2.

5.4.2 Results: Cortisol in 1-year-old children

Table 5-3 presents descriptive data for the main cortisol variables. Data of 42 one-year-old children were included in the current analyses. In Table 5-3 comparisons of mean cortisol levels collected during baseline and stress suggest higher cortisol levels during stress.

Table 5-3 Mean cortisol levels and standard deviations (in ng/ml) for each subgroup for the baseline and stress conditions

	baseline		**stress**	
	Mean	**SD**	**Mean**	**SD**
girls (n =19)	2.2	1.5	2.4	1.6
boys (n =23)	1.6	0.8	2.8	1.8
young (n =25)	2.0	1.3	2.3	1.3
old (n =17)	1.7	1.0	3.1	2.2

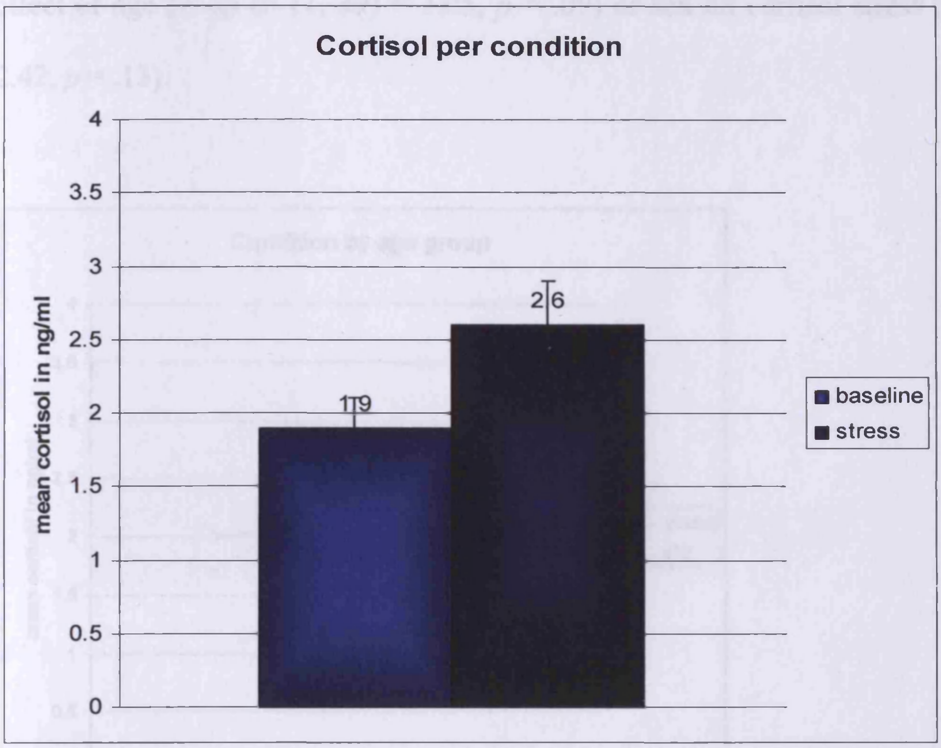

Figure 5-1 Mean cortisol in 1-year-old children during baseline and stress with error bars indicating SE's (n = 42).

Repeated measures ANOVAs revealed a significant effect of condition, with significantly higher levels of cortisol at the time of stress (F (1, 41) = 8.42, $p < 0.01$).

Analyses did not show a main effect of age group on cortisol (F (1, 40) = .05, p = .82), but there was a significant interaction between condition and age group on cortisol (F (1, 40) = 4.05, p = 0.05, see Figure 5-2) with older children having higher cortisol levels during stress. However, independent sample t-tests did not show an effect of age group on cortisol at baseline or during stress (baseline: t (40) = .93, p = .36; stress: t (40) = -1.22, p = .23). No main effect of sex (F (1, 40) = .03, p = .86), and no interaction between sex and condition on cortisol (F (1, 40) = 3.28, p = .08) were found. ANCOVAs adjusting for cortisol baseline did not show a significant effect of age group (F (1, 39) = 3.05, p = .09) or sex on cortisol stress (F (1, 39) = 2.42, p = .13).

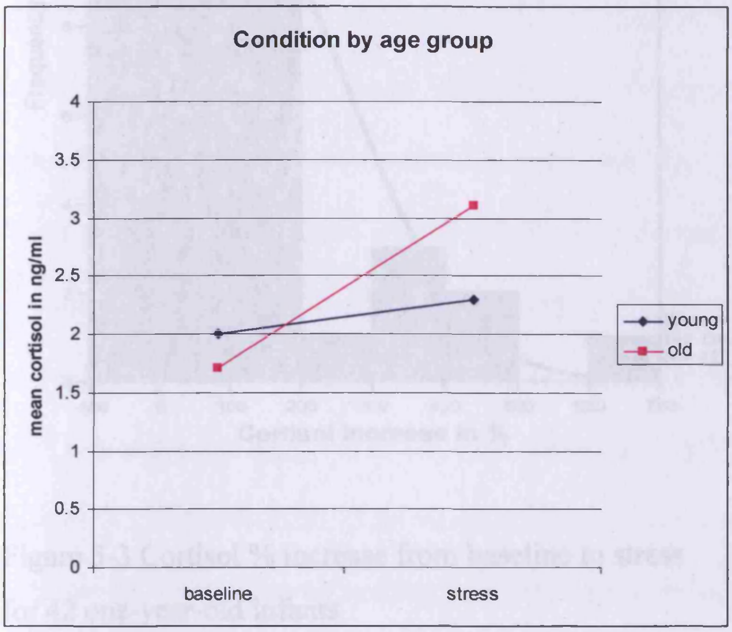

Figure 5-2 Mean cortisol in ng/ml for baseline and stress in younger (mean age = 8.0, sd = 1.0) and older infants (mean age = 11.7, sd = 1.5)

Cortisol reactivity

Next we examined cortisol reactivity as a result of stress exposure by calculating CortΔ (i.e., the highest cortisol value during the stress phase minus the mean cortisol at baseline) and cortisol %increase. Figure 5-3 shows the %increase in cortisol from baseline to stress for the group as a whole. Thirty two infants (76%) showed an increase in cortisol levels as a result of stress exposure. The mean %increase was 112.2% (sd = 158.83). The mean %decrease for the 10 children who did not respond with an increase was 27.3% (sd = 24.7).

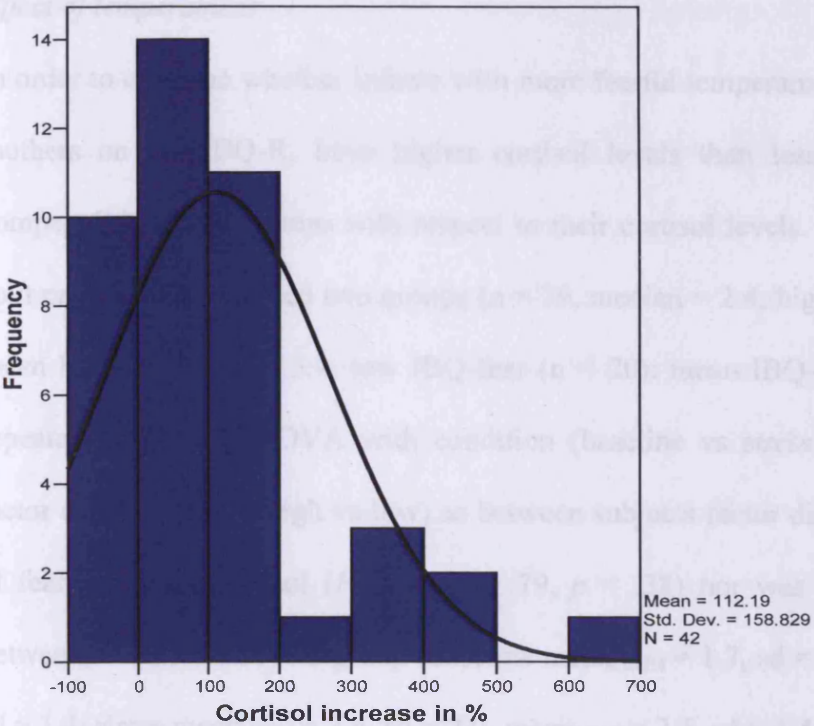

Figure 5-3 Cortisol % increase from baseline to stress for 42 one-year-old infants

Two separate independent samples t-tests were conducted to examine the effects of age group and gender on CortΔ and cortisol %increase. A significant difference was found between the two age groups on CortΔ ($mean_{YOUNG}$ = 0.7, sd = 2.0, $mean_{OLD}$ = 2.2, sd = 2.5; t (40) = -2.10, p< 0.05) and cortisol %increase ($mean_{YOUNG}$ = 81.0, sd = 161.2, $mean_{OLD}$ = 158.1, sd = 148.0; t (40) = -2.04, p< 0.05); with older infants showing stronger reactivity. However, no main effect of gender was found for CortΔ ($mean_{GILRS}$ = 0.8, sd = 2.1, $mean_{BOYS}$= 1.8, sd= 2.4; t (40) = 1.32, p = .19) or cortisol %increase ($mean_{GIRLS}$ = 68.0, sd = 101.0, $mean_{BOYS}$= 149.0, sd = 188.7; t (40) = 1.5, p = .15).

Effect of temperament

In order to examine whether infants with more fearful temperament, as rated by their mothers on the IBQ-R, have higher cortisol levels than less fearful infants we compared these two groups with respect to their cortisol levels. Following a median split procedure we created two groups (n = 39, median = 2.4; high IBQ-fear (n = 19): mean IBQ-fear score = 3.4, low IBQ-fear (n = 20): mean IBQ-fear score = 1.9). A repeated measures ANOVA with condition (baseline vs stress) as within subjects factor and fear group (high vs low) as between subjects factor did not show an effect of fear group on cortisol (F (1, 37) = .79, p = .38) nor was there an interaction between condition and fear group (baseline $mean_{HIGH}$ = 1.7, sd = 0.9, $mean_{LOW}$ = 2.1, sd = 1.4; stress $mean_{HIGH}$ = 2.6, sd = 2.0, $mean_{LOW}$ = 2.5, sd = 1.4; F (1, 37) = .37, p = .55). ANCOVAs controlling for variation in baseline cortisol could not be performed because the assumption of homogeneity of regression slopes was violated. Therefore, in order to examine whether individual differences in children's baseline levels influenced the relations between IBQ-fear and cortisol reactivity partial correlations were run (r = .06, ns). The relation between IBQ-fear and cortisol reactivity when adjusting for cortisol baseline was not significant. Independent samples t-tests also did not show a significant difference between the high and low fear groups in CortΔ or cortisol %increase (CortΔ: $mean_{HIGH}$ = 1.6, sd = 2.3, $mean_{LOW}$ = 1.0, sd = 2.4; t (37) = -1.24, p = .22; cortisol %increase: $mean_{HIGH}$ = 106.0, sd = 123.2, $mean_{LOW}$ = 115.7, sd = 198.7; t (37) = -1.15, p = .26).

Finally, we assigned our infants to a high (n = 16, mean distress score = 102.8) or low (n = 25, mean distress score = 25.2) distress group based on their Lab-TAB composite distress scores (n = 41, median distress score = 40.0). A repeated measures ANOVA did not show a significant effect of distress group on cortisol (*F* (1, 39) = .08, *p* = .78), but there was a significant interaction between condition and distress group (baseline $mean_{HIGH}$ = 1.5, sd = 0.8, $mean_{LOW}$ = 2.1, sd = 1.4; stress $mean_{HIGH}$ = 3.3, sd = 2.2, $mean_{LOW}$ = 2.1, sd = 1.2; F (1, 39) = 11.70, $p < 0.01$, see Figure 5-4). Further independent samples t-tests revealed that the interaction between distress group and condition was attributable to a significantly higher cortisol stress level in the high distress group (t (39) = -1.89, $p < 0.05$, one-tailed). The significant effect of distress group on cortisol stress level remained when we controlled for cortisol baseline (F (1, 38) = 4.67, p = .04).

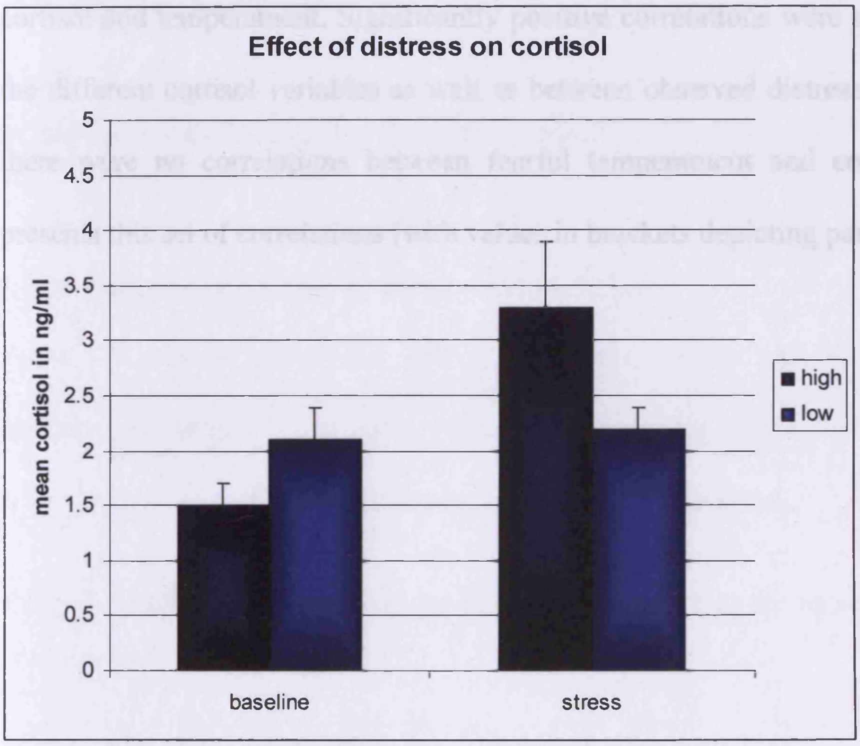

Figure 5-4 Mean cortisol in ng/ml for baseline and stress in high and low distress groups with error bars indicating the SE's

Independent sample t-tests also revealed a significant difference between the high and low distress groups in CortΔ and cortisol %increase ($mean_{HIGH}$ = 2.8, sd = 2.6, $mean_{LOW}$ = 0.4, sd = 1.5; *t* (39) = -3.1, $p< 0.01$; and $mean_{HIGH}$ = 217.6, sd = 202.0, $mean_{LOW}$ = 40.4, sd = 79.6; *t* (39) = -2.7, $p< 0.01$, respectively). Infants high in observed distress had a larger increase in cortisol as a result of stress exposure.

Correlations

Since previous analyses revealed age group differences in CortΔ, cortisol %increase, IBQ-fear and Lab-TAB composite distress scores, partial correlations were performed for these and the main cortisol variables when examining for associations between cortisol and temperament. Significantly positive correlations were observed between the different cortisol variables as well as between observed distress and cortisol, but there were no correlations between fearful temperament and cortisol. Table 5-4 presents this set of correlations (with values in brackets depicting partial correlations).

Table 5-4 Correlations between cortisol, fearfulness (IBQ-R) and observed distress (Lab-TAB) in 42 infants

	cortbase[a]	cortstress[b]	cortΔ[c]	cort%[d]	fear[e]	distress[f]
cortbase		.39*	-.42**	-.37*	-.05	-.15
			(-.34*)	(-.52**)	(.05)	(.02)
cortstress			.63**	.26	.03	.37*
			(.63**)	(.49**)	(.04)	(.43**)
cortΔ				.57**	.19	.47**
				(.95**)	(.13)	(.38*)
cort%					.18	.39*
					(.11)	(.26)
fear						.14

[a] mean cortisol level during baseline [b] mean cortisol level during stress
[c] peak cortisol stress value minus mean cortisol baseline [d] cortisol %increase
[e] IBQ-fear [f] composite distress score

*.Correlation is significant at the 0.05 level (two-tailed)
**. Correlation is significant at the 0.01 level (two-tailed)

5.4.3 Results: Cortisol in 2-year-old children.

Table 5-5 presents descriptive data for the main cortisol variables collected in 28 children. A comparison of mean cortisol levels during baseline and stress suggests relatively higher cortisol levels during stress in all comparisons.

Table 5-5 Mean cortisol and standard deviations (in ng/ml) for each subgroup during baseline and stress

	baseline		stress	
	Mean	**SD**	**Mean**	**SD**
girls (n = 13)	1.4	0.7	2.0	0.9
boys (n = 15)	1.2	0.7	2.5	3.1
young (n = 13)	1.4	0.6	2.7	2.7
old (n = 15)	1.3	0.8	1.8	1.8

Repeated measures ANOVAs showed a significant main effect of condition, with significantly higher cortisol levels during stress than during baseline (F (1, 27) = 6.05, $p < 0.05$).

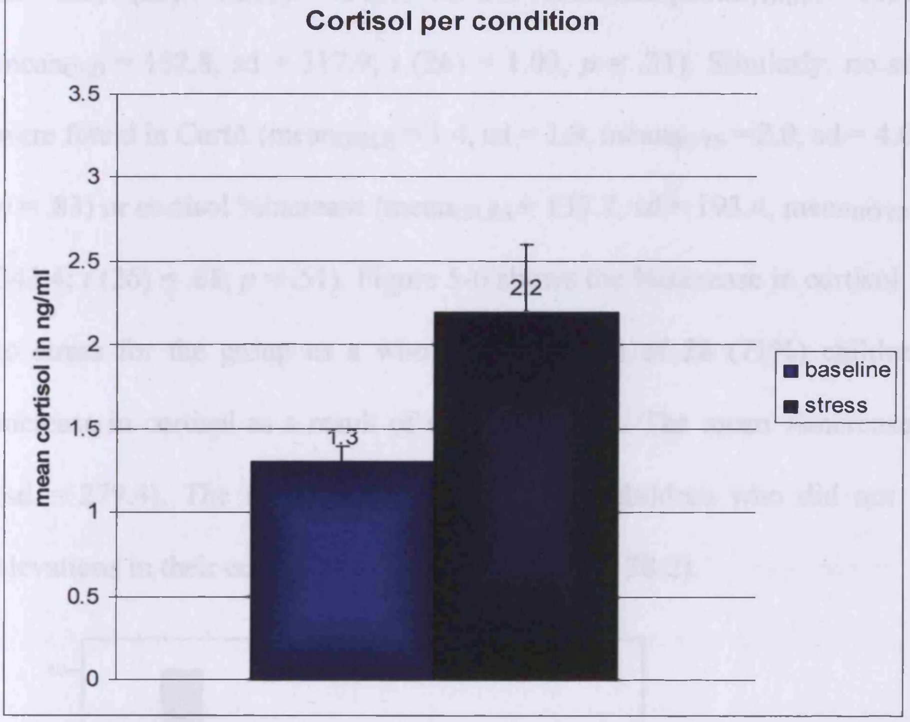

Figure 5-5 Mean cortisol (and sd's) at baseline and stress in 2-year-old children (n = 28).

Further repeated measures ANOVAs showed no main effect of age group (F (1, 26) = 2.23, p = .15) and no interaction between age group and condition (F (1, 26) = .86, p = .36), nor was there a main effect of sex (F (1, 26) = .74, p = .40) or an interaction between sex and condition (F (1, 26) = .02, p = .89) on cortisol. ANCOVAs controlling for cortisol baseline confirmed this result for age group (F (1, 25) = 1.62, p = .22) and sex (F (1, 25) = .17, p = .69).

Cortisol reactivity

Two separate independent samples t-tests were conducted to examine for differences between age and gender groups in CortΔ and cortisol %increase. Younger and older children did not differ in terms of CortΔ ($mean_{YOUNG}$ = 2.2, sd = 3.2, $mean_{OLD}$ = 1.3, sd = 3.1; t (26) = 1.20, p = .24) or cortisol %increase ($mean_{YOUNG}$ = 180.7, sd = 239.3, $mean_{OLD}$ = 152.8, sd = 317.9; t (26) = 1.03, p = .31). Similarly, no sex differences were found in CortΔ ($mean_{GIRLS}$ = 1.4, sd = 1.9, $mean_{BOYS}$ = 2.0, sd = 4.0; t (26) = .22, p = .83) or cortisol %increase ($mean_{GILRS}$ = 137.7, sd = 193.4, $mean_{BOYS}$ = 190.1, sd = 342.4; t (26) = .68, p = .51). Figure 5-6 shows the %increase in cortisol from baseline to stress for the group as a whole. Twenty out of 28 (71%) children showed an increase in cortisol as a result of stress exposure. The mean %increase was 165.8% (sd = 279.4). The mean %decrease for the 8 children who did not respond with elevations in their cortisol levels was 27.6% (sd = 28.2).

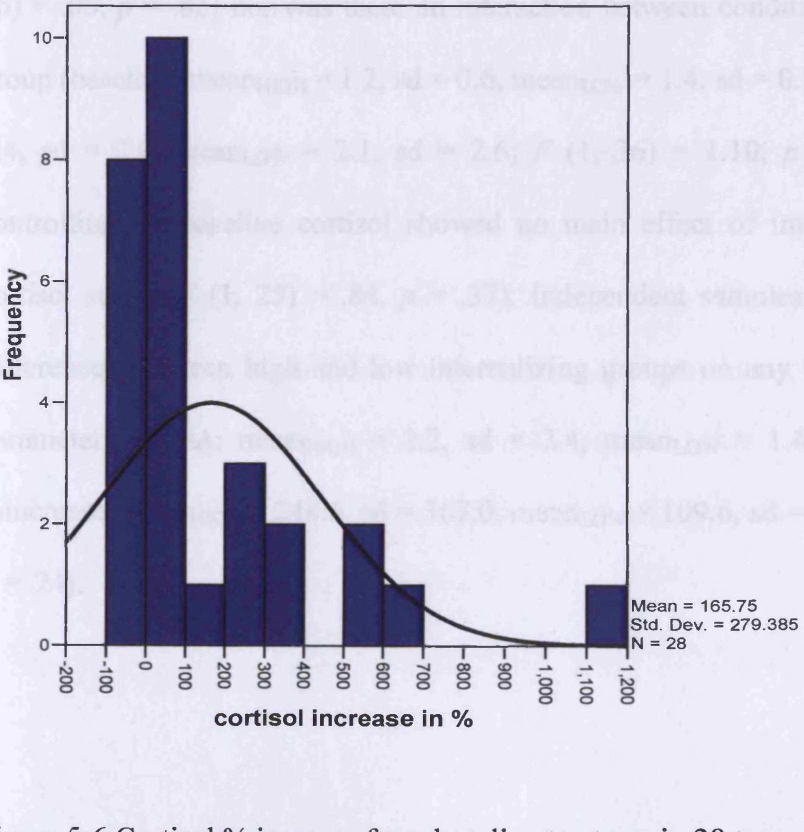

Figure 5-6 Cortisol % increase from baseline to stress in 28 two-year-olds

Effect of temperament

Next we examined whether children higher in CBCL externalizing or internalizing behaviour would have different physiological patterns than the ones with lower scores on these dimensions. Following a median split procedure two groups based on these CBCL dimension were created (n = 28, median CBCL internalizing score = 43.0, high CBCL internalizing group (n = 12): mean CBCL internalizing score = 56.4, low CBCL internalizing group (n = 16): mean CBCL internalizing score = 37.1; median CBCL externalizing score = 51.0, high CBCL externalizing group (n = 15): mean CBCL externalizing score = 57.4, low CBCL externalizing group (n = 13): mean CBCL externalizing score = 44.8). A repeated measures ANOVA with condition (baseline vs stress) as within subjects factor and internalizing group (high vs low) as between subjects factor did not show an effect of internalizing group on cortisol (F (1, 26) = .06, p = .82) nor was there an interaction between condition and internalizing group (baseline: $mean_{HIGH}$ = 1.2, sd = 0.6, $mean_{LOW}$ = 1.4, sd = 0.8; stress: $mean_{HIGH}$ = 2.4, sd = 2.0, $mean_{LOW}$ = 2.1, sd = 2.6; F (1, 26) = 1.10, p = .31). ANCOVAs controlling for baseline cortisol showed no main effect of internalizing group on cortisol stress (F (1, 25) = .84, p = .37). Independent samples t-tests indicated no differences between high and low internalizing groups on any of the other cortisol parameters (CortΔ: $mean_{HIGH}$ = 2.2, sd = 2.4, $mean_{LOW}$ = 1.4, sd = 3.0; cortisol %increase: $mean_{HIGH}$ = 244.6, sd = 367.0, $mean_{LOW}$ = 109.6, sd = 177.9; t (26) = -1.2, p = .24).

The same analyses were applied with respect to the high and low CBCL externalizing groups. A repeated measures ANOVA did not show an effect of externalizing group on cortisol (F (1, 26) = 3.0, p = .10) nor was there an interaction between condition and externalizing group (baseline: $mean_{HIGH}$ = 1.2, sd = 0.7, $mean_{LOW}$ = 1.5, sd = 0.7; stress: $mean_{HIGH}$ = 1.6, sd = 1.0, $mean_{LOW}$ = 2.9, sd = 3.1; F (1, 26) = .03, p = .85). After adjusting for cortisol baseline no effect of externalizing groups on cortisol stress was found (F (1, 25) = .61, p = .44). No differences were observed between high and low externalizing groups on any of the other cortisol parameters (CortΔ: $mean_{HIGH}$ = 1.1, sd = 1.7, $mean_{LOW}$ = 2.5, sd = 4.2; t (26) = .67, p = .51; cortisol %increase: $mean_{HIGH}$ = 132.3, sd = 214.1, $mean_{LOW}$ = 208.0, sd = 341.5; t (26) = -.28, p = .78).

Finally, we assigned the children to high (n = 15, mean distress score = 130.3) or low (n = 13, mean distress score = 40.6) distress subgroups based on their Lab-TAB composite distress scores (n = 28, median distress score = 98.5). Repeated measures ANOVAs did not show a significant effect of distress group on cortisol (F (1, 26) = .49, p = .49) nor was there a significant interaction between condition and distress group (baseline: $mean_{HIGH}$ = 1.5, sd = 0.8, $mean_{LOW}$ = 1.1, sd = 0.4; stress: $mean_{HIGH}$ = 2.2, sd = 1.8, $mean_{LOW}$ = 2.3, sd = 2.9; F (1, 26) = .08, p .78). ANCOVAs controlling for cortisol baseline also did not show an effect of distress group on cortisol stress (F (1, 25) = .001, p = .99). Independent sample t-tests did not show differences between the high and low distress groups on the other cortisol parameters (CortΔ: $mean_{HIGH}$ = 1.6, sd = 3.4, $mean_{LOW}$ = 1.8, sd = 3.0; t (26) = .55, p = .59; cortisol %increase: $mean_{HIGH}$ = 175.8, sd = 334.6, $mean_{LOW}$ = 161.8, sd = 210.8; t (26) = .75, p = .46).

Correlations

Significantly positive correlations were observed between the different cortisol variables, but no significant correlations were found between cortisol, on the one hand, and CBCL/Lab-TAB dimensions, on the other. Table 5-6 shows this set of correlations.

Table 5-6 Cortisol correlations with temperament

	cortbase[a]	cortstress[b]	cortΔ[c]	cort%[d]	CBCL-Int[e]	CBCL-Ext[f]	distress[g]
cortbase		.38*	-.13	-.25	-.09	-.25	.10
cortstress			.80**	.60**	.22	-.02	.30
cortΔ				.85**	.21	-.08	.13
cort%					.22	.04	.12
CBCL-Int						.52**	.19
CBCL-Ext							.03
distress							

[a] mean cortisol level during baseline year 2 [b] mean cortisol level during stress year 2

[c] peak cortisol stress value minus mean cortisol baseline year 2

[d] cortisol % increase year 2 [e] CBCL internalizing dimension

[f] CBCL externalizing dimension [g] composite distress score year 2

*.Correlation is significant at the 0.05 level (two-tailed)

**. Correlation is significant at the 0.01 level (two-tailed)

5.5 Results of comparisons year1 and year 2: Developmental effects

As mentioned in previous chapters, 2-year-olds were more difficult to test than one-year-olds. This was evidenced by the significant increase in observed distress in year 2 as compared to year 1 (distress_1 = 57.5, sd = 39.75, distress_2 = 88.7, sd = 47.9, t (32) = -2.84, $p< 0.01$). Table 5-7 presents the mean cortisol levels for the two year groups for both conditions and times.

Table 5-7 Cortisol means and standard deviations for the groups across time and condition

	Year 1				Year 2			
	baseline		stress		baseline		stress	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group (n = 26)	2.0	1.3	2.5	1.9	1.3	0.7	2.4	2.3

To examine whether there would be an effect of time and condition on cortisol, a repeated measures ANOVA was conducted with condition (baseline vs stress) and time (year 1 vs year 2) as the within subjects factors. Gender was not used in this analysis since our previous results showed no gender differences in this respect. To test for individual consistency in cortisol across time, Pearson's correlations were employed because all cortisol variables were log $_{10}$ transformed.

The repeated measures ANOVA showed a marginally significant effect of time (F (1, 25) = 3.9, p = .06) and a significant effect of condition (F (1, 25) = 7.70, p < 0.05) but no significant interaction between time and condition (F (1, 25) = 2.37, p = .14).

Paired samples t-tests revealed that cortisol baseline was lower in year 2 than in year 1(baseline_1 = 2.0, sd = 1.3, baseline_2 = 1.3, sd = 0.7; t (25) = 2.36, p< 0.05) but there was no significant difference between year 1 and year 2 cortisol stress levels (stress_1 = 2.5, sd = 1.9, stress_2 = 2.4, sd = 2.3; t (25) = .89, p = .38, see Figure 5-7). With respect to cortisol reactivity, CortΔ was significantly higher in year 2 (CortΔ_1 = 1.2, sd = 2.3; CortΔ_2 = 1.6, sd = 3.3; t (25) = 2.30, p< 0.05), but there was no difference in cortisol %increase between year 1 and year 2 (cortisol %increase_1 = 86.2, sd = 121.5; cortisol %increase_2 = 184.2, sd = 333.9; t (25) = -1.04, p = .31).

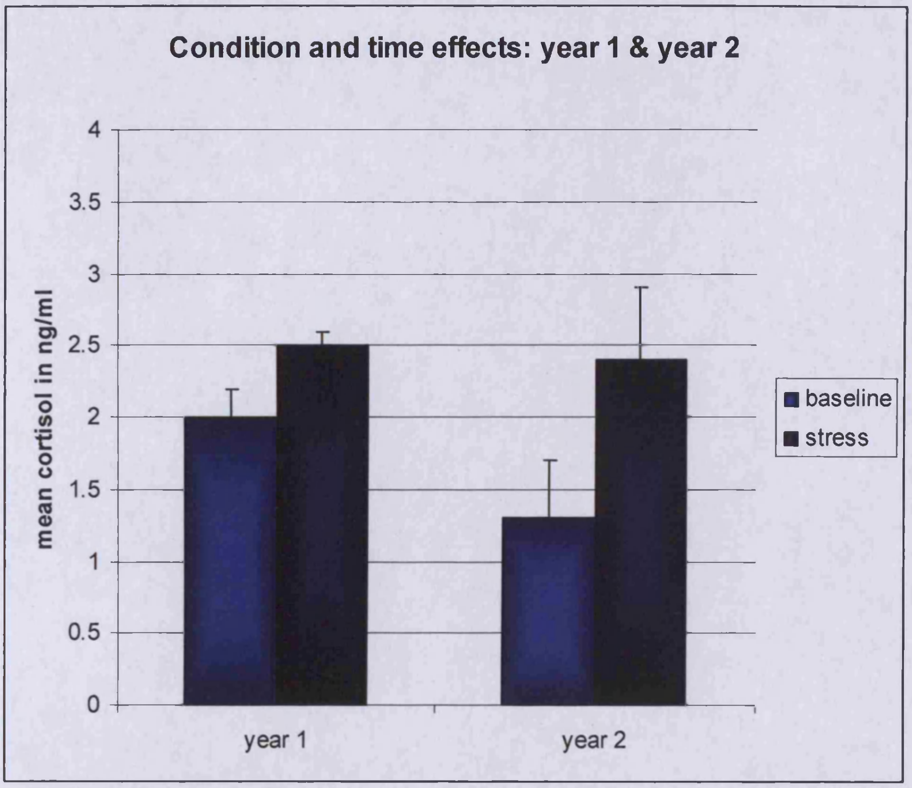

Figure 5-7 Mean cortisol in ng/ml (with error bars indicating the SE) during baseline and stress, for year 1 and year 2 (n = 26).

Correlations

Since previous analyses revealed age group effects on CortΔ _1, cortisol %increase_1, IBQ-R fear and the Lab-TAB composite distress score_1, partial correlations were performed between these variables and the main cortisol variables. Cortisol stress_1 was found to be significantly correlated with cortisol stress_2 (r = .51, $p < 0.01$) and CortΔ_2 (r = .39, $p < 0.05$). Some significant correlations were also observed between cortisol and temperament over time. Table 5-8 presents this set of correlations (with values in brackets depicting partial correlations).

Table 5-8 Correlations between cortisol parameters and temperament dimensions over time

	cortbase_1[a]	cortstress_1[b]	cortΔ_1[c]	cort%_1[d]	fear[e]	distress[f]
cortbase_2[g]	.05	-.11	-.09 (.09)	-.09 (.10)	.11 (.44*)	-.27 (-.06)
cortstress_2[h]	.28	.51**	.15 (.27)	.08 (.19)	-.07 (.19)	-.10 (.07)
cortΔ_2[i]	.19	.39*	.08 (.06)	.06 (.03)	-.17 (-.39)	-.004 (.05)
cort%_2[j]	.08	.32	.11 (.05)	.18 (.12)	-.25 (-.33)	.13 (.13)
CBCL-Int[k]	.39*	.30	-.02 (-.03)	-.12 (-.05)	-.02	.13
CBCL-Ext[l]	.22	.14	-.12 (-.06)	-.18 (-.04)	-.14	-.09
distress_2[m]	.49**	.25	-.12 (.21)	-.28 (-.36)	-.33	.04

[a] mean cortisol level during baseline year 1 [b] mean cortisol level during stress year 1

[c] peak cortisol stress value minus mean cortisol baseline year 1

[d] cortisol% increase year 1 [e] IBQ-R fear dimension

[f] composite distress score year 1 [g] mean cortisol level during baseline year 2

[h] mean cortisol level during stress year 2

[i] peak cortisol stress value minus mean cortisol baseline year 2 [j] cortisol %increase year 2

[k] CBCL internalizing dimension [l] CBCL externalizing dimension [m] composite distress score year 2

*.Correlation is significant at the 0.05 level (two-tailed)

**. Correlation is significant at the 0.01 level (two-tailed)

5.6 Discussion

The goal of the current study was to examine cortisol reactivity in one-year-old infants in response to a stressor, to examine relations between different cortisol parameters (i. e., resting level and stress response) and to explore the associations between this physiological system and behavioural distress and maternal reported temperament (IBQ-R/CBCL). The study was repeated once more when children were 24 months old to examine stability in these parameters and their relationships over time. To our knowledge, not many studies have examined time effects on cortisol in the 1st two years of life. Subsidiary goals were to find out whether there were age (in months) and sex differences in cortisol levels. This section will be structured according to the 7 initial hypotheses.

We expected to successfully induce stress in young children as reflected in a change in their cortisol levels. Specifically, 32 infants out of 42 (76%) in the 1st year (see Figure 5-3) and 20 children out of 28 (71%) in the 2nd year (see Figure 5-6) showed an increase in cortisol level from baseline to stress. According to Kirschbaum and Hellhammer (1989) a subject can be classified as a cortisol responder when there is an increase in cortisol of at least 15% from a previous value. In the 1st year there were 30 cortisol responders (71%) and in the 2nd year there were 18 cortisol responders (64%). Results of repeated measures ANOVA tests confirmed that cortisol levels were significantly higher during stress than during baseline in both years ($p < 0.01$ year 1, $p < 0.05$ year 2). These results render our experimental manipulation successful and enabled us to investigate individual differences in cortisol reactivity in infancy and early childhood.

Other hypotheses were related to the effects of gender and age. With respect to gender, data from previous research are inconclusive. Gunnar et al. (1989) found that newborn boys had higher cortisol levels following physical examinations as compared to girls, who also habituated faster, while studies in adults report that women and men differ in cortisol response in relation to different types of stressors. However, most studies failed to report whether they found gender differences in cortisol. Therefore, one of our goals was to test for gender differences in cortisol level and reactivity. We found no gender differences in year 1 or year 2. One explanation might be related to the establishment of the diurnal rhythm of cortisol secretion, and factors such as feeding, sleep-wake patterns and other psychological factors prior to testing, which have been reported to affect basal cortisol concentrations (de Weerth & van Geert, 2002). As mentioned in the Introduction, evidence regarding the appearance and establishment of a cortisol circadian rhythm has been inconclusive. A circadian rhythm in cortisol secretion in human infants has been detected as early as the 3rd month of age (Spangler, 1991), while others suggest that it is not there till the 9th month of age (de Weerth & van Geert, 2002). Also, even when there is a circadian rhythm the nature of the rhythm is different in children compared to adults. For example Gunnar and Donzella (2002) concluded that the adult cortisol pattern of higher mid-morning levels and lower mid-afternoon levels cannot be reliably detected until children are 4 years old. We don't know whether there are gender differences in the development of a cortisol diurnal rhythm. The incoherent activation of the HPA axis in young children might have affected our ability to detect gender differences in cortisol levels across both times of assessment. Although we always collected cortisol in the morning (to control for diurnal variation) and ANCOVAs showed cortisol baseline to be a non-significant covariate in the relationship between gender and cortisol reactivity, we do not know whether factors such as the sleep-wake pattern, feeding, and any psychological factors prior to testing differed between boys and girls, and had an effect on the cortisol data, since we were not able to control for these. Secondly, Kirschbaum, Wust and Hellhammer (1992) suggested that gender differences in cognitive appraisal and emotion may underlie gender differences in cortisol secretion. Thus, differences in how boys and girls appraise distressing psychological situations could be reflected in their cortisol levels. However, as stated in Chapter 3, in the current study there were no gender differences in maternal-rated temperament or level of distress as observed in the laboratory. Thus the absence of gender differences in emotionality is in line with the absence of gender differences in cortisol secretion.

We also tested for age differences, both within each year and across years. Gunnar et al. (1996) reported a decrease in cortisol reactivity from 2 to 4 months and between the 1st and the 2nd year of life despite an increase in behavioural distress. Contrary to these results, in our 1st study older infants (mean age = 11.7 months, sd = 1.5) showed stronger cortisol reactivity (CortΔ and cortisol %increase) than younger infants (mean age = 8.0 months, sd = 1.0). However, this result was not replicated in the 2nd study; older children (mean age = 25.0 months, sd = 2.1) did not have higher cortisol stress levels than younger ones (mean age = 19.5 months, sd = 1.8). As mentioned in previous sections the age range of the sample employed in year 2 was restricted with most children falling in the 21-24 month age range.

Although Gunnar et al. (1996) observed a general decrease in cortisol levels in the 1st two years of life we found that two-year-olds indeed had significantly lower cortisol baselines as compared to their baselines in year 1 ($p < 0.05$; see Figure 5-7), but had a stronger cortisol reactivity (CortΔ). This result was as predicted and is in line with our other findings (see section 3.4.4 & 4.7.5) of higher levels of observed distress and SCL in 2- as compared to 1-year-olds. As stated previously, the latter finding could be attributed to developmental factors, specifically the increasing cognitive abilities that emerge by the end of the 1st and extend into the 2nd year of life (Gartstein & Rothbart, 2003). The decrease in cortisol baseline level as the child gets older but maybe also a decrease in cortisol stress reactivity upon mild stress provocation (see Gunnar et al., 1996) has been discussed in relation to the increase in nighttime sleep, the establishment of attachment relations (e.g., parental presence may decrease cortisol response) and the development of memory capacity (i.e., the reduction of novelty because of repetition) (Gunnar et al., 1996).

Based on earlier studies (de Haan et al., 1998; Kagan et al., 1987; Schmidt et al., 1997) we predicted positive correlations between fearful temperament and distress behaviour, on the one hand, and cortisol parameters, on the other. Fearful temperament (IBQ-fear) was not significantly correlated with cortisol baseline or cortisol stress in year 1, nor was internalizing behaviour (CBCL) related to cortisol baseline or stress in year 2. Nonetheless, significant associations were found between temperament and cortisol over time. Specifically, when controlling for age in months IBQ-fear was significantly positively correlated with cortisol baseline in year 2 (rho = .44). A significant positive association was also observed between cortisol baseline in year 1 and internalizing behaviour in year 2 (rho = .39; see Table 5-8). It is somewhat surprising that cortisol baseline rather than cortisol stress was related to temperament over time. As mentioned above, 76% of the children in the 1st year and 71% of the children in the 2nd year responded with an increase in cortisol in response to fear exposure. The fact that so many children reacted with an increase in cortisol level might have hindered our ability to detect a relation between stress and fearful temperament, since the individual variation in fearful temperament might have been masked because of the general increase in distress. Moreover, variations in the timing of the cortisol peak stress response have been reported among infants (Goldberg et al., 2003; Ramsay & Lewis, 2003). We collected two cortisol samples, one after termination of stress and another one 20 min. later. Given that some studies reported a peak in cortisol response 40 min. after the termination of the stressor (Goldberg et al., 2003), it is possible that we failed to obtain important information in children with a more slowly reacting HPA axis system. It could well be that more fearful children have a differently organised HPA axis system, with differences in level and speed of cortisol reactivity and/or recovery. To circumvent this problem, future studies should obtain multiple post-stress cortisol samples. Furthermore, as stated above, basal cortisol levels can be affected by the child's biobehavioural organization (sleep, feeding, physical condition and daily experiences) (de Weerth, Zijl & Buitelaar, 2003). This sensitivity might have had an impact on our data, since we did not control for individual sleep-wake patterns, naps, feeding prior to experiment and the car trip, all of which could affect cortisol level.

Our assessment of basement or resting cortisol level was also different from the one used in other studies. We took two saliva samples, 20 min. apart, after the child had got used to the new situation and was quietly playing. Other studies collected saliva at home. Several studies reported that basal cortisol collected at home rather than stress levels better predict behavioural inhibition and internalizing behaviour (Kagan et al., 1987; Schmidt et al. 1997). Finally, Smider et al. (2002) found that high home afternoon cortisol level predicted later internalizing behaviour and social wariness as reported by teachers.

The unexpected time-lagged associations between maternal reports of children's temperament and baseline cortisol levels (i.e., temperament not being related to current cortisol, but significantly correlated with year 2 (IBQ) or year 1 (CBCL) cortisol) might be explained by the nature of the information derived from questionnaires, as discussed in previous chapters. Parents change their impressions of their children's temperament over the course of development, and cannot always reliably report on their children's temperament for personal (e.g., social desirability) or situational reasons. This is also supported by the fact that we found no significant relations between maternal ratings in the 1st (IBQ-R) and the 2nd year (CBCL; rho = -.02 for internalizing, rho = -.14 for externalizing, see section 3.4.4).

In addition to questionnaire ratings of children's temperament we also used observed behavioural ratings of distress when they were challenged. Highly distressed infants had higher cortisol stress levels and showed a larger increase in cortisol from baseline to stress (i.e., higher CortΔ and cortisol %increase). However, there was no correlation between cortisol baseline and observed distress. These results were not replicated when the children were 2 years old; highly distressed 2-year-olds did not differ in cortisol from less distressed children, nor were distress levels correlated with cortisol baselines or cortisol stress levels. At the moment we have no explanation for the absence of correlations between cortisol and distress levels in year 2. Interestingly, cortisol baseline in year 1 was positively correlated with Lab-TAB-distress scores in year 2 (r = .49; see Table 5-8). We mentioned before that other studies found that basal cortisol levels predicted later behavioural inhibition problems (Kagan et al., 1987).

Although, higher levels of cortisol have been reported in more outgoing and surgent children (Donzella et al., 2000) and lower levels in highly externalizing ones (Van Goozen et al., 2000) the results of the current study did not support these findings. It is important to emphasize once more that the current analyses were based on median split procedures and not on absolute CBCL values. Due to the nature of our sample, the ranges of the CBCL internalizing and externalizing scores were restricted (see Figure 3-4 & Figure 3-5) with only one child scoring in the clinical range on both the externalizing and internalizing scales, and a further one child scoring in the clinical range on the internalizing scale. None of the children fell in the borderline range. Finally, as mentioned in the results of Chapter 3 (section 3.4.3) the two CBCL scales were highly correlated (rho = .52, $p < 0.01$; seeTable 3-6), which might have contributed to the current results. According to Achenbach and Rescorla (2000) these two behavioural profiles are not mutually exclusive and co-exist in children.

Finally, we predicted consistency in cortisol values across time with, for example, relatively reactive one-year-olds predicted to be also more reactive in year 2. Cortisol stress levels in year 1 were significantly correlated with cortisol stress levels in year 2, as were CortΔ in year 1 and year 2. However, cortisol baseline levels were not correlated across time. Goldberg et al. (2003) found 1 week stability in baseline cortisol levels in 12 to 18-month-old infants. The significantly higher cortisol baseline levels in the 1st year as compared to the 2nd year might have contributed to the lack of correlation between baseline levels across time. Moreover, it is possible that the lack of correlation between the year 1 and 2 basal measures of cortisol can be accounted for by the developing HPA axis' circadian rhythm in the 2nd year of life, which mainly affects basal and not stress cortisol levels. As stated previously, basal cortisol levels are affected by sleep-wake patterns, feeding and psychological conditions, and these factors might have differed between the year 1 and year 2 collections. The current finding of individual consistency in cortisol stress reactivity over time, but not in resting level, underlines the importance of collecting both sets of data in order to obtain a better understanding of the development and functioning of the HPA axis in young children.

Does fear increase with age?

We found clear effects of age on cortisol baseline (with decreasing levels across time) and cortisol stress (with increasing levels across time).

Are there gender differences in fearful temperament?

No effects of gender were observed with respect to any cortisol variable in year 1 or year 2.

What are the consequences of a more or less fearful temperament?

In this study we only found clear relationships between cortisol and emotional distress in year 1. Although there were no within-year relations between questionnaire measures of temperament and cortisol, cortisol baseline in year 1 correlated significantly with CBCL internalizing behaviour, and observed distress in year 2 and IBQ-fear in year 1 correlated with cortisol baseline in year 2. Externalizing behaviour was not correlated with any cortisol parameter.

Stability of the HPA axis activity

Cortisol stress in year 1 was significantly correlated with cortisol stress in year 2, as were CortΔ in year 1 and year 2. However, cortisol baseline levels were not correlated across time.

For the purposes of the current hypotheses, multiple comparison tests were performed on this set of data. This could have increased the probability of making Type I errors and should be noted as a limitation to the current results.

As mentioned in the Introduction to the current chapter cortisol has been found to be a reliable parameter in studies of child temperament. While the number of studies measuring cortisol in young children has increased, the findings are inconsistent. For example, although higher cortisol levels have been reported in inhibited and internalizing children similar patterns have also been observed in more outgoing and surgent children (Donzella et al., 2000; Gunnar et al., 1997; de Haan et al., 1998). In this study we found clear relationships between emotional distress and cortisol in year 1, but no relations between cortisol and externalizing behaviour. One reason for the inconsistency in previous results could be related to the quality of the experimental designs used. In the current study we used a strong structured stressor, and both in year 1 and in year 2 the majority of the children showed a significant increase in cortisol as a result of exposure to this stressor.

Stress reactivity and cortisol release are necessary for survival. However, individuals differ in their vulnerability to stressful events, with some being more easily and/or more intensely stressed than others, even in situations of minor provocation (Gunnar & Quevedo, 2007). Both stress hyper-reactivity and hypo-reactivity have been linked to adverse outcome (Gunnar & Donzella, 2002). However, although non-human animal research clearly shows the effects of early HPA axis programming for later cognitive, emotional and social development, similar research in humans is – for obvious reasons – relatively scarce (Huizink, Mulder, & Buitelaar, 2004, ; Meaney & Szyf, 2005).

The current study is part of a longitudinal study on HPA axis development in normal healthy children and focuses specifically on stability and change in HPA axis reactivity to stress. More waves of measurement together with a larger sample will enable us to use more sophisticated statistical methods to identify those children who react more and those who react less strongly to stress. Children with more extreme patterns of cortisol reactivity might - over time - be found to be on a trajectory of internalizing or externalizing behavioural problems.

Inconsistencies have also been reported with respect to the issue of gender and cortisol (Gunnar et al, 1996; Kirschbaum & Hellhammer, 1994; Lewis & Ramsay, 1999). No effect of gender was observed with respect to cortisol in year 1 or year 2. Social factors that have been implicated in the development of gender differences in temperament, such as more fearfulness in girls, seem to influence temperament at a later stage in development and not before the 3rd year of life (Hay, 2007). This could explain our results.

Still, more work needs to be done. It would be interesting to find out whether these developmental effects and relations with temperament continue to exist in the 3rd year of life. We discussed that cortisol basal levels in young children are influenced by daytime stressors, meals, naps, and the development of the cortisol circadian rhythm. Although it is advisable to take morning samples to reduce these potential sources of variation (which is what we did) it is difficult to evaluate the magnitude of these effects on the cortisol baseline concentrations that we reported. Future work should also focus on determining the intra-individual variation in cortisol in infants at short but frequent time intervals.

Chapter 6 Associations between fearfulness, sustained attention and effortful control

6.1 Effortful control as a temperament construct

Effortful control (EC), the ability to suppress a dominant response in order to perform a subdominant response (Posner & Rothbart, 2000; Rothbart & Ahadi, 1994; Rothbart & Bates, 1998), is a key element of executive functioning and has been generally studied as a temperament construct (Kochanska, Murray & Harlan, 2000). EC emerges between 6 and 12 months of age along with the maturation of attentional mechanisms (Rothbart, Derryberry & Posner, 1994), becomes increasingly coherent during the second year of life, develops rapidly between the ages of 3 and 6 years (Carlson, 2005; Carlson & Wang, 2007; Reed, Pien & Rothbart, 1984) and has been shown to formalise as a personality trait by 45 months of age (Kochanska & Knaack, 2003). EC involves the voluntary control of both attentional and behavioural processes. For example, individual differences in EC have been associated with individual differences in sustained attention (Kochanska et al., 2000; Posner & Rothbart, 2000) and behavioural inhibition (Kochanska & Knaack, 2003). It has been suggested that early temperament characteristics like reactivity to stress and orientation are integrated into or regulated by the later developing temperament construct of EC (Hill-Soderlund & Braungart-Rieker, 2008).

The importance of understanding the early antecedents of EC has been underlined by recent studies reporting relations between impulsivity, low EC and attentional disorganisation, on the one hand, and externalizing problems, on the other (Olson, Sameroff, Kerr, Lopez & Wellman, 2005). EC is known to be a protective factor against the development of aggressive and antisocial behaviour (Oldehinkel, Hartman, Ferdinand, Verhulst & Ormel, 2007; Olson et al., 2005; Kochanska & Knaack, 2003). Thus, a better understanding of the development of EC in normal individuals will ultimately help with the diagnosis, prevention and possibly treatment of neurodevelopmental disorders such as ADHD (Posner & Rothbart, 2000).

6.1.1 Links between sustained attention and EC

Two attentional temperament dimensions have been identified: distractibility and attention span/persistence (Thomas & Chess, 1977). Distractibility refers to how easily the attention of the child is drawn away from ongoing behaviour. Attention span or persistence refers to the length of time a child is occupied with an activity or is able to sustain activity or attention in the face of distraction. In the current study we will focus on the attention span dimension.

In young children researchers usually measure duration of orienting (Rothbart, 1981) and sustained attention (Kochanska, Murray & Harlan, 2000), with both variables being related to attention span. A novel stimulus causes an initial stimulus orienting and then sustained attention. Stimulus orienting involves the detection of a change (stimulus) in the environment (Choudhury & Gorman, 2000) and sustained attention is associated with information processing related to the stimulus; it is voluntary and controlled by the individual (Stroganova, Orekhova & Posikera, 1998). By the age of 3 months infants can usually engage in only five- to ten- seconds of sustained attention. Decreases in sustained attention have been reported between 3 and 6 months of age, with a subsequent increase from 7 months onwards (Colombo, 2002). Ruff and Lawson (1990) reported a significant increase in sustained attention over the first three years of life. Sustained attention has been suggested to develop earlier in girls than in boys (Greenberg & Waldmant, 1993). Studies of sustained attention usually employ complex three-dimensional stimuli within interactive and play-based situations (Choudhury & Gorman, 2000; Courage, Reynolds & Richards, 2006). Colombo et al. (2001) state that the amount and level of sustained attention can be more reliably assessed during presentations of dynamic stimuli rather than static presentations. Attentional mechanisms have long been linked to the development of self-regulation, an important characteristic of temperament, which involves the ability of the child to selectively regulate incoming information from stimuli (Rothbart & Derryberry, 1981). Orienting and sustained attention early in development are believed to become integrated in the developing EC later on (Rothbart, Derryberry & Hershey, 2000).

Kochanska et al. (2000) examined sustained attention as an early contributor to EC in 106 children at 22 and 33 months of life. Sustained attention was measured at 9 months of age by using an episode from Lab-TAB (Goldsmith & Rothbart, 1992). EC at 22 months was assessed through tasks such as 'snack delay', 'wrapped gift', 'gift in the bag' etc.; at 33 months children were able to perform more EC tasks including delaying, slowing down motor activity, and suppressing/initiating activity to signal tasks. Sustained attention at 9 months correlated with EC at 22 but not at 33 months. Kochanska et al. (2000) attributed this finding to cognitive and experiential factors that influenced development between 22 and 33 months. Generally, EC increased from 22 to 33 months, there was significant convergence between EC questionnaire and observational measures, and EC was better in girls across most tasks at both times.

Hill and Braungart-Rieker (2002) examined links between attention regulation and EC, as the behavioural antecedent of self-regulation. Attention regulation (object orientation) was tested with the still-face procedure in 70 4-month-old infants. Self-regulation was assessed when the children were 36 months of age, through observation of compliance – non-compliance, when they had to stop playing with desirable toys and start cleaning up the play area. Results showed that infants who were able to direct their attention away from the frustrating still face of their mothers could better regulate their impulses during the clean up episode and needed less prompts from their mothers to remain on task.

Davis, Bruce and Gunnar (2002) investigated the relationship between performance on tasks that involve the anterior cingulate gyrus (ACC), which is part of the anterior attention system, and EC in normally developing 6-year-old children. The ACC has been proposed to modulate attention and executive function and is at the core of the ability to regulate behaviour in an effortful way (Davis, Bruce & Gunnar, 2002). Two neuropsychological tasks which have been shown to tap into the anterior attentional system were used in this study: a 'Go, No-Go' task, which measures the ability to inhibit a predominant motor response, and an attentional control task, which assesses the ability to inhibit or direct attention to different sensory stimuli (e.g., colours, shapes). Children also performed two delay of gratification tasks as measures of EC, while parents completed questionnaires. The CBQ dimension of inhibitory control but not performance on the neuropsychological tasks was related to the ability to delay gratification. The authors attributed the lack of association between the delay of gratification tasks and the other neuropsychological tasks to the multifaceted nature of the ACC, because this is not only involved in executive functioning and EC, but also active in detection and planning (Rothbart, Ahadi & Evans, 2000).

In addition to these behavioural data, neurobiological correlates of EC have been identified. A recent case study showed that a 14-month-old boy (PF1), who sustained damage to the Prefrontal Cortex (PFC) when he was 3-days old, had significant impairments in attention engagement and emotion regulation (Anderson, Aksan, Kochanska, Damasio, Wisnowski & Afifi, 2007). Fear regulation has been widely studied in individuals with callous-unemotional traits and/or antisocial behaviour. For example, Fairchild, Van Goozen, Stollery & Goodyer (2008) reported a reduced startle response and deficits in fear conditioning in conduct disordered youngsters which could be linked to the amygdala and PFC. Since both EC and emotion regulation are linked to the PFC and predict developmental outcome, it is plausible that effortful regulation of reactivity to fear-provoking episodes in early life, may be another predictor of socialisation outcome.

6.1.2 Links between EC and fearfulness

Although EC is usually seen as an active inhibitory temperament dimension and fearfulness as a more passive one, they have been reported to share some underlying characteristics (Kochanska & Knaack, 2003). For example, it has been shown that EC regulates negative reactivity or distress in order to accomplish other internal goals, and both temperament dimensions are important in the development of conscience (Rothbart, 2007). Not many studies to date have examined the association between fearfulness and EC in very young children and the few findings that exist are contradictory.

Kochanska and Knaack (2003) induced fear by using the 'masks' episode from the Lab-TAB (Goldsmith & Rothbart, 1996), in which an unfamiliar experimenter wears different fear-inducing masks close to the infant's face, and measured EC by using tasks involving delaying gratification, slowing down motor activity, suppressing/initiating activity to a signal, effortful attention, and lowering voice. Their results showed that infants who expressed stronger novelty fear at 14- and 22-months demonstrated better EC performance at 22-, 33- and 45-months-of-age. Parental reports of infant fear at 22-months correlated positively with EC, and girls showed better EC at all times. On the other hand, Kochanska and Knaack (2003) found a relation between poor EC and the emergence of externalizing problems (see, for similar results, Barkley, 1997; Mischel, Shoda & Rodriguez, 1989; Olson, Schilling & Bates, 1999).

To the contrary, Hill-Soderlund and Braungart-Rieker (2008) observed negative correlations between fearfulness and the later development of EC. The authors used the stranger approach paradigm (Lab-TAB; Goldsmith & Rothbart, 1996) to test for fearful reactivity in infants at 8-, 12-, and 16-months-of-age. When the participants were between 4.5 and 5.5 years old, they were tested for their EC ability through a battery of tasks measuring delay of gratification, slowing down motor activity, suppressing/initiating activity to a signal and effortful attention. Results showed that infants with larger increases in fear reactivity between 8 and 16 months of age had lower EC ability when they were about 5 years old. The authors hypothesised that fearful infants find their environments more threatening and therefore concentrate more effort on avoiding such situations. Consequently, these children have fewer opportunities to learn effective coping techniques to deal with fear, and ultimately suffer in their development of self-regulation and EC (Hill-Soderlund & Braungart-Rieker, 2008).

6.1.3 Relations between sustained attention, EC and fearfulness in the current study

The current study was designed to examine the proposed associations between EC and sustained attention. A longitudinal design was employed with measurements of sustained attention being taken in the first- and second-year of life, and EC being measured when the children were 2 years old. A subsidiary goal was to examine whether fearful temperament as assessed during fear exposure and through maternal reports was related to EC and/or the ability to sustain attention.

When we reviewed the literature we showed that only a few studies have examined the relation between early sustained attention and EC, and we pointed out that the results are contradictory [see, for example, Kochanska et al., (2000) for a positive, and Davis, Bruce and Gunnar (2002) for negative findings]. Moreover, although positive correlations have been reported between observational measures of EC and fearfulness some recent evidence points towards a negative association (Hill-Soderlund & Braungart-Rieker, 2008).

6.2 Hypotheses

Since research (Courage, Reynolds & Richards, 2006) has shown that complex or interactive stimuli increase look duration we predicted that (1) children would show more sustained attention during a manipulative task than a non-manipulative task.

With respect to age (2) one-year-old children were expected to have lower sustained attention scores than 2-year-old children, and we explored whether similar effects would be observed when comparing younger and older children within-year groups. We did not measure EC in year one, but predicted that relatively older children in year 2 would demonstrate better EC, as this has been reported to increase with age (Kochanska & Knaack, 2003). Moreover, in line with previous research findings (3) girls were expected to perform better on EC tasks than boys (Kochanska et al., 2000), while the same was not expected for sustained attention. (4) We predicted that sustained attention in year 1 would be positively correlated with sustained attention in year 2, and that both would be positively related to EC in year 2. Finally, (5) more fearful children were predicted to show better EC and more sustained attention, whereas more externalizing children were predicted to do worse on EC and sustained attention tasks (Kochanska & Knaack, 2003; Murray & Kochanska, 2002).

6.3 Methods

6.3.1 Participants

Year 1 sample

Participants were 50 infants (mean age in months = 9.7, SD = 2.2) and their mothers; all participants had been born in Cardiff, Wales. For analysis purposes the sample was divided into four subgroups: boys (n = 27, mean age = 9.9, sd = 2.0) and girls (n = 23, mean age = 9.5, sd= 2.5), younger (n = 27, mean age = 8, sd = 1.0) and older (n = 23, mean age = 11.7, sd = 1.0). Of the 27 boys 14 belonged to the younger (mean age = 8.3, sd = 0.9) and 13 belonged to the older (mean age = 11.5, sd = 1.4) group, and of the 23 girls 13 belonged to the younger (mean age = 7.7, sd = 0.9) and 10 to the older group (mean age = 11.9, sd = 1.5). A chi-square test for independence indicated no significant association between gender and age (χ^2 (1) = .11, p = .74). Boys and girls did not differ in age (t (48) = -.55, p = .59).

Year 2 sample

Approximately 12 months after the first assessment the families were contacted again by mail and telephone and asked whether they were willing to participate in a follow-up study. Of the original sample (N = 50) 10 participants could not be located or did not respond to our letter or phone calls. Therefore, 40 children (80%) returned to the laboratory for a visit in their 2nd year of life.

Of the 40 participants, 7 were unable to complete the 2nd study[5] and could therefore not be used in the analyses. These 7 children did not differ from the other participants on attention as measured in year 1 (music box: p = .60; wheel: p = .46; see pp 10-11 for a description), nor did they differ from the participating children in mother-rated IBQ-R (fear: U = 126.500, p = .84) or on the Lab-TAB composite distress score (t (47) = 10, p = .31). So in the end, 33 participants (66%) completed the 2nd study (mean age in months = 22.3, sd = 3.2). This sample was again divided into the same four subgroups; boys (n = 18, mean age = 22.6, sd = 3.4) and girls (n = 15, mean age = 21.9), young (n = 18, mean age = 19.9, sd = 2.1) and old (n = 15, mean age = 25.1, sd = 1.9). Of the 18 boys 8 belonged to the young group (mean age = 19.8, sd = 2.5) and 10 to the old group (mean age = 24.9, sd = 1.8), and of the 15 girls 10 belonged to the young (mean age = 20.1, sd = 1.8) and 5 to the old group (mean age = 25.4, sd = 2.1). A chi-square for independence indicated again no significant association

[5] Seven infants did not complete the experiment due to fussiness in response to the demands of the experimental procedure, specifically the placement of the electrodes.

between gender and age (χ^2 (1) = 1.63, p = .20). Boys and girls again did not differ in age (t (31) = -.65, p = .52).

6.3.2 Procedure

a) Baseline

As described in section 3.3.2, the experimenter interacted with the mother and the infant for about 20 minutes prior to testing in order for the dyad to get used to the laboratory environment. Next, the infant was carefully placed in a car seat and fastened safely by the mother in order for the sustained attention episode to take place.

b) Sustained attention

In this study we employed two dynamic stimuli for studying sustained attention in one-year-old infants. The first stimulus, a 'music box', was a colourful object which upon activation attracts the child's attention by the appearance of a moving prince revolving around himself whilst the music is playing. The music box was placed on a small table in front of the child, but out of reach. The other stimulus, a 'spinning wheel', was an age appropriate colourful toy consisting of a spinning wheel with little round balls which the child could manipulate. The little balls contained different colourful moving patterns, such as a little round face, a little mirror etc. Each of the two stimuli was presented for two minutes. In year two, the spinning wheel toy was replaced by a wooden toy consisting of small colourful cones that could be hammered into their designated holes.

Sustained attention coding

Sustained attention was scored following instructions of the Lab-TAB manual (see Appendix 3; Goldsmith & Rothbart, 1999). Each stimulus was presented for 120 sec. with 4 epochs of 30 sec. each. For the first stimulus (music box) the 'duration of looking' (0-3), the 'presence of gestures' (0, 1), the 'presence of vocals' (0, 1), and the 'intensity of facial interest' (0-2) were scored (with Cronbach's alpha's = .64 and .77 for year 1 and 2 respectively). For the second stimulus (spinning wheel/wooden toy) the 'duration of looking' (0-3), the 'manipulations of stimuli' (0-3) and the 'intensity of facial interest' were scored (Cronbach's alpha's = .75 and .96 for year 1 and 2 respectively). The sum score over these behaviours in each epoch was calculated to create a composite score for each stimulus. The intra-correlation coefficient between two coders was calculated for 10 of the 50 children in year 1, and for 10 of the 33 children in year 2, and ranged from .65 to .80 in both years.

c) Effortful control

1) Delay of gratification (Kochanska, Murray & Harlan, 2000).

The child was fastened into a chair and a table was placed directly in front of him/her. The primary caregiver was seated in a chair next to the child, outside his/her visual field, at a distance of approximately 0.5 metres. The experimenter knelt beside the child and produced a Petri dish filled with raisins (or another favourite snack as chosen by the mother). The experimenter then said the following:

> *Hello [infant's name], I have some snacks here for you. [Experimenter eats a snack]. Mmm, that tastes delicious! Would you like one [infant's name]? Sorry, but you are not allowed one until I ring this bell. I will put this snack under the cup [experimenter*

puts one raisin under a transparent plastic cup], and when you hear this bell ring you can take it. Do you understand? You can only eat the raisin when I ring the bell.

According to Garon, Bryson and Smith (2008) it is important to select a 'simple' response inhibition (or EC) task that would not put a high demand on the child's working memory given their young age. The instructions were reiterated until the children understood what they had to do. If English was not the infant's first language, the mother was asked to repeat the instructions once more in the infant's first language. The hidden snack was placed in front of the child and the dish filled with snacks was left next to the transparent cup. Time was recorded by stopwatch and time registration started after the instructions had been given and the experimenter moved away from the child. The bell was rung after 30 seconds or earlier if the child consumed the snack. The child was allowed to eat the snack and was given some water. This method was repeated twice more.

Snack delay coding

Each trial was coded by generating two different variables. The *snack delay-1,* which was adopted from Kochanska, Murray and Harlan (2000) was scored on a scale of 1 to 3 *(1 = consumed the snack before the experimenter rang the bell; 2 = touched the snack before the experimenter rang the bell, but did not eat it; 3 = did not touch or eat the snack until the experimenter rang the bell).* In the analysis the mean score of the *snack delay-1* was used and it was calculated across the 3 trials. The second variable, *snack delay-2,* which was adopted from Carlson (2005), was the number of trials on which the child delayed eating the snack until the experimenter had rung the bell (score range 0-3). Coding was done via video recordings. Inter-rater reliability between two coders was calculated for 7 of the 33 participants and was high (intra-class correlation coefficients: snack delay-1 = 1.00, snack delay-2 = .99).

2) Gift task (Kochanska, Murray & Harlan, 2000)

The child was again fastened into a chair and a table was placed directly in front of him/her. The primary caregiver was seated on a chair next to the child, outside his/her visual field, at a distance of approximately 0.5 metres. The familiar experimenter brought a colourful gift box and said to the child that it contained a gift for him/her. The child was asked to wait and not to touch the box until the experimenter came back into the room (180 sec).

Gift task coding

Scores for behaviour involving the gift box ranged from 1 (opens the box and takes the gift), to 2 (opens the box, takes the gift, and then puts it back in the box as if nothing happened), to 3 (opens to peak), to 4 (touches the box, but does not open it) and 5 (does not touch the box at all) as based on Kochanska, Murray and Harlan (2000). This variable was called gift delay. The latency to touch in seconds, if any of these behaviours was performed, was also coded (variable 'gift latency'). Coding was done post-testing via video recordings. Inter-rater reliability between two coders was calculated for 7 of the 33 participants and was high (intra-class correlation coefficient for gift delay = .98, for gift latency = .77).

d) Presentation of Unpredictable Toy

This episode and its coding procedure have already been described in previous chapters (see section 3.3.2). For the purposes of the current analyses the Lab-TAB composite distress scores created by accumulating infant's ratings on the behavioural variables across the fear episode, were used (see section 3.3.2).

e) Maternal reports

Year 1: Infant Behaviour Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003)

As mentioned previously in section 3.3.2 the IBQ-R subscale assessing fearfulness was used in the current analyses. An example of a fearfulness item is: *"how often during the last weeks did the baby cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)?".*

Year 2: Child behaviour checklist and language development (CBCL/1.5-5; Achenbach & Rescorla, 2000)

Only the composite scales of internalizing and externalizing behavioural problems were used for the purposes of the current study (see section 3.3.2).

6.4 Results: Sustained attention

6.4.1 Statistical analyses

The data from one participant in year 1 could not be analysed due to a malfunctioning video camera; therefore n = 49. Preliminary analyses showed that data on the manipulative tasks (the spinning wheel and the wooden toy) were highly negatively skewed (see Figure 6-2 & Figure 6-4 in the Results section) with all children regardless of the year in which they were tested showing excellent ability to sustain attention. Because individual differences in performance were larger on the music box task, only the data on the music box task were used in further analyses. T-tests for independent samples were performed to test for age, gender and temperament differences (IBQ-fear/Lab-TAB/CBCL) on sustained attention in both years. Spearman's correlations were calculated to examine consistencies between the different tasks used in each year. Spearman's correlations were employed when exploring relations between sustained attention and temperament.

6.4.2 Results: Sustained attention in 1-year-old children

Sustained attention task and between subjects effects

Figure 6-1 and Figure 6-2 display the frequency distributions of the two different types of tasks presented in year 1. Obvious in Figure 6-2 is the non-normal distribution obtained during presentation of the manipulative task, the 'spinning wheel' toy.

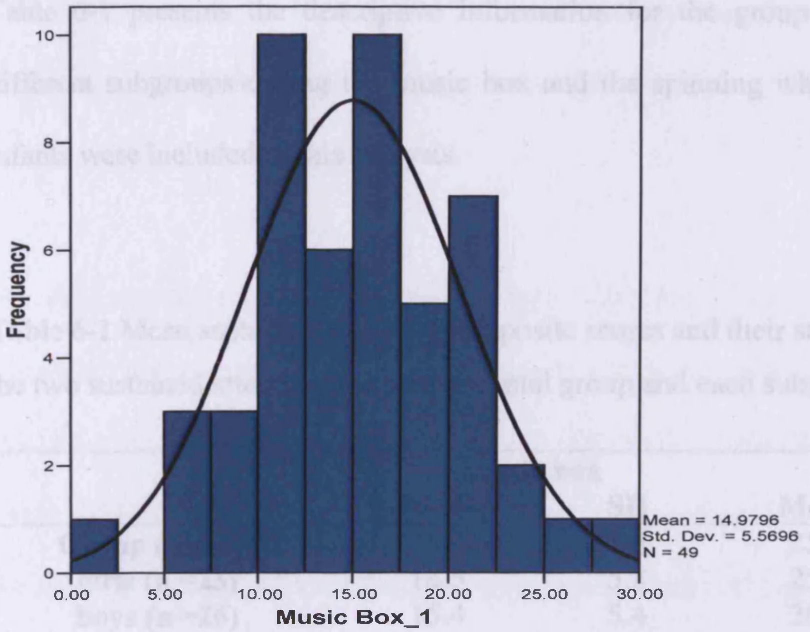

Figure 6-1 Frequency distribution of mean sustained attention composite scores during the 'music box' in 49 1-year-olds.

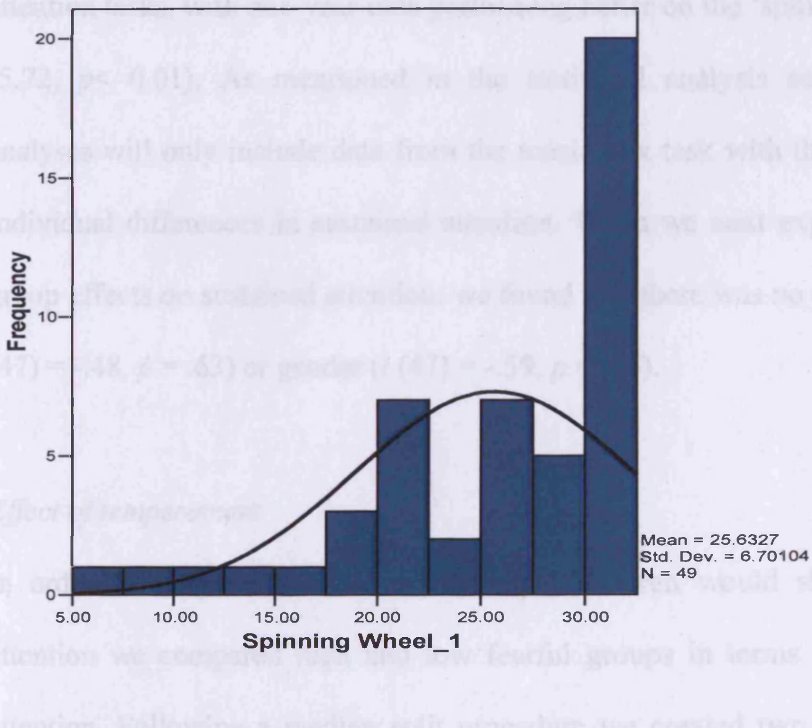

Figure 6-2 Frequency distribution of mean sustained attention composite scores during the 'spinning wheel' in 49 1-year-olds

Table 6-1 presents the descriptive information for the group as a whole and the different subgroups during the music box and the spinning wheel tasks. Data of 49 infants were included in this analysis.

Table 6-1 Mean sustained attention composite scores and their standard deviations for the two sustained attention tasks for the total group and each subgroup.

	music box		wheel	
	Mean	**SD**	**Mean**	**SD**
Group (n = 49)	15.0	5.6	25.6	6.7
girls (n =23)	14.5	5.8	25.2	7.4
boys (n =26)	15.4	5.4	26.0	6.2
young (n =26)	14.6	5.7	26.5	6.2
old (n =23)	15.4	5.6	24.7	7.3

A Wilcoxon nonparametric test revealed a significant difference between the two attention tasks, with one-year-olds performing better on the 'spinning wheel' task ($z = -5.72$, $p < 0.01$). As mentioned in the statistical analysis section above, further analyses will only include data from the music box task with the goal of identifying individual differences in sustained attention. When we next explored for significant group effects on sustained attention, we found that there was no effect of age group (t (47) = -.48, $p = .63$) or gender (t (47) = -.59, $p = .56$).

Effect of temperament

In order to examine whether more fearful children would show better sustained attention we compared high and low fearful groups in terms of level of sustained attention. Following a median split procedure we created two groups based on the median IBQ-R fear score (median IBQ-fear score = 2.6): a high (n = 23, mean IBQ-fear score = 3.4) and a low fear group (n = 21, mean IBQ-fear score = 1.9).

T-test did not show a significant difference between the fear groups on sustained attention (music box: $mean_{HIGH}$ = 16.6, sd = 4.9, $mean_{LOW}$ = 13.9, sd = 6.4; t (42) = -1.58, p = .12).

We also explored whether children with high Lab-TAB composite distress scores (n = 24, mean distress score = 100.8) showed better sustained attention than children low in distress (n = 25, mean distress score = 25.2; median Lab-TAB composite distress score = 53.0). Independent sample t-tests showed no significant difference between the distress groups on sustained attention (music box: $mean_{HIGH}$ = 15.0, sd = 5.4, $mean_{LOW}$ = 15.0, sd = 5.8; t (47) = -.03, p = .98).

Correlations

Since previous analyses revealed age group effects on the IBQ-fear and Lab-TAB composite distress scores, partial correlations controlling for age were calculated between these dimensions and the sustained attention variables. No significant correlations were observed between sustained attention and temperament. Table 6-2 presents this set of correlations (with values in brackets depicting partial correlations).

Table 6-2 Correlations between sustained attention and temperament

	fear[a]	distress[b]
music box	.18 (.17)	.03 (-.07)
fear		.14 (.04)

[a] IBQ-fear

[b] Lab-TAB composite distress score

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

6.4.3 Results: Sustained attention in 2-year-old children

Sustained attention task and between subjects effects

Figure 6-3 and Figure 6-4 display the frequency distributions for the two attention tasks in the 2nd year. Data of the 'wooden toy' task were found to be highly positively skewed.

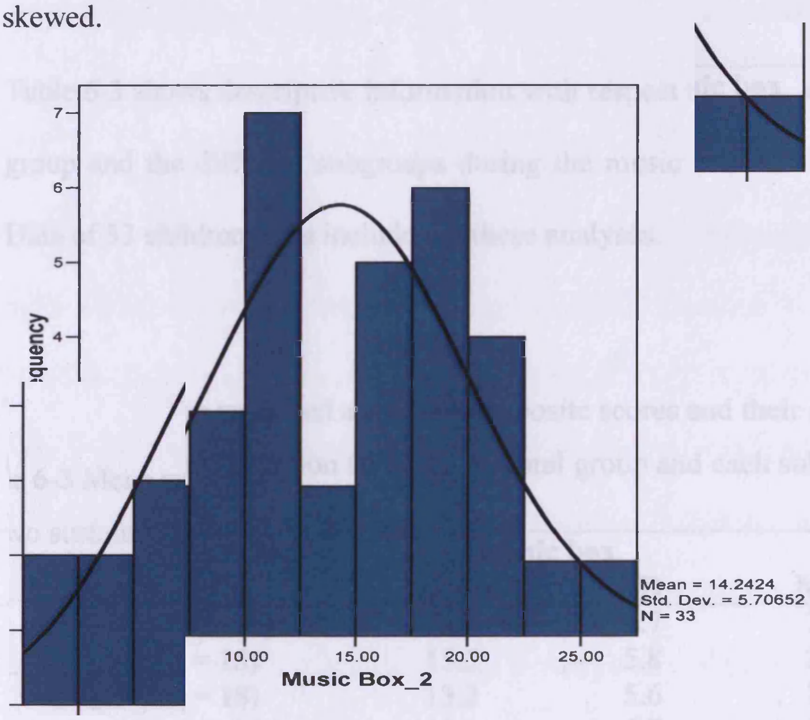

Figure 6-3 Frequency distribution of mean sustained attention composite scores during the 'music box' in 33 2-year-olds.

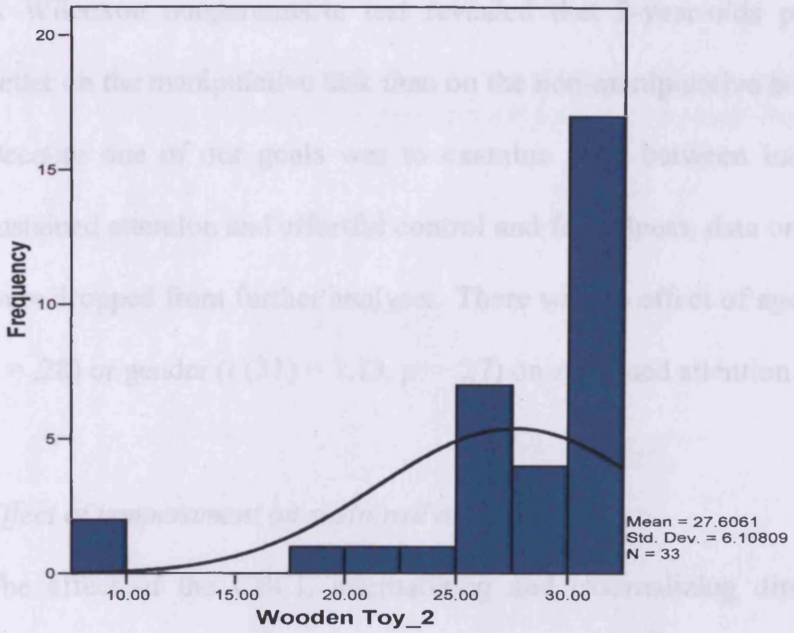

Figure 6-4 Frequency distribution of mean sustained attention composite scores during the 'wooden toy' in 33 2-year-olds.

Table 6-3 shows descriptive information with respect to sustained attention in the total group and the different subgroups during the music box and the wooden toy tasks. Data of 33 children were included in these analyses.

Table 6-3 Mean sustained attention composite scores and their standard deviations for the two sustained attention tasks for the total group and each subgroup

	music box		**wooden toy**	
	Mean	**SD**	**Mean**	**SD**
Group (n = 33)	14.2	5.7	27.6	6.1
girls (n = 15)	15.5	5.8	28.8	4.1
boys (n = 18)	13.2	5.6	26.6	7.4
young (n = 16)	13.1	5.7	26.8	7.6
old (n = 17)	15.3	5.6	28.4	4.4

A Wilcoxon nonparametric test revealed that 2-year-olds performed significantly better on the manipulative task than on the non-manipulative task (z = -4.99, $p < 0.01$). Because one of our goals was to examine links between individual differences in sustained attention and effortful control and fearfulness, data on the 'wooden toy' task were dropped from further analyses. There was no effect of age group (t (31) = -1.10, $p = .28$) or gender (t (31) = 1.13, $p = .27$) on sustained attention.

Effect of temperament on sustained attention

The effect of the CBCL internalizing and externalizing dimensions on sustained attention was next explored. Following median split procedures we created two subgroups for the internalizing dimension (median CBCL internalizing score = 43.0; high CBCL internalizing group (n = 19): mean CBCL internalizing score = 52.2, low CBCL internalizing group (n = 12): mean CBCL internalizing score = 35.7), and two subgroups for the externalizing dimension (median CBCL externalizing score = 51.0; high CBCL externalizing group (n = 16): mean CBCL externalizing score = 57.0, low CBCL externalizing group (n = 15): mean CBCL externalizing score = 45.7). No main effect of internalizing group on sustained attention was found (music box: $mean_{HIGH} = 14.9$, sd = 6.3, $mean_{LOW} = 13.1$, sd = 5.1; F (1, 29) = .92, $p = .35$), nor was there an interaction between internalizing group and sustained attention (F (1, 29) = .000, $p = .99$). Similarly, there was no effect of externalizing group on sustained attention (music box: $mean_{HIGH} = 16.3$, sd = 5.9, $mean_{LOW} = 12.0$, sd = 5.1; F (1, 29) = 1.8, $p = .19$) but this time there was a marginally significant interaction between externalizing group and sustained attention (F (1, 29) = 2.9, $p = .10$). An independent samples t-test showed that the high externalizing group did significantly better on the 'music box' than the low externalizing group (t (29) = -2.22, $p < 0.05$, see Figure 6-5).

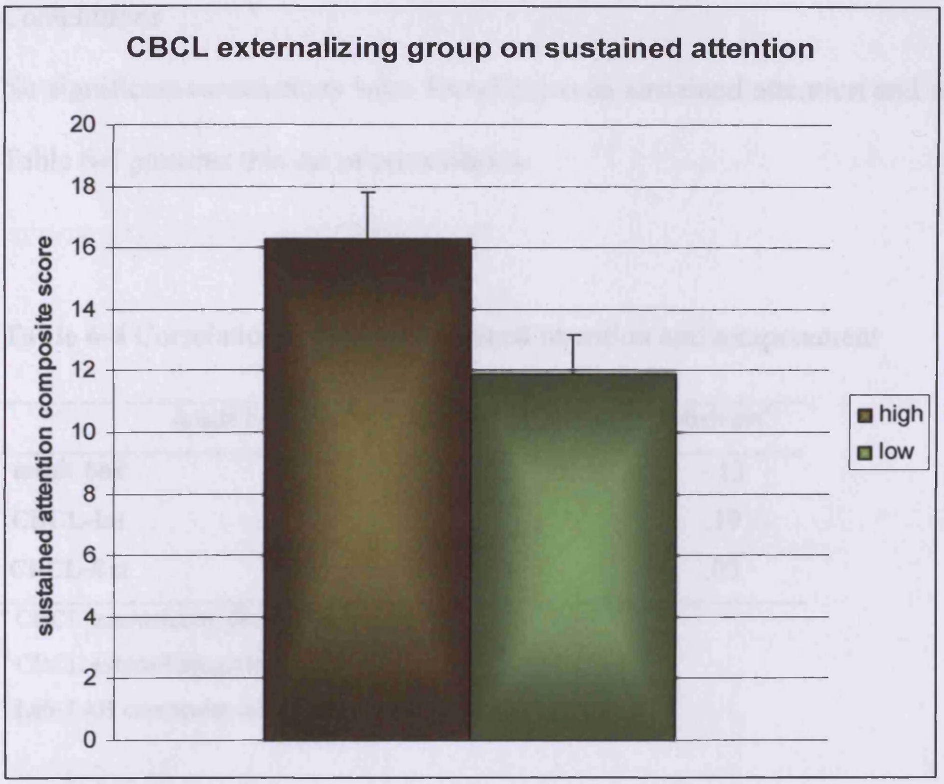

Figure 6-5 Sustained attention in high and low CBCL externalizing groups during the music box task, with bars indicating the SE's

Next, we explored the effect of distress behaviour on sustained attention. Following a median split procedure (median Lab-TAB composite distress score = 98.0) a high distress (n = 16, mean distress score = 131.8) and a low distress (n = 17, mean distress score = 49.1) group were created. Independent sample t-tests did not show a significant difference between the distress groups on sustained attention (music box: $mean_{HIGH} = 13.6$, sd = 5.8, $mean_{LOW} = 16.1$, sd = 6.0, $t\ (31) = .66$, $p = .52$).

Correlations

No significant associations were found between sustained attention and temperament. Table 6-4 presents this set of correlations.

Table 6-4 Correlations between sustained attention and temperament

	music box	CBCL-Int[a]	CBCL-Ext[b]	distress[c]
music box		.13	.26	-.13
CBCL-Int			.52**	.19
CBCL-Ext				.03

[a] CBCL internalizing dimension

[b] CBCL externalizing dimension

[c] Lab-TAB composite distress score

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

6.5 Results: Effortful control

6.5.1 Statistical analyses

Independent sample t-tests were employed to examine differences between age and gender subgroups on the snack delay task. Mann-Whitney nonparametric tests were used for the same purpose on the gift task variables, which were found to be skewed. Independent samples t-tests were also used to examine differences between Lab-TAB distress groups and the CBCL (internalizing-externalizing) groups on the snack delay variables, while for the same purpose Mann-Whitney nonparametric tests were used on the gift task variables. Pearson's correlations were employed when examining the relations between snack delay and the Lab-TAB distress score in both years, while Spearman's correlations were employed in all other cases.

Between subjects effects

Table 6-5 presents the descriptive information for the total group and the different subgroups on the snack and gift delay tasks.

Table 6-5 Means for the snack and gift task variables and their standard deviations, for the total group and the different subgroups

	snack delay-1		**snack delay-2**		**gift delay**		**gift latency**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Group(n = 33)	2.2	0.8	1.7	1.3	3.1	1.5	45.4	65.0
girls (n = 15)	2.3	1.0	2.0	1.4	3.3	1.4	43.2	66.1
boys (n = 18)	2.0	0.8	1.5	1.2	2.9	1.6	47.2	65.9
young (n = 16)	2.1	0.8	1.6	1.3	3.5	1.2	38.9	54.7
old (n = 17)	2.2	0.9	1.9	1.3	2.7	1.7	51.4	74.6

No significant differences were found between younger and older children on snack delay-1 (t (31) = -.31, p = .76) or snack delay-2 (t (31) = -.70, p = .49). Similarly, no gender differences were found on snack delay-1 or snack delay-2 (t (31) -1.0 p = .32; t (31) = -1.1, p = .28, respectively).

Mann-Whitney nonparametric tests did not show a difference between the two age groups on the gift task ('gift delay': U = 105.000, p = .25, 'gift latency': U = 130.000, p = .83), nor were there gender differences ('gift delay': U = 113.500, p = .42, 'gift latency': U = 121.000, p = .61).

Effect of temperament

Next we explored the effects of distress group (Lab-TAB) and CBCL internalizing and externalizing groups on effortful control (see pp. 19-20 for details). The two distress groups did not differ on the snack delay task (snack delay-1: $mean_{HIGH} = 2.1$, sd = 0.8, $mean_{LOW} = 2.2$, sd = 0.9; $t\,(31) = .51$, $p = .61$; snack delay-2: $mean_{HIGH} = 1.8$, sd = 1.3, $mean_{LOW} = 1.7$, sd = 1.4; $t\,(31) = -.17$, $p = .87$). Mann-Whitney nonparametric tests also did not show a difference between the two distress groups on the gift delay task (gift delay: $mean_{HIGH} = 3.5$, sd = 1.5, $mean_{LOW} = 2.7$, sd = 1.5, U = 92.500, $p = .11$; gift latency: $mean_{HIGH} = 57.4$, sd = 71.8, $mean_{LOW} = 34.1$, sd = 57.8, U = 107.000, $p = .29$).

The two internalizing groups did not differ on the snack delay task (snack delay-1: $mean_{HIGH} = 2.2$, sd = 0.9, $mean_{LOW} = 2.0$, sd = 0.8; $t\,(30) = -.63$, $p = .54$; snack delay-2: $mean_{HIGH} = 1.7$, sd = 1.5, $mean_{LOW} = 1.5$, sd = 1.2; $t\,(30) = -.30$, $p = .77$), nor did the two externalizing groups (snack delay-1: $mean_{HIGH} = 2.2$, sd = 0.8, $mean_{LOW} = 2.0$, sd = 0.9; $t\,(30) = -.82$, $p = .42$; snack delay-2: $mean_{HIGH} = 1.8$, sd = 1.4, $mean_{LOW} = 1.5$, sd = 1.3; $t\,(30) = -.52$, $p = .61$). Mann-Whitney nonparametric tests revealed a significant difference between the internalizing groups on gift delay, but not on gift latency (gift delay: $mean_{HIGH} = 3.6$, sd = 1.5, $mean_{LOW} = 2.3$, sd = 1.3, U = 61.500, $p < 0.05$; gift latency: $mean_{HIGH} = 60.4$, sd = 76.6, $mean_{LOW} = 26.8$, sd = 39.7, U = 103.000, $p = .43$). More internalizing children were better able to delay opening and/or touching the present than less internalizing children. The same analyses done for the two externalizing groups showed no differences between these groups on the gift task (gift delay: $mean_{HIGH} = 3.3$, sd = 1.5, $mean_{LOW} = 2.8$, sd = 1.5, U = 104.500,

p = .36; gift latency: $mean_{HIGH}$ = 53.7, sd = 67.5, $mean_{LOW}$ = 39.8, sd = 64.9, U = 99.500, p = .28).

Next, two groups were created based on year 1 IBQ-fear scores (n = 30, median IBQ-fear = 2.6; high fear group (n = 14): mean IBQ-fear score = 3.5, low fear group (n = 16): mean IBQ-fear score = 1.9) and year 1 Lab-TAB composite distress scores (n = 32, median distress score = 47.0; high distress group (n = 16): mean distress score = 87.9, low distress group (n = 16) mean distress score = 23.2). Independent sample t-tests revealed a significant difference between the IBQ-fear groups with more fearful children showing better ability to delay on snack delay-1, but not on snack delay-2 (snack delay-1: $mean_{HIGH}$ = 2.4, sd = 0.7, $mean_{LOW}$ = 1.8, sd = 0.9; t (28) = -2.1, $p <$ 0.05; snack delay-2: $mean_{HIGH}$ = 2.0, sd = 1.2, $mean_{LOW}$ = 1.3, sd = 1.3; t (28) = -1.62, p = .12, see Figure 6-6). Mann-Whitney nonparametric tests showed no differences between these groups on the gift task (gift delay: $mean_{HIGH}$ = 2.9, sd = 1.6, $mean_{LOW}$ = 3.1, sd = 1.5, U = 14.500, p = .73; gift latency: $mean_{HIGH}$ = 34.6, sd = 60.6, $mean_{LOW}$ = 52.9, sd = 65.1, U = 84.500, p = .24).

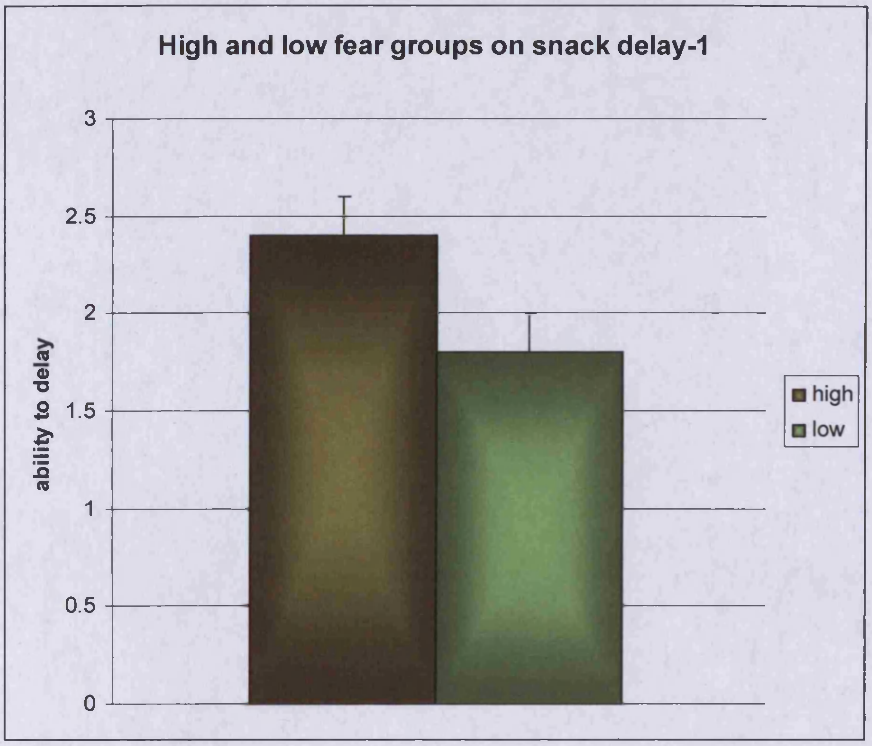

Figure 6-6 Snack delay-1 in high and low IBQ-fear groups, with error bars indicating the SE's

No significant differences were found between the two distress groups on the snack delay task (snack delay-1: $mean_{HIGH}$ = 2.2, sd = 0.9, $mean_{LOW}$ = 2.2, sd = 0.8; t (30) = .07, p = .94; snack delay-2: $mean_{HIGH}$ = 1.6, sd = 1.4, $mean_{LOW}$ = 1.9, sd = 1.2; t (30) = .68, p = .50) or the gift task (gift delay: $mean_{HIGH}$ = 3.4, sd = 1.6, $mean_{LOW}$ = 2.9, sd = 1.3, U = 99.000, p = .26; gift latency: $mean_{HIGH}$ = 66.9, sd = 77.5, $mean_{LOW}$ = 26.5, sd = 44.8, U = 109.000, p = .47).

Correlations

Since previous analyses revealed age group effects on the IBQ-fear and Lab-TAB year 1 composite distress scores, additional partial correlations controlling for age were computed between these variables and the EC variables. Snack delay-1 was significantly correlated with snack delay-2 (r = .94, $p < 0.01$), and with gift delay and gift latency (rho = .55, $p < 0.01$; rho = .37, $p < 0.05$, respectively). Snack delay-2 was significantly correlated with gift delay and gift latency (rho = .52, $p < 0.01$; rho = .40, $p < 0.05$, respectively). Gift delay and gift latency were also highly correlated (rho = .73, $p < 0.01$). With respect to temperament, a significant correlation was observed between CBCL internalizing scores and gift delay (rho = .43, $p < 0.05$) but no significant correlations were observed between the IBQ and Lab-TAB distress scales, on the one hand, and EC, on the other (seeTable 6-6). Finally, in line with Hill-Soderlund and Braungart-Rieker's (2008) research, the difference between Lab-TAB composite distress scores in year 1 and year 2 was calculated with this score reflecting the relative increase or decrease in observed distress from year 1 to year 2. We expected that this difference score would be positively related to EC, but the correlation between EC and this score turned out to be non-significant

Table 6-6 Correlations between EC and temperament dimensions of years 1 and 2

	fear[a]	CBCL-Int[b]	CBCL-Ext[c]	distress_1[d]	distress_2[e]	dif-score[f]
delay-1	.35 (.25)	.08	-.02	.08 (-.16)	-.02	-.06
delay-2	.29 (.25)	.03	-.08	.03 (-.22)	.04	-.002
gift delay	.01	.43*	.26	.25	.28	.12
gift latency	-.14	.15	.29	.20	.13	-.003

[a] IBQ-fear [b] CBCL internalizing dimension [c] CBCL externalizing dimension

[d] composite distress score year_1

[e] composite distress score year_2

[f] composite distress year_2 minus composite distress year_1

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

6.5.2 Developmental effects in sustained attention and associations with effortful control

Table 6-7 presents the descriptive information with respect to sustained attention for the total group across time. To examine whether there would be a main effect of time on sustained attention a paired sample t-test was performed on the music box task between the years. Pearson's correlations were performed to explore for consistency between the years. Spearman's correlations were also calculated when examining relations between the IBQ-fear, the CBCL scales and sustained attention.

Table 6-7 Mean sustained attention composite scores and their standard deviations in year 1 and year 2

	Year 1				***Year 2***			
	music box		**wheel**		**music box**		**wooden toy**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Group (n = 32)	15.0	5.8	25.7	7.2	14.2	5.8	27.8	6.0

Paired sample t-tests did not show a main effect of time (t (31) = -.52, p = .61) on sustained attention.

No significant correlations were found between music box_1 and music box_2 (r = -.27, ns). Table 6-8 presents this set of correlations (with values in brackets depicting partial correlations).

Table 6-8 Correlations between sustained attention and temperament in year 1 and 2

	music box_1[a]	IBQ-fear[b]	distress_1[c]
music box_2[d]	-.27	-.02 (-.05)	.12 (-.06)
CBCL-Int[e]	-.23	-.02	.19
CBCL-Ext[f]	-.10	-.14	.03
distress_2[g]	-.36	-.33	.04

[a] music box_ [b] IBQ-fear

[c] Lab-TAB distress score_1 [d] music box _2 [e] CBCL internalizing dimension

[f] CBCL externalizing dimension [g] Lab-TAB distress score_2

*= Correlation is significant at the 0.05 level

**= Correlation is significant at the 0.01 level

We next examined whether there was a relation between sustained attention (in year 1 and/or year 2) and effortful control. Pearson's correlations were employed with respect to the delay task, while Spearman's correlations were used with respect to the gift task. Table 6-9 presents this set of correlations. Unexpectedly, a significant negative association was found between music box_1 and gift delay (rho = -.42, $p <$ 0.05). No other correlations were observed between sustained attention and EC.

Table 6-9 Correlations between EC and sustained attention in year 1 and 2.

	music box_1[a]	music box_2[b]
delay-1	-.26	.000
delay-2	-.24	-.02
gift delay	-.42*	-.01
gift latency	-.20	.04

[a] music box_1 [b] music box_2

*.Correlation is significant at the 0.05 level (two-tailed)

**. Correlation is significant at the 0.01 level (two-tailed)

6.6 Discussion

The current study was designed to examine the proposed association between sustained attention and effortful control (EC). A longitudinal design was employed with measurements of sustained attention being taken in the 1st and 2nd years of life and the ability to delay gratification being measured in year 2. We also examined whether fearful temperament had an effect on EC and/or the ability to sustain attention. Until now, only a few studies examined the relation between sustained attention and EC, and the results were contradictory. Thus, although positive correlations have been reported between EC and fearful temperament (Kochanska & Kanack, 2003), some recent evidence indicated a negative association (Hill-Soderlund & Braungart-Rieker, 2008). In what follows we will review the evidence in support of the 5 hypotheses mentioned in the Introduction to the current chapter.

Because it has been claimed that sustained attention can be more reliably assessed using dynamic, complex, interactive and play-based situations than static ones (Choudhury & Gorman, 2000; Colombo et al., 2001) we used two different stimuli, one which allowed manipulation and the other which was merely observational, and expected to find an effect of stimulus type on sustained attention. This prediction was confirmed, with children in year 1 and 2 showing much better sustained attention during the dynamic stimulus than during the static one. However, because the data on the dynamic stimulus showed ceiling effects in both years, further analyses only involved data on the non-manipulative task.

Sustained attention increases with age (Choudhury & Gorman, 2000) and we expected to find more sustained attention in older children. This hypothesis could not be confirmed, because we found no differences in sustained attention between older and younger children within the year groups, nor was there better sustained attention in year 2 as compared to year 1. Sustained attention has been shown to increase from 7 months onwards. Ruff and Lawson (1990) found that sustained attention increased significantly from the 1st to the 2nd and the 3rd year of life. They measured sustained attention during free-play episodes. Kochanska, Murray and Harlan, (2000) also used a free play episode for measuring sustained attention at 9 months of age, which lasted for up to 5 minutes. We provided the child with 2 toys for the duration of 2 minutes each, and the toy we used in our analyses did not involve free-play. Some children persistently asked their mums to bring the toy closer to them, which they were not allowed to do. This restriction might have had a negative impact on the children's interest in the music box, especially in year 2. While look duration has been reported to increase with age during presentation of an interactive stimulus (Choudhury & Gorman, 2000) information processing is faster and look duration is shorter as a result of cognitive development in older children (Courage, Richards & Reynolds, 2006). The failure to find a significant increase in sustained attention across years could thus be related to more efficient information processing skills in the older children. Another factor that might have limited our ability to detect developmental effects in sustained attention is related to individual differences in the development of sustained attention. We observed a non-significant negative correlation between sustained attention in year 1 and 2 ($r = -.27$). It is possible that over time some children showed an increase in sustained attention and others a decrease.

We also predicted age effects with respect to EC. It has been stated that EC emerges between 6 and 12 months of age (Rothbart, Derryberry & Posner 1994) and becomes increasingly coherent during the 2nd year of life with rapid developmental increases between the ages of 3 and 6 years (Carlson, 2005; Carlson & Wang, 2007; Reed, Pien & Rothbart, 1984). Although we predicted better ability to delay in relatively older children no differences between older and younger age groups were observed. Although the mean age in the younger group was 19.7 months and 24.8 months in the older group, most children fell within the 21 to 24 months age range, which might have made it difficult to detect differences in EC between age groups. Kochanska et al. (2000) found developmental effects between 22 and 33 months of life, with 33-month-olds doing better on EC tasks.

As for gender, several studies have reported better EC in girls (Kochanska et al., 2000; Kochanska & Knaack, 2003). Following this evidence girls were expected to be better in EC but not in sustained attention. Our analyses did not show any gender differences in effortful control or sustained attention. Kochanska et al (2000) found gender differences on the snack delay and gift tasks at 22 and 33 months. Kochanska et al.'s sample was larger, consisting of 106 22-month-olds and 104 33-month-olds, and the power of the observed effects was weak (with d ranging between 0.2 - 0.4). As for sustained attention, Greenberg and Waldmant (1993) found gender differences in sustained attention in older groups (6-16 years). It might be the case that gender differences in sustained attention might become more apparent at a later age.

Another important goal of the study was to examine the association between sustained attention and EC. Kochanska et al. (2000) showed that sustained attention at 9 months predicted better EC at 22 months ($r = .27, p < 0.01$) but not at 33 months ($r = 0.05$, ns). We predicted that sustained attention in year 1 would be positively correlated with sustained attention in year 2, and that both would be related to effortful control in year 2. Unexpectedly, sustained attention in year 1 and 2 were not correlated (see Table 6-8) and inversely related to EC (with correlations ranging between -.20 and -.42, see Table 6-9). We already discussed the absence of an increase in sustained attention over time. The role of developmental differences in explaining the lack of stability in sustained attention over time could also be part of the explanation for the negative relation between sustained attention and EC. This inverse correlation could also be seen in a different light. Hill and Braungart-Rieker (2002) found a positive relation between the ability to shift attention away from a frustrating source (i.e., attentional orienting) and later self-regulatory abilities. Thus, the ability to refocus, disengage attention rather than sustained attention might be more related to later developing EC.

Fearfulness has been associated with better EC. Kochanska and Knaack (2003) showed that infants who expressed stronger novelty fear at 14 and 22 months demonstrated better EC performance at 22-, 33- and 45-months-of-age. Also, parental reports of infant fear at 22-months correlated positively with EC. Therefore, we predicted that more fearful children (based on IBQ-fear and Lab-TAB ratings) would show better EC and more sustained attention. We found that more fearful one-year-olds showed better ability to delay when they were 2 years old (see Figure 6-6). The current results also showed a significant positive association between internalizing temperament (measured by the CBCL internalizing scale) and delay behaviour on the gift task (rho = .43, $p<0.05$), but no other significant associations were found between temperament and EC or sustained attention. Developmental analyses in Chapter 3 showed us that although distress increased significantly over time, no individual stability was observed between year 1 and year 2. Therefore, developmental differences might have contributed to the absence of association between distress behaviour and EC. Moreover, fearfulness is sometimes viewed as a passive inhibitory dimension while EC is seen as an active one. These temperament dimensions could thus be unrelated (Kochanska & Knaack, 2003; Posner & Rothbart, 2000). As stated by Murray and Kochanska (2002) the modest relations often obtained in studies of EC probably mean that EC must be viewed as a complex characteristic, one that cannot be reliably assessed by using a single dimension. Finally, negative relations between fearfulness and EC have also been observed. Hill-Soderlund and Braungart-Rieker (2008) speculated that fearful children find their environments more threatening and concentrate their efforts on avoiding such situations. Consequently, they have fewer opportunities to learn effective coping techniques to deal with fear and ultimately suffer in their development of self-regulation and EC.

Poor EC has generally been linked to externalizing behaviour in school-aged children (Mischel, Shoda & Rodriguez, 1989). Although few empirical data exist in very young children, we predicted that more externalizing behaviour would be associated with less sustained attention and EC (Mischel, Shoda & Rodriguez, 1989; Olson et al., 2005; Kochanska & Knaack, 2003). Contrary to these predictions, children relatively high in externalizing behaviour were found to perform significantly better on the music box task in year 2 (see Figure 6-5) and were better in delaying on the gift task. As mentioned above, internalizing behaviour was also positively correlated with ability to delay on the gift task and part of these results might be explained by the significant positive correlation between the two CBCL scales (rho = .52; as reported in section 3.4.3). Another factor that might explain the results is the fact that all children (except one) scored in the normal range on the CBCL externalizing scale.

To summarize our findings, children showed more sustained attention during the stimulus they could manipulate than during the one they could only observe.

Do sustained attention (SA) and effortful control (EC) increase with age?

No effect of age on sustained attention or effortful control was found.

Are there gender differences in SA and EC?

No gender differences in sustained attention or effortful control were found.

Is there an association between SA and EC?

The current study failed to find a positive relation between sustained attention and effortful control. We attributed this unexpected result to the developmental of sustained attention in the first two years of life and to the nature of the attention and effortful control tasks we used. However, we found a positive relation between fearful temperament and EC as predicted, but no relation between distress and EC. Finally, and contrary to our predictions, children relatively high in externalizing behaviour were found to be better in sustained attention and delay of gratification.

It should be noted that performing multiple comparison tests on the current data might have increased the chances of committing Type I error.

Very few studies have examined the relation between EC and the emergence of behavioural problems in young children. It will be interesting to find out whether EC as an ability and temperament dimension continue to develop over time and whether early assessments of EC can reliably predict later EC. Given that individual differences in internalizing and externalizing problems also become more pronounced around the ages of 3-4 years, relations between EC and emotional and behavioural problems could possibly be better observed then.

The current study is one of the very few that aimed to study the association between fearfulness, sustained attention and later development of EC in very young, normally developing children. We pointed out that our sample might have been too small and too young to observe clear relations and that it will be interesting to examine how these constructs and their associations continue to develop in the 3rd year of life.

Chapter 7 General discussion

This thesis examined the development of fearful temperament in a sample of healthy infants during the first two years of life. Fearful temperament was investigated from a behavioural and physiological perspective and examined for its relations with sustained attention and effortful control. Other aims were related to investigating individual differences in fearful temperament, specifically to find out whether individual differences in behaviour and physiology were positively related and stable over time. The following sections describe and discuss the main findings of this research.

7.1 Individual differences in temperament: Main findings and limitations

We studied fearfulness in the first two years of life. In year 1 parents reported on their children's fearful temperament via the IBQ-R (Gartstein & Rothbart, 2003) while in year 2 they filled out the CBCL questionnaire (Achenbach & Rescorla, 2000). In both years the mechanical toy episode from the Lab-TAB (Goldsmith & Rothbart, 1999) was used to induce fear and behavioural distress. Skin conductance (SCA) and cortisol were measured in both years as the physiological indices of fear and stress. Sustained attention was measured behaviourally in both years, while effortful control (EC) was only measured in year 2 because one-year-olds have not yet developed the ability to control their responses effortfully (Kochanska et al., 2000).

Does fear increase with age?

Based on parental reports, previous research has shown that fearfulness tends to increase by the end of the first year of life and onwards (Gartstein & Rothbart, 2003). For example, infants appear fearless in novel or fear-eliciting situations that scare them later in life. Cognitive development, including learning and memory, but also specifically the ability to recognise and appraise unfamiliar events as entailing potential danger, emerges by the end of the 1st year (Ollendick et al., 2001). We therefore expected to find age effects in behavioural and parent-reported fearfulness, as well as in cortisol and SCA reactivity. In confirmation of this hypothesis, relatively older infants (mean age 11.7 months) obtained higher scores on mother-rated fearfulness (IBQ-fear), were observed to be more distressed (Lab-TAB) and showed stronger cortisol reactivity than younger infants (mean age 8.0 months). Similar age effects were not observed in year 2. However, 2-year-olds showed higher levels of behavioural distress, had a higher amplitude and frequency of SCRs, and higher levels of SCL and cortisol during stress than 1-year-olds. Different in pattern, but similar to what is known about the development of heart rate in young children (Bar-Haim, Marshall & Fox, 2000), was the finding that resting levels of cortisol and SC were lower in year 2 as compared to year 1. Developmental effects related to more extensive experience, greater memory capacity, the development of language enabling the child to express emotions vocally (i.e. call the mother when distressed) and the formation of attachment relations by the end of the 1st extending into the 2nd year all might have contributed to the finding of greater fearfulness in older children.

Although we also expected relatively older infants (as compared to younger ones) and 2-year-olds children (as compared to one-year-olds) to be better in sustained attention and EC, no such effects were observed. This was ascribed to the limited age range in the 2-year-olds (affecting results on EC) and the type of attention task used (being only observational). Thus while sustained attention has been reported to improve with age (Ruff & Lawson, 1990), greater cognitive ability could have resulted in the children processing the attentional stimuli more quickly.

Are there gender differences in fearful temperament?

Although women are generally reported to be more fearful than men, inconsistent gender differences have been reported in younger age groups. We therefore did not expect to find gender differences on any of our measures, apart from a gender difference in EC (Kochanska, Murray & Harlan, 2000). We found indeed no differences between boys and girls in parent-rated fearful temperament, distress behaviour, fear physiology, sustained attention or EC. Although the latter finding was unexpected, closer inspection of previous results (Kochanska, Murray, & Harlan, 2000) revealed that the effect sizes were weak and the significant results could be attributed to the large samples size.

Social factors are clearly important in the development of gender differences and it is therefore not surprising that gender differences are not yet pronounced in one- and two-year-olds (Hay, 2007). As mentioned in Chapter 3, children enter into gendered social worlds that provide them with social roles that are clearly different for males and females. Thus, for example, children develop from an early age the view that girls are fearful and boys are fearless (Brody, 2000). The family system and peer group are the main social sources that dictate these gender stereotypes to children. However, these social influences seem to emerge later in life, after the 2nd year of life, when children are starting to become more cognitively and psychologically prepared to support and occupy these roles (Hay, 2007). The fact that no gender differences in fear distress or physiology were observed is in line with the absence of gender differences in cortisol and SCA (Collins & Frankenhaeuser, 1978; Jorgenson et al., 1988).

What are the consequences of a more or less fearful temperament?

In light of previous evidence showing stronger behavioural and physiological reactivity in more fearful children (Fowles, Kochanska & Murray, 2000; Kagan, 1994; Kagan, Reznick & Snidman, 1987) we predicted similar effects in our children. One-year-old children, who were observed to be more distressed, had higher SC and cortisol levels during stress, showed a larger increase in SCL and cortisol from baseline, and showed a larger number of SCRs during our habituation test. Similar relations were not observed in year 2. Mother-rated fearfulness in year 1 or year 2 was not significantly correlated with any behavioural or physiological stress index. Despite a few positive findings, we discussed in the individual chapters that mostly weak or non-significant associations have been reported between temperament questionnaires and observational (Carranza et al., 2000) or physiological measures (Strelau, 1998). This has been attributed to the nature of the questionnaire methodology, specifically the parents' inclination to give socially desirable answers and their inability to predict their children's behaviour in non-domestic, novel situations (Kagan, 1994). Interestingly, prior to our fear exposure 85% of the mothers - regardless of the age of her child - predicted that her child would *not* be distressed during the robot episode.

Although observed levels of distress were related to stronger physiological reactivity in the 1st year, the same was not found in year 2. The absence of an association between physiology and observed distress in year 2 was attributed to developmental effects, because there was no stability in behaviour or physiology across the years. Cognitive development and maturation of the HPA axis and SNS continue throughout the 2nd year of life and consistency between these correlates of fearfulness might therefore be difficult to obtain at this point in time.

We also examined the relation between fearful temperament and effortful control. More fearful children in year 1 and more internalizing children in year 2 - as rated by their mothers - were better able in delaying gratification, thus supporting the link between fearfulness and EC. This finding is important, because it has been shown that EC regulates negative reactivity or distress in order to accomplish other internal goals, and because both temperament dimensions play an important role in the development of conscience (Rothbart, 2007). On the other hand, observed fearfulness was not associated with EC. Fearfulness is sometimes viewed as a passive inhibitory dimension while EC is seen as an active one. These temperament dimensions could thus be unrelated (Kochanska & Knaack, 2003; Posner & Rothbart, 2000). Moreover, the fact that distress behaviour (observed fearfulness) increased significantly with age, in combination with the possibility that fearful children concentrate their efforts on avoiding fearful situations, thus having fewer opportunities to learn effective coping techniques to deal with fear and consequently suffer in their development of self-regulation and EC (Hill-Soderlund & Braungart-Rieker, 2008), might also explain this result.

Finally, in line with previous studies in older children (Snoek et al., 2004; Van Goozen et al., 2000) we investigated whether less fearful, more externalizing children would show less distress, have lower levels of physiological arousal, and perform worse on EC. Only a difference in baseline SCL was found with more externalizing children having lower SCL baselines. Low resting levels of physiological arousal have been hypothesized to reflect sensation seeking motivation, which in turn may predispose to externalizing types of behavioral problems such as aggression and antisocial behavior (Van Goozen et al., 2000). Follow-up research in our sample will need to examine not only whether this finding can be replicated when the children are older, but also what the behavioural implications of low resting SCLs are.

Some limitations

The results of our studies should be interpreted with caution. Given the small sample size and low range in phenotypical and physiological scores, we used median split procedures rather than absolute temperament values to compare groups. Although dichotomizing a variable simplifies and facilitates data presentation, it has been suggested that it reduces statistical power and increases the risk of a positive result being a false positive (MacCallum, Zhang, Preacher, & Rucker, 2002). Moreover, many comparison tests were carried out on the same set of data, which might have increased the probability of committing Type I errors. However, in addition to using median split procedures, we also calculated correlations to overcome this limitation to some extent.

Although repeated measurement analyses over two points in time could provide an estimation of the amount of change, developmental research ideally uses more than two time points to study the shape and rate of individual developmental trajectories (Duncan & Duncan, 2004). One such methodology that models growth trajectories at both the group and individual level, and examines specific factors as predictors of change and rate of development, is the latent growth model (LGM) (Duncan & Duncan, 2004). The current study is part of a larger longitudinal study. By incorporating more waves of measurement and a larger sample of children we will be able in the future to use more sophisticated statistical methods to identify developmental trajectories of fearfulness.

Studies that succeeded in finding relations that we failed to observe either used larger samples (Gartstein & Rothbart, 2003; Kochanska & Knaack, 2003), involved older children (Fowles, Kochanska, & Murray, 2000; Scarpa et al., 1997), or included children with more clinically significant behavioural problems (Herpertz et al., 2005; Van Goozen et al., 2000). Finally, as stated in the Introduction to the current thesis, although recent evidence has increased interest in the genetic influences on temperament, the current study focused specifically on the physiological correlates of fearful temperament because these have been shown to hold a prominent role in explaining patterns of normal and abnormal development. As mentioned before, increasing our sample size and extending our research into the 3rd year of life might enable us to draw firmer conclusions regarding the course and consequences of fearful temperament in early life.

Stability of temperament

Stability in temperament from year 1 to year 2 was not found on any of our measures. There are several reasons for this result. Firstly, not many studies in very young children have found positive correlations between temperament measures over time (Carnicero et al., 2000), or when these were found they turned out to be low (e.g. Rothbart, Derryberry & Hershey [2000] found cross age stabilities in observed fear ranging from .22 to .31). Secondly, we already mentioned that cognitive (i.e., memory and language ability) and emotion development (including the development of coping skills and the ability to regulate emotion), the establishment of attachment relationships and emerging motor skills, which take place by the end of the 1st and in the 2nd year of life (Gartstein & Rothbart, 2003), might have contributed to the absence of stability in our findings. Thirdly, changes in the biobehavioural organisation of the child in the 2nd year, such as the appearance of a circadian rhythm in sleep and HPA axis functioning, and possibly a similar developmental organisation of the SNS (Hernes et al., 2002) also could have played a role. Only cortisol stress reactivity showed stability over time ($r = .51$, $p < 0.01$) and it was therefore suggested that cortisol baseline levels were more affected by the establishment of an HPA axis' circadian rhythm and changes in biobehavioural organisation than cortisol stress responses.

Is there an association between sustained attention and effortful control?

Although previous research has identified positive associations between the abilities of sustained attention and effortful control, we found a negative association between both temperament dimensions. Individual differences in the development of sustained attention, with some children showing a decrease and others an increase, might explain this finding. Specifically, the suggestion that some older children showed greater cognitive efficacy and processed the stimuli faster (resulting in lower levels of sustained attention) might have contributed to the observed inverse relation between sustained attention and EC. It has been proposed that measures of attention disengagement, rather than look duration, might be better related to the concept of EC (Hill & Braungart-Rieker, 2002). Finally, EC is a complex temperament construct, which probably cannot be reliably assessed by using only one type of task, which is what we did (Murray & Kochanska, 2002). Extension of the current sample size and a follow-up study in the 3rd year of life might enable us to get a better insight in the relation between these temperament dimensions and how they develop over time.

7.2 Clinical implications and future research

An investigation of early temperament is important for our understanding of the socio-emotional development of children later in life, because temperament is an important factor in the formation of personality, and in normal and abnormal development. Research in developmental psychopathology reports that stable behavioural problems in early childhood, or even problems in pregnancy, may lead to psychopathology later in life (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003; Murray & Kochanska, 2002). Although temperament scholars have acknowledged that temperament has a biological basis, it is only fairly recently that studies have started to examine the role of biological factors in children's development. It is therefore not surprising that information regarding the development of temperament's physiology is lacking. Our study was one of the first to simultaneously measure infant behaviour and physiology during stress, and to compare this information with maternal reports of temperament to gain a more complete understanding of the temperament construct and its correlates across development.

Understanding abnormal development requires first of all a proper understanding of how normal development proceeds. Since not much is known about the development of the HPA axis and ANS system in normal healthy developing young children the current research might contribute to this hiatus in knowledge.

A relatively recent line of research has drawn attention to the important influence of the perinatal environment in neurobiological development. Research on prenatal stress in animals and humans supports the view that early stress has a programming influence on the foetal brain and might impose a general susceptibility to psychopathology (Huizink, Buitelaar, & Mulder, 2004). In addition, early adverse postnatal experiences have a similar programming influence on the brain and comparable outcomes (e.g., Halligan, Herbert, Goodyer, & Murray, 2004) and the quality of maternal care has become a tremendously important topic in developmental psychobiology (Albers, Riksen-Walraven, Sweep, & de Weerth, 2008; Sapolsky, 1997).

With respect to the activation of the HPA axis a stress hyporesponsive period (SHRP) has been reported to gradually develop over the first year of life, when it is difficult to produce elevations in cortisol levels in response to stressors. This period has been reported to protect the infant's developing brain from the impact of elevated glucocorticoids (Gunnar, & Fisher, 2006; Sapolsky & Meaney, 1986). Was there evidence of a SHRP in our research?

The current study found that physiological baseline levels were higher in year 1 than in year 2 and in both years children showed clear cortisol stress responses when challenged (with a mean cortisol %increase of 112% in year 1 and of 166% in year 2). Similar findings were observed with respect to SCL under stress (with a mean SCL %increase of 64% in year 1 and of 201% in year 2). Therefore, although strong physiological stress responses were elicited in most children in both years, the intensity of the physiological stress reactions was stronger in year 2. Specifically, two-year-olds showed significantly greater cortisol and SCL reactivity levels (Δ) and more behavioural distress than one-year-olds. We speculated that two-year-old children were more affected by the stressor due to their greater cognitive abilities and that one-year-olds were relatively protected because of their limited ability to appraise novel/ fearful situations. Thus our results support the idea of a stress hyporesponsive period emerging in the first year of life, even though strong physiological stress responses were observed in both age groups.

Thus although nature seems to protect young infants from experiencing early stress, the same does not hold in case of early, intense and frequent stress exposure. Early neglect and maltreatment have been associated with either hypo-cortisolism or hyper-cortisolism. Hypocortisolism consists of the production of low early morning levels of cortisol and a relatively flat pattern of cortisol production over the course of the day (Gunnar & Donzella, 2002). On the other hand, frequently or chronically elevated levels of glucocorticoids are likely to result in hyper-responsivity, high resting levels of cortisol, inefficient downregulation of hypothalamic CRH, and slow stress recovery (Gunnar & Fisher, 2006).

There is emerging evidence that the SHRP is maintained under conditions of sensitive and responsive caregiving (Albers et al., 2008). However, if this buffering mechanism is disturbed through low quality and insensitive caregiving high cortisol baselines and reactivity levels are observed (Blair, Granger, Willoughby, & Kivlighan, 2006) together with slow stress recovery (Albers et al., 2008). Finally, insecurely attached children have been shown to produce higher cortisol and SCA levels than their securely attached counterparts (Gillisen, 2007; Nachmias et al., 1996).

These studies demonstrate that even normal variations in the mother-infant relationship play an important role in children's psychobiological development with important implications for later psychological functioning. Thus another goal of future research will be to assess maternal sensitivity and responsivity and find out how mothers influence their children's experience and expression of emotion over time.

In Chapter 5 (p. 133), we described how long-term or intense stress can affect the development of the brain, including the HPA axis system, and ultimately have pathological effects for the organism (Sapolsky, Romero & Munck, 2000). We also noted that chronically elevated glucocorticoid levels can result in hyper-reactivity and inefficient down-regulation of hypothalamic CRH (i.e., hyper-cortisolism) or in counter-regulatory downregulation, which results in a flattening of the daytime rhythm (i.e., hypo-cortisolism) (Gunnar & Quevedo, 2007).

Fearful temperament has often been linked to stronger physiological reactivity. According to Kagan, Reznick and Snidman (1987) inhibited children display heightened physiological reactivity under stressful events and heightened physiological reactivity may lead to future anxiety disorders (Buss et al., 2004). On the other hand, lower physiological resting and reactivity levels have been found in children with externalizing behavioural problems (Herpertz et al., 2005; van Goozen et al., 2000). However as mentioned before, the physiological results are not always consistent and the patterns sometimes differ for normally or clinically defined children.

Whether or not variations in stress reactivity early in life are a marker for psychopathology later in life can only be tested in a large group of children who are tested multiple times with a paradigm that has been shown to reliably reflect individual differences in cortisol/stress reactivity. We have described that the current research has limitations and is only part of a more extensive, longer term line of research on the role of neurobiological factors in developmental psychology and psychopathology. The clinical relevance of this research therefore remains unclear at this point in time.

In the future it would be interesting to conduct a longitudinal study in a group of high risk children and expose them repeatedly and relatively frequently to the same stressor in order to find out how their behavioural and physiological re-activity develop over time, and what the consequences of these early patterns are for later emotional development and outcome. Among the children who are at risk and who have been shown to have relatively abnormal behavioural and physiological patterns of reactivity are those who have been neglected and maltreated (Gunnar & Fischer) and children who have been exposed to postnatal depression (Halligan, Herbert, Goodyer,

& Murray, 2004). Although the results of these studies are important, they also have some methodological limitations. For example, Gunnar, Morison, Chisholm, and Schuder (2001) examined the daily cortisol levels of 6 to 12-year-old children who had been adopted 6 and half years ago. Similarly, Halligan et al. (2004) measured the cortisol levels of 13-year-old children who had been exposed to maternal depression. However, in none of these studies the developmental trajectory of these children's physiological levels was studied prospectively.

Another group of children who are at risk for developing behavioural and emotional problems are children who live in deprived and low-income environments (Keenan, Gunthrope & Young, 2001) or who are exposed to parental psychopathology or relationship problems. Although these children are faced with adverse experiences and chronic stress not many studies have examined how these children cope with stress over time and whether their stress regulation is systematically related to development of psychopathology. It would be interesting to examine these children prospectively by exposing them at regular time intervals to a reliable stressor to examine their behavioural and physiological development over time.

The studies as reported in this thesis provide insight into the mechanisms that underlie variation in temperamental differences among children. As such they cast light on a substantively important question relating to factors that predict and explain change and continuity in children's temperamental differences, which are known to be precursors of children's long-term behavioural development.

References

Achenbach, T. M. & Rescorla, L. A. (2000). *Manual for the ASEBA preschool forms and profiles*. Burlington: University of Vermont Department of Psychiatry.

Ahadi, S., Rothbart, M. & Ye, R. (1993). Children's temperament in the U.S. and China: Similarities and differences. *European Journal of Personality, 7*, 359-377.

Ahadi, S. A. & Rothbart, K., M. (1994). Temperament, development, and the Big Five. In J. In C. F. Halverson, G. A. Kohnstamm & R. P. Martin (Eds.), *The developing structure of temperament and personality from infancy to adulthood* (pp. 189-207). Hiilsdale, NJ: Erlbaum.

Ainsworth, M. D. S. & Wittig, B. A. (1969). Attachment and the exploratory behavior of one-year-olds in a strange situation. In B. M. Foss (Ed), *Determinants of infant behaviour* (Vol. 4, pp. 113-136). London: Methuen.

Ainsworth, M. D. S., Blehar, M. C., Waters, E. & Wall, S. (1978). *Patterns of attachment: A psychological study of the Strange Situation*. Hillsdale, NJ: Erlbaum.

Albers, E. M., Riksen-Walraven, J. M., Sweep, F. C. G. J. & de Weerth, C. (2008). Maternal behavior predicts infant cortisol recovery from a mild everyday stressor. *Journal of Child Psychology and Psychiatry, 49 (1)*, 97-103.

Anderson, S.W., Aksan, N., Kochanska, G., Damasio, H., Wisnowski, J. & Afifi, A. (2007). The earliest behavioral expression of focal damage to human prefrontal cortex. *Cortex, 43*, 806-816.

Bar-Haim, Y., Marshall, P. & Fox, N. (2000). Developmental changes in heart periods and high frequency heart period variability from 4 months to 4 years of age. *Developmental Psychobiology, 37*, 44-56.

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin, 121*, 65-94.

Bartels, M., de Geus, E. J. C., Kirschbaum, C., Sluyter, F. & Boomsma, D.I. (2003). Heritability of daytime cortisol levels in children. *Behavior Genetics, 33*, 421-433.

Bee, H. (1997). *The Developing Child* (8^{th} Ed.). Harper Collins College Publishers.

Berk, L. E. (2009). *Child Development (*8th Ed). Pearson International Edition: USA.

Bernstein, A., Frith, C., Gruzelier, J., Patterson, T., Straube, E., Venables, P. & Zahn, T. (1982). An analysis of the skin conductance orienting response in samples of American, British, and German schizophrenics. *Biological Psychology, 14,* 155-211.

Blair, C. (2002). Early intervention for low birth weight, preterm infants: The role of negative emotionality in the specification of effects. *Development and Psychopathology, 14*, 311-332.

Blair, C., Granger, D., Willoughby, M., Kivlighan, K. & The family life project investigators (2006). *Annals of the New York Academy of Sciences, 1094 (Resilience in Children),* 263-267.

Brody, L. R. (2000). The *socialization of gender differences in emotional expression: Display rules, infant temperament, and differentiation.* In Fischer, A.H. (Ed.), Gender and emotion: Social psychological perspectives (pp. 24-47). Cambridge University Press: UK

Bugental, D. B., Martorell, G. A. & Barazza, V. (2003). The hormonal costs of subtle forms of infant maltreatment. *Hormones and Behavior, 43,* 237-244.

Buss, A. H. & Plomin, R. (1975). *A temperament theory of personality development.* New York: Wiley-Interscience.

Buss, K. A. & Goldsmith, H. H. (1998). Fear and anger regulation in infancy: Effects on the temporal dynamics of affective expression. *Child Development, 69*, 359-374.

Buss, A. H., Schumacher, J. R., Dolski, I., Kalin, N. H., Goldsmith, H. H., & Davidson, R. J. (2003). Right frontal brain activity, cortisol, and withdrawal behaviour in 6-month-old. *Behavioral Neuroscience, 117*, 11-20.

Buss, K. A., Davidson, R. J., Kalin, N. H. & Goldsmith, H. H. (2004). Context-specific freezing and associated physiological reactivity as a dysregulated fear response. *Developmental Psychology, 40*, 583-594.

Calkins, S. D. & Fox, N. A. (1992). The relations among infant temperament, security of attachment and behavioral inhibition at 24 months. *Child Development, 63,* 1456-1472.

Calkins, S. D., Fox, N. A. & Marshall, T. R. (1996). Behavioral and physiological antecedents of inhibition in infancy. *Child Development, 67*, 523-540.

Calkins, S. D. & Dedmon, S. E. (2000). Physiological and behavioral regulation in two-year-old children with aggressive/destructive behaviour problems. *Jouranl of Abnormal Child Psychology, 28,* 103-118.

Calkins, S. D., Dedmon, S. E., Gill, K. L., Lomax, L. E. & Johnson, L. M. (2002). Frustration in infancy: Implications for emotion regulation, physiological processes, and temperament. *Infancy, 3*, 175-197.

Carlson, S.M. (2005). Developmentally sensitive measures of executive function in preschool children. *Developmental Neuropsychology, 28,* 595-616.

Carlson, S.M. & Wang, T.S. (2007). Inhibitory control and emotional regulation in preschool children. *Cognitive Development, 22,* 489-510.

Carranza, J. A., Perze-Lopez, J., Salinas, M. G., & Martinez-Fuentes, M.T. (2000). A longitudinal study of temperament in infancy: Stability and convergence of measures. *European Journal of Personality, 14,* 21-37.

Cattell, R. B. (1965). *The Scientific Analysis of Personality.* Harmondsworth, England: Penguin Books.

Choudhury, N. & Gorman, S., K. (2000). The relationship between sustained attention and cognitive Performance in 17-24-Month Old Toddlers. *Infant and Child Development, 9,* 127-146.

Cicchetti, D. & Cohen, D. J. (1995). Perspectives on developmental psychopathology. In D. Cicchetti & D. J. Cohen (Eds.), *Developmental Psychopathology: Volume 1. Theory and Methods* (pp. 3-20). New York: Wiley.

Collins, A. & Frankenhaeuser, M. (1978). Stress responses in male and female engineering students. *Journal of Human Stress, 4,* 43-48.

Colombo, J., W, R. A., Shaddy, J. D., Greenhoot, F. A. & Maikranz, M. J. (2001). Heart-rate-defined phases of attention, look duration, and infant performance in the paired-comparison paradigm. *Child Development, 72*, 1605-1616.

Colombo, J. (2002). Infant attention grows up: The emergence of a developmental cognitive neuroscience perspective. *Current Directions in Psychological Science, 11*, 196-200.

Courage, M. L., Reynolds, G. D. & Richards, J. E. (2006). Infants' attention to patterned stimuli: Developmental change from 3 to 12 months of age. *Child Development, 77,* 680-695.

Crockenberg, S. C. & Leerkes, E. M. (2006). Infant and maternal behavior moderate reactivity to novelty to predict anxious behaviour at 2.5 years. *Development and Psychopathology, 18*, 17-34.

Davis, E. P., Bruce, J. & Gunnar, M. R. (2002). The anterior attention network: Associations with temperament and neuroendocrine activity in 6-year-old children. *Developmental Psychobiology, 40*, 43-56.

Davison, G. C. & Neale, J. (2001). *Abnormal Psychology.* New York: Wiley & Sons.

Dawson, M. E., Schell, A. M. & Catania, J. J. (1977). Autonomic correlates of depression and clinical improvement following eletcroconvulsive shock therapy. *Psychophysiology, 14*, 569-578.

Dawson, M. E., & Filion, D. L. & Schell, A. M. (1989). Is elicitation of the autonomic orienting response associated with allocation of processing resources? *Psychophysiology, 26,* 560-572

Dawson, M. E., Schell, A. M. & Filion, D. L. (1990). The electrodermal system. In J. T. Cacioppo & L. G. Tassinary (Eds.), *Principles of psychophysiology: Physical, social, and inferential elements* (pp. 295-324). New York: Cambridge University Press.

Dawson, M. E., Schell, A. M. & Filion, D. L. (2000). *The electrodermal system.* In J. T. Cacioppo, L. G. Tassinary & G. G. Berntson (Eds.), Handbook of Psychophysiology (2nd ed., pp. 200-223). Cambridge: Cambridge University Press.

de Haan, M., Gunnar, M. R., Tout, K., Hart, J. & Stansbury, K. (1998). Familiar and novel contexts yield different associations between cortisol and behavior among 2-year-olds. *Developmental Psychobiology, 31,* 93-101.

de Weerth, C. & van Geert, P. (2002). A longitudinal study of basal cortisol in infants: Intra-individual variability, circadian rhythm and developmental trends. *Infant Behaviour & Development, 25,* 375-398.

de Weerth, C., Zijl, R. H. & Buitelaar, K. J. (2003). Development of cortisol circadian rhythm in infancy. *Early Human Development, 73*, 39-52.

Dettling, A. C., Gunnar, M. R. & Donzella, B. (1999). Cortisol levels of young children in full-day childcare centres: relations with age and temperament. *Psychoneuroendocrinology, 24*, 519-536.

Diamond, S. (1957). *Personality and temperament.* New York: Harper.

Donzella, B., Gunnar, M. R., Krueger, W. K. & Alwin, J. (2000). Cortisol and vagal tone responses to competitive challenge in preschoolers: Associations with temperament. *Developmental Psychobiology, 37*, 209-220.

Duncan, T.E., Duncan, S. C. (2004). An introduction to Latent Growth Curve Modeling. *Behavior Therapy, 35,* 333-363

Edelberg, R. (1967). Electrical properties of the skin. In C. C. Brown (Ed.), *Methods in psychophysiology* (pp. 1-53). Baltimore: Williams & Wilkins.

El-Sheikh, M. (2005). The role of emotional responses and physiological reactivity in the marital conflict-child functioning link. *Journal of Child Psychology and Psychiatry, 46*, 1191-1199.

Eysenck, H. J. (1970). *The structure of human personality* (3rd Ed.). London: Methuen.

Eysenck, H. J. (1990). Genetic and environmental contributions to individual differences: The three major dimensions of personality. *Journal of Personality, 58*, 245-261.

Fairchild, G., Van Goozen, S.H., Stollery, S.J. & Goodyer, I.M. (2008). Fear conditioning and affective modulation of the startle reflex in male adolescents with early-onset and adolescence-onset conduct disorder and healthy control subjects. *Biological Psychiatry*, 63, 279-285.

Fere, C. (1888). Note on changes in electrical resistance under the effect of sensory stimulation and emotion. *Comptes Rendus des Seances de la Societe de Biologie, (Ser. 9)*, 217-219.

Fowles, D. C., Kochanska, G. & Murray, K. (2000). Electrodermal activity and temperament in preschool children. *Psychophysiology, 37*, 777-787.

Fung, M. T., Raine, A., Lynam, D. R., Venables, P. H., Loeber, R. & Steinhauer, S. R. (2005). Reduced electrodermal activity in psychopathy-prone adolescents. *Journal of Abnormal Psychology, 114*, 187-196.

Gao, Y., Raine, A., Dawson, M. E., Venables, P. H. & Mednick, S. A. (2007). Development of skin conductance orienting, habituation, and reorienting from ages 3 to 8 years: A longitudinal latent growth curve analysis. *Psychophysiology, 44*, 855-863.

Garon, N., Bryson, S.E. & Smith, I.M. (2008). Executive function in preschoolers: A review using an integrative framework. *Psychological Bulletin, 134,* 31-60.

Gartstein, A., M & Rothbart, M. K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behaviour & Development, 26,* 64-86.

Gilissen, R., Koolstra, C. M., van Ijendoorn, M. H., Bakermans-Kranenburg, M. J. & van der Veer, R. (2007). Physiological reactions of preschoolers to fear-inducing film clips: Effects of temperamental fearfulness and quality of the parent-child relationship. *Developmental Psychobiology, 49,* 187-195.

Gladman, G. & Chiswick, M. L. (1990). Skin conductance and arousal in newborn. *Archives of Disease in Childhood, 65*, 1063-1066.

Goldberg, S., Levitan, R., Leung, E., Masellis, M., Basile, V., Nemeroff & Atkinson, L. (2003). Cortisol concentrations in 12- to 18-month-old inbfants: stability over time, location, and stressor. *Biological Psychiatry, 54,* 719-726.

Goldsmith, H. H. (1987). *The Toddler Behavior Assessment Questionnaire: A preliminary manual.* Eugene, OR: University of Oregon, Department of Psychology.

Goldsmith, H. H., Buss, A. H., Plomin, R., Rothbart, K., M, Thomas, A. & Chess, S. (1987). Roundtable: What is temperament? Four approaches. *Child Development, 58*, 505-529.

Goldsmith, H. H. & Campos, J., J. (1982). Toward a theory of infant temperament. In I. R. N. E. R. J. Harmon (Ed.). *The development of attachment and affiliative systems* (pp. 161-193). New York: Plenum.

Goldsmith, H. H. & Rothbart, M. K. (1988). *Manual for the Laboratory Temperament Assessment Battery (version 1)*: Unpublished manuscript.

Goldsmith, H. H. & Campos, J. J. (1990). The structure of temperamental fear and pleasure in infants: A psychometric perspective. *Child Development, 61*, 1944-1964.

Goldsmith, H. H. & Rothbart, M. K. (1992). *The laboratory Temperament Assessment Battery (LAB-TAB): Locomotor Version 3.0* [Technical manual] Eugene: University of Oregon, Department of Psychology.

Goldsmith, H. H., Reilly, H. H., Lemery, K. S., Longley, S. & Prescott, A. (1994). *Manual for the Preschool Laboratory Temperament Assessment Battery (Lab-TAB):* Unpublished manuscript, University of Wisconsin-Madison.

Goldsmith, H. H. & Rothbart, M. K. (1996). *The Laboratory Temperament Assessment Battery (Lab-TAB)*: Locomotor, Version 3.0. [Technical manual] Madison, WI: Department of Psychology, University of Wisconsin.

Goldsmith, H. H. & Rothbart, M. K. (1999). *The Laboratory Temperament* Assessment Battery; Description of Procedures. Locomotor Version.

Goldsmith, H. H., Lemery, K. S., Aksan, N. & Buss, K. A. (2000). Temperamental Substrates of Personality Development. In V. J. Molfese & D. L. Molfese (Eds.), *Temperament and personality development across the life span* (pp. 1-29). Mahwah, NJ: Lawrence Erlbaum Associates Publishers.

Goldsmith, H. H. Hewitt, E. C. (2003). Validity of parental report of temperament: Distinctions and needed research. *Infant Behavior & Development, 26,* 108-111.

Gray, J. A. (1991). The neuropsychology of temperament. In I. J. S. A. Angleitner (Ed.), *Explorations in temperament: International perspectives on theory and measurement* (pp. 105-128). New York.

Greenberg, L. M. & Waldmant (1993). Developmental normative data on the Test of Variables of Attention (T.O.V.A.). *Journal of Child Psychology and Psychiatry, 34,* 1019-1030.

Gunnar, M. R., Leighton, K. & Peleaux, R. (1984). Effects of temporal predictability on the reactions of 1-year-olds to potentially frightening toys. *Developmental Psychology, 20*, 449-458.

Gunnar, M. R., Connors, J., Isensee, J. & Wall, L. (1988). Adrenocortical activity and behavioral distress in human newborns. *Developmental Psychobiology, 21*, 297-310.

Gunnar, M. R., Connors, J. & Isensee, J. (1989). Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. *Developmental Psychobiology, 22,* 221-233.

Gunnar, M. R., Mangelsdorf, S., Larson, M. & Hertsgaad, L. (1989). Attachement, temperament, and adrenocortical activity in infancy: A study of psychoendocrine regulation. *Developmental Psychology, 25*, 355-363.

Gunnar, M. R. (1992). Reactivity of the hypothalamic-pituitary-adrenocortical system to stressors in normal infants and children. *Pediatrics, 90*, 491-497.

Gunnar, M. R., Brodersen, L., Krueger, K. & Rigatuso, J. (1996). Dampening of adrenocortical responses during infancy: normative changes and individual differences. *Child Development, 67*, 877-889.

Gunnar, M. R., Tout, K., de Haan, M., Pierce, S. & Stansbury, K. (1997). Temperament, social competence, and adrenocortical activity in preschoolers. *Developmental Psychobiology, 31*, 65-85.

Gunnar, M. R., Morison, S.J., Chisholm, K., & Schuder, M. (2001). Salivary cortisol levels in children adopted from Romanian orphanages. *Development and Psychopathology,* 13, 611-628

Gunnar, M. R. & Vasquez, D. M. (2001). Low cortisol and flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology, 13*, 515-538.

Gunnar, M. R. & Donzella, B. (2002). Social regulation of the cortisol levels in early human dvelopment. *Psychoneuroendocrinology, 27*, 199-220.

Gunnar, M. R. (2006). Social regulation of stress in early child development. In K. McCartney & D. Phillips (Eds.), *The Blackwell handbook of early childhood development* (pp. 106-125): Blackwell Publishing.

Gunnar, M. R., Fisher, P.A. & The early experience, stress, and prevention network (2006). Bringing basic research on early experience and stress neurobiology to bear on preventive intervention for neglected and maltreated children. *Development and Psychopathology, 18,* 651-677.

Haley, D. W. & Stansbury, K. (2003). Infant stress and parent responsiveness: regulation of physiology and behavior during still-face and reunion. *Child Development, 74*, 1534-1546.

Halligan, S. L., Herbert, J., Goodyer, I. M. & Murray, L. (2004). Exposure to postnatal depression predicts elevated cortisol in adolescent offspring. *Biological Psychiatry, 55,* 376-381.

Ham, J. & Tronick, E. (2006). Infant resilience to the stress of the still-face: Infants and maternal psychophysiology are related. *New York Academy of Sciences 1094*, 297-302.

Harrison, D., Boyce, S., Loughnan, P., Dargaville, P., Storm, H. & Johnston, L. (2006). Ski conductance as a measure of pain and stress in hospitalised infants. *Early Human Development, 82,* 603-608.

Hay, D. F. (2007). The gradual emergence of sex differences in aggression: alternative hypotheses. *Psychological Medicine, 37,* 1527-1537

Hellerud, B. C. & Storm, H. (2002). Skin conductance and behavior during sensory stimulation of preterm and term infants. *Early Human Development, 70*, 35-46.

Hernes, G, Morkrid, L., Fremming, A., Odegarden, S., Martinsen, O. & Storm, H. (2002). Skin conductance changes during the first year of life in full-term infants. *Paediatric Research 52,* 837-843.

Herpertz, S. C., Mueller, B., Qunaibi, M., Lichterfeld, C., Konrad, K. & Herpertz-Dahlmann, B. (2005). Response to emotional stimuli in boys with conduct disorder. *American Journal of Psychiatry, 162*, 1100-1107.

Hibel, L. C., Granger, D. A., Kivlighan, K. T., Blair, C. & The family life project investigators (2006). Individual differences in salivary cortisol: Associations with common over-the-counter and prescription medication status in infants and their mothers. *Hormones and Behavior, 50,* 293-300.

Hightower, A. D., Spinell, A. P. & Lotyczewski, B. S. (1989). *T-CRS Guidelines.* Rochester, NY: Primary Mental Health Project.

Hill, A. L. & Braungart-Rieker, J. M. (2002). Four-month attentional regulation and its prediction of three-year compliance. *Infancy, 3*, 261-273.

Hill-Soderlund, A.L. & Braungart-Rieker, J.M. (2008). Early individual differences in individual temperamental reactivity and regulation: Implications for effortful control in early childhood. *Infant Behavior and Development 31,* (386-397).

Huizink, A. C., Robles de Medina, P. G., Mulder, E. J. H., Visser, G. H. A., & Buitelaar, J. K. (2003). Stress during pregnancy is associated with developmental outcome in infancy. *Journal of Child Psychology and Psychiatry, 44,* 810-818.

Huizink, A. C., Buitelaar, J. K. & Mulder, E. J. H. (2004). Prenatal stress and risk for psychopathology: Specific effects or induction of general susceptibility?, *Psychological Bulletin, 130,* 115-142

Johnson, M. H., Posner, M. I. & Rothbart, K., M. (1991). Components of visual orienting in early infancy: Contigency learning, anticipatory looking, and disengaging. *Journal of Cognitive Neuroscience, 3*, 335-344.

Jorgenson, R. J., Salinas, C. F., Dowben, J. S. & John, D. L. S. (1988). A population study on the density of palmar sweat pores. *Birth Defects Original Article Series, 24*, 51-63.

Jung, C. G. (1923). *Psychological types.* London: Routledge & Kegan Paul.

Kagan, J., Reznick, J. S., Clarke, C., Snidman, N. & Garcia-Coll, C. (1984). Behavioral inhibition to the unfamiliar: *Child Development, 55,* 2212-2225.

Kagan, J., Reznick, S. & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development, 58*, 1459-1473.

Kagan, J. (1989). The concept of behavioral inhibition to the unfamiliar. In J. S. Reznick (Ed.), *Perspectives on behavioral inhibition (*pp. 1-23). Chicago: University of Chicago Press.

Kagan, J. (1994). *Galen's Prophecy: Temperament in human nature.* NY: Basic Books.

Kagan, J., Snidman, N. & Arcus, D. (1995). The role of temperament in social development. *Annals of the New York Academy of Sciences, 771*, 485-490.

Kagan, J. (1998). Biology and the child. In W. Damon (Series Ed.) & N. Eisenberg (vol. Ed.), *Handbook of child psychology: Vol. 3. Social, emotional and personality development* (5th ed., pp. 177-235). New York: Wiley.

Kagan, J. & Snidman, N. (1999). Early childhood predictors of adult anxiety disorders. *Biological Psychiatry, 46*, 1536-1541.

Kagan, J. & Saudino, K. J. (2001). Behavioral inhibition and related temperaments. In R. N. Emde, J. K. Hewitt & J. Kagan (Eds.), *Infancy to Early Childhood: Genetic and Enviromental Influences on Developmental Change* (pp. 111-122). US: Oxford University Press.

Kalin, N. H., Larson, C., Shelton, S. E. & Davidson, R. J. (1998). Asymmetric frontal brain activity, cortisol, and behaviour associated with fearful temperament in Rhesus monkeys. *Behavioral Neuroscience, 112*, 286-292.

Keenan, K., Gunthorpe, D., & Young, D. (2001). Patterns of cortisol reactivity in African-American neonates from low-income environments. *Developmental Psychobiology, 41,* 265-276.

Kirschbaum, C. & Hellhammer, D. (1989). Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology, 22*, 150-169.

Kirschbaum, C., Wust, S. & Helhammers, D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine, 54,* 648-657.

Kirschbaum, C. & Hellhammer, D. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology, 19*, 313-333.

Kirschbaum, C., Klauer, T., Filipp, S.H. & Hellhammer, D.H. (1995). Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosomatic Medicine, 57,* 23-31.

Kochanska, G., Coy, K. C., Tjebkes, T. L. & Husarek, S. J. (1998). Individual differences in emotionality in infancy. *Child Development, 64*, 375-390.

Kochanska, G., Murray, K. T. & Harlan, E. T. (2000). Effortful control in early childhood: continuity and change, antecedents, and implications for social development. *Developmental Psychology, 36*, 220-232.

Kochanska, G., Coy, K. C. & Murray, K. T. (2001). The development of self-regulation in the first four years of life. *Child Development, 72*, 1091-1111.

Kochanska, G. & Knaack, A. (2003). Effortful control as a personality characteristic of young children: Antecedents, correlates, and consequences. *Journal of Personality, 71*, 1087-1112.

Komsi, N. R., Raikkonen, K., Pesonen, A. K., Heinonen, K., Keskivaara, P., Jarvenpaa, A. L. & Strandberg, T. E. (2006). Continuity of temperament from infancy to middle childhood. *Infant Behavior & Development, 29*, 494-508.

Krueger, R. F., Caspi, A., Moffitt, T. E., White, J. & Stouthamer-Loeber, M. (1996). Delay of gratification, psychopathology, and personality: Is low self-control specific to externalizing problems? *Journal of Personality, 64,* 107–129.

Kugler, J., Reintjes, F., Tewes, V. & Schedlowski, M. (1996). Competition stress in soccer coaches increases salivary. Immunoglobin A and salivary cortisol concentrations. *Jouranl of Sports Medicine & Physical Fitness, 36,* 117-120.

Lawson, K. R. & Ruff, H. A. (2004). Early attention and negative emotionality predict later cognitive and behavioural function. *International journal of behavioral development, 28*, 157-165.

Lewis, M. & Ramsay, D.S., (1995). Developmental change in infants' responses to stress. *Child Development, 66,* 657-670.

Lewis, M. & Ramsay, D. S. (1999). Effect of maternal soothing on infant stress response. *Child Development, 70*, 11-20.

Lykken, D. T. & Venables, P. H. (1971). Direct measurment of skin conductance: A proposal for standardization. *Psychophysiology, 8*, 656-672.

MacCallum, R. C., Zhang, S., Preacher, J., & Rucker, D.D. (2002). On the practice of dichotomization of quantitative variables. *Psychological Methods, 7,* 19-40.

Magano. C. L., Diamond, E. J. & Gardner, J. M. (1989). Use of salivary cortisol measurements in young infants: A note of caution. *Child Development, 60,* 1099-1101

Malatesta, C. Z., Culver, C., Tesman, J. & Shepard, B. (1989). The development of emotion expression during the first two years of life. *Monographs of the Society for Research in Child Development, 54*, 1-104.

Matheny, A. P., Jr., Wilson, R. S. Thoben, A. S. (1987). Home and mother. Relations with infant temperament. Developmental Psychology, 23, 323-331.

Meaney, M., & Szyf, M. (2005). Environmental programming of stress responses through DNA methylation: Life at the interface between a dynamic environment and a fixed genome. *Dialogues in Clinical Neuroscience, 7,* 103-123.

McCall, R. B. (1986). Issues of stability and continuity in temperament research. In I. R. P. J. Dunn (Ed.), *The study of temperament: Changes, continuities, and challenges* (pp. 13-26). Hillsdale, NJ: Erlbaum.

Mishel, W., Shoda, Y. & Rodriguez, M. I. (1989). Delay of gratification in children. *Science, 244,* 933-938.

Munro, L. L., Dawson, M. E., Schell, A. M. & Sakai, L. M. (1987). Electrodermal lability and rapid performance decrement in a degraded stimulus continuous performance task. *Journal of Psychophysiology, 1*, 249-257.

Murray, K. T., Kochanska, G. (2002). Effortful control: Factor structure and relation to externalizing and internalizing behaviors. *Jouranl of Abnormal Child Psychology, 30,* 503-514.

Nachmias, M., Gunnar, M., Mengelsdorf, S. & Parritz, R. H. (1996). Behavioral inhibition and stress reactivity: The moderating role of attachment security. *Child Development, 67*, 508-522.

Netter, P. (1991). Biochemical variables in the study of temperament: Purposes , approaches, and selected findings. In J. Strelau & A. Angleitner (Eds.), *Explorations in Tempermanent: International Perspectives on Theory and Measurement* (pp 147-161). New York: Plenum.

Oldehinkel, A. J., Hartman, C. A., Ferdinand, R. F., Verhulst, F. C. & Ormel, J. (2007). Effortful control as modifier of the association between negative emotionality and adolescents' mental health problems. *Development and Psychopathology, 19*, 523-539.

Ollendick, T. H., Langley, A. K., Jones, R. T. & Kephart, C. (2001). Fear in Children and adolescent: Relations with negative life events. Attributional style, and avoidant coping. *Journal of Child Psychology and Psychiatry,42*, 1029-1034.

Olson, S. L., Schilling, E. M. & Bates, J. E. (1999). Measurement of impulsivity: construct coherence, longitudinal stability, and relationship with externalizing problems in middle childhood and adolescence. *Journal of Abnormal Child Psychology, 27*, 151-165.

Olson, S. L., Sameroff, A. J., Kerr, D. C. R., Lopez, N. L. & Wellman, H. M. (2005) Developmental foundations of externalizing problems in young children: The role of effortful control. *Development and Psychopathology, 17*, 25-45.

Pfeifer, M., Goldsmith, H. H., Davidson, R. J. & Rickman, M. (2002). Continuity and change in inhibited and uninhibited children. *Child Development, 73*, 1474-1485.

Posner, M. I. & Rothbart, M. K. (2000). Developing mechanisms of self-regulation. *Development and Psychopathology, 12*, 427-441.

Ramsay, D. & Lewis, M. (2003). Reactivity and regulation in cortisol and behavioral responses to stress. *Child Development, 74*, 456-464.

Raine, A. (1997). Autonomic nervous system factors underlying disinhibited, antosocial, and violent behavior: Biosocial perspectives and treatment implications. *Annals of the New York Academy of Sciences, 794*, 46-59.

Raine, A., Bihrle, S., Venables, P. H., Mednick, S. A. & Pollock, V. (1999). Skin conductance orienting deficits and increased alcoholism in schizotypal criminals. *Journal of Abnormal Psychology, 108*, 299-306.

Reed, M. A., Pien, D. L. & Rothbart, K., M. (1984). Inhibitory self-control in preschool children. *Merrill-Palmer Quarterly, 30*, 131-147.

Rescorla, L. A. (2005). Assessment of young children using the Achenbach System of Empirically Based Assessment (ASEBA). *Mental Retardation and Developmental Disabilities Research Reviews, 11*, 226-237.

Rosenthal, R. & Jacobson, L. (1968). *Pygmalion in the Classroom: Teacher Expectation & Pupils' Intellectual Development*. New York: Rinehart & Winston.

Rothbart, M. K. (1981). Measurement of temperament in infancy. *Child Development, 52*, 569-578.

Rothbart, M. K. & Derryberry, D. (1981). Development of individual differences in temperament. In M. E. Lamb & A. L. Brown (Eds.), *Advances in Developmental Psychology* (Vol. 1, pp. 37-86). Hillsdale, NJ: Erlbaum.

Rothbart, K., M. (1986). Longitudinal home observation of infants temperament. *Developmental Psychology, 22*, 356-365.

Rothbart, M. K. (1989). Biological processes in temperament: In G. A. Kohnstamm, J. E. Bates. & M. K. Rothbart (Eds.), Temperament in Childhood (pp. 77-110) Chichester, England: Wiley.

Rothbart, M.K., & Ahadi, S.A. (1994). Temperament and the development of personality. *Journal of Abnormal Psychology, 103,* 55-66.

Rothbart, K., M, Ahadi, S. A. & Hershey, K. L. (1994). Temperament and social behavior in childhood. *Merrill-Palmer Quarterly, 40*, 21-39.

Rothbart, M. K. & Bates, J. E. (1998) Temperament. In W. Damon (Series Ed.), & N. Eisenberg (Vol. Ed), *Handbook of Child Psychology: Volume 3. Social, Emotional and Personality Development,* 5th ed. (pp. 105-176). New York: Wiley.

Rothbart, M. K., Derryberry, D. & Posner, M. I. (1994). A psychobiological approach to the development of temperament. In J. E. Bates & T. D. Wachs (Eds.), *Temperament: Individual differences in biology and behavior* (pp. 83-116). Washington, DC: American Psychological Association.

Rothbart, K. M., Ahadi, S. A. & Evans, D. E. (2000). Temperament and personality: Origins and outcomes. *Journal of Personality and Social Psychology, 78*, 122-135.

Rothbart, M. K., Derry-Berry, D. & Hershey, R. (2000). Stability of temperament in childhood: Laboratory infant assessment to parent report at 7 years. In V. J. Molfese & D. L. Molfese (Eds.). *Temperament & Personality Development Across the Lifespan (pp. 85-119).* Mahwah, NJ: Erlbaum.

Rothbart, M. K., Hwang, J. (2002). Measuring infant temperament. *Infant Behavior & Development, 25, 113-116.*

Rothbart, K., M. (2007). Temperament, development, and personality. *Current Directions in Psychological Science, 16*, 207-112.

Ruff, H. A. & Lawson, K. R. (1990). Development of sustained, focused attention in young children during free play. *Developmental Psychology 26,* 85-93.

Rutter, M. (1985). Resilience in the face of adversity: Protective factors and resistance to psychiatric disorder. *British Journal of Psychiatry, 147,* 598-611

Sanchez, M. M. (2006). The impact of early adverse care on HPA axis development: Nonhuman primate models. *Hormones and Behavior, 50, 623-631.*

Sanson, A. & Rothbart, M. K. (1995). Child temperament and parenting. In M. H. Bornstein (Ed.), *Handbook of Parenting: Volume 4. Applied and Practical Parenting* (pp. 299-321). Mahwah, NJ: Erlbaum.

Sapolsky, R. M. & Meaney, M. J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain research, 396,* 65-76.

Sapolsky, R. M. (1997). Behavior: The importance of a well-groomed child. *Science, 277,* 1620-1621.

Sapolsky, R. M. (2004). *Why zebras don't get ulcers* (3rd ed.). New York: Henry Hold and Company, LLC.

Sapolsky, R. M., Romero, L. M., & Minck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21,* 55-89.

Saudino, K. J. (2005). Behavioral genetics and child temperament. *Journal of Developmental and Behavioral Pediatrics, 26,* 214-223.

Scarpa, A., Venables, P. H., Raine, A. & Mednick, S. (1997). Heart rate and skin conductance in behaviorally inhibited Mauritian children. *Journal of Abnormal Psychology, 106*, 182-190.

Schmidt, L. A., Fox, N. A., Rubin, K. H., Sternberg, E. M., Gold, P. W. & Smith, C. C. (1997). Behavioral and neuroendocrine responses in shy children. *Developmental Psychobiology, 30*, 127-140.

Schmidt, L. A. & Fox, N. A. (1998). Fear-potentiated startle responses in temperamentally different human infants. *Developmental Psychobiology, 32*, 113-120.

Schmidt-Reinwald, A., Pruessner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schurmeyer, T. H. & Kirschbaum, C. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences, 64,* 1653-1660.

Schwartz, E. B., Granger, D. A., Susman, E. J., Gunnar, M. R. & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development, 69,* 1503-1513.

Seeman, T. E., Singer, B., Wilkinson, C. W. & McEwen, B. (2001). Gender differences in age-related changes in HPA axis reactivity. *Psychoneuroendocrinology, 26*, 225-240.

Seifert, K. L., Hoffnung, R. J., & Hoffnung, M. (2000). *Lifespan Development* (2nd Ed.). Houghton Mifflin Company.

Singh, J. P., Larson, M. G., O' Donnel, C. J., Tsuji, H., Evans, J. C., Levy, D. (1999) Heritability of heart rate variability: The Framingham heart study. *Journal of the American Heart Association, 99,* 2251-2254.

Smider, N. A., Essex, M. J., Kalin, N. H., Buss, K. A., Klein, M. H., Davidson, R. J. & Goldsmith, H. H. (2002). Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective Study. *Child Development, 73,* 75-92.

Smith, P. K. & Hart, C. H. (2004). *Blackwell Handbook of Childhood Social Development.* Blackwell Publishing.

Snoek, H., van Goozen, S. H. M., Matthys, W., Buitelaar, J. K. & Engeland, H. V. (2004). Stress responsivity in children with externalizing behavior disorders. *Development and Psychopathology, 16,* 389-406.

Spangler, G. (1991). The emergence of adrenocortical circadian function in newborns and infants and its relationship to sleep, feeding and maternal adrenocortical activity. *Early Human Development, 25,* 197-208.

Spangler, G. & Schieche, M. (1998). Emotional and adrenocortical responses of infants to the strange situation: The differential function of emotional expression. *International Journal of Behavioral Development, 22,* 681-706.

Strelau, J. (1998). *Temperament: a Psychological perspective.* New York: Plenum Press.

Storm, H. (2000). Skin conductance and the stress response from heel stick in preterm infants. *Archives of Disease in Childhood Fetal Neonatal 83,* F143-F147.

Storm, H., Myre, K., Rostrup, M., Lien, M. D. & Raeder, J. C. (2002). Skin conductance correlates with perioperative stress. *Acta Anaesthesiologica Scandinavica, 46,* 887-895.

Stroganova, A. T., Orekhova, V. E., & Posikera, N. I. (1998). Externally and internally controlled attention in infants: an EEG study. *International Journal of Psychophysiology, 30,* 339-351.

Stroud, L.R., Salovery, P. & Epel, E.S. (2002). Sex differences in stress responses:social rejection versus achievement stress. *Biological Psychiatry, 52,* 318-327.

Thomas, A., Chess, S., Birch, H. G., Hertzig, M. E. & Korn, S. (1963). *Behavioral individuality in early childhood.* New York: New York University Press.

Thomas, A. & Chess, S. (1977). *Temperament & Development.* New York: Bruner/Mazel.

Tout, K., de Haan, M., Campbell, E. & Gunnar, M. R. (1998). Social behavior correlates of adrenocortical activity in daycare: gender differences and time-of-day effects. *Child Development, 69*, 1247-1262.

Tranel, D., Fowles, D. C. & Damasio, A. R. (1985). Electrodermal discrimination of familiar and unfamiliar faces: A methodology. *Psychophysiology, 22*, 403-408.

Tranel, D. & Damasio, H. (1994). Neuroanatomical correlates of electrodermal skin conductance responses. *Psychchophysiology, 31,* 427-438.

Tronick, E. Z., Als, H., Adamson, L., Wise, S. & Brazelton, T. B. (1978). The infant's response to entrapment between contradictory messages in face-to-face interaction. *Journal of American Academy of Child Psychiatry, 17*, 1-13.

Uhart, M., Chong, R. Y., Oswald, L., LinP. & Wand, G. S. (2005). Gender differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity. *Psychoneuroendocrinology, 31,* 642-652.

van Beijsterveldt, C. E., Molenaar, P. C., de Geus, E. J., & Boomsma, D. I. (1996). Heritability of human brain functioning as assessed by electroencephalography. *American Journal of Human Genetics, 58,* 562-573.

van Goozen, S. H. M., Matthys, W., Cohen-Kettenis, P. T., Buitelaar, J. K. & van Engeland, H. (2000). Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *Journal of American Academy of Child and Adolescence Psychiatry, 39*, 1438-1448.

Vaughn, B. E., Taraldson, B. J., Cuchton, L., Egeland, B. (1981). The assessment of infant temperament: A critique of the Carey Infant temperament Questionnaire. *Infant Behavior & Development, 4,* 1-17.

Vaughn, B. E., Taraldson, B. J., Cuchton, L., Egeland, B. (2002). The assessment of infant temperament: A critique of the Carey Infant temperament Questionnaire. *Infant Behavior & Development, 25,* 98-112.

Venables, P. H. & Christie, M. J. (1980). Electrodermal activity. In I. Martin & P. H. Venables (Eds.), *Techniques in Psychophysiology* (pp. 3-67). Chichester, UK: Wiley.

Wilcox, R. R. (2002). *Applying contemporary statistical techniques*. San Diego, CA: Academic Press.

Wolfe, C. D. & Bell, A. M. (2003). Working memory and inhibitory control in early childhood: Contributions from physiology, temperament, and language. *Developmental Psychobiology, 44*, 68-83.

Zimmerman, M. A., Arunkumar, R. (1994). Resiliency research: Implications for schools and policy. Social Policy report. *Society for research in child development, 8,* 1-18.

Zuckerman, M. (1991c). *Psychobiology of Personality.* New York: Cambridge University Press.

Apendix 1

Emotions and Coping in Children

We would like to invite you and your child to take part in a study of children's emotions and early coping behaviour. This would take up about 1 hour of your time, and would involve a visit to a specially designed playroom in the School of Psychology on Park Place, in central Cardiff. We can arrange parking or pay your bus or taxi fare.

We would like to find out why some children are born with a more easy going temperament than others. Temperamental differences in children are generally seen from birth onwards but especially also when children start to crawl and explore their environments. You will probably know that some children approach new people or new toys quite readily, whereas others prefer to stay closely to their parent and take time before cautiously approaching or responding. The reason is that children differ in fearlessness. Children who are less fearful react less strongly to novel events, and are more prepared to approach new objects and take risks.

We are particularly interested in the development of fear and risk taking in 8-12 months old children. If you decide that you want to participate, we will video your child's behaviour and record his/her bodily responses while we present him/her with different objects. We measure sweat reaction by attaching two metal stickers to the sole of your child's foot.

We also want to collect some saliva from your child. Some children are less likely than others to produce certain hormones that help them deal with new situations. We can measure that from their saliva.

In the session we will record your child's reaction to calming music, to novel sounds and to a robot toy, that slowly approaches your child.

To show our appreciation for your participation, we will give your child a present and give you a £10 gift voucher. In addition, we will pay for your travel to and from the School of Psychology.

For further details, you may ring the project coordinator at Cardiff University on 029 20 876191. Thank you for taking the time to read this information sheet. We hope that you will be able to help us.

CARDIFF UNIVERSITY PRIFYSGOL CAERDYDD

Appendix 2

Unpredictable Toy Scoring
EC: 1,2

Subject #________________ Scorer __________
Subject Name _____________ Date Scored _______
Visit 1 or 2 Tape # ________ Episode Order ______
Counter # ________ Experimenter Code ____
DOB ______ DOV ______

Latency to fear response
T1 _____s
T2 _____s
T3 _____s

Latency to sadness response
T1 _____s
T2 _____s
T3 _____s

	Trial 1				Trial 2			
Time Begin/End				Avg.				Avg.
Intensity of facial fear (0-3)								
Intensity of facial sadness (0-3)								
Intensity of vocal distress (0-5)								
Intensity of bodily fear (0-3)								
Presence of startle response 0=no 1=yes								
Intensity of escape behavior (0-3)								

	Trial 3			
Time Begin/End				Avg.
Intensity of facial fear (0-3)				
Intensity of facial sadness (0-3)				
Intensity of vocal distress (0-5)				
Intensity of bodily fear (0-3)				
Presence of startle response 0=no 1=yes				
Intensity of escape behavior (0-3)				

Parent behavior:_______

Baseline state:_______

Missing episode code _______

# of observed epochs _______

Unpredictable Toy
Version 3.10

Appendix 3

Coding Sheet For Music Box

	1 (30s):	2 (30s):	3 (30s):	4 (30s):	Average
Duration of looking (0-3)					
Presence of gestures (0,1)					
Presence of vocals (0,1)					
Intensity of facial interest (0-2)					

Coding Sheet for the rattle or the wooden toy

	1 (30s):	2 (30s):	3 (30s):	4 (30s):	Average
Duration of looking (0-3)					
Manipulations of stimuli (0-3)					
Intensity of facial interest (0-2)					