Frontal Cortex Functioning in Infancy

A thesis submitted for the degree of Doctor of Philosophy

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Declaration

I, Karla Holmboe, declare that the work presented in this thesis is my own.

A small part of the data reported in this thesis is based on work undertaken during my MSc in Cognitive Neuropsychology at University College London/Birkbeck in 2002-2003. This work involved testing a group of 24 9-month-old infants on two tasks, the Freeze-Frame task and the A-not-B task. This work was written up in my MSc dissertation, supervised by Dr. Pasco Fearon from University College London (now at University of Reading). During the first year of my PhD I followed up the same group of children, administering additional tasks when the children were 2 years old. The interpretation of the data collected at 2 years relies heavily on the data collected on the same children at the earlier age. These data have therefore been incorporated in my PhD thesis (Chapter 3). The remaining data reported in the thesis have been collected during the course of my PhD (Chapters 4-7).

Tuesday 9th of December 2008

Abstract

The functional status of the frontal cortex in infancy and early childhood is relatively poorly understood, and not much is known about the relationships between the few tasks developed to assess such functioning. The work presented in this thesis investigated these questions using an individual differences approach. Two cohorts of children were tested longitudinally on a set of tasks that have been associated with the frontal cortex in an attempt to establish whether these tasks share common functional mechanisms. Cohort 1 ($n = 24$) was tested at 9 and 24 months of age, and Cohort 2 ($n = 104$) was tested at 4 and 9 months of age. Furthermore, a new task, termed the Freeze-Frame task, was developed to assess inhibitory functioning in infancy using eye movements as the dependent measure. The relationship between performance on infant frontal cortex tasks and measures of temperament and general development was also investigated. Finally, a common genetic variation, the COMT Val¹⁵⁸Met polymorphism, which has been specifically associated with variation in the neurobiology of the frontal cortex, was investigated in relation to performance on the infant frontal cortex tasks. The results indicated that frontal cortex tasks administered at the same age shared functional mechanisms. Performance indices from the new Freeze-Frame task were associated with performance on several other infant and toddler frontal cortex tasks. Associations between frontal cortex tasks administered at 9 months were stronger than between the tasks administered at 4 months. Longitudinal associations were generally lacking between 4 and 9 months, but some strong associations were found between 9 and 24 months of age. At 4 months of age frontal cortex tasks tended to be associated with better self-regulation as assessed by a parent-report temperament questionnaire. However, at 9 and 24 months of age this pattern had reversed indicating that more reactive children tended to perform better on frontal cortex tasks. Some of the frontal cortex tasks were associated with measures of general cognitive development. However, the associations found between frontal cortex tasks could generally not be accounted for by variation in general cognitive ability. The COMT Val¹⁵⁸Met polymorphism was found to be related to a selective aspect of performance on the new Freeze-Frame task, but not to any of the other frontal cortex tasks. The results are discussed in terms of their implications for our understanding of frontal cortex functioning in infancy and early childhood.

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CHAPTER 1

General Introduction

Overview

The current thesis investigates the functions of the frontal cortex in infancy and early childhood. The frontal cortex is a part of the brain which is associated with higher cognitive functions, and it is also the part of the brain that undergoes the most prolonged anatomical development (e.g., Fuster, 2001; Huttenlocher, 1990). This makes it interesting in developmental research since important changes can be expected to occur continuously throughout development. The end result is a set of quite sophisticated, interrelated functions necessary for successful adaptation to a complex environment.

The fact that the functioning of the frontal cortex is complex and undergoes an extended period of development compared to other areas of the brain means that great care has to be taken when trying to assess its functions, particularly during development where there are several methodological constraints that need to be taken into account. Furthermore, the developing frontal cortex may function differently to the adult frontal cortex. This chapter will review what is presently known about the frontal cortex in terms of its anatomy, functions and development. Whereas our knowledge about the adult frontal cortex is extensive (though not always conclusive) and involves many cognitive domains, we know relatively little about the status of the frontal cortex in the infant.

First, a brief overview of the gross neuroanatomy of the frontal cortex will be presented. This is followed by a review of the development of the anatomical micro structure and

metabolism of the frontal cortex. An understanding of this area is important as the prolonged anatomical and metabolic development of the frontal cortex is likely to impact on behavioural measures of frontal cortex functioning at different points in time. Third, theories of adult frontal cortex functioning will be presented and discussed. This area of research is large and involves several long-standing controversies. Therefore, the review is far from exhaustive, and the focus is on some of the most influential theories and findings.

Fourth, an overview of what is known about frontal cortex functioning in childhood and infancy will be presented. Whereas, it is possible to adapt adult frontal cortex measures for use with children, studying frontal cortex functions in infancy is more challenging and requires additional methods and converging data. One way to approach this issue is to investigate individual differences in task performance. This method can be used to establish the relationship between different tasks developed to assess frontal cortex functioning in infancy within and across age in the same group of children. This approach provides the opportunity to test whether tasks developed to assess frontal cortex functioning share the same underlying functional mechanism, which in turn is an important step in accumulating converging evidence for frontal cortex functioning at this early age. Alternatively, direct indices of individual differences in frontal neurotransmitter systems, such as different genotypes, can be related to performance on frontal cortex tasks.

Surprisingly, these methods have only been used to a limited extent in the investigation of infant frontal cortex functioning. The last part of this chapter reviews the existing literature implementing these approaches, followed by an outline of the main research questions of this thesis.

Neuroanatomy of the frontal cortex

The cerebral cortex, i.e., the outer, convoluted layer of the brain, consists of four major divisions or *lobes*, the frontal lobe, the temporal lobe, the parietal lobe and the occipital lobe. These divisions are illustrated in Figure 1.1. The frontal lobe is separated from the parietal lobe by the central sulcus and from the temporal lobe by the Sylvian fissure.

Figure 1.1. The four lobes of the cerebral cortex (Source: Gazzaniga, Ivry, & Mangun, 2002).

The frontal lobe is further subdivided. These subdivisions are illustrated in Figure 1.2. Cortical areas based on Brodmann's cytoarchitectonic maps are presented in Figure 1.3. The most posterior part of the frontal lobe is the primary motor cortex. Anterior and ventral to the primary motor cortex are the secondary motor areas. These areas include the lateral premotor cortex and supplementary motor area (Brodmann area 6), the frontal eye fields (Brodmann area 8), Broca's area (Brodmann area 44/45), and the posterior part of the cingulate cortex (Brodmann area 23). The rest of the frontal cortex is termed the *prefrontal* cortex (PFC) (Gazzaniga et al., 2002; Jacobsen, 1935).

Figure 1.2. Subdivisions of the frontal lobe (Source: Gazzaniga et al., 2002).

Figure 1.3. Brodmann areas of the cerebral cortex (Source: Gazzaniga et al., 2002).

Subdivisions of the prefrontal cortex, as well as the names of these subdivisions, vary between accounts, but in general three main areas can be identified: the lateral prefrontal cortex (often divided into a dorsal and a ventral part), the ventral or ventromedial prefrontal cortex, and the anterior cingulate (Figure 1.2). In contrast to some posterior areas, such as the visual cortex, the functions of the prefrontal cortex often do not map neatly onto these anatomical divisions. Therefore, some theorists prefer to divide the prefrontal cortex on a functional basis. Some of these functional divisions will be discussed below in the section on adult frontal cortex functioning.

Structural and neurophysiological development of the frontal cortex

The frontal cortex develops more slowly than other cortical areas of the brain. For example Huttenlocher (1990) has demonstrated that several aspects of neural development such as changes in neuronal density, dendritic length and synaptogenesis show a much slower time course in the frontal cortex than in the visual cortex. In particular, synaptogenesis, the development of connections between neurons, shows a protracted development, with the number of synapses reaching its maximum around one year of age and subsequently showing a slow decrease until around 16 years of age. This decrease in synaptic density in the frontal cortex only starts to become evident around 7 years of age. In comparison, the number of synapses in the visual cortex reaches its maximum at 4 months of age and is close to adult levels at 7 years of age (Huttenlocher, 1990). More recently, Glantz and colleagues found that synaptogenesis in the frontal cortex continues even longer, until about 10 years of age, and is followed by a decline in synaptic density until adulthood (Glantz, Gilmore, Hamer, Lieberman, & Jarskog, 2007).

The structural development of the frontal cortex is paralleled by metabolic changes. For example, Chugani and colleagues have shown that glucose utilisation starts to increase around 6-

8 months of age in the frontal cortex and continues to increase until 3-4 years of age (Chugani, Phelps, & Mazziotta, 1987). In comparison, some subcortical areas and somatosensory cortex show high rates of glucose metabolism from birth (Chugani & Phelps, 1986; Chugani et al., 1987). These findings have been replicated by Chiron and colleagues (1992) in a study investigating relative changes in cerebral blood flow (rCBF: % of total CBF) during postnatal development. The latter study found that the frontal cortex reached adult levels of rCBF later (approx. 1-2 years of age) than primary sensory cortices (approx. 6 months of age) (Chiron et al., 1992).

More recently, Franceschini and colleagues used an optical imaging method termed frequency-domain near-infrared spectroscopy (FD-NIRS) to establish changes in cerebral blood flow and oxygen metabolism during the first year of life (Franceschini et al., 2007). This group found a linear increase in cerebral blood volume (closely correlated with oxygen metabolism) in the frontal cortex from birth onwards until it reached a plateau around 8-9 months of age. Interestingly, the linear increase in cerebral blood volume observed in the frontal cortex was in contrast to a steeper increase in the visual cortex occurring earlier, around 3-4 months of age. This developmental pattern is comparable to the structural changes observed by Huttenlocher (1990).

Recent magnetic resonance imaging evidence indicates that the frontal cortex, especially the dorsolateral prefrontal cortex, continues to develop into adolescence and even early adulthood (reviewed in Toga, Thompson, & Sowell, 2006). For example, Lenroot and Giedd (2006) have presented evidence that the cortical thickness of the dorsolateral prefrontal cortex reaches adult values relatively late during the adolescent years. Sowell and colleagues have shown that structural changes in grey matter density between childhood and adolescence primarily affect

posterior cortical areas (Sowell, Thompson, Holmes, Batth et al., 1999), whereas structural changes between adolescence and early adulthood take place in the prefrontal cortex and the striatum (Sowell, Thompson, Holmes, Jernigan, & Toga, 1999). Late adolescent maturation of dorsal prefrontal areas has also been found in a longitudinal study of cortical brain development between 4 and 21 years of age (Gogtay et al., 2004). Thus, the frontal cortex clearly undergoes a prolonged neuroanatomical development.

Functions associated with the frontal cortex in the adult brain

In the adult brain the frontal lobes have been implicated in a broad range of higher cognitive functions, and this area of the brain has puzzled neurologists and scientists for centuries (for historical reviews see Luria, 1966; B. L. Miller, 2007). Several theorists within the field point to the complexity of the role of the frontal lobes in human cognition as well as the difficulties associated with defining and segregating frontal cortex functions (e.g., Duncan, 2001; Gazzaley & D'Esposito, 2007; Luria, 1966). Some of these difficulties are clearly associated with differences in lesion location in neuropsychological studies and differences in methodology and aim in behavioural and neuroimaging studies. It is therefore important to clarify at this stage the definition of “frontal cortex function” as it is used here. The following will review some of the important functional divisions of the frontal cortex and discuss their relevance for this thesis.

The various theoretical and empirical accounts of frontal cortex function use a plethora of different anatomical and functional terms. However, most accounts separate the motor and premotor areas from the prefrontal cortex (see Figure 1.2). The motor cortex is involved in fine motor coordination and the premotor cortex in overall organisation of movement (B. L. Miller, 2007). Despite this distinction, several authors use the terms “prefrontal” and “frontal” interchangeably, referring to functions that go beyond simple motor coordination. Furthermore,

the link between motor functions and higher cognitive functions mediated by the prefrontal cortex has been emphasised by some authors. For example, Gazzaley and D'Esposito (2007) have suggested that the prefrontal cortex is the highest level of the motor system in both evolutionary and functional terms. However, according to this account, the prefrontal cortex has an overall role in guiding and controlling behaviour within several domains (Gazzaley & D'Esposito, 2007). Thus, even these accounts seem to regard the role of the prefrontal cortex in motor function as of a higher order than basic motor coordination.

In this thesis the focus is mainly on what would be defined as *prefrontal* cortex functions (see below), as opposed to basic motor skills, but since we know very little about the actual functional status of the frontal lobes in infancy and early childhood, the broader term “frontal” is used to refer to these functions. Even after excluding the primary and secondary motor areas, the range of functions ascribed to this area of the brain is diverse, and different accounts argue for different levels of unity within the prefrontal cortex.

A common functional division of the prefrontal cortex is that between the ventral (or ventromedial) and lateral prefrontal cortex (e.g., B. L. Miller, 2007; Stuss, 2007) (see Figure 1.2). The ventral prefrontal cortex has been associated with emotional processing, especially emotional self-regulation and goal-directed behaviour (Cummings & Miller, 2007; Damasio, 1994; Gazzaley & D'Esposito, 2007; Stuss, 2007). Damage to this area leads to a distinct pattern of disinhibited behaviour, social inappropriateness, poor judgement, and lack of empathy (Cummings & Miller, 2007). The most famous case involving damage to the ventromedial prefrontal cortex is probably Phineas Gage, a railway worker who went through a profound personality change after sustaining damage to the ventral prefrontal cortex (for a detailed account of this case, see Damasio, 1994).

Some accounts of the ventral prefrontal cortex further separate out the orbitofrontal or polar prefrontal cortex (i.e., the tip of the prefrontal cortex). For example Stuss (2007) defines the ventral prefrontal cortex as mediating self-regulatory and decision making processes, whereas the orbitofrontal cortex is proposed to be involved in metacognitive processes, thus integrating processes mediated by other prefrontal regions. A similar integrative role of the most anterior part of the PFC has been suggested by Fletcher and Henson (2001). The rostral prefrontal cortex, which is also located in the most anterior part of the PFC (area 10 in Figure 1.3), has recently been suggested to be involved in mentalising, the ability to understand other people's minds (Gilbert et al., 2007).

Compared to ventral frontal areas, the lateral surface of the prefrontal cortex has traditionally been associated with more cognitive functions. Such functions include generation, planning, working memory, overcoming habitual responses, and the ability to shift flexibly between response sets. In neuropsychology these functions are often referred to as *executive functions* and testing of frontal lobe patients usually involves measures of such functioning. However, the link between executive functions and the frontal lobes is not undisputed, and neither are the tests developed to measure executive functions (e.g., Kramer & Quinlan, 2007; Stuss, 2007). Furthermore, the definition of "executive" is not consistent across accounts. For example, Stuss (2007) defines executive functions in a strict cognitive sense, dissociated from regulatory/emotional aspects of frontal cortex function (associated with the ventral frontal cortex), whereas Gazzaley and D'Esposito (2007) use the term *executive control* to refer to the top-down control of both motor functions, thought processes (cognition), and emotion. Thus, even though some form of executive control is considered a key function of the prefrontal cortex in most theoretical accounts (Gazzaley & D'Esposito, 2007; Kerns et al., 2004; B. L. Miller,

2007; E. K. Miller & Cohen, 2001; Norman & Shallice, 2000), there is some disagreement as to whether this is primarily a function of the lateral surface (Stuss, 2007) or a more general functional principle (Gazzaley & D'Esposito, 2007; E. K. Miller & Cohen, 2001).

Even though the relationship between executive functions and the neuroanatomy of the prefrontal cortex is not entirely clear cut, there is consistent evidence that the dorsal part of the lateral prefrontal cortex, i.e., the dorsolateral prefrontal cortex, is involved in working memory, an essential aspect of executive function (B. L. Miller, 2007). Working memory implies a function that is more complex than simple short term storage of information, though such storage is part of the working memory system (D'Esposito & Postle, 1999). In particular, working memory involves manipulating and transforming information in order to plan and guide behaviour (D'Esposito & Postle, 2002; Goldman-Rakic, 1987). A classic task requiring such active maintenance of information is the *delayed-response* task. This task has been extensively investigated in non-human primates. It requires the participant to keep track of an object which is placed in one of two wells across repeated trials. After each hiding event a delay is imposed to tax the participant's working memory. Non-human primates as well as human adults with lesions in the dorsolateral prefrontal cortex fail on this task even at relatively short delays (Freedman & Oscar-Berman, 1986; Goldman-Rakic, 1987), indicating a difficulty in keeping the location information online in order to guide subsequent behaviour. Furthermore, such individuals typically show a striking behavioural pattern in that they tend to search the initial hiding location repeatedly (Diamond & Goldman-Rakic, 1989; Goldman-Rakic, 1987). Such behaviour has been termed *perseveration* and may indicate a lack of ability to inhibit a learnt or automatic response (Diamond, 1985, 2002).

As pointed out by Diamond and Goldman-Rakic (1989), the association between delayed-response performance and the dorsolateral prefrontal cortex is one of the most established brain-behaviour relationships in cognitive neuroscience. It has been replicated many times using several different methodological approaches such as ablation studies in monkeys (Goldman & Rosvold, 1970; Jacobsen, 1935, 1936), cortical cooling (Fuster & Alexander, 1970), single-cell recordings (Funahashi, Bruce, & Goldman-Rakic, 1989; Fuster & Alexander, 1971; Wilson, Scalaidhe, & Goldman-Rakic, 1993) and neuropsychological studies of patients with damage to the dorsolateral prefrontal cortex (D'Esposito & Postle, 1999; Freedman & Oscar-Berman, 1986; Verin et al., 1993) (for review see Fuster, 1997). Therefore, the dorsolateral prefrontal cortex is clearly involved in the active maintenance of memory representations and the ability to carry out actions based on these representations even when strong habitual responses exist.

Another area of the frontal cortex that has been extensively investigated is the anterior cingulate cortex/superior medial prefrontal cortex (Figure 1.2 and Figure 1.3, Brodmann areas 24, 25 and 32 [Gazzaniga et al., 2002]). This area has been related to controlled attention in neuroimaging research. Classic tasks involving the detection and resolution of conflicting information and response tendencies have been shown to activate the anterior cingulate, such as the Stroop task (Carter et al., 2000; Fan, Flombaum, McCandliss, Thomas, & Posner, 2003; Pardo, Pardo, Janer, & Raichle, 1990; Peterson et al., 2002), the flanker task (Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999; Fan et al., 2003), the Go-NoGo task (Liddle, Kiehl, & Smith, 2001), and the Simon/Spatial Conflict task (Fan et al., 2003; Peterson et al., 2002). All of these tasks involve *conflict* in that participants are confronted with interfering stimuli and / or strong response tendencies that need to be ignored or controlled in order to perform the correct response. However, some controversy exists as to whether the anterior cingulate primarily

monitors conflict, actively selects appropriate actions, or serves a more general evaluative function (for review, see Botvinick, Cohen, & Carter, 2004; Jonides, Badre, Curtis, Thompson-Schill, & Smith, 2002). It is also noteworthy that other areas of the frontal cortex (as well as some posterior areas) are often activated during conflict and interference tasks in conjunction with the anterior cingulate, and one study even found that the anterior cingulate modulated control processes carried out by the dorsolateral prefrontal cortex (Kerns et al., 2004).

Patients with damage to the anterior cingulate and superior medial prefrontal cortex are apathetic and lack motivation (Cummings & Miller, 2007; Stuss, 2007). These patients generally have difficulty generating and maintaining actions and mental processes. Stuss (2007) refers to such damage as leading to “disorders of energization and drive” (p. 294). For example, these patients are slow in simple reaction time tasks that require continuous and consistent responding (Stuss, 2007). These findings again emphasise the role of the anterior cingulate in active attentional processes.

Despite evidence that certain areas of the frontal cortex mediate specific functions, a meta-analysis by Duncan and Owen (2000) of more than 20 neuroimaging studies showed that the same areas, primarily the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex and the anterior cingulate, were activated in a wide range of different tasks involving functions such as working memory, response conflict, novelty detection, and perceptual difficulty. Duncan (2001) subsequently suggested that prefrontal neurons, across subdivisions, work in an adaptive way, adjusting flexibly to changing task demands. Apart from presenting evidence for the potentially unique way in which frontal neurons function, Duncan and Owen’s (2000) meta-analysis highlights the fact that the frontal cortex is involved in almost every kind of higher cognitive functioning.

In this thesis, the focus is mainly on the control aspect of frontal cortex functioning. As mentioned above, very little is known about the functional status of the frontal cortex in infancy, but the few studies that have provided evidence at this early age have used tasks associated primarily with the dorsolateral prefrontal cortex (e.g., Diamond, 1985; Diamond & Goldman-Rakic, 1989; Gilmore & Johnson, 1995; Johnson, 1995). Furthermore, there seems to be general agreement in the literature that the frontal cortex is important for controlling and planning behaviour. Some authors conceptualise highly separate, almost modular control processes specific to the subregions and subcortical connections of the frontal cortex (Cummings & Miller, 2007). Others advocate a much broader definition of control processes where frontal neurons in different areas take on a wide range of functional tasks (Duncan, 2001). Finally, some authors argue for a conceptualisation somewhere in between these two extremes. For example Gazzaley and D'Esposito (2007) suggest that the frontal cortex is composed of relatively discreet regions that form specific networks with posterior parts of the brain, but that the same governing principle (executive control) applies to all of these regions of the frontal cortex.

In infancy and early childhood the investigation of such control processes and their relationship to the frontal cortex have only just started to gather pace in the last three decades. Therefore, the exact neural substrate (within the frontal cortex) of these control processes in infancy is not a core question in this thesis, though it is one of the ultimate aims of this research endeavour. Instead, a broad range of control processes are investigated using tasks that have been developed specifically to assess processes similar to those that have been investigated in adult studies of frontal cortex functioning. Because substantially more is known about processes such as working memory and inhibitory control in infancy, the focus of this work is mainly on the more cognitive control processes associated with the lateral prefrontal cortex, whereas there is

less focus on areas associated with emotional processing and moral decision making, such as the ventral and orbitofrontal cortex.

Frontal cortex functioning in childhood

Given the complexity and diversity of the functional divisions of the frontal cortex discussed above, it is hardly surprising that the behavioural measurement of such functions is complex even in adults (e.g., Cummings & Miller, 2007; Kramer & Quinlan, 2007; Stuss, 2007). The difficulties involved in measuring such functions are greater still in children and infants because of the methodological limitations associated with research in this age group. Apart from needing to tap into different functions associated with the frontal cortex, frontal tasks designed for young children have to be adjusted to their limited language and motor skills. This is somewhat easier in preschool and school age children, and several tasks appropriate for this age group have been developed in recent years. These tasks are usually adaptations of adult frontal cortex tasks that have been modified to an age appropriate level. For example, Zelazo, Fry and Rapus (1996) have demonstrated developmental progression on a simplified version of the Wisconsin Card Sort Task in 3- to 5-year-old children. In this task children are required to shift flexibly between two sorting dimensions (shape and colour).

Similarly, Diamond and colleagues (Diamond & Taylor, 1996; Gerstadt, Hong, & Diamond, 1994) have shown that 3-year-olds have more problems inhibiting a prepotent response than older children in a modified version of the Stroop task. In this task, termed the *Day-Night task*, children have to say “day” to a picture of a moon and stars, and “night” to a picture of a sun, thereby inhibiting the most strongly associated response. Work on the Day-Night task has recently been extended by Simpson and Riggs (2005) who reported a steep non-linear increase in

performance on the inhibitory component of the task and a more linear trajectory on the working memory component of the task between 3.5 and 5 years of age.

Finally, recent studies have shown that 4-year-old children have great difficulty performing the executive attention component of the Attention Network Test (ANT), but that children reach adult performance levels around 7 years of age (Rueda, Fan et al., 2004; Rueda, Posner, Rothbart, & Davis-Stober, 2004; Rueda, Rothbart, McCandliss, Saccomanno, & Posner, 2005).

Thus, most of the preschool frontal cortex tasks involve an inhibitory component, i.e., overcoming an automatic or dominant response in order to perform an alternative response. Several of these tasks also involve a working memory component because they require the child to keep one or more rules in mind. The general finding from this research is that performance on such paradigms shows significant improvement during the preschool and early school years.

Frontal cortex functioning in infancy

Development of appropriate tasks for toddlers and infants has proven somewhat harder. One task that has been hypothesised to measure frontal cortex functioning in children as young as 2 years of age is the *Spatial Conflict task* (Gerardi-Caulton, 2000; Rothbart, Ellis, Rueda, & Posner, 2003), which has been shown to activate prefrontal areas and the anterior cingulate in adults (Fan et al., 2003; Peterson et al., 2002). In this task children have to match a picture at the top of a computer screen to one of two pictures at the bottom of the screen. The top picture appears either above the matching response option (congruent trials) or above the incorrect response option (incongruent trials) (Rothbart et al., 2003). Even adults have slower reaction times on incongruent trials compared to congruent trials (Fan et al., 2003), but young children show particularly strong interference effects. Thus, Gerardi-Caulton (2000) and Rothbart and colleagues (2003) demonstrated that 2-, 2.5- and 3-year-old children made more errors in the

incongruent condition than in the congruent condition, and that this accuracy interference decreased with age. Gerardi-Caulton (2000) also found a steady decrease in interference measured by reaction time between ages 2, 2.5 and 3. Rothbart et al. (2003) generally replicated this finding, but did not find a reaction time difference between congruent and incongruent trials in the 2-year-old group, perhaps due to the very long reaction times found at this age.

Both of the above studies also found that performance on the Spatial Conflict task was related to measures of Effortful Control as measured by parental questionnaires (Gerardi-Caulton, 2000; Rothbart et al., 2003). Effortful Control is a temperament dimension that has been consistently extracted in factor analytic work (Putnam, Ellis, & Rothbart, 2001; Putnam, Gartstein, & Rothbart, 2006), and has been hypothesised by Rothbart and colleagues to be associated with a frontal/anterior cingulate neural network mediating executive function (Rothbart et al., 2003). The general trend found in these studies was for children who showed large interference effects in the Spatial Conflict task to have less Effortful Control. These children also tended to express more negative affect (Gerardi-Caulton, 2000; Rothbart et al., 2003).

In infants, one of the few well-studied tasks which have been linked to the frontal cortex is the *A-not-B task*. The conceptualisation of the task was originally formulated by Piaget (1954) and later formalised by Diamond (1985). In the A-not-B task, an attractive object is hidden in one of two (or several) target wells, with the location where the object is hidden being varied over repeated trials depending on the infant's performance. Thus, the A-not-B task is very similar to the delayed-response task described above, the only difference being that the hiding pattern is pseudorandom (i.e., unrelated to performance) in the delayed-response task. In the A-not-B task infants around 8 months of age tend to search the initial hiding location repeatedly

despite the fact that they have seen the toy being hidden at the other location more recently (Diamond, 1985; Fox, Kagan, & Weiskopf, 1979; Gratch, Appel, Evans, LeCompte, & Wright, 1974). This behavioural pattern is often referred to as the *A-not-B error* or *perseveration* (see also section on “Functions associated with the frontal cortex in the adult brain” above). Between 7 and 12 months of age infants improve on the A-not-B task, sustaining increasingly longer delays without perseverating (Bell & Fox, 1992; Diamond, 1985). A similar developmental pattern has been observed on the delayed-response task in infants (Diamond & Doar, 1989).

The A-not-B task has been related to the functioning and integrity of the frontal cortex, in particular the dorsolateral prefrontal cortex, in several studies. For example, prefrontal damage in both humans and non-human primates leads to the perseverative pattern typically seen in young infants (Diamond & Goldman-Rakic, 1986, 1989; Freedman & Oscar-Berman, 1986; Verin et al., 1993). Furthermore, the task has been related to the frontal cortex in neuroimaging studies using EEG and optical imaging methodology (Baird et al., 2002; Bell, 2001; Bell & Fox, 1992, 1997).

Several other studies suggest that the frontal cortex is functional during the first year of life, perhaps as early as 4 months of age. Studies using eye movements as the dependent measure have shown some promise in tapping into frontal functioning at an early age. For example, Hofstadter and Reznick (1996) have shown that 7- to 11-month-old infants performed better on a two-location delayed-response task when gaze direction was used as the dependent measure instead of reaching. This method was even used with infants as young as 5 months of age, though at this age infants showed the classic perseverative pattern typically seen in older infants’ reaching behaviour (Hofstadter & Reznick, 1996). Bell and colleagues have validated a looking version of the A-not-B task by comparing performance on the looking version and reaching

version of the task in 8-month-old infants (Bell, 2001; Bell & Adams, 1999). However, unlike Hofstadter and Reznick (1996) they found equivalent performance levels for the two outcome measures. These results indicate that eye movements are a feasible measure of early frontal cortex functioning, which have the potential to assess such functioning at an earlier age than measures involving reaching or similar behaviours.

More evidence for frontal functioning before 7 to 8 months of age comes from Gilmore and Johnson (1995), who devised an oculomotor delayed-response task and showed that infants as young as 6 months of age may possess at least some working memory abilities. This technique was adapted from that developed by Funahashi and colleagues (e.g., Funahashi et al., 1989), who demonstrated that performance on the task by non-human primates was related to activity patterns in prefrontal neurons (see also Wilson et al., 1993).

Similarly, Johnson (1995) devised a task to measure the inhibition of automatic saccades. The task was designed in such a way that inhibiting an automatic saccade to a peripheral cue allowed infants to detect an interesting animated stimulus more rapidly. Results from the study indicated that infants as young as 4 months of age were able to inhibit automatic saccades. This result suggests that, when eye movements constitute the dependent measure, very young infants can perform tasks that require inhibitory control, a function that has been extensively related to the frontal cortex.

Whereas both studies on the functional and metabolic development of the frontal cortex indicate that important maturational changes occur during the second half of the first year of life (Chugani et al., 1987; Diamond, 2002; Franceschini et al., 2007), the functional role of the frontal cortex before 6 months of age is less well understood, and until more recently this area was considered “silent” due to its immature state in infancy (Johnson, 2005). As discussed

above, a couple of behavioural studies have suggested that 4- to 6-month-old infants have at least some abilities associated with the frontal cortex (Gilmore & Johnson, 1995; Johnson, 1995). Furthermore, a couple of recent studies using fMRI and near-infrared spectroscopy (NIRS) have shown activation in the frontal cortex during language processing and processing of novel stimuli as early as 3 months of age (Dehaene-Lambertz, Dehaene, & Hertz-Pannier, 2002; Nakano, Watanabe, Homae, & Taga, 2008).

There is also additional direct evidence from psychophysiological studies that the frontal cortex is functionally active from an early age. These studies have used event-related potentials (ERPs) derived from the electroencephalogram (EEG) to investigate the neural correlates of saccade planning in young infants (e.g., Csibra, Tucker, & Johnson, 1998, 2001; Richards, 2005). Compared to the aforementioned behavioural studies, which focused on demonstrating skills that have been specifically related to the frontal cortex in adults, the infant ERP studies have looked at very basic aspects of infants' ability to control their visual behaviour. Nevertheless, these studies have provided evidence that the frontal cortex, despite its prolonged development, is active from a very early age.

Two saccadic control tasks in particular have been associated with frontal ERP components. In the *Gap task* infants are first presented with a central stimulus on a computer screen. When the infant has fixated the central stimulus, a peripheral target is presented. The essential manipulation in the task is whether the central stimulus remains on the screen while the target is presented, or whether it disappears shortly before target presentation. Infants as well as adults consistently look faster to the target when the central stimulus disappears shortly before target onset (Csibra, Johnson, & Tucker, 1997; Csibra et al., 1998; Hood & Atkinson, 1993), and this effect has been termed the *Gap effect*. Furthermore, Hood and Atkinson (1993) found that 1.5-

month-old infants showed a significantly larger Gap effect than 3- to 6-month-old infants, indicating that a crucial development in the ability to disengage from the central stimulus occurs between 1.5 and 3 months of age. Interestingly, Csibra, Tucker and Johnson (1998) found a left frontal positivity in response to fixation offset in an ERP version of the Gap task with 6-month-old infants. The authors interpreted this effect as frontally mediated disinhibition of saccade-generating areas of the superior colliculus. A similar effect was found in adults in an earlier study (Csibra et al., 1997), and was later replicated in another study by Csibra and colleagues (2001)¹.

Another paradigm that has been associated with frontal ERP components is the *Anticipation task*. This paradigm was developed by Haith, Hazan and Goodman (1988), and involves presenting the infant with a predictable sequence of stimuli. Pictures are presented alternating in left and right target locations; a sequence which is not affected by the infant's looking behaviour. Haith and colleagues (1988) found that 3.5-month-old infants were able to move their eyes either before the next stimulus in the sequence appeared (*anticipation*) or faster after stimulus onset (*facilitation*) in the alternating sequence compared to a less predictable sequence. This effect has been extensively replicated in infants of different ages (Canfield, Smith, Brezsnyak, & Snow, 1997; Canfield, Wilken, Schmerl, & Smith, 1995; Csibra et al., 1997).

Haith and others have hypothesised that in order to be able to anticipate, the infant has to form an expectancy and act rapidly on this expectancy so as to foveate the new stimulus

¹ Even though most authors investigating the early development of visual attention agree that cortical areas gain control over a more primitive system for saccadic control during the first few months of life (Atkinson, 1984a, 1984b; Bronson, 1974; Johnson, 1990), the specific cortical areas and connections involved in this process are not fully established. The different theories on early cortical development in relation to eye movement control will be discussed in more detail in Chapter 4.

(Canfield et al., 1997; Haith et al., 1988). Similarly, Csibra et al. (2001) hypothesised that anticipatory saccades require the infant to make a volitional saccade to the new target location and that this process requires differential frontal cortex activation. Accordingly, in their ERP study with 4-month-old infants, Csibra et al. (2001) found that instead of the left-frontal positivity in response to target offset before reactive saccades (replicating Csibra et al., 1998), anticipatory saccades were preceded by a strong right frontal positivity. Even though the laterality of the effect was unexpected, this clearly suggests that the frontal cortex plays an active role in saccade planning as early as 4 months of age.

Individual differences in frontal cortex functioning

Despite encouraging evidence that the frontal cortex is functionally active early in infancy, studies in this area are limited to relatively crude localisation of neural activity. The ERP methodology used in the studies reviewed above has excellent temporal resolution, but relatively low spatial resolution. Some authors have used source modelling on EEG data to address this issue (e.g., Richards, 2005), and the recent development of near-infrared spectroscopy is starting to provide more information on the functional status of the frontal cortex in infancy (Homae, Watanabe, Nakano, & Taga, 2007; Nakano et al., 2008; Taga, Asakawa, Maki, Konishi, & Koizumi, 2003). Future studies using these recently developed neuroimaging methods in infancy will allow us to gain a better understanding of frontal cortex development. However, even with better localisation of neural activity in the frontal cortex, an understanding of the underlying processes is needed.

Another way of addressing the question of frontal functioning during infancy is through the study of individual differences in performance on frontal cortex tasks. When tasks are designed to assess frontal functioning in infancy and early childhood this is usually done on the basis of

theories of which cognitive processes are required to perform those tasks. In the case of older children it seems reasonable to assume that a virtually identical task measures the same function in children as in adults (e.g., Diamond & Taylor, 1996; Gerstadt et al., 1994). However, in infant research the situation is somewhat uncertain, and converging evidence is needed to demonstrate that similar processes are measured by different tasks developed to assess frontal cortex functioning.

One way to approach this issue is to collect data from the same group of children performing different purported frontal cortex tasks. If the tasks do indeed measure the same underlying function we would expect performance across tasks to correlate. This evidence would be even stronger if we can demonstrate that individual differences in task performance early in infancy predict performance later in infancy or, even better, during childhood and adulthood, where we are equipped with more established frontal cortex measures. Correlations among tasks within the same age would confirm that the tasks are tapping into the same underlying function. Even though we cannot be 100% sure that the measured function is frontal, we can approach this conclusion on the basis of converging evidence. To obtain such evidence we ideally need tasks that have a strong likelihood of measuring frontal cortex functioning, i.e., tasks that either have a strong theoretical basis and/or have been directly associated with the frontal cortex in neuroimaging and/or lesion studies.

Even though longitudinal correlations represent particularly strong converging evidence that a set of tasks tap into the same functioning, it is important to realise that a lack of such correlations does not preclude that tasks at each individual age measure the specific function. Longitudinal correlations require that individual differences in the measured function are stable across time, which might not be the case. However, in cases where longitudinal correlations are

found, this provides us not only with evidence that the function we are interested in is present from an early age, but also that it is relatively stable across development.

Another advantage of the individual differences approach is that it can elucidate component processes that are not obvious in the group data. For example, infants at a particular age may not show much evidence for frontal cortex functioning overall in a specific task. Nevertheless, it is possible that a small proportion of infants perform well on the task and that these infants also perform well on other frontal cortex tasks within and across age. This would indicate that some infants had acquired the skill early on and that the acquisition of the skill was associated with better performance on frontal cortex tasks in general.

Given the promise of this method, it is surprising that research on individual differences in frontal cortex functioning during development has been relatively sparse. Bell and colleagues have reported correlations between task performance on presumed frontal cortex tasks and frontal EEG activity in both infants and young children (Bell, 2001; Bell & Fox, 1992, 1997; Wolfe & Bell, 2004, 2007b). More recently Wolfe and Bell (2007a) did not find longitudinal stability of frontal cortex functioning. However, they did find that temperament characteristics in infancy and early childhood were related to frontal task performance at the later age (Wolfe & Bell, 2007a). This evidence indicates that individual differences in frontal cortex functioning exist from an early age, though stability of individual differences in such functioning is uncertain at this point.

Another way of tackling the question of individual differences in frontal cortex functioning during development is through the study of specific neurotransmitter systems in the frontal cortex and their functional correlates. For example Diamond, Prevor, Callender and Druin (1997) investigated children treated early and continuously for phenylketonuria (PKU) and found that

estimated dopamine levels in the frontal cortex affected children's performance on frontal cortex tasks in infancy and early childhood.

Related to this line of research, genetic variations impacting on neurotransmitter levels in the frontal cortex have been investigated as a way of understanding differences in frontal cortex functioning. This is a large area of research in psychiatry (e.g., schizophrenia and ADHD [Attention Deficit Hyperactivity Disorder] research), and some well-studied candidate genes, such as the Catechol-*O*-methyltransferase (COMT) gene, have also been related to differences in typical adult populations (e.g., Barnett, Jones, Robbins, & Müller, 2007). One study found an association between variation in the COMT gene and frontal cortex performance in children (Diamond, Briand, Fossella, & Gehlbach, 2004). Given the promising results from these studies, and the relatively well-understood and biologically plausible mechanisms of some of these candidate genes, it is surprising that no studies so far have investigated potential associations between genetic polymorphisms affecting neurotransmitters in the frontal cortex and frontal task performance in infancy.

Aims and framework of this thesis

The general aim of the work presented in this thesis was to provide converging evidence for frontal cortex functioning in infancy and early childhood. To elucidate this area, an individual differences approach was taken and implemented in a longitudinal design. The advantage of this approach is not only that it allows an estimation of which tasks tap into the same presumed frontal cortex processes, but that it allows us to track the stability of these processes during early development. Very little systematic work adopting this approach exists within the area of frontal cortex functional development (but see Wolfe & Bell, 2007a). Chapter 2 provides further details and discussion of this approach.

In order to investigate individual differences in frontal cortex functioning in infancy and early childhood, two cohorts of children were tested. The first cohort (Cohort 1) consisted of a small group of children ($N = 24$) tested at 9 and 24 months of age. The second cohort (Cohort 2) consisted of a large group of children ($N = 104$) tested at 4, 6 and 9 months of age. Only data from the 4- and 9-month assessments are reported here. The 6-month assessment involved collection of behavioural as well as EEG and ERP data; these data have not been analysed yet due to time constraints.

At 4 months two tasks were administered, the Gap task (e.g., Csibra et al., 1998; Hood & Atkinson, 1993) and the Anticipation task (e.g., Canfield et al., 1997; Csibra et al., 2001; Haith et al., 1988). These tasks were chosen because they have both been related directly to the frontal cortex in infant ERP studies (Csibra et al., 1998, 2001), and measure important aspects of eye movement control, processes which have been associated with the frontal cortex (e.g., Guitton, Buchtel, & Douglas, 1985; for review, see Johnson, 2005). At 9 months, two infant frontal cortex tasks, the A-not-B task (reviewed in Diamond, 2002) and a new task, the *Freeze-Frame task* (Holmboe, Fearon, Csibra, Tucker, & Johnson, 2008), were administered. Finally, at 24 months, a toddler frontal cortex task, the Spatial Conflict task (Gerardi-Caulton, 2000; Rothbart et al., 2003), was administered. Further details on these tasks can be found in Chapters 3 to 5.

A second aim of this thesis was the development of a new infant frontal cortex task, the Freeze-Frame task. As a first step towards gaining a better understanding of frontal cortex functioning in infancy, this new task was developed to measure aspects of inhibitory control using eye movements as the dependent measure. Based on previous research (Gilmore & Johnson, 1995; Johnson, 1995) it was hoped that a frontal cortex task using a very simple response such as eye movements would allow the assessment of such functioning more easily

and eventually at an earlier age. Few such tasks are currently available, and the tasks that have been developed so far (Gilmore & Johnson, 1995; Johnson, 1995) do not provide sensitive measures of individual performance differences. Therefore, the development of the new task was an important addition to the relatively small number of frontal cortex tasks suitable for infants. Chapter 3 reports on the initial validation study of the new task.

Given previous research findings indicating strong associations between frontal cortex task performance and temperament in infancy and early childhood (e.g., Gerardi-Caulton, 2000; Johnson, Posner, & Rothbart, 1991; McConnell & Bryson, 2005), a third aim of the work presented here was to establish the validity of claims that good frontal cortex task performance is associated with better self-regulatory abilities in infancy and early childhood (Posner & Rothbart, 1998; Rothbart et al., 2003). This is a recurrent theme throughout Chapters 3 to 6.

Relatedly, another important variable that needs to be considered is general development. Investigating the relationship between general development and frontal cortex task performance in infancy is important for two reasons. Firstly, some studies of adult populations have indicated that performance on frontal cortex tasks is correlated with measures of general intelligence (Duncan, Burgess, & Emslie, 1995; Obonsawin et al., 2002). Secondly, in a largely correlational design such as the one used here, it is important to rule out the most likely confounds of potential relationships between frontal cortex tasks. That is, it is important to establish that an association between two frontal cortex tasks cannot be entirely accounted for in terms of general motor and cognitive development. Therefore, the relationship between frontal cortex task performance and measures of general development during infancy and early childhood was investigated in both cohorts. The Mullen Scales of Early Learning, AGS Edition (Mullen, 1995) was used to assess general development at 9 months (Cohort 2), whereas the Bayley Scales of Infant Development,

2nd Edition (Bayley, 1993) was used to assess general development at 24 months (Cohort 1).

These analyses are presented in Chapters 3, 5 and 6.

Finally, an alternative way of assessing individual differences in frontal cortex functioning is the use of data on genetic variation. As discussed above, such data provide feasible neurobiological models of differences in frontal cortex function in both adults and children.

Furthermore, such data are important in order to establish a direct association between infant frontal cortex tasks and the neurobiology of the frontal cortex. Chapter 7 adopts this method and presents gene-behaviour associations found across the two cohorts.

Chapter 8 provides an overview and general discussion of the findings from Chapters 3 through 7.

Summary of Chapter 1

- The frontal cortex is associated with higher cognitive functions such as working memory, inhibitory control, attention, and decision making.
- The frontal cortex undergoes a prolonged anatomical and metabolic development with some structural changes taking place into early adulthood.
- Frontal cortex tasks developed for children are usually adaptations of adult frontal cortex tasks, and children's performance on these tasks improves considerably during the pre-school and early school years.
- Only a few frontal cortex tasks have been developed for infants. The most studied infant frontal cortex task is the A-not-B task.
- Studies using eye movements as the dependent measure indicate that the frontal cortex may already be functional during the first year of life (despite its immaturity).
- Investigating correlations between infant frontal cortex tasks enables us to establish whether tasks measure the same underlying functions. Longitudinal testing enables us to establish whether individual differences in task performance are stable over time.
- Data on genetic polymorphisms is a way of establishing a more direct link between infant frontal cortex tasks and the neurobiology of the frontal cortex.
- The work presented in this thesis investigated the relationships between frontal cortex tasks developed for infants and young children, as well as frontally related gene-behaviour associations, in two cohorts of children tested longitudinally.

CHAPTER 2

General Methods

Introduction

This chapter provides an overview of the general methodology and analyses used in the studies reported in this thesis. Specific details on the individual paradigms are reported in the relevant chapters. The first part of the chapter will address some general aspects of the approach taken to the study of frontal cortex functioning in infancy. These include the use of looking behaviour in infant research, investigating frontal cortex functioning via individual performance differences, longitudinal relationships, and the use of genetics in such research.

The second part of the chapter will discuss specific methodological aspects of the reported studies. First, an overview of what data were collected at each age will be presented, as well as the recruitment procedure and laboratory setups. Then, various issues associated with sample size, standardised tests, parent-report questionnaires, coding procedures, and the use of a correlational design will be addressed.

General methodological background

Looking behaviour as a measure of infant cognition

Chapter 1 introduced some of the difficulties involved in assessing frontal cortex functioning in infancy. In fact the methodological challenge of assessing frontal cortex functioning in infancy is not unique to this particular area. Since the 1950s developmental psychologists have been

using simple behavioural responses such as visual fixation to estimate many aspects of infants' knowledge and cognitive abilities (Aslin, 2007). Using methodologies such as the *violation-of-expectation* paradigm, it soon became apparent that even very young infants may possess considerable knowledge about the physical world. For example, evidence was presented indicating that 3- to 5-month-old infants could maintain object representations in memory and were surprised by violations of these representations, an ability which was previously assumed to develop much later in infancy (Baillargeon, 1987; Baillargeon, Spelke, & Wasserman, 1985).

These findings were soon challenged however. Some developmental scientists argued that interpretations of looking time behaviour as indicating that the infant possessed specific expectations, beliefs, reasoning and representational abilities were too "rich". That is to say, they believed that the same findings could be more parsimoniously explained by simpler processes such as perceptual discrimination (e.g., Haith, 1998). This spurred a fierce debate over what looking time was a measure of, and what conclusions could be made based on such measures (Aslin, 2000; Munakata, 2000).

This debate highlights the difficulties involved in infancy research. As Haith has pointed out "linguistic incompetence is key" (1998, p. 167). We simply cannot ask infants what they think and therefore have to rely on elicited behaviours. Given the fact that infants can see from birth and develop the ability to move their eyes flexibly within a few months (for review, see Bronson, 1974; Hood, 1995; Johnson, 1990), with manual skills emerging only later (Diamond, 1991), looking behaviour seemed the obvious tool with which to measure early conceptual development. However, as Aslin (2007) comments, different cognitive processes may very well underlie apparently similar looking behaviour (for example attentive looking vs. a blank stare), and often converging measures such as heart rate or event-related potentials are needed to

separate out the cognitive components during looking tasks (e.g., Courage, Reynolds, & Richards, 2006; Richards, 2005). Consequently, establishing what the infant knows about the world requires a long and meticulous process of establishing looking patterns in response to relevant events (such as object occlusion) and ruling out alternative accounts of such looking patterns (such as perceptual discrimination and more basic novelty effects).

However, looking behaviour has also been used in other research domains than that of infant conceptual development. For example, a considerable literature now exists on the development of attentional skills during infancy based on eye movement and looking time data. Within this area, some of the issues haunting research on infants' conceptual development are less prominent. This is because the development of infants' looking behaviour becomes the object of the research itself (Colombo, 2002). Such studies have looked at factors influencing infants' orienting responses during visual competition (Dannemiller, 1998, 2000), developmental changes in infants' ability to disengage attention (Atkinson, Hood, Wattam-Bell, & Braddick, 1992; Hood, 1995; Hood & Atkinson, 1993; Johnson et al., 1991), and developmental changes in habituation and looking time across the first year of life (Colombo, 2002; Colombo, Shaddy, Richman, Maikranz, & Blaga, 2004; Courage et al., 2006). These studies have helped us gain an understanding of some of the general characteristics of infants' looking behaviour as well as individual differences in such behaviour and the correlates of these differences (Colombo et al., 2004; Frick, Colombo, & Saxon, 1999). Furthermore, such studies are often based on specific hypotheses about the neural substrate of the observed behaviours (e.g., Csibra et al., 1998; Hood & Atkinson, 1993).

Thus, studies of infant visual attention have created a new area of research where infants' looking behaviour is not primarily used as a tool to assess knowledge (Colombo, 2002). Instead,

looking patterns are used to gain insight into how infants allocate their attentional resources and the neural substrate of these mechanisms. Findings from these studies have indicated that infants take longer to process stimuli and have more problems disengaging from stimuli early in the first year of life (Atkinson et al., 1992; Blaga & Colombo, 2006; Colombo, 2002; Hood, 1995; Hood & Atkinson, 1993). Towards the end of the first year, infants start to actively engage in more interesting stimuli, whereas they lose interest in less dynamic displays (Courage et al., 2006), a phenomenon that Colombo (2002) has interpreted as the emergence of endogenous or sustained attention. Developmental differences in individual infants' look durations have also been found to be predictive of later cognitive functioning (Colombo et al., 2004).

An area related to research on infant attention is the direct investigation of infants' ability to *control* their eye movements. As discussed in Chapter 1, infants only start to be able to perform coordinated gross motor actions, such as reaching, relatively late during the first year of life. However, the eye movement system is comparatively mature from an early age, providing an opportunity to investigate potential cognitive control processes. Furthermore, the control of eye movements has been directly related to cortical structures such as the frontal cortex in adult participants (Guitton et al., 1985; O'Driscoll et al., 1995), making the study of this type of behaviour particularly relevant to frontal cortex functioning in infancy.

This approach has already been adopted with some success in studies investigating infants' ability to perform visual versions of the A-not-B task (Bell & Adams, 1999; Hofstadter & Reznick, 1996). Such work has demonstrated that eye movements may provide a better, or at least equivalent, measure of inhibitory control in infancy. However, only a couple of studies have systematically explored infants' ability to inhibit automatic saccades (Johnson, 1995;

Nakagawa & Sukigara, 2007), and more work is needed to establish the emergence and development of this ability.

In trying to establish functions such as inhibitory control using eye movements, it is important to take several issues into account. Firstly, the role of individual differences in basic attentional mechanisms needs to be addressed and ideally controlled for in experimental paradigms assessing the inhibition of saccadic eye movements. This is particularly important in studies with young infants, where the ability to disengage visual attention is not yet fully developed (Atkinson et al., 1992; Hood & Atkinson, 1993; Johnson et al., 1991). For example, it is important to establish that infants are able to disengage from a visual stimulus with relative ease before assessing their ability to inhibit looks away from the same stimulus (see also Hood, 1995, for a similar point regarding looking time and visual preference measures). This approach was used in a new infant inhibition task presented in Chapter 3 (Holmboe et al., 2008). In this task infants went through a calibration phase in order to establish adequate detection and response to a peripheral stimulus before inhibitory aspects of task performance were assessed.

Secondly, it is important to obtain enough data from the majority of infants. This is particularly important in studies investigating individual differences in the ability to control eye movements using a categorical outcome on each trial (e.g. look or no look to a cue or target). One problem with the study by Johnson (1995, see Chapter 3 for a detailed review of this study) was that only half of the infants were included in the analyses. The other half was excluded because they did not make enough saccades to a visual cue in the first place.

Thirdly, the infant needs to be provided with appropriate motivation to inhibit looking towards a particular stimulus. In Johnson's (1995) study this was done by giving infants the opportunity to look for longer at a more interesting stimulus if they managed to ignore the initial

contralateral cue. In studies with non-human primates a food reward is often used (Fuster, 1997), but in infant studies a visual reward is more practical and ethical.

Finally, given the overwhelming evidence for infant habituation (e.g., Colombo, 2002), it is important to establish that a decrease in automatic saccades to distracting stimuli is due to the infant's effort to inhibit these saccades, not a passive learning mechanism. Ways to address this issue will be further discussed in Chapter 3.

The individual differences approach

Even within infancy research there are several ways to investigate the functional status of the frontal cortex. The early studies by Diamond and colleagues approached the question by looking at developmental progression on tasks that had been related to the frontal cortex in animal research (Diamond & Doar, 1989; Diamond & Goldman-Rakic, 1989; Diamond, Zola-Morgan, & Squire, 1989). Though this method provided strong evidence for functional development of the frontal cortex during infancy, it was still indirect. Later studies by Bell and colleagues started to incorporate neuroimaging methods such as EEG in an effort to more definitively associate performance on tasks such as the A-not-B with the frontal cortex (Bell, 2001; Bell & Fox, 1992, 1997). Findings from these studies confirmed a role of the frontal cortex in A-not-B performance.

The studies conducted by Bell and colleagues were also some of the first to take advantage of an individual differences approach to frontal cortex functioning in infancy. These studies demonstrated not only changes in the EEG between rest and task performance, but also that infants who performed better on the task showed different EEG power and coherence values over the frontal cortex as well as a differential pattern of development in these power values during the second half of the first year of life (Bell, 2001; Bell & Fox, 1992, 1997). This highlighted the

potential of investigating individual differences in frontal cortex task performance, especially in combination with neuroimaging techniques.

However, within the area of early frontal cortex function it is not only research using neuroimaging methods that has benefited from the individual differences approach. Diamond's initial studies focused largely on group effects (Diamond, 1985; Diamond & Doar, 1989; Diamond & Goldman-Rakic, 1985, 1989), but later she and her colleagues carried out a large study based on the individual differences method. This study investigated performance differences on frontal cortex tasks in infants and children treated early and continuously for phenylketonuria (PKU), a metabolic disorder affecting dopamine transmission in the brain (Diamond et al., 1997). Since dopamine is a particularly important neurotransmitter in the frontal cortex (e.g., Goldman-Rakic, Muly, & Williams, 2000), even small changes in available dopamine should disproportionately affect this cortical region. Diamond and colleagues elegantly demonstrated that the amount of dopamine in the frontal cortex, as determined by how well the PKU condition was managed, had a direct relationship with frontal cortex task performance in these children (Diamond et al., 1997).

Despite many investigations of the A-not-B task, and relatively consistent evidence that this task involves frontal cortex functioning during infancy, surprisingly few studies have related performance on the task to performance on other frontal cortex tasks. Furthermore, other tasks developed to assess some aspect of frontal cortex functioning, such as Johnson's (1995) saccade inhibition task, are yet to be compared with performance on other potential infant frontal cortex tasks. Establishing that such tasks measure similar functions is important since this provides converging evidence for frontal cortex functioning in infancy. Many infant and early childhood frontal cortex tasks are based on purely theoretical assumptions and not much is known about

whether they measure the same functions. Establishing consistency of frontal cortex performance across tasks and across age is therefore an important step towards understanding frontal cortex functioning in infancy. If shared variance between tasks (i.e., consistency of individual differences across tasks) can be established, this will provide a first step towards a more thorough investigation of the neural substrates of these shared functions using neuroimaging and other techniques.

Using genetic polymorphism data to investigate frontal cortex functioning

Even though establishing the relationship between the few infant tasks thought to involve frontal cortex functioning is an important step forward, this kind of evidence will only provide evidence that the tasks measure some of the same functions. As hinted above, further converging evidence is ultimately needed to establish that this shared functionality is indeed associated with the frontal cortex. In tasks for older children which closely resemble adult tasks we can perhaps be more certain that the same frontal processes are involved (e.g., Diamond & Taylor, 1996; Gerstadt et al., 1994; Rueda, Fan et al., 2004). However, in infancy, where new paradigms have to be developed to target infants' limited behavioural repertoire, such as the eye movement system, even a correlation between tasks hypothesised to engage the frontal cortex does not ensure that the common functional mechanism is in fact frontal. Of course certain obvious confounds can be controlled for, such as general speed of processing or general cognitive functioning, but this still does not guarantee that the measured commonality between tasks is directly associated with the frontal cortex.

One way to address this concern is to use neuroimaging methods to assess the engagement of the frontal cortex during task performance. Recently, the development of source modelling in EEG paradigms and of optical imaging methods has enabled researchers to address questions of

cortical localisation of function in infant populations (e.g., Baird et al., 2002; Homae et al., 2007; Nakano et al., 2008; Richards, 2005; Taga et al., 2003). This is an important area of future research.

Alternatively, performance on infant frontal cortex tasks can be related to genetic variation impacting directly on the neurobiology of the frontal cortex. This method is similar to the approach adopted by Diamond and colleagues in their study of children treated early and continuously for PKU (Diamond et al., 1997). By investigating a specific biochemical mechanism impacting on the level of dopamine available in the frontal cortex, Diamond et al. (1997) were able to demonstrate that children with less dopamine available in the frontal cortex performed worse on behavioural tasks designed to tap into frontal cortex functions. Similarly, in genetic variation studies a specific variation in a gene is typically associated with changes in neurotransmission, sometimes in specific parts of the brain such as the frontal cortex. This enables information about genotype to be used to make direct predictions about which infants are likely to perform better on a task thought to involve frontal cortex functioning, at least on the assumption that variation in the gene has an influence that is strong enough to affect individual performance differences.

Using genetic variation data is therefore another way to establish a more direct link between the biology of the frontal cortex and performance on behavioural tasks developed to assess frontal cortex functioning in infancy. However, it is important to make clear that only genetic variation which has been linked to *specific* biochemical mechanisms impacting on brain function, and which have already been shown to affect behaviour in the relevant domain are likely to produce interesting findings in an infant population. This is because a single genetic variation tends to have a relatively small effect on behaviour (e.g., Egan et al., 2001; Munafò,

2006). Furthermore, infant data are generally noisier than adult data, potentially making it harder to detect a subtle genetic effect.

Summary of methodological approach

To summarise, the studies to be reported in this thesis attempt to use individual differences to more thoroughly establish frontal cortex functioning in infancy. Several tasks use eye movements as the dependent measure because there is strong evidence that this method, though not perfect, enables the collection of data relevant to infants' cognitive functioning from an early age. Furthermore, this thesis attempts to establish the relationship between several different tasks thought to assess frontal cortex functioning. This is an important step in research relying on simple behavioural measures such as eye movements as it can potentially create the foundation for further investigations into the frontal nature of common mechanisms across tasks and across ages. Finally, the relationship between genetic variation which is likely to impact directly on the frontal cortex is investigated in relation to infant frontal cortex tasks in order to add to the converging evidence that these tasks engage the frontal cortex.

The studies in this thesis

Overview

Two groups of infants were tested. The first cohort (Cohort 1) was a small group of children tested at 9 and 24 months of age. At 9 months of age these children completed two tasks: the Freeze-Frame task (Holmboe et al., 2008) and the A-not-B task (Diamond, 1985; Piaget, 1954). At 24 months of age parents filled in the Early Childhood Behavior Questionnaire (ECBQ) (Putnam et al., 2006), and the children completed two tasks: the Spatial Conflict task (Gerardi-Caulton, 2000; Rothbart et al., 2003) and the Bayley Scales of Infant Development, 2nd Edition (Bayley, 1993). The data from this study are presented in Chapter 3.

The second cohort of infants (Cohort 2) was considerably larger than the first cohort. More than 100 infants were recruited for this study. Infants visited the laboratory three times, at 4, 6, and 9 months. However, only data collected at 4 and 9 months are reported in this thesis due to time constraints (data from the 6-month visit has not been fully coded and analysed yet). At 4 months, parents filled in a form on their educational and occupational background. At this age, parents also filled in the Revised Infant Behavior Questionnaire (IBQ-R) (Gartstein & Rothbart, 2003). At the 4-month visit to the laboratory infants completed two behavioural tasks: the Gap task (Hood & Atkinson, 1993) and the Anticipation task (Haith et al., 1988). Furthermore, a cotton cheek swab was used to collect cheek cells for genotyping. At 9 months, parents again filled in the IBQ-R. At the 9-month laboratory visit infants completed three tasks: the Freeze-Frame task, the A-not-B task and the Mullen Scales of Early Learning, AGS Edition (MSEL-AGS) (Mullen, 1995). Results from Cohort 2 are presented in Chapters 4, 5 and 6.

Data from the two cohorts are pooled in Chapter 7, which investigates the relationship between genetic variation and the frontal cortex measures. Ethical approval for the studies was granted by the School of Psychology ethics committee at Birkbeck College (ref. no. 2248).

Recruitment

Infants were recruited from various sources. The majority of infants were recruited through advertising in parent magazines, leaflets in baby accessory shops, and through the lab website. When parents contacted the lab, they were provided with an extensive information pack about the lab and its studies. All parents who contacted the lab were phoned by a lab member before receiving the information pack. This was done to ensure that parents had the opportunity to ask any questions they might have before volunteering to participate with their infant. If parents were happy to participate after receiving information about the lab, the infant's details were entered into a secure database stored on a lab computer.

For recruitment into the specific studies reported in this thesis, parents who had volunteered to participate with their infants (i.e., the infant's details were stored on the lab database) were contacted when the infant approached the age range for the study. To be eligible for the study, infants had to be either full-term (gestation 36 to 42 weeks) or weigh at least 5 lbs (2270 g) at birth, with no major birth complications or health issues. Parents were given comprehensive information about the study before agreeing to participate, including details of the genetic aspects of the study.

If the parent agreed to participate, the visit was scheduled within a week of the infant's ideal age. That is, if the infant was recruited for testing at 4 months, the visit was scheduled ± 1 week of the infant's 4-month birthday. If possible, the visit was scheduled a few days before the infant's monthly birthday to accommodate for re-scheduling. This procedure was adopted

throughout to avoid infants getting too old for a study when re-scheduling was necessary. The great majority of infants were tested within the 1-week age range at every visit, but, if no other scheduling options were possible, infants were occasionally allowed to be slightly out of the age range on the day of testing. This protocol was adopted to minimise data loss at the follow-up visits. Descriptive statistics for the age variable, including the standard deviation, is reported in the individual chapters. Each follow-up visit was scheduled on the previous visit, except in Cohort 1 where the follow-up visit took place 15 months later. Appointments were confirmed by phone 1 to 3 days before the visit.

Questionnaire data were collected by sending the form to parents approximately one week before the visit. Parents were asked to bring the questionnaire with them at the visit. If parents had forgotten the questionnaire or had not had time to fill it in, they were given a pre-paid envelope and asked to return the questionnaire within a week of the visit. On receipt of the questionnaire, the experimenter checked that all items had been rated. If ratings were missing or ambiguous (e.g., two different ratings had been given for the same item), the experimenter asked the parent to complete or correct the rating. If the questionnaire was received by post this procedure was completed over the phone or via email.

Anonymous participant numbers were allocated to each infant at the point of recruitment and never changed afterwards. This was done in order to ensure that each child's data were associated with the same identification number throughout. When the parent and child came to visit the lab they were welcomed by the experimenter and the testing procedure for each study was explained to them. Parents were asked to sign a consent form before participating in the study with their child. Parents did not receive payment for their participation, but after each study the child received an "Infant Scientist" diploma, and children who visited the lab more

than once received a small present, such as a bib, t-shirt or toy. Parents were reimbursed for their travel expenses to and from the laboratory.

Testing rooms

Three different testing room setups were used. Two testing rooms were set up for computerised procedures. The first testing room was used for testing infants at 4 months of age on the Gap and Anticipation tasks, and for testing children on the Spatial Conflict task at 24 months of age. This room was painted a dark grey colour and had a dimmer switch to dim the light before running an experiment. The dimmer switch was set to the same position in each test session. The room was divided in two by a large grey screen in the middle of the room. The child sat in front of a monitor on the right side of this screen, and the experimenter controlled the experiment from a computer on the left side of the screen. An infrared camera was mounted above the monitor so that it captured the child's face, and was used by the experimenter to monitor eye movements and other behaviours. The picture from the camera filming the child's face was mixed with a picture of the stimulus screen and a half-frame timer for later offline coding.

The second testing room was used for computerised administration of the Freeze-Frame task at 9 months of age. This room was similar to the first testing room. The main difference was that instead of having a divider in the room, the experimenter controlled the experiment from an adjacent room. In the second testing room the camera filming the infant's face was positioned just below the monitor instead of above the monitor. The experimenter had similar equipment available to monitor and control the experiment. A video titler displaying the participant number and stimulus events was superimposed on the video recording of the infant's face.

A third testing room was used for more interactive testing such as the A-not-B task and standardised test batteries. The room was quiet, had minimal décor to distract the child, and had enough space on the floor for the child to crawl or walk during standardised test administration.

Sample size

Sample size is an important factor in the interpretation of any study. The two cohorts reported on in this thesis were very different in terms of size. The reason for this is that the studies conducted on each cohort had different scopes. The initial study was based on a small sample of 24 infants (Cohort 1). This study was mainly aimed at validating a new task developed to assess frontal cortex functioning in infancy using eye movements as the dependent measure (Chapter 3). However, some caution is warranted given the small n in many of the analyses.

The studies conducted in Cohort 2 employed a much larger sample size ($N > 100$) for three important reasons. Firstly, the studies aimed at replicating findings from the initial study in a sample that was large enough to firmly establish the previously found relationships. Secondly, any longitudinal study suffers from attrition, even when every effort is made to retest the participants. How many participants will drop out during a study is difficult to assess and depends on many factors, so recruiting slightly more participants than may be needed at the end of the study is the safer option. Finally, and most importantly, studies conducted across the two cohorts involved an investigation of genetic polymorphism data. Such data tend to produce large within-group differences (that is to say, even participants with the same genotype will perform very differently) and effect sizes are generally small (Barnett et al., 2007; Egan et al., 2001; Munafò, 2006). Therefore, it was important to increase the sample size of Cohort 2.

Even though a large sample size increases the power of statistical comparisons, there are also caveats associated with having a large sample. Smaller effects can be detected, but it has to be

borne in mind that very small effects may have a minimal (though statistically significant) effect on the individual child's performance and therefore little practical relevance. In a large sample it is therefore particularly important to consider the size of any significant effects. Larger effects are more likely to influence the child's performance in a meaningful way. In contrast, in small samples effects tend to be less reliable overall.

Standardised tests

An important part of the studies presented in this thesis is the inclusion of measures of general development. These measures were included partly to establish the relationship between the frontal cortex tasks and general motor and cognitive development, and partly to test whether possible relationships between frontal cortex tasks were still present after controlling for individual differences in developmental level. This is important because the main factor underlying correlations between frontal cortex tasks could be general development. For example, if cognitive ability was found to account for most of the correlation between infant frontal cortex tasks, this would seriously question the specificity of the frontal cortex tasks. Of course it is possible that the frontal cortex tasks *are* measures of general cognitive functioning as some researchers have suggested (Duncan et al., 1995; Obonsawin et al., 2002). However, leaving this controversy aside (for discussion see Chapters 5 and 6), it is at least important to establish the relationship between the two sets of measures.

Two standardised test batteries were used in the studies to be reported here, the Bayley Scales of Infant Development, 2nd Edition (Bayley, 1993) was administered at 24 months of age in the initial study (Chapter 3), and the Mullen Scales of Early Learning, AGS Edition (Mullen, 1995) was used at 9 months in the larger study presented in Chapters 4 to 6. These two tests were selected because they have been rigorously standardised in large populations of infants and

children and have been shown to have good reliability and validity (Bayley, 1993; Mullen, 1995). Standardised tests were administered in accordance with the published manuals. A discussion of the differences between the two tests, and the reason for choosing different tests for the two cohorts, can be found in Chapter 5.

The use of parent-report questionnaires

The studies presented in this thesis involved the collection of temperament data based on parent report. This method is not uncontroversial. In particular, some researchers have raised concerns about parental bias in parent reports of infant temperament (Seifer, 2002; Vaughn, Taraldson, Cuchton, & Egeland, 2002). Vaughn et al. (2002) have even claimed that scores on an early temperament instrument, the Carey Infant Temperament Questionnaire (Carey, 1970, 1973), primarily reflect the caregiver's characteristics and subjective perceptions, not the infant's temperament. However, their study involved a high-risk sample of young mothers who were likely to experience more anxieties and difficulties than the general population, potentially confounding the results. More importantly, since Vaughn et al.'s study (which was originally published in 1981, and reprinted in 2002) more accurate and reliable parent-report measures of infant temperament have been developed.

Whereas the Carey ITQ categorised infant temperament into abstract dimensions such as “easy” and “difficult”, more recent questionnaires developed by Rothbart and colleagues (Gartstein & Rothbart, 2003; Putnam et al., 2001; Putnam et al., 2006) have taken a more fine-grained and concrete approach. The two questionnaires used in the work reported in this thesis, the IBQ-R and the ECBQ, have both been extensively validated (Gartstein & Rothbart, 2003; Parade & Leerkes, in press; Putnam et al., 2006). The questionnaire items ask parents to rate the occurrence (from “never” to “always”) of a particular behaviour or reaction to everyday events

during the last week or two (e.g., “When placed on his/her back, how often did the baby fuss or protest?”). This procedure ensures that parents are not asked abstract or comparative questions about the infant’s temperament. Furthermore, because the items address recent events, parents’ ability to recall the events is likely to be a minimal confound (Gartstein & Rothbart, 2003). Finally, good internal consistency, a similar factor structure across ages, and at least moderate correlations between primary and secondary caregiver ratings have been found for both instruments (Gartstein & Rothbart, 2003; Parade & Leerkes, in press; Putnam et al., 2006).

Despite the successful validation of the Rothbart questionnaires, the point made by Vaughn and colleagues (2002) and Seifer (2002) that ratings made in parent-report questionnaires reflect the caregiver’s perception of the infant is worth taking into account. Essentially, each infant’s score is based on the parent’s assessment of the infant’s behaviour. The assumption is that, as long as the questionnaire manages to elicit accurate responses from the parent (who usually spends most time with the infant), the ratings will reflect the infant’s *actual* temperament. However, a strict interpretation of such questionnaire data would be that any correlation between a scale or factor score and other measures (in the current study frontal cortex measures) is essentially a correlation between the parent’s description of the infant and those measures. This is also a relevant consideration in longitudinal studies, such as the studies presented here; changes in parent-report questionnaire scores over time likely reflect changes in the child’s behaviours and reactions, but they may also to a certain extent reflect changes in the parent’s perception of the child.

The validity of the parent-report method and alternative ways of assessing infant temperament in relation to the current studies are further discussed in Chapter 8.

Coding methods and reliability

A large proportion of the work reported in this thesis involved offline coding of infants' eye movements and other behaviours. This method is common in infant research because it is sometimes difficult for the experimenter to accurately judge the infant's behaviour online. In particular, older infants rarely sit perfectly still and observe the stimuli on a screen.

For measures such as saccadic reaction time, offline coding is essential because the difference in saccadic reaction time between different conditions can be very small (e.g. 10-20 ms). If the experimenter had to judge saccade onset online, a large amount of variability would be introduced by the experimenter's reaction time, potentially weakening the small effects of the experimental conditions on the infant's saccadic reaction time. This problem would be even more serious if different experimenters tested the infants.

The studies reported in this thesis were often concerned with individual differences in measures of saccadic eye movements. Sometimes the number of trials that an infant completed was small, making these measures less accurate in terms of estimating the infant's performance. This is different from group-based studies where an effect of condition will tend to shine through even when the data from individual infants are noisy. For these reasons great care was taken in setting up and testing the coding protocols used to analyse the video recordings of infants' looking (and other) behaviour. This was done in order to obtain the best possible estimate of each infant's performance in the administered tasks.

Most of the coding protocols used for the work reported in the following chapters have focused on two important aspects of the infant's behaviour in relation to the stimuli presented. The first aspect is the actual dependent measure, such as saccadic reaction time, accuracy of response (correct or incorrect), and occurrence of the behaviour of interest (e.g., a look to the

distractor). The second aspect is the validity of the trial. In order to obtain data that are as clean and representative of each infant's behaviour, it is important only to include trials where the infant was attending to the presented stimuli and where factors other than the stimulus display were unlikely to affect their performance in any important way. Thus, for example, if a peripheral target is presented, and we are interested in the infant's latency (or accuracy) in making a saccade to this new target, it is essential that the infant is attending to a set fixation point before moving his or her eyes to the new target. If the infant makes a saccade to the new target from another location than the set fixation point, it is likely that the infant's response is affected by the different distance between the stimuli, and thus the basic parameters of the trial setup have been violated.

Several steps were taken to make the coding of the raw data reliable and consistent. A coding protocol was developed for each task, specifying how to code the infant's response and listing specific criteria for the validity of each trial. Coders were trained on these protocols and inter-coder reliability was assessed after this training. Inter-coder reliability was assessed independently, that is, coders did not discuss or adjust their coding before running statistical analyses to assess reliability. If inter-coder reliability was not satisfactory, further training was provided or the coding protocol was clarified, and a new set of trials was coded by each coder.

It is noteworthy that many articles in the infant eye movement literature do not report inter-coder reliability, or they refer to vague assessments of inter-coder reliability (e.g., Haith et al., 1988). In particular, the reliability of the validity assessment, i.e., the assessment of whether a trial should be included or not, is often ignored. This may be because reliable coding of trial validity is harder to obtain than reliable coding of an overt behaviour, such as the onset of an eye movement. Consistent with these observations, reliability of validity coding in the studies

reported in this thesis were generally lower than reliability for the dependent measure. However, this is not a problem as reliability of the validity coding was generally high.

Using a correlational design

The studies reported in this thesis are largely analysed using correlation analyses. This approach has been chosen because the main research question is whether performance on various frontal cortex measures during infancy is an expression of a common underlying function. Thus, the research question itself is correlational in nature.

There are several limitations of a correlational design that need to be taken into account.

Causality. Firstly, it is well known that causality cannot be inferred on the basis of a correlation between two measures. This is less of a problem for the studies reported here because the primary aim was to establish shared variance between the measures, not to establish whether performance on one measure *caused* certain outcomes on other measures. In terms of longitudinal analyses it is reasonable to conclude that, when a correlation is found across ages, the early measure *predicts* the later outcome, though the exact causal mechanisms involved in this relationship may not be direct. For example, early differences in a specific function or characteristic may lead to differential developmental pathways caused by the infant's exploration of the environment or caregiver reactions. Such developmental pathways are further discussed in Chapters 6 and 8.

The third-variable problem. Secondly, bivariate correlations suffer from the *third-variable* problem (Field, 2005), that is, the shared variance between two measures may be explained by a different factor than the one hypothesised by the researcher. This is a more serious problem here. With a purely correlational design we cannot be 100% certain that correlations between the tasks are indeed due to frontal cortex functioning – they could be due to various other factors such as

general cognitive ability. This problem has been dealt with in several ways in the work presented here. Firstly, the tasks chosen were tasks that have been associated with the functioning of the frontal cortex in previous research, making it more *likely* that these measures would tap into such functioning. Secondly, the effects of the most common factors thought to affect performance on a range of tasks, such as reaction time and general intelligence, were partialled out in analyses assessing the correlation between frontal cortex measures. Finally, the genetic variation approach adopted in Chapter 7 enabled a more direct, biologically plausible, assessment of the frontal nature of the chosen tasks.

Range restriction. Thirdly, in order to establish correlations between performance on different frontal cortex tasks, infants need to differ in their performance on these tasks. That is, a certain range of performance differences is needed. If the range of performance is restricted, associations may not be detected when in fact they do exist. The range may be restricted due to factors such as sample characteristics (e.g., only participants from a particular group in society are sampled) and lack of sensitivity of the measures used (e.g., if a categorical measure is used where a continuous measure would be more appropriate). In this thesis these issues will be addressed when a limited range of performance is evident.

Multiple comparisons. A final issue is the use of multiple comparisons in the presented analyses. The number of Type 1 errors (false positives) is likely to increase with number of analyses carried out. Such *multiplicity* is common in the behavioural sciences and needs to be addressed (Sankoh, Huque, & Dubey, 1997). As discussed by Sankoh et al. (1997) there are two ways to control the probability of a false rejection of the null-hypothesis (and thereby the false acceptance of the experimental hypothesis) when testing multiple hypotheses in a set of analyses. One method is to control for the individual Type 1 error rates at a given significance level (e.g., p

$< .05$) for each hypothesis. In this way the experimenter controls for what is called the *comparisonwise* error rate. The advantage of this method is that it provides a more powerful test of each hypothesis. However, by testing each hypothesis at a set significance level, the likelihood of finding a significant effect when no true effect exists (i.e., a Type 1 error) increases with each additional comparison. Thus, the comparisonwise method is essentially uncorrected for multiplicity.

A more stringent approach is to control the *experimentwise* error rate. This method involves controlling the Type 1 error rate at a given significance level (e.g., $p < .05$) across *all* hypotheses tested in the analysis. This is the method used in a Bonferroni correction, which controls the experimentwise error rate by dividing the pre-specified significance level by the number of comparisons. For example, in an analysis involving 5 tests with an experimentwise significance level of $p < .05$, only tests obtaining a significance level of $p < .01$ (because $0.05 \div 5 = 0.01$) would survive the correction. Thus, the Bonferroni correction and similar correction methods provide excellent protection against Type 1 errors. However, this method often leads to very restrictive corrected significance levels, potentially resulting in the rejection of correct experimental hypotheses (i.e., Type 2 errors) (Sankoh et al., 1997).

In this thesis the issue of multiplicity has been dealt with by providing both the comparisonwise p -value and a corrected p -value for analyses involving more than one comparison. In corrections of the experimentwise error rate the Dubey/Armitage-Parmer (D/AP) procedure was used. This Bonferroni-based procedure is suitable because it takes the mean correlation between all measures in the analysis into account. In the standard Bonferroni procedure the correlation between a set of measures expected to be associated with another set of measures is not taken into account, resulting in an overly restrictive correction. However, in the

D/AP procedure the adjustment equals the Bonferroni correction if the mean correlation coefficient is zero, and the adjusted and unadjusted p -values are identical if the mean correlation coefficient is one (Sankoh et al., 1997, p. 2534). This provides an adequate correction when a set of correlated measures from one task is expected to correlate with a set of correlated measures from another task, which is often the case in the current thesis. Findings presented in the following chapters which survive the correction for multiplicity can generally be considered robust, whereas findings that do not survive the correction have to be interpreted more cautiously and should ideally be replicated in the future.

The issue of multiplicity has also been dealt with from a theoretical perspective. In particular, associations between the measures most relevant to the aims of this thesis are the main focus of the analyses. For example, the relationship between infant temperament and general cognitive development was not investigated because it was not part of the overall research questions of the thesis. Including these analyses would have vastly increased the number of comparisons. Finally, the effect sizes and significance levels of the reported findings are taken into account in the discussion of results.

Summary of Chapter 2

- Chapter 2 described the general methodological approach taken in this thesis and provided an overview of the studies.
- Various measures of looking behaviour have been used to gauge infants' cognitive abilities and to investigate the development of visual attention itself.
- The individual differences approach as applied to research into infant frontal cortex functioning was reviewed.
- The potential of investigating genetic variation linked to *specific* biochemical mechanisms impacting on the frontal cortex was discussed.
- Specific methodological considerations in relation to the current studies were discussed, including sample size, the inclusion of standardised test batteries, the use of parent-report questionnaires, coding protocols and reliability, and issues pertaining to using a correlational design and correcting for multiplicity.

CHAPTER 3

Freeze-Frame: A new infant inhibition task and its relation to frontal cortex tasks during infancy and early childhood

Introduction

Why the development of infant frontal cortex tasks is important

As discussed in Chapter 1, relatively few infant frontal cortex tasks have been developed so far. There are several likely reasons for this. Firstly, even adult frontal cortex assessment is complex, and theorists as well as clinicians do not always agree on the definition and functional divisions of the frontal cortex (Gazzaley & D'Esposito, 2007; Kramer & Quinlan, 2007; Stuss, 2007). Secondly, there is strong evidence that the frontal cortex develops slower than other parts of the brain (Chugani & Phelps, 1986; Chugani et al., 1987; Franceschini et al., 2007; Glantz et al., 2007; Gogtay et al., 2004; Huttenlocher, 1990; Sowell, Thompson, Holmes, Jernigan et al., 1999), and early research focussed mainly on developmental changes occurring during the second half of the first year of life (e.g., Diamond, 1985), sometimes on the assumption that the frontal cortex was too immature to play a significant functional role earlier in infancy (Diamond, 1991; for discussion see Johnson, 2005).

If the frontal cortex does indeed play a minimal role in early infancy, then there would be little reason for studying this area. However, the position that the frontal cortex is relatively “silent” before 7-8 months of age has been challenged by studies indicating the existence of simple frontal cortex abilities, such as inhibitory control, early in infancy, as well as by the

finding of frontal cortex activation in neuroimaging studies with young infants (Csibra et al., 1998, 2001; Dehaene-Lambertz et al., 2002; Grossmann, Johnson, Farroni, & Csibra, 2007; Johnson, 1995; Richards, 2005). A third reason why few infant frontal cortex tasks have been developed is the issue of adequate dependent measures. In particular, when testing young infants, outcome measures have to depend largely on simple behaviours such as eye movements or looking time, given infants' limited motor skills. Furthermore, since infants cannot be instructed to perform specific actions, task development depends on measures that exploit infants' natural preferences.

Despite the challenges of developing frontal tasks for young infants, this is a necessary step for answering important questions regarding frontal cortex functioning in general. Such tasks are necessary for an understanding of the development of frontal functioning throughout the life span. Adult performance on frontal cortex tasks is the result of a long development of core abilities such as working memory and inhibitory control (reviewed in Chapter 1). Understanding the developmental origin and trajectory of these abilities is necessary, not only to improve our understanding of such functions in general, but also to understand why individuals differ in these abilities.

Infant frontal cortex tasks using eye movements as the dependent measure

A few studies have already attempted to develop and evaluate frontal cortex tasks for infants that use eye movements as the dependent measure. Hofstadter and Reznick (1996) and Bell and Adams (1999) have tested visual versions of the A-not-B task. Both groups were successful in obtaining individual performance measures based on the infants' responses. Hofstadter and Reznick (1996) found that infants performed better in the visual version of the task, whereas Bell and Adams (1999) found similar performance levels for reaching and looking measures. The

discrepancy in these results indicate that, even though the visual version of the A-not-B task provides adequate individual performance measures, small differences in the task setup make a difference in terms of infants' performance. The visual version of the A-not-B task also has the limitation that a fairly limited number of trials can be administered given the relatively long duration of each trial (approx. 20-30 s). Furthermore, the A-not-B task is highly interactive, even in the visual version, making it a less objective measure than computerised tasks.

A related approach to assessing frontal cortex functioning in infancy has been adopted by Johnson (1995). Johnson argued that since infants develop the ability to control their eye movements at an earlier age than other motor systems, such as those needed for reaching, they may be able to perform tasks that depend on frontal cortex systems at an earlier age. He tested this prediction by administering an infant version of the anti-saccade task. In the adult version of the task participants have to make an eye movement in the direction opposite to a peripherally presented cue (Hallett, 1978). The anti-saccade task has been related directly to the frontal cortex in research with adult participants. For example Guitton, Buchtel and Douglas (1985) found that adult patients with frontal lobe lesions had difficulty suppressing reflexive responses to the cue.

In Johnson's (1995) version of the anti-saccade task, 4-month-old infants were first presented with a brief peripheral cue, quickly followed by an attractive target on the opposite side of the screen. Thus, infants had to learn the association between the cue and the target. More importantly, infants had to inhibit looking to the peripheral cue if they wanted to observe the attractive target for its full duration. Johnson (1995) found that infants looked less to the peripheral cue and faster to the target later in the experiment compared to the beginning of the experiment, indicating that not only did infants detect the association between cue and target, they were also able to learn to inhibit saccades to the less interesting peripheral cue. In a control

version where there was no relationship between the cue and the target, infants did not decrease their looking to the peripheral cue, indicating that the decrease in looking to the cue in the first experiment was not simply due to habituation.

Even though Johnson's (1995) study provides evidence that infants as young as 4 months of age possess some abilities to inhibit saccades to a peripheral cue, the task is not appropriate for assessing individual differences in inhibitory function. Johnson (1995) had to exclude half of the tested infants in the analyses because these infants rarely looked to the cue even in the beginning of the experiment. This means that the results obtained in the study were only representative of the subsample of infants who had high rates of looking to the cue initially.

Freeze-Frame: A new infant frontal cortex task

This chapter reports an initial study carried out to validate a new infant frontal cortex task. The main aim of the study was to develop a task to measure aspects of inhibitory control in infancy using eye movements as the dependent measure. Like Johnson's (1995) task, the new task had the advantage that many trials could be presented in a short period of time, thus overcoming some of the problems with the visual version of the A-not-B task. Furthermore, a calibration phase was included to accommodate for individual differences in sensitivity to peripheral distractors so that most infants could obtain a score in the task (for discussion of this issue, see Chapter 2). Finally, to simplify the task, and thus gain a wider range of data, infants did not have to make an eye movement to obtain the visual reward (the target in Johnson's [1995] study). Instead, keeping the eyes on the centre of the screen, while competing distractors were being presented in the periphery, elicited the reward.

The new task, termed the *Freeze-Frame task*, was designed to index inhibition of automatic saccades. Infants were presented with a series of dynamic and changing cartoon stimuli in the

centre of their field of view. On each trial a peripheral distractor was briefly presented. The duration of the distractor was calibrated individually for each infant until the infant started looking to the distractors. This was done in order to ensure that the infant was able to detect the distractors before assessing inhibitory function. In order to motivate the infant to inhibit saccades to the peripheral distractors, the central stimulus was frozen for a brief period of time if the infant made a saccade toward a distractor.

In order to differentiate inhibition effects, every other trial restricted the motivation to inhibit looks toward the distractor by presenting a repetitive central stimulus instead of the more complex and variable one. These repetitive trials were termed *boring trials*, and the trials presenting a variable central stimulus were termed *interesting trials*. The stimulus chosen for the boring trials, a simple rotating orange star, was less engaging for infants to look at in several respects: it was a single colour, it only moved in one direction (rotation), and it was shown again and again. Even though infants will initially find this stimulus engaging it was expected that they would find it inherently less interesting and quickly habituate to it. In contrast, the interesting trials presented a set of changing cartoon-like animations, many of them involving human-like and animal-like figures. These stimuli were chosen because infants were likely to find them immediately engaging and would stay engaged for a longer period of time. Based on these stimulus characteristics, it was expected that infants would be particularly motivated to inhibit looks to the distractors in the interesting trials compared to the boring trials. Thus, having two trial types, involving stimuli of differential interest to the infant, enabled the comparison of performance when the infant was highly engaged to performance when the infant had no particular motivation to stay focused on the central stimulus.

Three types of inhibitory effect were anticipated. (a) *General Inhibitory Learning* – as defined by a general decrease in looks to the distractors across both trial types during the course of the experiment. This effect was hypothesised to be a type of learning during the experiment which was inhibitory in nature, but non-selective. General Inhibitory Learning could be an active process involving effort or a more passive habituation to the distractors. (b) *Selective Inhibition* – as defined by an initial difference in looks to the distractors between the two trial types, resulting in a lower rate of looking to the distractors during the interesting trials than during the boring trials. Selective Inhibition was hypothesised to be a low-level effect driven by the stimulus competition between the central stimulus and the distractor before any significant learning had taken place. (c) *Selective Inhibitory Learning* – as defined by a greater decrease in looks to the distractors in the interesting trials than in the boring trials during the course of the experiment. This effect was hypothesised to involve actively learning to inhibit looks to the distractors during the more engaging trials where the motivation to inhibit would be greater. Such an effect would be strong evidence for inhibitory control in infancy, a function which is likely to be mediated by the frontal cortex.

Based on these predicted effects, individual performance indices of General Inhibitory Learning, Selective Inhibition, and Selective Inhibitory Learning were calculated, and it was investigated whether individual differences in performance on these indices was correlated with performance on other frontal cortex tasks at the same and older ages. This was done in order to validate the Freeze-Frame task as a frontal cortex task. Furthermore, in order to explore the specificity of the inhibitory indices, three additional indices were calculated assessing general distractibility in the task and in the two trial types separately.

In accordance with the predicted group effects, the Freeze-Frame General Inhibitory Learning index was expected to measure general learning during the test session. The Freeze-Frame Selective Inhibition index was hypothesised to measure the individual infant's sensitivity to stimulus competition and baseline relative distractibility. Finally, the Freeze-Frame Selective Inhibitory Learning index was hypothesised to be a measure of higher-level inhibitory control as evidenced by the individual infant's selective learning pattern in the two trial types during the test session. A high level of such selective inhibitory learning would be characterised by a faster decrease in looking to the distractors in the interesting trials compared to the boring trials. On the basis of this task structure it was predicted that individual differences in the Selective Inhibitory Learning index would best predict performance on other frontal cortex tasks.

Study outline

These hypotheses were tested by administering a set of frontal cortex tasks to the same group of children at 9 and 24 months of age. The study protocol is briefly reviewed below, and a more detailed description of each frontal cortex task used for comparison with the Freeze-Frame task can be found in Chapter 1 (see the section "Frontal cortex functioning in infancy").

The first age, 9 months, was chosen in order to investigate the relationship between the Freeze-Frame task and the A-not-B task (Diamond, 1985; Piaget, 1954). The A-not-B task is probably the most well-studied infant frontal cortex task, and therefore investigating the relationship between this task and the Freeze-Frame task was the natural place to start. Whereas the Freeze-Frame task could easily be administered to infants younger than 9 months of age, infants only just start to improve on the A-not-B task at this age, providing the opportunity to observe large individual performance differences (e.g., Diamond, 1985). Though it is somewhat controversial as a measure of inhibitory function (e.g., Smith, Thelen, Titzer, & McLin, 1999;

Thelen, Schöner, Scheier, & Smith, 2001), performance on the A-not-B task has been extensively related to the functioning and integrity of the frontal cortex in both animal and human research (Baird et al., 2002; Bell, 2001; Bell & Fox, 1992, 1997; Diamond & Goldman-Rakic, 1986, 1989; Freedman & Oscar-Berman, 1986; Verin et al., 1993).

The second age was chosen because 2 years is the earliest point at which children can be instructed adequately, making it possible to administer a manual response task. At 24 months children completed a touch screen version of the Spatial Conflict task (Gerardi-Caulton, 2000; Rothbart et al., 2003), and parents filled in the Early Childhood Behavior Questionnaire (ECBQ), a measure of toddler temperament (Putnam et al., 2006; Rothbart et al., 2003). The Spatial Conflict task was chosen because it is one of the very few toddler tasks that have been specifically hypothesised to be related to frontal cortex functioning (Rothbart, 2003). The ECBQ was administered in order to investigate the relationship between the Freeze-Frame task (and other frontal cortex tasks) and temperament in early childhood generally. Of particular interest was that fact that performance on the Spatial Conflict task had previously been related to Effortful Control as assessed by the ECBQ (Gerardi-Caulton, 2000; Rothbart, 2003), indicating that the two measures have similar functional mechanisms, possibly associated with the frontal cortex.

Finally, the Bayley Scales of Infant Development, 2nd Edition (BSID-II), a measure of general development (Bayley, 1993), was administered at 24 months. This measure was included in order to investigate the independence or otherwise of the Freeze-Frame measures from general motor and cognitive development at age 2.

Method

General procedures

Children were recruited from the greater London area. The initial sample consisted of 24 infants: 13 girls and 11 boys. Twenty-one infants came from a white ethnic background, one infant came from a black ethnic background, and two infants had mixed ethnic backgrounds. At the 9-month visit the children were between 264 and 270 days old ($M = 267.96$, $SD = 1.81$). All infants performed the Freeze-Frame task first, and then the A-not-B task after a short break. This order of presentation was chosen because pilot studies indicated that infants tended to get quickly bored with the computerised Freeze-Frame task if they performed the more interactive A-not-B task first.

Twenty children from the original sample (12 girls, 8 boys) participated in the follow-up study when they were 2 years old. The age range was 24 months $\pm$ 10 days ($M = 731.75$, $SD = 3.45$). First the children performed the Spatial Conflict task, and then the Bayley Scales of Infant Development, 2nd edition (BSID-II) was administered. In six cases BSID-II testing had to be discontinued either because the child refused to continue or otherwise stopped cooperating. In these cases a home visit was arranged in order to complete testing. All home visits were made within 3 days of the visit to the laboratory.

The Freeze-Frame task

A schematic of the procedure and stimuli in the Freeze-Frame task are presented in Figure 3.1 (stimuli are not in actual proportions).

Apparatus and stimuli. Infants sat on their parent's lap at a 60-cm distance from a 19-inch

colour monitor in a darkened room. The experimenter controlled stimulus presentation via a computer in an adjacent room. The infant's behaviour was monitored and recorded via a remote controlled video camera placed just below the monitor. Stimuli consisted of (a) colourful animated cartoon figures at the centre of the monitor, with a maximum size of 13 cm $\times$ 16 cm ($12.4^\circ \times 15.2^\circ$ of visual angle) (interesting animations), (b) a rotating orange star at the centre of the monitor, with a 10.5-cm diameter (10° of visual angle) (boring animation), and (c) 3.5 cm $\times$ 3.5 cm (3.2° of visual angle) white squares at either side of the monitor (vertically aligned with the animations) at a 14-cm distance from the centre (13.5° of eccentricity) (distractors). Stimuli appeared on a dark grey background. If necessary, the infant's attention was drawn to the monitor by computer-generated alerting sounds (such as an animal sound, a bell, etc.) The experimenter could also speak to the infant via a microphone in order to regain the infant's attention.

Figure 3.1. Schematic of the Freeze-Frame procedure.

Procedure. Initially, interesting animations were presented at the centre of the screen, changing every 2 s until the infant fixated on them. Once the experimenter judged that the infant was fixated on the animations, she pressed a key on the keyboard to start the first trial. In each trial, the animation froze after a delay of 1000 to 2000 ms (varied to avoid anticipatory eye movements) and at the same time a distractor appeared randomly on either the right or left side of the screen. The duration of the distractor presentation was calibrated individually (see below). The experimenter indicated whether the infant made a saccade towards the distractor stimulus by releasing the key on the keyboard. If the infant made a saccade towards the distractor stimulus within 600 ms (as indicated by the experimenter's key release and regardless of whether the distractor was still on the screen), the animation remained frozen for 3000 ms, and then a new trial was started. If the infant did not make a saccade towards the distractor within 600 ms (and

the experimenter did not release the key), the animation continued for another 2400 ms before the next trial was presented. The even-numbered trials presented new interesting animations changing every 2 s (interesting trials), whereas the odd-numbered trials always presented the same uninteresting rotating orange star (boring trials). The infant was encouraged to complete 60 trials, but the experiment was stopped if the infant became fussy or stopped looking at the screen.

Calibration. Pilot experiments as well as previous research (Johnson, 1995) indicated that the distractor duration needed to elicit automatic saccades varied greatly among infants. Therefore, the initial phase of the experiment was used to calibrate the duration of the distractor to the individual participant. Initial distractor duration was set to 200 ms by the computer and was automatically increased in 40 ms steps in every trial where the infant did not look to the distractor. If the infant looked to the distractor the experimenter released a key on the keyboard to indicate to the computer that the infant had looked to the distractor. On the following trial the computer presented the distractor at the same duration, and if the infant again looked to the distractor (and the experimenter released the key to indicate to the computer that the infant had looked to the distractor), the computer set the distractor presentation to this duration for the rest of the experiment. Thus, infants had to look to the distractor on two consecutive trials before distractor duration became fixed. Results were analysed from two trials before the calibration trial in order to include the first trials where the infant started to look to the distractors.

Data analyses. Video recordings of infants' looking behaviour were analysed offline. If the infant was not looking at the central stimulus at distractor onset or if he/she looked away while the distractor was being presented, the trial was considered invalid and was not included in the analyses. For the purposes of analysis, the experiment was divided into three phases with 16 trials in each phase. This was done in order to investigate differences in looking to the distractors

during the beginning, middle, and end of the experiment. Most infants completed enough trials to provide data for all three phases of the experiment (see Results section below). After dividing trials into phases, invalid trials were excluded, and the proportion of looks to the distractors was calculated for the interesting and boring trials separately within each phase. Inter-coder reliability for looking behaviour (look vs. no look to the distractor) and trial validity was high (looking behaviour: $\kappa = .93$; trial validity: $\kappa = .87$; based on data from 7 participants).

The A-not-B task

Apparatus. Infants sat in their parent's lap in front of a table containing two wells. The wells were 11.2 cm in diameter and positioned 18 cm apart. The distance between each well and the edge of table was 18.5 cm. The parents were asked to keep the infant centred in front of the table. The experimenter was seated across the table, facing the child. A collection of small colourful toys was available out of the infant's view. Two brown cardboard squares of a similar colour to the table were used as covers for the wells. The infant's behaviour was video-recorded and was also monitored by another experimenter who hid behind a divider in the room. This second experimenter controlled the computer that displayed the delay period, adjusted the delay period according to the success or failure of the infant, and indicated to the first experimenter where to hide the toy and when the delay period was over.

Procedure. A trial began with the experimenter drawing the infant's attention to the toy. When the infant was focused on the toy, the experimenter put the toy into one of the wells and covered the wells simultaneously. The side where the toy was hidden in the first trial was counterbalanced across participants. A trial was only considered valid if the infant saw the toy being hidden in the well and if the toy was completely hidden in the well. As soon as the toy was in the well and the wells had been covered, the delay period started. During the delay period the

infant's attention was distracted to prevent him or her from looking at the correct well during the delay. The distraction consisted of talking in an exaggerated voice to the infant, saying his or her name, waving etc. Furthermore, the parents were asked to hold their infant back to prevent him or her from reaching towards the wells during the delay. At the end of the delay period the experimenter said, "Okay," and this was the signal to the parent to let the infant reach.

The initial delay was set to 2 s and adjusted depending on the infant's performance (see below). At the 0 s delay infants were allowed to reach immediately without any attempt to distract them. If the infant reached to the correct well, he or she was praised and allowed to play with the toy briefly as a reward. If the infant reached to the incorrect well, the experimenter drew the infant's attention to the toy in the correct well and started a new trial without letting the infant touch the toy. If the infant reached to both wells simultaneously, did not reach at all, or if it was otherwise difficult to establish whether the response was correct, the trial was repeated. The experimenter continued to hide the toy in the same well until the infant reached to the correct well on two consecutive trials. At this point, the toy was hidden in the other well (*change trial*), and then was repeatedly hidden in that well until the infant had completed another two successful trials consecutively. If two consecutive trials ended in failure, the delay period was decreased by 2 s. If two consecutive *change trials* ended in success, the delay period was increased by 1 s. The experiment was continued until the infant had completed approximately 30 trials or stopped attending to the task.

Data analyses. In terms of A-not-B performance, the individual variation in the length of delay which infants could sustain and still succeed in change trials was of particular interest. Although the delay period was varied systematically with the performance of the infant, the *observed* delay between hiding and search (on change trials: $M = 4.04$, $SD = 2.56$) was usually

longer than the *intended* delay (on change trials: $M = 1.70$, $SD = 1.79$) because infants sometimes had difficulty disengaging from the experimenter who distracted them during the delay period. Therefore, the observed delay in successful change trials was measured from the video-recordings and these values were used in the further analyses. The observed delay was measured from when the wells were completely covered to when the child touched one of the wells. The maximum observed delay was highly correlated with the maximum intended delay, $r = .756$, $p < .001$, and double coding of data from 7 participants indicated that the measured delay was consistent across coders, $r = .992$, $p < .001$.

The maximum observed delay which an infant could sustain was somewhat sensitive to the infant being distracted from the task or forgetting the task on a single change trial. For example, an infant might be distracted for several seconds after the delay period had finished and then by chance choose the correct well. Therefore a cumulative measure was calculated. This measure took into account the number of times an infant succeeded at various delay durations on change trials and the total number of trials the infant completed. The cumulative measure was calculated by adding the observed delays for all successful change trials during the task for each infant and then dividing the result by the total number of trials completed by that infant. In this way the measure took into account both how consistently an infant succeeded on change trials, the magnitude of the sustainable delays, and how many “chances” the infant had to be successful in the task. The A-not-B cumulative delay correlated highly with both the maximum intended delay, $r = .933$, $p < .001$, and the maximum observed delay, $r = .725$, $p < .001$, on a change trial.

The Spatial Conflict task

The paradigm used for the Spatial Conflict task was based on the paradigm developed by Gerardi-Caulton (2000) and Rothbart et al. (2003).

Apparatus. Children sat in a booster seat, or, if a child refused to sit alone, on the parent's lap. An Elo 15 inch infrared touch screen was placed on a table in front of the child. The base of this monitor was covered by a green plate positioned at a slight angle towards the child. This plate had two yellow plastic hand shapes attached to it. Children were encouraged to keep their hands on this plate between trials.

The session was recorded by two cameras: one in front of and above the child, the other behind the child to the right. In this way Camera 1 recorded the child's face and shoulders, whereas Camera 2 recorded the child's hands and the touch screen. The input from the two cameras was fed into a mixer and shown on a split screen. This combined picture was recorded on videotape for offline coding.

The testing room was divided into two compartments by a large grey screen. One experimenter sat next to the child, while the other experimenter controlled the experiment from the other side of the screen. Parents usually sat on a chair a few metres behind the child.

Procedure. As soon as the child was comfortably in the booster seat, the experiment was started by the second experimenter. The first phase of the experiment consisted of practice trials. On each practice trial a cartoon figure was presented at the top-centre of the screen. This presentation was accompanied by a brief swooping sound in order to catch the child's attention. One second after this target stimulus had been presented two houses appeared at the bottom of the screen, one on each side. One house contained a figure that matched the target stimulus, while the other house contained a different figure. Presentation of the houses with figures was accompanied by a low sound similar to the sound of flipping a page.

At this point Experimenter 1 encouraged the child to touch the house with the matching picture. If the child did not touch the screen at all, the experimenter demonstrated the task by

touching the correct house. A correct response always initiated a short animated sequence inside the correct house combined with a tune. An incorrect response made all stimuli disappear from the screen combined with a low buzzing sound. After demonstrating the correct response the same pair of stimuli was presented, and Experimenter 1 encouraged the child to try again. If the child did not respond by touching one of the houses within 15 s, the practice trial was terminated and repeated.

To start, six practice trials were presented. If at any point the child responded correctly on two consecutive practice trials (one on the left side and one on the right side of the screen), the program proceeded to the experimental phase after the first practice block (six trials). However, if the child did not respond correctly on two consecutive trials during the first practice block, another six practice trials were presented and so forth. If a child responded incorrectly on a practice trial, the same trial was presented repeatedly until the child produced the correct response. This procedure was chosen in order to ensure that the child understood the task before proceeding to the experimental trials.

After the practice phase 20 experimental trials were presented. The experimental trials were presented in one of two pseudo-random sequences, counterbalanced across participants. On these trials the target was presented at the top of the screen either on the same side as the correct house (*non-conflict trials*) or on the other side (*conflict trials*). Five different stimulus pairs were used in the houses and each target stimulus always appeared once in a conflict trial and once in a non-conflict trial. The instructions were the same, but no demonstration was offered at any point during the experimental phase. Labelling of the target was minimised as much as possible (e.g., Experimenter 1 would say, “and where does this one go?” when the target was presented). No trials were repeated during this phase. The child was praised for correct responses.

After the experimental trials were completed, the child was given a short break (1-2 minutes), and then another session was administered. The second session was identical to the first session, but trial types and stimulus locations were counterbalanced, i.e., if a picture appeared in the left house in a specific trial during the first session, that picture would appear in the right house in the equivalent trial during the second session, and the same was the case for target pictures. With one exception, all children completed both sessions.

Data analyses. Accuracy data were collected by the computer, but the video recordings were coded offline in order to exclude invalid trials and to verify the accuracy of the child's responses. Trials were coded as invalid if the child did not look at the target before choosing a house, looked away from the screen for more than 2 s at any one point during the trial, touched both houses simultaneously, touched one of the houses accidentally, or had his/her hand close to the chosen house at target onset. Practice trials as well as trials where the child did not respond to the houses within 15 s were also excluded from the analyses. Occasionally the touch screen did not detect the child's response; the accuracy of such responses was corrected in the offline coding and included in the analyses. If a child first touched one house and then attempted to touch the other house before the stimuli disappeared, the score was based on the child's first response. Inter-coder reliability based on all trials from 5 participants was high (Accuracy: $\kappa = .97$; Validity: $\kappa = .90$).

For correlational analyses comparing performance on the Spatial Conflict task with other measures, a difference score was calculated. Proportion measures were first calculated by dividing the number of correct responses in each trial type (conflict and non-conflict) by the number of valid trials of that trial type. The difference score was then obtained by subtracting the proportion of correct responses on conflict trials from the proportion of correct responses on non-

conflict trials. This score is presumed to be a measure of conflict interference and is identical to the accuracy measure used by Rothbart et al. (2003).

The Early Childhood Behavior Questionnaire

The Early Childhood Behavior Questionnaire is a parent-report measure of toddler temperament. It consists of 18 scales based on a total of 201 items. The Early Childhood Behavior Questionnaire has been extensively validated in factor analytic work (Putnam et al., 2001; Putnam et al., 2006). The instrument has good internal consistency and adequate inter-rater reliability (based on ratings from primary and secondary caregivers). A recent factor analysis of data on more than three hundred 1.5- to 3-year-old children suggested a three-factor structure of Surgency/Extraversion, Negative Affectivity and Effortful Control (Putnam et al., 2006).

In the current study, the Early Childhood Behavior Questionnaire was sent to parents about a week before the 24-month visit. Parents brought the completed questionnaire with them at the visit or returned the questionnaire by post within a week of the visit.

The Bayley Scales of Infant Development, 2nd Edition (BSID-II)

The BSID-II was administered according to the instructions specified in the BSID-II manual. The BSID-II has been standardised on a sample of 1700 US children aged between 1 and 42 months (17 age groups, 100 children in each group). The BSID-II has been extensively validated as a reliable measure of general cognitive and motor development in infants and young children; see the BSID-II manual for more details (Bayley, 1993).

The BSID-II session was recorded on video. Performance on the BSID-II Mental Scale and Motor Scale was scored during and immediately after testing. Test scores were confirmed by offline analysis of the videos by an independent scorer.

Results

Freeze-Frame group results

The duration of distractor presentation was calibrated within an average of 5.70 trials ($SD = 5.13$), and the calibrated distractor duration varied between 200 ms and 920 ms ($M = 322.00$, $SD = 159.20$). Three infants did not calibrate (i.e., did not look to the distractor on two consecutive trials at any point), and one infant had to be excluded because the experimenter calibrated the distractor duration too late (more than 15 trials after the distractor duration should have been calibrated). These four infants were not included in any analyses involving the Freeze-Frame task.

The mean number of trials that infants completed, counting from two trials prior to calibration, was 52.60 ($SD = 10.91$), and the mean number of valid trials that infants completed from two trials prior to calibration was 47.05 ($SD = 10.17$). The mean proportion of valid trials was .90 ($SD = .06$).

The proportion of looks to the distractors was entered into a repeated measures ANOVA with Trial Type (2 levels) and Phase (3 levels) as within-subjects factors. Each phase consisted of 16 trials, 8 boring and 8 interesting. If an infant had fewer than 50% valid trials within a particular Trial Type $\times$ Phase cell (i.e., less than 4 valid trials to base the proportional measure on) data from that cell were removed since the proportion of looks to the distractors in that cell was unlikely to be meaningful. As a result, four infants did not have complete data from all three phases of the experiment. One infant only had data from Phase 1 (both trial types) and the boring trials in Phase 2, one infant only had data from Phases 1 and 2 (both trial types), one infant had data from all phases except the boring trials in Phase 2, and one infant had data from all phases except the boring trials in Phase 3. These infants were excluded from the repeated measures

ANOVA, but included in the correlational analyses reported below.

Table 3.1 presents descriptive statistics for the proportion of looks to the distractors in each phase and trial type. As can be seen from the table, there was a trial type difference in looking to the distractors already in the first phase of the experiment. Furthermore, for both trial types there was a decline in looking to the distractors during the course of the experiment, with the largest decrease seen between Phases 1 and 2.

Table 3.1. Freeze-Frame descriptive statistics for the proportion of looks to the distractors in each phase split by trial type.

	<i>n</i>	<i>Mean</i>	<i>SD</i>
Boring - Phase 1	16 (20)	0.67 (0.65)	0.19 (0.19)
Boring - Phase 2	16 (19)	0.45 (0.40)	0.25 (0.27)
Boring - Phase 3	16 (17)	0.43 (0.45)	0.19 (0.21)
Interesting - Phase 1	16 (20)	0.39 (0.37)	0.22 (0.21)
Interesting - Phase 2	16 (19)	0.21 (0.18)	0.16 (0.16)
Interesting - Phase 3	16 (18)	0.18 (0.17)	0.22 (0.21)

Note: Numbers in brackets are the equivalent values when the sample included infants who did not have complete data from all three phases.

The repeated measures ANOVA confirmed this picture of the data. There were significant main effects of Trial Type, $F(1,15) = 24.77, p < .001, \eta_p^2 = .62$, and Phase, $F(2,30) = 22.07, p < .001, \eta_p^2 = .68$, but no significant interaction, $F(2,30) = .13, p > .20, \eta_p^2 = .02$. Post-hoc tests using a Bonferroni correction revealed that the proportion of looks to the distractors differed significantly between Phases 1 and 2, and between Phases 1 and 3 ($p < .001$ in both cases), whereas the proportion of looks to the distractors in Phases 2 and 3 did not.

The results of the repeated measures ANOVA were confirmed by a linear mixed model analysis (LMM) using an unstructured covariance structure. Phase and Trial Type were entered as repeated measures and proportion of looks was entered as the dependent measure. The

advantage of LMM is that data from participants with missing data points, in this case missing data from one or more phases of the experiment, can be included in the analysis (Garson, 2008). The LMM confirmed significant effects of Trial Type, $F(1,18.81) = 32.56, p < .001$, and Phase, $F(2,17.51) = 21.31, p < .001$, but no significant interaction, $F(2,17.04) = .44, p > .20$. Again, post-hoc tests using a Bonferroni correction revealed that the proportion of looks to the distractors differed significantly only between Phases 1 and 2, and between Phases 1 and 3 ($p < .001$ in both cases).

Thus, a significant difference between boring and interesting trials and a decrease in looks to the distractors during the experiment was found. However, infants did not stop looking to the distractors faster in one trial type than the other.

Freeze-Frame performance measures

According to the predictions outlined at the beginning of this chapter, three of the performance measures from the Freeze-Frame task are expected to tap into different aspects of inhibitory function. Definitions of these measures can be found in Table 3.2. The first performance measure, General Inhibitory Learning, was calculated by subtracting the proportion of looks to the distractors in Phase 3 from the proportion of looks to the distractors in Phase 1 of the experiment (across both trial types). A positive score on this index indicated more looking to the distractors in the beginning of the experiment, whereas a negative score indicated more looking to the distractors at the end of the experiment. The mean General Inhibitory Learning score was .21 ($n = 20, SD = .20$), indicating that on average infants stopped looking to the distractors during the experiment.

The Selective Inhibition index was calculated by subtracting the proportion of looks to the distractors in the interesting trials from the proportion of looks to the distractors in the boring

trials in Phase 1 for each infant. This measure was based on Phase 1 only because the index was intended to measure the initial effect of trial type. A positive score indicated that the infant looked more to the distractors in the boring trials, a score near zero indicated equal amounts of looking, and a negative score indicated a higher level of looking to the distractors in the interesting trials. The mean Selective Inhibition score was .28 ($n = 20$, $SD = .26$), indicating that infants generally looked less to the distractors in the interesting trials than the boring trials in the first phase of the experiment.

According to the main prediction of this study, good inhibitory function should be particularly strongly associated with the Selective Inhibitory Learning index. Good performance on this measure was expected to be characterised by a faster decrease in looks to the distractors in the interesting trials than in the boring trials. In order to obtain a measure of such performance the difference in looks to the distractors between Phase 1 and Phase 3 was calculated for each trial type. This calculation provided the decrease in looking in the boring trials and interesting trials separately. Subsequently, these two measures were subtracted from each other in order to obtain the final score on the Selective Inhibitory Learning index. A positive score on this index indicated a larger comparative decrease in the interesting trials, whereas a negative score indicated a larger comparative decrease in the boring trials. In this study a positive score was considered to indicate better selective inhibitory learning. The mean Selective Inhibitory Learning score was -.01 ($n = 17$, $SD = .39$), indicating that on average infants showed a similar decrease in the two trial types. However, large individual performance differences were present with scores ranging from -.63 to .58.

Table 3.2. Definitions of Freeze-Frame task terminology and measures.

Freeze-Frame Definitions	
Phase:	Test session divided into 3 phases. Each phase contains 16 trials (8 boring trials and 8 interesting trials)
Boring trial:	Central stimulus is always the same rotating orange star.
Interesting trial:	Central stimulus is always changing, dynamic animation.
General Inhibitory Learning Index:	(Proportion of looks to distractor, Phase 1) – (Proportion of looks to distractor, Phase 3).
Selective Inhibition Index:	(Proportion of looks to distractor, Phase 1, boring trials) – (Proportion of looks to distractor, Phase 1, interesting trials).
Selective Inhibitory Learning Index:	(Proportion of looks to distractor, Phase 1, interesting trials – Proportion of looks to distractor, Phase 3, interesting trials) – (Proportion of looks to distractor, Phase 1, boring trials – Proportion of looks to distractor, Phase 3, boring trials).
General Distractibility Index:	Proportion of looks to distractor, all trials.
General Distractibility Index, boring trials:	Proportion of looks to distractor, all boring trials.
General Distractibility Index, interesting trials:	Proportion of looks to distractor, all interesting trials.

Additional Freeze-Frame measures

Though the main predictions of the study concerned the three Freeze-Frame indices hypothesised to tap into different aspects of inhibitory functioning, three additional Freeze-Frame measures were calculated (see Table 3.2, bottom panel). These three measures were considered to be background measures and were included to investigate whether any general aspects of performance on the Freeze-Frame task were associated with performance on the other

frontal cortex tasks. The inclusion of these measures was important to assess the specificity of the inhibitory indices.

The first additional measure was simply the overall proportion of looks to the distractors, i.e., the number of trials where the infant looked to the distractor in relation to the number of trials where the infant remained fixated on the central stimulus. This measure was termed the Freeze-Frame General Distractibility index. The proportional measure was based on all the available valid trials for each infant (i.e., the data were not separated into phases and then combined). This measure can be conceived of as estimating the infant's overall sensitivity to the peripheral distractors. The mean overall proportion of looks to the distractors was .36 ($n = 21$, $SD = .13$). This indicates that overall infants looked to the distractors on slightly more than a third of the trials.

The second and third additional measures were the overall proportions of looks to the distractors in the boring trials and the interesting trials calculated separately. These two measures were termed the Freeze-Frame General Distractibility index for boring trials and interesting trials, respectively. The measures were calculated separately on the basis of all available valid trials for the two trial types. The overall proportion of looks to the distractors in the boring trials was not expected to be significantly related to the frontal cortex tasks, since the motivation to inhibit looks to the distractors in these trials was low. However, the overall proportion of looks to the distractors in the interesting trials could be related to performance on the other frontal cortex tasks, since these were the trials where the motivation to inhibit looks to the distractors was greatest. The mean proportion of looks to the distractors in the boring trials was .50 ($n = 21$, $SD = .17$), indicating that on average infants looked to distractors on approximately half of the boring trials. The mean proportion of looks to the distractors in the interesting trials was .24 ($n =$

21, $SD = .16$), indicating that on average infants looked to distractors on approximately a quarter of the interesting trials.

The A-not-B task

On average, infants completed 26.09 trials ($SD = 8.20$) in this task. The average success rate across all valid trials was 60%. This is a modest performance level, especially considering the short duration of the initial delay (2 s) and the fact that the delay was decreased if the infant failed persistently. However, large individual differences in performance were still evident. The maximum observed delay at which infants succeeded on change trials varied between 0 and 8.61 s, with the average being 4.00 s ($SD = 2.56$). The average cumulative delay (see Method section) was 0.43 s ($SD = 0.40$), ranging from 0 to 1.26 s. The latter measure was used in the correlational analyses reported below.

The Spatial Conflict task

Twenty children completed the Spatial Conflict task at 24 months. One child only completed half of the trials (Session 1), and was excluded from analyses involving the Spatial Conflict task. Mean accuracy was .77 ($SD = .15$) on non-conflict trials and .65 ($SD = .17$) on conflict trials. The mean difference in accuracy between the two conditions was .12 ($SD = .21$). A paired-samples t test showed a significant difference in accuracy between the two trial types, $t(18) = 2.57$, $p = .019$, two-tailed, $r = .52$, replicating the results of Gerardi-Caulton (2000) and Rothbart et al. (2003).

Previous studies have found that not only do 2-year-olds perform quite poorly on the Spatial Conflict task, there is also considerably more data loss in this age group than with older children, and it could be argued that the strong interference effects were driven by the particularly mature

2-year-olds who managed to perform the task. Thus, in Gerardi-Caulton's (2000) study, data from two thirds of the 24-month-olds were excluded. The exclusion criterion was strict; each child had to provide at least 75% valid trials in order to be included in the analyses (Gerardi-Caulton, 2000). In Rothbart et al.'s (2003) study 34% of the 24-month-olds were excluded using an exclusion criterion of less than 50% valid trials (Rothbart et al., 2003). In the current study all children except one (this child only completed one session and was excluded from the analyses) had more than 50% valid trials, i.e., 19 out of 20 children provided usable data using Rothbart et al.'s criterion. Sixteen out of 20 children had more than 75% valid trials (Gerardi-Caulton's criterion), and the overall proportion of valid trials was 82%.

On this basis it seems safe to conclude that the children in the current study were at least capable of attending to and performing the task. It was not the case that only a small proportion of the children completed enough trials to derive a useable measure of their performance. Furthermore, the results clearly replicated the overall results reported by Gerardi-Caulton (2000) and Rothbart et al. (2003). Specifically, the current study showed a significant difference between the two trial types (conflict vs. non-conflict). Similarly, both the overall accuracy level and accuracy on the two trial types was similar across all three studies. This evidence suggests that the Spatial Conflict task is appropriate for use with 24-month-olds when accuracy is used as the dependent measure.

The Early Childhood Behavior Questionnaire

Early Childhood Behavior Questionnaire data were collected for 19 children, (one parent did not return the questionnaire.) Score on the Effortful Control composite was the main variable of interest for the correlational analyses. However, scores on all three factors identified by Rothbart et al. (2003) were calculated in order to assess whether effects were specific to the Effortful

Control measure. All three measures were calculated using the same method as Rothbart et al. (2003).

The Effortful Control score was calculated by averaging the mean scores of the following scales: Attentional Focusing, Attentional Shifting, Inhibitory Control, Low Intensity Pleasure and Perceptual Sensitivity. The mean score on the Effortful Control composite was 4.42 ($SD = 0.44$). The lowest score on the Effortful Control composite was 3.68 and the highest score was 5.48.

The Surgency score was calculated by averaging the mean scores of the following scales: High Intensity Pleasure, Activity Level and Positive Anticipation. The mean score on the Surgency composite was 4.87 ($SD = .75$). The lowest score on the Surgency composite was 3.75 and the highest score was 6.11.

The Negative Affect score was calculated by averaging the mean scores of the following scales: Anger/Frustration, Sadness, Shyness (termed Social Fear in Rothbart, 2003) and reverse-scored² Soothability/Falling Reactivity. The mean score on the Negative Affect composite was 2.76 ($SD = .51$). The lowest score on the Negative Affect composite was 1.77 and the highest score was 3.80.

² Each item was rated on a 7-point Likert scale with 1 being the lowest rating and 7 the highest. In order to reverse the scale score, this score was subtracted from 8. In this way, an infant obtaining a score of 2 on the Soothability/Falling Reactivity scale (indicating that the infant is difficult to soothe and takes a long time to calm down) would get a reversed score of 6. Thus, a score of 6 on the reversed Soothability/Falling Reactivity scale would tend to *increase* the Negative Affect factor score.

The BSID-II

All children performed the BSID-II at 24 months. Data from the Motor Scale were missing for two children; one child refused too many items to yield a valid score, and data were lost from one child due to equipment malfunction. The mean Mental Scale score was 104.70 ($SD = 9.23$). The mean Motor Scale score was 102.39 ($SD = 13.82$).

Correlations

Correlations between measures taken at 9 and 24 months are presented in Table 3.3 and a graphic representation of the relationships can be found in Figure 3.1. All infants who calibrated in the Freeze-Frame task were included in the correlational analyses, including wherever possible the four infants who did not have complete data from all three phases of the experiment. Given the varying number of data points for each measure, n is indicated for the following analyses.

Table 3.3. Correlations between measures taken at 9 and 24 months of age.

	9 months: FF Selective Inhibition	9 months: FF Selective Inhibitory Learning	9 months: FF General Distractibility	9 months: FF General Distractibility: Boring Trials	9 months: FF General Distractibility: Interesting Trials	9 months: A- not-B Performance	24 months: Spatial Conflict Interference	24 months: ECBQ Effortful Control	24 months: ECBQ Surgency	24 months: ECBQ Negative Affect	24 months: BSID-II Mental Scale	24 months: BSID-II Motor Scale
9 months: FF General Inhibitory Learning	$r = -.379$ $p = .110$ $n = 19$	$r = .139$ $p = .595$ $n = 17$	$r = .022$ $p = .927$ $n = 20$	$r = -.343$ $p = .139$ $n = 20$	$r = .309$ $p = .185$ $n = 20$	$r = .048$ $p = .841$ $n = 20$	$r = -.065$ $p = .810$ $n = 16$	$r = -.126$ $p = .643$ $n = 16$	$r = .098$ $p = .719$ $n = 16$	$r = .304$ $p = .252$ $n = 16$	$r = .234$ $p = .384$ $n = 16$	$r = .171$ $p = .543$ $n = 15$
9 months: FF Selective Inhibition	—	$r = -.657^{**}$ $p = .004$ $n = 17$	$r = .153$ $p = .521$ $n = 20$	$r = .566^{**}$ $p = .009$ $n = 20$	$r = -.297$ $p = .204$ $n = 20$	$r = .456^{*}$ $p = .043$ $n = 20$	$r = .531^{*}$ $p = .041$ $n = 15$	$r = -.514^{*}$ $p = .042$ $n = 16$	$r = -.305$ $p = .250$ $n = 16$	$r = .450^{†}$ $p = .080$ $n = 16$	$r = .011$ $p = .968$ $n = 16$	$r = -.242$ $p = .385$ $n = 15$
9 months: FF Selective Inhibitory Learning	—	—	$r = -.354$ $p = .163$ $n = 17$	$r = -.262$ $p = .311$ $n = 17$	$r = -.222$ $p = .391$ $n = 17$	$r = -.356$ $p = .161$ $n = 17$	$r = -.656^{*}$ $p = .015$ $n = 13$	$r = .432$ $p = .141$ $n = 13$	$r = .219$ $p = .472$ $n = 13$	$r = -.339$ $p = .257$ $n = 13$	$r = -.005$ $p = .986$ $n = 13$	$r = -.004$ $p = .990$ $n = 12$
9 months: FF General Distractibility	—	—	—	$r = .742^{**}$ $p < .001$ $n = 21$	$r = .831^{**}$ $p < .001$ $n = 21$	$r = -.061$ $p = .794$ $n = 21$	$r = .272$ $p = .309$ $n = 16$	$r = -.103$ $p = .693$ $n = 17$	$r = -.326$ $p = .202$ $n = 17$	$r = -.005$ $p = .986$ $n = 17$	$r = .013$ $p = .959$ $n = 17$	$r = -.509^{*}$ $p = .044$ $n = 16$
9 months: FF General Distractibility: Boring Trials	—	—	—	—	$r = .258$ $p = .259$ $n = 21$	$r = .165$ $p = .474$ $n = 21$	$r = .291$ $p = .274$ $n = 16$	$r = -.235$ $p = .364$ $n = 17$	$r = -.361$ $p = .155$ $n = 17$	$r = .040$ $p = .878$ $n = 17$	$r = .017$ $p = .949$ $n = 17$	$r = -.603^{*}$ $p = .013$ $n = 16$
9 months: FF General Distractibility: Interesting Trials	—	—	—	—	—	$r = -.237$ $p = .300$ $n = 21$	$r = .105$ $p = .699$ $n = 16$	$r = .023$ $p = .930$ $n = 17$	$r = -.218$ $p = .400$ $n = 17$	$r = -.041$ $p = .876$ $n = 17$	$r = .029$ $p = .911$ $n = 17$	$r = -.252$ $p = .347$ $n = 16$
9 months: A- not-B Performance	—	—	—	—	—	—	$r = .239$ $p = .339$ $n = 18$	$r = -.521^{*}$ $p = .027$ $n = 18$	$r = -.288$ $p = .246$ $n = 18$	$r = .250$ $p = .316$ $n = 18$	$r = .173$ $p = .478$ $n = 19$	$r = .147$ $p = .573$ $n = 17$
24 months: Spatial Conflict Interference	—	—	—	—	—	—	—	$r = .054$ $p = .832$ $n = 18$	$r = -.094$ $p = .709$ $n = 18$	$r = .087$ $p = .731$ $n = 18$	$r = .092$ $p = .707$ $n = 19$	$r = -.517^{*}$ $p = .034$ $n = 17$
24 months: ECBQ Effortful Control	—	—	—	—	—	—	—	—	$r = .153$ $p = .531$ $n = 19$	$r = -.579^{**}$ $p = .009$ $n = 19$	$r = .125$ $p = .610$ $n = 19$	$r = -.356$ $p = .161$ $n = 17$
24 months: ECBQ Surgency	—	—	—	—	—	—	—	—	—	$r = -.196$ $p = .421$ $n = 19$	$r = -.151$ $p = .539$ $n = 19$	$r = .393$ $p = .119$ $n = 17$
24 months: ECBQ Negative Affect	—	—	—	—	—	—	—	—	—	—	$r = .088$ $p = .720$ $n = 19$	$r = .114$ $p = .663$ $n = 17$
24 months: BSID-II Mental Scale	—	—	—	—	—	—	—	—	—	—	—	$r = -.218$ $p = .385$ $n = 18$

Note: FF = Freeze-Frame; ECBQ = Early Childhood Behavior Questionnaire; BSID-II = Bayley Scales of Infant Development, 2nd Edition.

****** $p < .01$, ***** $p < .05$, **†** $p < .10$. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity. D/AP correction was carried out separately for tests of the relationship between the frontal cortex tasks (Freeze-Frame, A-not-B task and Spatial Conflict) (28 tests: $p_{a28} < .005$), and for tests of these tasks in relation to ECBQ factor scores (27 tests: $p_{a27} < .004$) and BSID-II scale scores (23 tests: $p_{a23} < .003$).

Figure 3.2. Diagram of correlations between measures taken at 9 and 24 months of age.

Note: All correlations are significant at the .05 level or lower except for the correlation indicated by the dotted line, which is significant at trend level ($p < .10$).

Correlations between measures taken at 9 months. The Freeze-Frame General Inhibitory Learning index was not significantly related to any of the other measures taken at 9 months. However, The Freeze-Frame Selective Inhibition index was strongly negatively related to the Freeze-Frame Selective Inhibitory Learning index, $n = 17$, $r = -.657$, $p = .004$. This indicates that infants who initially looked relatively less to the distractors in the interesting trials than the boring trials (infants with a high Selective Inhibition score) tended to show a smaller decrease in looking to the distractors in the interesting trials than in the boring trials (i.e., they had a low Selective Inhibitory Learning score). Conversely, infants who initially looked more equally to the distractors in the two trial types (infants with a low Selective Inhibition score) showed a larger relative decrease in looks to the distractors in the interesting

trials than in the boring trials (i.e., they had a high Selective Inhibitory Learning score).

In terms of general distractibility, the Freeze-Frame General Distractibility index was highly correlated with both the Freeze-Frame General Distractibility index for boring trials, $n = 21$, $r = .742$, $p < .001$, and interesting trials, $n = 21$, $r = .831$, $p < .001$. This is unsurprising given the fact that the first measure is a composite of the two latter measures. However, it is noteworthy that the General Distractibility index for boring trials did not correlate with the equivalent index for interesting trials, indicating potentially separable mechanisms for distractibility when the central stimulus is interesting compared to boring. Finally, the Freeze-Frame Selective Inhibition index was significantly associated with the General Distractibility index for boring trials, $n = 20$, $r = .566$, $p < .009$, possibly indicating that a higher level of distractibility in the boring trials drives a considerable proportion of the individual differences in the Selective Inhibition index.

Of particular interest for the current study is the finding of a significant correlation between the Freeze-Frame Selective Inhibition index and A-not-B performance, $n = 20$, $r = .456$, $p = .043$. This results indicates that infants who initially looked less to the distractors in the interesting trials than in the boring trials tended to sustain longer delays in the A-not-B task. Selective Inhibition was the only Freeze-Frame index showing a significant association with A-not-B performance.

Correlations between measures taken at 24 months. At 24 months Spatial Conflict interference score was negatively correlated with BSID-II Motor Scale score, $n = 17$, $r = -.517$, $p = .034$, indicating that children with high levels of conflict interference had poorer motor skills. Furthermore, the ECBQ Effortful Control score was negatively related to the ECBQ Negative Affect score, $n = 19$, $r = -.579$, $p = .009$,

replicating previous findings (Putnam et al., 2006). No other measures taken at 24 months were significantly related.

Correlations between measures taken at 9 and 24 months. The cross-age correlations revealed several significant relationships between the 9- and 24-month measures. The General Inhibitory Learning index was not significantly related to any of the 24-month measures. However, the Selective Inhibition index was positively related to Spatial Conflict interference, $n = 15$, $r = .531$, $p = .041$, indicating that children who had a high level of Selective Inhibition in the Freeze-Frame task at 9 months showed more conflict interference at 24 months. The Freeze-Frame Selective Inhibition index was also negatively correlated with ECBQ Effortful Control at 24 months, $n = 16$, $r = -.514$, $p = .042$, and there was a trend towards a positive correlation between this index and ECBQ Negative Affect, $n = 16$, $r = .450$, $p = .080$.

The Freeze-Frame Selective Inhibitory Learning index was negatively correlated with Spatial Conflict interference at 24 months, $n = 13$, $r = -.656$, $p = .015$, indicating that infants with good selective inhibitory learning skills at 9 months showed less conflict interference later in development. This relationship is illustrated in Figure 3.2.

Figure 3.3. Scatter plot of correlation between the Freeze-Frame Selective Inhibitory Learning index at 9 months of age and Spatial Conflict interference at 24 months of age.

Note: $n = 13$, $r = -.656$, $p = .015$

A-not-B performance at 9 months was negatively correlated with Effortful Control at 24 months, $n = 18$, $r = -.521$, $p = .027$.

Finally, the Freeze-Frame General Distractibility index was negatively correlated with BSID-II Motor Scale score at 24 months, $n = 16$, $r = -.509$, $p = .044$. The Freeze-Frame General Distractibility index for boring trials, but not for interesting trials, was also negatively related to performance on the BSID-II Motor Scale, $n = 16$, $r = -.603$, $p = .013$. This indicates that overall distractibility level in the boring trials of the Freeze-Frame task predicted general motor performance at 2 years of age.

Correction for multiplicity. The only correlations which survived the D/AP correction for multiplicity were three strong correlations between the Freeze-Frame indices (see Table 3.3). This is likely due to the relatively small sample size of Cohort

1. The results should therefore be interpreted with caution, although some of these findings were replicated in Cohort 2 (see Chapter 5).

Overview of results. With the caveat regarding small sample size in mind, the results do seem to confirm the hypothesis that the three inhibitory indices derived from the Freeze-Frame task would be associated with performance on frontal cortex tasks during early development. As expected, the Selective Inhibitory Learning index was the measure most strongly associated with good inhibitory performance; this measure was a strong predictor of Spatial Conflict performance at 2 years.

Interestingly, the Selective Inhibitory Learning index was also significantly related to the Selective Inhibition index, indicating that early performance differences in the Freeze-Frame task were an important factor in infants' inhibitory learning during the task. Furthermore, the Selective Inhibition index was related to all the other frontal cortex tasks used in the study, though this measure was not always associated with better inhibitory performance. The General Inhibitory Learning index was not significantly associated with any of the other measures, indicating that general learning during the Freeze-Frame task was independent of the other components of the task as well as later general development and frontal task performance.

To summarise the most important points: it was mainly the inhibitory Freeze-Frame indices, rather than the General Distractibility indices, which showed significant associations with other frontal cortex tasks. Furthermore, these indices were not significantly associated with BSID-II scores at 2 years, suggesting that the inhibitory indices tap into more specific functioning. However, General Distractibility in the Freeze-Frame task, especially in the boring trials, did relate to general motor performance at 2 years and also to one of the inhibitory indices (Selective Inhibition).

Discussion

A new task to measure inhibitory function in infancy

The main aim of the current study was to develop a new easily administered task to measure inhibitory function in infancy. The new task, called the Freeze-Frame task, was developed on the basis of previous work suggesting the existence of frontal cortex functioning in young infants using eye movements as the dependent measure (Gilmore & Johnson, 1995; Johnson, 1995). In order to validate this novel task, possible associations with a set of measures that have been related to frontal cortex functioning in infancy and early childhood were investigated.

In the Freeze-Frame task infants had to inhibit looking to peripherally presented distractors in order to keep a central cartoon animated. On every other trial the central cartoon was new and changeable (interesting trials), whereas the remaining trials presented a repetitive animated stimulus (boring trials). Three effects were anticipated: (a) A general decrease in looking to the distractors during the experiment across the two trial types (General Inhibitory Learning), (b) a lower rate of looking to the distractors during interesting trials than during boring trials already in the first phase of the experiment (Selective Inhibition), and (c) a larger decrease in looking to the distractors in the interesting trials where the motivation to inhibit would be greatest (Selective Inhibitory Learning).

Individual performance indices were calculated based on these components of the Freeze-Frame task. The first index, General Inhibitory Learning, refers to the infant's general learning during the experiment. This index, which is a measure of the overall decrease in looks to the distractors during the test session, may be equivalent to basic habituation or may constitute a more active process. The second index, Selective

Inhibition, was hypothesised to be a measure of differences in sensitivity to stimulus competition early in the experiment, before learning had taken place. Finally, the third index, Selective Inhibitory Learning, was hypothesised to be a measure of infants' ability to learn to selectively inhibit during the test session. It was expected that the latter index would be the most predictive of performance on frontal cortex tasks.

In addition to the main Freeze-Frame indices, three additional Freeze-Frame measures were included in the correlational analyses. These measures were included to explore whether the inhibitory Freeze-Frame indices were related to more general aspects of task performance. The three additional measures were the proportion of looks to the distractors for the experiment overall, termed General Distractibility, and for boring and interesting trials separately.

Findings at the group level

The group findings from the Freeze-Frame task provided evidence for the first and second hypothesised inhibitory effects, i.e., infants generally learned to inhibit looks to the distractors during the experiment, and they also looked less to the distractors in the interesting trials right from the beginning of the experiment. However, as a group there was no overall interaction between trial type and phase, indicating that on average the decrease in looking to the distractors was similar for the two trial types.

These results confirm that by 9 months of age most infants are able to learn to inhibit in a general, non-selective way. Consistent with this, there is considerable evidence in the literature that this ability develops early in infancy. For example, Colombo and colleagues have shown that look durations to repeated stimuli decrease between 3 and 9 months of age (Colombo, 2002; Colombo et al., 2004). In addition,

whereas young infants habituate faster with age in response to most types of stimuli, (though the average looking time is always shortest for the least interesting stimuli,) from around 6 months onwards infants' looking times to more engaging stimuli actually start to increase again, whilst looking times to less interesting stimuli stabilise (Courage, Reynolds, & Richards, 2006). These effects have been hypothesised to be due to the emergence of endogenous attentional control during the second half of the first year of life (Colombo, 2002; Courage et al., 2006).

The group results from the study also indicate that the initial level of distractibility in the Freeze-Frame task is modulated by the attractiveness of the central stimulus. Such competition effects have been shown to exist from as early as 2 to 3 months of age, though they tend to decrease with age (Blaga & Colombo, 2006; Dannemiller, 1998, 2000).

Individual differences in Freeze-Frame performance and their association with performance on early frontal cortex tasks

For the purposes of the current study, individual differences in Freeze-Frame performance were of particular interest. This is because individual differences in task performance provide additional important information about task structure and enable us to compare Freeze-Frame performance to performance on other hypothesised frontal cortex tasks. For example, while there was no overall group evidence for selective inhibitory learning in the Freeze-Frame task at 9 months, there was considerable variation between individuals. In fact, this index had the largest standard deviation of all the Freeze-Frame indices. Thus, the fact that no interaction was found in the group data does not indicate that all the infants performed the task in the same way. On the contrary, infants showed very different learning patterns in the two trial

types across the test session. It was hypothesised that variation in this measure would be a good predictor of performance on other frontal cortex tasks. In particular, it was expected that infants who demonstrated faster learning in the interesting trials relative to the boring trials would perform better on the frontal cortex tasks. Thus, following up on the group results, the predictive value of the Freeze-Frame indices was investigated.

General Distractibility. The three measures assessing general distractibility during the Freeze-Frame task were not significantly associated with performance on the frontal cortex tasks, though further investigation is warranted given that one of these general measures (General Distractibility in the boring trials) was associated with one of the inhibitory indices (Selective Inhibition).

General Inhibitory Learning. Of the inhibitory Freeze-Frame indices, the General Inhibitory Learning index turned out to be the least predictive. This measure did not correlate with any of the other measures, indicating that general learning during the experiment was a poor predictor of performance on the frontal cortex tasks selected for the current study. This learning component of the task was also found to be unrelated to the two other inhibitory Freeze-Frame indices, indicating that general inhibitory learning may constitute a somewhat different process from the selective inhibitory components of the task.

This finding is also important when considering the learning aspect of the Freeze-Frame task. Learning is clearly an integral component of the task; since we cannot ask infants to inhibit looks to the distractors, we have to rely on their ability to learn to select the most engaging stimuli. However, the fact that measures based on differences between the two trial types (Selective Inhibition and Selective Inhibitory Learning) were relatively independent of the overall decrease in looking to the

distractors during the session strongly suggests that the task does not simply measure general learning. Instead, infants produce a selective response to the two types of stimuli, and they show differential patterns of learning in response to these stimuli, which correlate with performance on other tasks associated with inhibitory function (discussed further below).

Selective Inhibition. The Freeze-Frame Selective Inhibition index turned out to be a strong predictor, though good performance on the index was not always associated with good inhibitory performance on the frontal cortex tasks. At 9 months the Selective Inhibition index was positively correlated with A-not-B performance, a result that seems to indicate that an early difference in distractibility in the two trial types is associated with good inhibitory performance. However, the Selective Inhibition index was negatively related to Effortful Control and positively related to Spatial Conflict interference at 24 months. It also tended to correlate positively with Negative Affect at 24 months. That is, infants with a high score on the Selective Inhibition index tended to have high levels of interference in the Spatial Conflict task, low levels of Effortful Control, and possibly express more Negative Affect, later in development. This counter-intuitive result indicates that apparently good performance on the Selective Inhibition index was in fact predictive of poor performance on the later frontal cortex measures used in the current study. However, the result was not entirely specific to the Freeze-Frame Selective Inhibition index since A-not-B performance at 9 months was also negatively related to Effortful Control at 24 months. I will return to possible explanations for these findings below.

Selective Inhibitory Learning. Importantly, the Freeze-Frame Selective Inhibitory Learning index was strongly related to performance on the Spatial Conflict task at 2 years. Infants with a high Selective Inhibitory Learning score at 9 months showed less

conflict interference later in development (see Figure 2). This finding confirms the hypothesis that the Selective Inhibitory Learning index would be a strong predictor of frontal task performance. It also demonstrates that a purely visual task such as the Freeze-Frame task has the potential to tap into individual differences in inhibitory functioning at an early age, and that such functioning is predictive of later performance on a frontal cortex task. The Freeze-Frame task is calibrated to each individual infant and provides sensitive performance indices over a short period of time. This is in contrast to earlier visual inhibition tasks, which have provided important evidence for frontal functioning in early infancy, but are limited in terms of deriving individual performance indices (Johnson, 1995).

The strong association between the Freeze-Frame Selective Inhibitory Learning index and later Spatial Conflict performance also emphasises the power of using an individual differences approach to the study of frontal cortex functioning in infancy and early childhood. Despite the lack of interaction at the group level, infants' individual patterns of relative decrease in looks to the distractors in the two Freeze-Frame trial types demonstrated the strongest correlation with one of the later frontal cortex measures out of the three inhibitory Freeze-Frame indices (see Table 3.3). Thus, the lack of interaction at the group level conceals the fact that infants showed considerable differences in their inhibitory learning patterns during the task. Some infants showed relatively faster learning in the interesting trials, whereas other infants showed the opposite pattern, resulting in a mean Selective Inhibitory Learning score near zero. Therefore, even though *on average* (i.e., at the group level) infants did not show selective inhibitory learning by 9 months of age, infants who did show evidence of this performance pattern had superior performance on a frontal cortex task later in development. Furthermore, as discussed above, this effect is unlikely to be due to a

general learning mechanism since the overall decrease in looking to the distractors (the General Inhibitory Learning index) was not associated with performance on the selected frontal cortex tasks. Such effects can only be revealed by sensitive individual performance measures and a longitudinal approach.

The relationship between Selective Inhibition and Selective Inhibitory Learning.

The association between the Selective Inhibitory Learning index and later frontal cortex performance suggests that this index may be a sensitive measure of inhibitory functioning in infancy. However, as can be seen from Figure 1, the Selective Inhibitory Learning index was negatively related to the Selective Inhibition index. That is, the relative difference in the decrease in looks to the distractors in the two trial types across the test session was dependent on the initial difference in looks to the distractors. Specifically, infants who initially looked a lot less to the distractors in the interesting trials than the boring trials tended to decrease more in the boring trials, whereas infants who initially had a smaller difference between the two trial types, or looked more to the distractors in the interesting trials, tended to decrease more in the interesting trials. Thus, infants who showed initial evidence of selective inhibition were *not* those who showed evidence of *learning* to selectively inhibit during the course of the test session.

Interpretation of the Selective Inhibition index. The latter result raises the question of what functional mechanism underlies the Freeze-Frame Selective Inhibition index. The expectation was that the Selective Inhibition index would be related to low-level processes, such as sensitivity to stimulus competition and relative distractibility before learning had taken place. This stands in contrast to Selective Inhibitory Learning, which was hypothesised to be a more active inhibitory control process. Whereas, clear predictions about the association between Selective Inhibitory

Learning and the frontal cortex measures were made from the start, no specific predictions were made about how Selective Inhibition would be related to these measures. It turned out that this measure was in fact strongly predictive of poor performance on frontal cortex tasks later in development, consistent with its reverse relationship to the Selective Inhibitory Learning index.

One interpretation of this result is that having a large initial difference in distractibility between the two trial types in the Freeze-Frame task indicates a lack of flexibility in the infant's looking behaviour. It is possible that adaptive performance early in the task is in fact associated with a fair amount of distractibility, across trial types, allowing the infant to gradually gain more control over his or her eye movements, (as evidenced by improved selective inhibitory learning in the more distractible infants). In contrast, infants who are less distractible when the stimulus is engaging may experience problems disengaging their visual attention; these infants may only be able to gain control over their eye movements during the more repetitive trials. Thus, according to this interpretation the infant needs to have a baseline level of distractibility in order to learn to selectively inhibit during the task.

Not much is known about how differences in distractibility and sensitivity to stimulus competition are related to performance on frontal cortex tasks. If the Freeze-Frame Selective Inhibition index is indeed related to infants' baseline distractibility, then the results indicate that such factors are associated with performance on frontal cortex tasks. The mechanism behind this possible relationship needs to be further explored. However, the results from the current study seem to suggest that at least some flexibility in the infant's visually-guided behaviour is necessary for successful inhibitory performance, both in infancy and later in development. Interestingly, A-not-B performance was also negatively associated with one of the 24-month frontal

cortex measures (Effortful Control), so whatever mechanism underlies the Freeze-Frame Selective Inhibition index may also play a role in A-not-B performance.

Baseline relative distractibility. Given the potential role of baseline relative distractibility in the Freeze-Frame Selective Inhibition index, it is worth considering the prevalence and correlates of such differences. Previous research has demonstrated that infants show clear patterns of stimulus-driven behaviour. For example, Dannemiller has demonstrated that infants' likelihood of orienting to a contra-lateral moving stimulus is modulated by the number, colour and position of distractors in the visual field (Dannemiller, 1998, 2000). Furthermore, a recent study by Blaga and Colombo (2006) showed that infants' ocular latencies to peripheral targets were affected by the novelty of the central stimulus. Little is known about the significance of individual differences in such stimulus-driven competition effects. However, large individual differences in the ability to *disengage* from centrally presented stimuli in fixation shift paradigms have been established (Atkinson et al., 1992; Hood & Atkinson, 1993; Johnson et al., 1991), and these differences have been found to be related to other aspects of infant development, such as temperament (Johnson et al., 1991; McConnell & Bryson, 2005).

In the Freeze-Frame task the most likely point where such disengagement effects would play a role in the infant's performance would be in the beginning of the experiment before the infant has had a chance to learn to selectively inhibit looks to the distractors. Therefore the Freeze-Frame Selective Inhibition index is likely to be associated with the infant's baseline capacity to disengage from the central stimulus as a function of the attractiveness of that stimulus.

With regard to this possibility, it is interesting to note that individual differences in the ability to disengage visual attention, as measured by experimental paradigms,

has been related to self-regulatory function in both infants (Johnson et al., 1991; McConnell & Bryson, 2005) and adults (Compton, 2000). In general these studies have found that individuals who can easily disengage attention have better self-regulatory function. Thus, if the Freeze-Frame Selective Inhibition index is related to stimulus-dependent disengagement of attention this may explain the negative association between this measure and later Effortful Control. That is, infants who are relatively less distractible when the stimulus is engaging may experience problems regulating their behaviour more generally. This interpretation is also consistent with the positive correlation between the Freeze-Frame Selective Inhibition index and later levels of Negative Affect, though this association was only significant at trend level.

Even though the above evidence only suggests a link between disengagement and temperamental factors related to self-regulation, a couple of very recent studies support a direct link between infant temperament and performance on the A-not-B task. Thus, Bell and colleagues found that the 8- to 10-month-old infants who performed *better* on a looking version of the A-not-B task were those who took longer to calm down, had higher activity levels, and showed more distress as measured by the Infant Behavior Questionnaire (Bell, 2005; Bell, Wolfe, & Adkins, 2007; Bell, Wolfe, Morasch, & Cardell, 2006). This indicates that factors such as self-regulatory function may play an important role in individual performance differences even in well-established frontal cortex tasks such as the A-not-B task. However, even though the results of this initial study suggest possible direct links between visual disengagement, self-regulation, and frontal cortex performance, further research is needed to thoroughly establish the association between these functions, in particular the relationship between stimulus-dependent visual disengagement and frontal cortex

performance. The studies reported in the following chapters investigate some of these questions.

Freeze-Frame performance and general development

Considering the overall pattern of results it is noticeable that only a few of the frontal cortex measures was related to general development as indexed by the BSID-II (see Table 3.3). Spatial Conflict interference was related to BSID-II Motor Scale score at 2 years of age, indicating that children who had large conflict interference effects had poorer motor skills. General Distractibility in the Freeze-Frame task at 9 months, especially in the boring trials, was also associated with BSID-II Motor Scale score at age 2. Though the latter effect is not specifically inhibitory in nature, the results do indicate that general motor skills may play a role in the Freeze-Frame and Spatial Conflict tasks, a question that warrants further investigation. However, none of the measures was related to BSID-II Mental Scale score, suggesting that both the 9- and 24-month frontal cortex tasks, including the Freeze-Frame indices, were relatively independent of general cognitive development at 2 years of age. Thus, the Freeze-Frame task and the frontal cortex tasks associated with it seem to tap into components of cognitive functioning that are relatively separate from general cognitive development in toddlerhood.

Task selection

Given the complexity of the results from this study, especially as regards the Freeze-Frame Selective Inhibition index, it seems appropriate to consider whether the administered tasks were adequate measures of frontal cortex functioning. As discussed in the introduction to this chapter, very few frontal cortex tasks have been developed for infants and toddlers. The aim of this study was to compare the new

Freeze-Frame task to some of the few well-studied infant and toddler tasks developed to measure frontal cortex functioning.

In infancy the obvious choice was the A-not-B task, since this task has been related to the frontal cortex in several previous studies (e.g., Baird et al., 2002; Bell & Fox, 1992; Diamond & Goldman-Rakic, 1989). It has to be acknowledged that the mechanism behind A-not-B performance, in particular perseveration, is a matter of debate. The A-not-B task has been shown to be sensitive to many contextual factors (Smith et al., 1999; Thelen et al., 2001), and the role of inhibition, versus other potential mechanism of task performance, such as memory and motor programming, remains controversial (e.g., Clearfield, Diedrich, Smith, & Thelen, 2006; Munakata, 1998). However, even though the A-not-B task is not yet fully understood, it seems feasible to conclude that the results of the current study cannot be adequately explained by a set of contextual influences. In the current study the canonical version of the task was used. Infants' scores on the task were not based on a single trial, which would be sensitive to various contextual effects. Instead, infants had to consistently reach correctly across trials to obtain a high score. More importantly, the measures found to be correlated with the A-not-B task, such as the Freeze-Frame Selective Inhibition index and later Effortful Control, were administered in very different contexts and at different points in time; therefore at least a significant proportion of the variance in A-not-B performance is likely to be related to the individual child's functioning.

A similar issue arises from using the Spatial Conflict task. This task is difficult for 2-year-olds, and previous studies have had considerable data loss in this age group (Gerardi-Caulton, 2000; Rothbart et al., 2003). However, as discussed in the Results section, the current study had less data loss than previous studies and clearly

replicated these studies in terms of overall performance and the effect of spatial conflict. It therefore seems safe to conclude that the Spatial Conflict task is adequate for use with 2-year-olds.

Conclusions

In conclusion, in this initial study it was found that the new Freeze-Frame task was associated with performance on several measures of frontal cortex function both within and across age. Previous studies have not investigated performance on this specific set of frontal cortex tasks in the same children across time, and thus have not established continuity or discontinuity of individual differences. However, the present study suggests that different aspects of inhibitory function are continuous across early development. It was found that the Freeze-Frame Selective Inhibitory Learning index predicted Spatial Conflict performance later in development; this demonstrates the promise of using visual inhibition tasks to measure early frontal functioning. Furthermore, other aspects of the Freeze-Frame task, such as the Selective Inhibition index, as well as the A-not-B task, tended to correlate negatively with later frontal cortex measures. Factors related to low-level inhibition, such as sensitivity to stimulus competition, distractibility, and disengagement of attention, may play an important role in these effects.

The following chapters report on a large longitudinal study carried out to replicate and follow up on these findings. In this second cohort infants were studied from an early age on a large set of measures, including frontal cortex tasks, temperament measures, and tests of general development.

Summary of Chapter 3

- This chapter reported on the development of a new infant inhibition task, the Freeze-Frame task, and its relationship to the few available infant and toddler frontal cortex tasks.
- The new task used eye movements as the dependent measure and was designed in a way that allowed collection of sensitive individual differences data in a short period of time.
- While engaged with a central stimulus of varying attractiveness ('boring' and 'interesting' trials), infants had to inhibit looks to peripheral distractors.
- It was found that two out of the three inhibitory indices from the Freeze-Frame task were correlated with performance on early frontal cortex tasks both within age and longitudinally.
- As expected, infants' ability to learn to selectively inhibit looks to peripheral distractors in the Freeze-Frame task (by showing a larger decrease in looks to the distractors in the more engaging trials) was a strong predictor of later frontal cortex task performance.
- Baseline differences in distractibility as a function of the attractiveness of the central stimulus were also associated with performance on early frontal cortex tasks. Some of these associations suggested that flexible disengagement of attention may be important for successful frontal cortex task performance in infancy.
- Freeze-Frame performance was relatively independent of general cognitive development.

CHAPTER 4

The relationship between frontal cortex tasks and temperament at 4 months

Introduction

To follow up on the initial study investigating the new Freeze-Frame task, a considerably larger longitudinal study was carried out. The initial study left some questions unanswered and also raised additional questions. Chapters 4, 5 and 6 of this thesis report on this large-scale study. The current chapter focuses on data collected when infants were 4 months old.

One area that the initial study did not address was the question of whether frontal cortex functioning can be reliably established at an age earlier than 9 months, and whether sensitive indices of individual differences in such functioning can be reliably established. In the first part of the current study a large group of infants were tested at 4 months of age. Infants completed two tasks that have been linked to frontal cortex activity in previous ERP studies (Csibra et al., 1998, 2001): the Gap task and the Anticipation task. However, because both of these paradigms rely on infants' ability to control their eye movements, it is pertinent to consider some general theories of cortical development in relation to visual attention during the first 6 months of life.

Cortical development and visual attention during the first 6 months of life

One of the first paradigms used to investigate young infants' ability to shift attention under different conditions was developed by Atkinson and colleagues (1992). In the *Fixation shift* paradigm a central stimulus fixated by the infant either disappears at the same time as a peripheral target appears on the screen

(noncompetition condition) or stays on during target presentation (competition condition). In a series of studies of both typically developing infants and neurologically impaired children (Atkinson et al., 1992; Braddick & Atkinson, 1988; B. Hood & Atkinson, 1990), Atkinson and colleagues found that both 1- and 3-month-olds were less accurate and slower in the competition condition compared to the noncompetition condition. However, compared to 3-month-olds, 1-month-olds had particular difficulty responding to targets in the competition condition as assessed by saccadic latencies. This effect is unlikely to be due to lower-level visual effects such as contrast sensitivity. Even though 1-month-olds had lower contrast sensitivity than 3-month-olds, they still performed worse than the 3-month-olds in the competition condition when contrast was adjusted to age-appropriate levels (Atkinson et al., 1992).

The findings of Atkinson et al. (1992) fit well with theories proposing that subcortical eye movement systems come under cortical control during the first few months of life (Atkinson, 1984a, 1984b; Braddick & Atkinson, 1988; Bronson, 1974; Johnson, 1990). In her early work Atkinson (Atkinson, 1984a, 1984b; Braddick & Atkinson, 1988) suggested that visual behaviour in infants younger than approximately 2 months of age is largely mediated by subcortical areas, such as the superior colliculus. From 2 months onwards cortical mechanisms start to mature, enabling infants to exert attentional control over their eye movements. A similar theory has been proposed by Bronson (1974), though this author also emphasises the development of the central retina as an important factor.

The cortical control theory has gained support from a study of hemispherectomised infants (Braddick et al., 1992; Hood, 1995). These infants were able to detect stimuli in the half-field contralateral to the lesion, indicating a

subcortical route for orienting to visual stimulation. However, during competition in the fixation shift paradigm one such infant (participant PP) failed to orient to the contralesional half-field, indicating that the cortex is needed to overcome visual competition.

There is some controversy as to which areas of the cortex gain control over the eye movement system during the first few months of life. Bronson (1974) suggested rapid development of the visual cortex as underlying the behavioural changes. Atkinson and colleagues (Braddick & Atkinson, 1988; B. Hood & Atkinson, 1990) have emphasised the parietal cortex and parieto-occipital connections due to the important role of the parietal cortex in visual attention (Mountcastle, 1978; Posner, Walker, Friedrich, & Rafal, 1984; Rafal, 1994). For example, patients with hemispatial neglect caused by lesions to the right parietal cortex have difficulty attending to peripheral stimuli in the affected visual field during competition from the normal visual field (Posner et al., 1984). This type of performance has been compared to that seen in very young infants (Atkinson et al., 1992; Braddick & Atkinson, 1988; B. Hood & Atkinson, 1990), thus implying immaturity of the parietal cortex in early infancy. However, Atkinson and colleagues have also pointed to the frontal cortex as a potentially important area during this early stage of visual development (Atkinson, 1984a; Atkinson & Braddick, 2003; Atkinson et al., 1992).

Based on a model proposed by Schiller (1985), Johnson (1990) suggested that four pathways mediating visual attention develop at different points over the first 6 months of life. Initially, eye movements are primarily controlled by the superior colliculus. This is followed by development of an inhibitory pathway from the substantia nigra to the superior colliculus around 1 month of age, which results in the disengagement problems described above. From 2-3 months onwards the maturation

of pathways between the colliculus and cortical areas such as the middle temporal area (MT) and the frontal eye fields (FEF) enables infants to move their eyes more flexibly and to anticipate visual events. Given the proposal that the maturation of the MT pathway primarily enables the smooth pursuit of objects during visual tracking, it would appear that Johnson (1990) attributes higher-level control of the eye movement system (such as anticipatory eye movements) to the frontal areas. However, Johnson (1990) also clearly points out that maturation of different layers of the primary visual cortex is the limiting factor in the development of these systems since all pathways, except the collicular pathway (which is functional at birth), involve the primary visual cortex.

In conclusion, most theorists agree that cortical areas take over control of visual attention during the first few months of life. Both the primary visual, parietal, and frontal cortices, as well as the connectivity between these areas and subcortical areas have been proposed as potential loci for this development. However, a few recent event-related potential studies have emphasised the frontal cortex as playing a particularly important role in saccadic control in early infancy. These studies will be reviewed in the following sections.

The Gap task

In the Gap task, infants are first presented with a central stimulus, followed by a peripheral stimulus. On some trials the central stimulus disappears just before the peripheral target is presented (Gap condition), whereas on the remaining trials the central stimulus stays on the screen while the peripheral target is presented (Overlap condition).

Thus, the main difference between the Fixation shift task described above and the Gap task is that the Gap task involves a condition where the central stimulus disappears just before the target is presented (i.e., not simultaneously with target presentation). The Gap condition is thought to help the participant disengage from the central stimulus and orient to the target (Fischer & Breitmeyer, 1987; Hood, 1995; Hood & Atkinson, 1993), possibly via an effect on attention systems in the parietal cortex (Csibra et al., 1997; Fischer & Breitmeyer, 1987; for discussion, see Hood, 1995) or eye movement systems in the frontal cortex (Csibra et al., 1998). It has been consistently found that participants take longer to make a saccade to the target in the Overlap condition compared to the Gap condition (Csibra et al., 1997; Csibra et al., 1998; Hood & Atkinson, 1993). Furthermore, consistent with earlier findings using the fixation shift paradigm, infants below 3 months of age have great difficulty disengaging from the central stimulus in the Overlap condition of the Gap task (Hood & Atkinson, 1993; Johnson et al., 1991).

As discussed above, this pattern of results likely indicates that the necessary cortical circuitry for controlling eye movements is not fully developed at this early age. Even though different authors agree that maturation of the cortex and connectivity between the cortex and subcortical areas are likely to underlie the increased flexibility in infant attention from about 2-3 months of age, very little is known about the specific areas involved. Studies of adult patients with parietal lesions have indicated that the parietal cortex is vital for enabling attention to parts of a display during visual competition (Posner et al., 1984). However, it has also been shown that patients with frontal cortex damage have difficulty controlling their eye movements when cued to a specific location (Guitton et al., 1985). Furthermore, different areas may play a larger role at different points in development.

Thus, the development of visual attention in young infants is likely to involve maturation of parietal and frontal cortical areas and the connections between these areas, primary visual cortex, and subcortical areas. However, evidence from electrophysiological studies has provided more consistent evidence for frontal involvement than parietal involvement in eye movement control, at least in infancy. Most importantly, by 6 months of age there is evidence that the frontal cortex plays a role in saccadic control in the Gap task. Csibra et al. (1998) found a left frontal positivity occurring just after target onset in the Gap condition. They interpreted this effect as equivalent to a central stimulus offset-related effect found in adults (Csibra et al., 1997), and speculated that the effect may be due to frontal disinhibition of collicular systems for saccade generation. In contrast, the parietal pre-saccadic positivity and spike potential observed in adults was not observed in the infant sample (Csibra et al., 1998).

In the current study, good performance on the Gap task was hypothesised to be associated with ease of disengagement. It was expected that optimal performance would be characterised by fast reaction times in the Overlap condition and a minimal “Gap effect” (the difference in reaction time between the Gap and Overlap conditions). Given the fact that young infants have difficulty disengaging when presented with competing visual stimuli, possibly due to the lack of frontal cortical control, it was expected that efficient disengagement in the task indicated more mature performance.

The Anticipation task

The second task employed in the 4-month study was a version of the visual expectation paradigm developed by Haith and colleagues (1988). In this simple task,

which will be referred to as the *Anticipation task*, infants are presented with an alternating left-right sequence of dynamic stimuli. When infants are presented with this kind of sequence they start to anticipate the next target; that is, they start moving their eyes to the next target location before it appears (Haith et al., 1988). Johnson (1990) has argued that anticipatory eye movements are enabled by the development of the frontal (FEF) pathway from around 3 months of age (for a more detailed description of Johnson's theory see above). In line with this, Csibra and colleagues (2001) carried out an ERP study using the anticipation paradigm with 4-month-old infants and found that anticipatory saccades were associated with a right frontal positivity following the offset of the previous stimulus. Though the mechanism behind the laterality of this effect is as yet unknown, this finding is further evidence that the frontal cortex plays a role in saccadic control as early as 4 months of age.

Study outline

Based on these results, it was hypothesised that good frontal cortex performance would be characterised by a high level of anticipation in the Anticipation task and ease of disengagement in the Gap task. Since both tasks have been related to the frontal cortex in relation to saccadic control, a correlation between the two tasks would indicate that the mechanism of such control is similar when the infant initiates a saccade in response to an external stimulus (the Gap task) and when the saccade is initiated by the infant in the absence of such stimulation (the Anticipation task).

The current study also involved an investigation of the Gap and Anticipation tasks in relation to infant temperament as assessed by the Revised Infant Behavior Questionnaire (Gartstein & Rothbart, 2003). The questionnaire was included because regulatory functioning has been related to task performance on potential early frontal

cortex tasks (Johnson et al., 1991; McConnell & Bryson, 2005; See also Chapter 3). Johnson et al. (1991) found that ease of disengagement on Overlap trials in the Gap task³ was associated with lower levels of Fear and Distress and with a higher level of Soothability in 4-month-old infants. Similarly, McConnell and Bryson (2005) found that infants who took longer to disengage showed more distress at 6 months. The latter study also found that infants who were slower in the Gap condition (but not the Overlap condition) at 4 months were more fearful. Finally, the study by Johnson et al. (1991) found that infants who anticipated more in their combined Gap and Anticipation task at 4 months had lower levels of Soothability as assessed by the IBQ.

Based on these results, it was expected that infants who performed well on the Gap task would have better self-regulatory skills. However, given the limited evidence on associations between the Anticipation task and temperament (the study by Johnson et al. [1991] had a small sample size and was not a simple Anticipation task), no specific predictions were made regarding this relationship.

In summary, the 4-month study had two main aims. Firstly, the study aimed at establishing whether performance on the Gap task and the Anticipation task was correlated, and whether these two tasks were associated with temperament measures of self-regulation that have previously been associated with frontal task performance.

Even though both tasks have been shown to activate the frontal cortex in ERP studies, it is important to realise that ERP methodology has a much coarser spatial resolution than imaging techniques such as PET and fMRI. Unfortunately, the latter techniques are either unethical or extremely difficult to use with an infant population (but see Dehaene-Lambertz et al., 2002, for a study using fMRI with very young

³ The task used by Johnson et al. (1991) was not a straightforward Gap task as it involved both anticipatory and disengagement components.

infants during sleep). Therefore, as with most other presumed infant frontal cortex tasks, the evidence that the Gap and Anticipation tasks do indeed measure aspects of early frontal cortex functioning is relatively weak compared to findings from adult studies in this area. One step towards establishing whether these tasks tap into frontal cortex functioning is to assess whether they are correlated with each other and with other early measures thought to have a frontal component, such as some aspects of infant temperament (e.g., Posner & Rothbart, 1998). This would provide us with at least some converging evidence that the tasks measure some of the same functions.

The second aim of the 4 months study was to investigate whether performance on the Gap and Anticipation tasks was related to Freeze-Frame and A-not-B performance at 9 months of age. This question will be addressed in Chapter 6.

Method

Sample characteristics

General details on the recruitment procedure have been described previously (Chapter 2). The group of infants tested in the current study is referred to as Cohort 2 in order to distinguish it from the group of infants reported in Chapter 3 (Cohort 1). Initially 113 infants were recruited for Cohort 2. Nine of the initial appointments were cancelled before the first visit leaving 104 infants in the final sample (51 boys and 53 girls). Approximately three quarters of the cohort came from a white ethnic background (74%); the remaining participants had primarily mixed ethnic backgrounds. The mean age at the 4-month visit was 122.78 days ($SD = 4.93$).

Demographic data such as parental age, years of education and family income are presented in Table 4.1. Given the sensitivity of such data, especially family income, it

was emphasised to parents that they were under no obligation to provide this information when participating in the study. Therefore, n is indicated in the table.

As can be seen from Table 4.1, the average family income in Cohort 2 was £60,825 per year. In comparison, the national average family income in 2006 was £32,779 (British Broadcasting Corporation, 2007). Given the fact that the average family income in London is more than 30% greater than the national average (National Statistics, 2006), the difference between the current sample and the general population may be somewhat inflated. Nevertheless, these data indicate that the families who participated in the study had above average income. In terms of educational achievement, both parents had spent an average of 17-18 years in education. Sixteen years of education is roughly equivalent to the attainment of a Bachelors degree. In the current sample 62.4% of parents had at least 16 years of education. In comparison, 31% of the London adult population had a degree or higher qualification according to the UK Census 2001 (National Statistics, 2001). Again, the difference in educational background may be partly due to the fact that most parents in the current sample were in their 30s, whereas the London average includes younger individuals. However, overall, the demographic data suggest that families participating in the present study were of middle or upper-middle class socio-economic status. This is common in infant opportunity samples (e.g., Courage et al., 2006; Diamond, 1985; Frick et al., 1999).

Table 4.1. Demographic data for Cohort 2.

	<i>n</i>	Mean	<i>SD</i>	Minimum	Maximum
Mother's age	98	34.4	5.23	21	49
Mother's years of education	95	17.9	3.54	9	30
Father's age	93	36.4	6.52	23	62
Father's years of education	89	17.3	3.41	11	30
Approx. family income after tax (£)	71	63,101	60,825	6,537	500,000

General procedure

Infants were first tested on the Gap task and subsequently on the Anticipation task. This testing order was chosen because infants generally enjoyed the Anticipation task more than the Gap task. There was always a 2-5 minute break after the Gap task. During this break the lights were turned on and the infant was entertained by the parent and experimenter until the experimenter judged that the infant was ready for the Anticipation task.

The Gap task

Apparatus and stimuli. The programme for running the Gap task was created using E-Prime software and run on a PC. A schematic of the stimuli and the two conditions of the task are presented in Figure 4.1. Stimuli were presented on a 51×67 cm plasma screen positioned 1 m from where the parent and infant sat. The stimuli consisted of 14.71×14.71 cm coloured squares, subtending $8.41^\circ \times 8.41^\circ$ of visual angle. The stimulus squares were presented on a black background. The distance between the centre of the screen and the nearest edge of the target square was 21.65 cm (12.30° of eccentricity). Each square was composed of four coloured triangles with the apex pointing towards the centre of the square. Adjacent triangles were of high contrast. On each trial, a new square containing a different set of coloured

triangles was presented. The triangles inside the square rotated in a clockwise or anti-clockwise direction (pseudo-randomised across trials), shifting position every 300 ms. This gave the square an animated appearance with the triangles rotating inside the square.

A camera mounted above the screen was used for monitoring the infant's face during the experiment. The picture from this camera and the picture from the screen used for stimulus presentation were fed into a mixer and displayed on a monitor positioned in front of the experimenter using a picture-in-picture method. This picture was also recorded on video for offline coding. Attractor sounds used to maintain the infant's attention were presented through a set of speakers positioned behind the stimulus monitor.

Procedure. Infants sat in their parent or caregiver's lap. The parents were asked to close their eyes during testing in order to avoid any potential movement cues. The parents were asked to hold the infant in an upright sitting position, letting the infant lean his or her head against their chest if the infant did not have sufficient neck control to sit unsupported. The experimenter controlled the experiment from behind a grey screen dividing the room.

On each trial an animated, colourful square containing four triangles, as described above, was presented in the centre of the screen; this was the central stimulus. As soon as the infant fixated the central stimulus, the experimenter pressed a key on the keyboard to present a peripheral colourful square, the target. The central stimulus and the target were identical, both in terms of colour and the direction of the triangle rotation. In the Overlap condition the central stimulus stayed on while the target was presented for 3 s (equivalent to 10 triangle rotations). In the Gap condition the central stimulus disappeared, leaving the screen blank for 200 ms before the target appeared,

again for 3 s. When the target appeared in the Gap condition the trial rotation had moved to the next position in order to keep the rotation continuous. If the infant looked down, the experimenter could attract his or her attention by playing computer-generated attractor sounds. These sounds were disabled during target presentation.

Figure 4.1. Stimulus presentation in the Gap task.

Forty-eight trials were presented in a pseudo-random sequence. The sequence was created in such a way that no more than two consecutive trials presented the same condition (Gap or Overlap), side of target (right or left) or rotation direction inside the square (clockwise or anti-clockwise). Due to the importance of collecting enough data from each infant (because of the study's longitudinal aspect), extra trials were run by restarting the experiment if the baby looked away from the screen on a large proportion of trials during the session.

Data analyses. Trial validity and saccadic latencies were coded offline. Saccadic reaction time (SRT) was coded as the time between target onset and the first visible eye movement towards the target. If the infant did not look at the central stimulus during the last 5 frames (200 ms) preceding target presentation, the trial was

considered invalid. Saccades that had a latency of 120 ms or less were also excluded from the analyses. Such saccades were uncommon in the Gap task and were most likely anticipatory (see below). The trial was also considered invalid if the infant looked from the central stimulus to some other location than the target or if the infant's eyes were out of view during target presentation. Trials where infants remained fixated on the central stimulus throughout target presentation were very rare (see below), and were excluded from subsequent SRT analyses. Inter-coder reliability for trial validity was good ($\kappa = .78$, based on all trials, $N = 240$, from 5 participants), and the correlation between SRTs for trials coded as valid by both coders was high ($r = .987$, $p < .001$). When the validity coding was inconsistent between coders, the final coding of the trial was determined by the author.

The Anticipation task

Apparatus and stimuli. The programme for running the Anticipation task was created using E-Prime software and run on a PC. Stimuli were presented on the same screen and infants sat at the same distance from the screen as for the Gap task. A schematic of the stimulus sequence and sample stimuli are shown in Figure 4.2. Stimuli consisted of animated cartoons presented in two 27.39×26.53 cm black squares positioned on each side of the screen. Each black square subtended $15.60^\circ \times 15.11^\circ$ of visual angle. The distance between the two black squares was 13.95 cm (7.98° of visual angle). The black squares were positioned at an equal distance from the top and bottom of the screen and were presented on a grey background. The cartoons inside the squares were colourful 3D-like animations of a similar size. The infant's behaviour was monitored and recorded with the same camera setup as that used for the Gap task.

Figure 4.2. Stimulus presentation in the Anticipation task.

Procedure. Infants sat in their parent or caregiver's lap. The parents were given the same instructions as for the Gap task. The experiment consisted of a set sequence of animations presented in the two stimulus locations on each side of the screen. A new animation was used for each trial. At the beginning of the experiment the first frame of the first animation blinked at a 200 ms rate in the stimulus location on the right. As soon as the infant looked at this location, the experimenter pressed a key to start the first animation. Each animation was presented for 1200 ms at a frame rate of 100 ms (i.e., 12 frames per animation). Animations were presented in an alternating sequence in the left and right target locations. Each animation was followed by an inter-trial interval of 800 ms where no animations were present in the two target locations. The animations were accompanied by attractor sounds. These attractor sounds were always presented in synchrony with the onset of a new animation. The experimenter could also attract the infant's attention by pulling a string attached to a small bell positioned behind the stimulus monitor.

Sixty trials were presented in a session, but due to the importance of collecting enough data from each infant, additional sessions were run by restarting the experiment if the baby looked away from the screen on a large proportion of trials during the first session.

Data analyses. Trial validity and saccadic latencies were coded offline. Trials were only considered valid if the infant looked from the previous target location to the new target location, but the trial was still coded as valid if the infant moved his or her

eyes to the new target location in several saccades (this looking pattern was common). SRT was coded on a frame-by-frame basis as the time between target onset and the first visible eye movement towards the new target. The smallest time unit was 1 half-frame, equivalent to 20 ms. Negative SRTs indicated anticipatory eye movements. Inter-coder reliability for trial validity was acceptable ($\kappa = .69$, based on all trials [$N = 540$] from 5 participants). This was slightly lower than for other tasks reported in this thesis, largely because of the difficulties associated with coding eye movements in a free viewing paradigm where the visual angle is large. As mentioned above, infants often moved their eyes in several stepped saccades to reach the target or moved their eyes beyond the target followed by small corrective eye movements to foveate the target. Such looking patterns sometimes made it difficult to judge whether the infant looked at the target or looked away from the screen. The correlation between SRTs for trials coded as valid by both coders was high ($r = .830, p < .001$). When the validity coding was inconsistent between coders, the final coding of the trial was determined by the author.

The Infant Behavior Questionnaire – Revised

The IBQ-R was originally developed by Rothbart (1981) and revised by Gartstein and Rothbart in 2003. It was designed to assess temperament in 3- to 12-month-old infants. The instrument contains 191 items contributing to 14 scales. Each item is rated on a 7-point Likert type scale, ranging from 1 (never) to 7 (always). Parents can also choose the option N/A if a particular behaviour has not been observed within the last one or two weeks. Good internal consistency and adequate inter-rater reliability has been established in previous research (Gartstein & Rothbart, 2003; Parade & Leerkes, in press). A factor analysis carried out by Gartstein and Rothbart (2003)

indicated three main factors which the authors labelled Surgency/Extraversion, Negative Affectivity, and Orienting/Regulation.

In the present study, the IBQ-R was sent to parents about a week before the visit. Parents brought the completed questionnaire with them at the visit or returned the questionnaire by post within a week of the visit.

Results

The Gap task

Group results. All infants completed the Gap task at 4 months of age. The mean number of trials completed by infants was 53.27 ($SD = 12.80$). Thus, on average infants completed slightly more than one session of 48 trials. This is because extra trials were administered if the experimenter was not sure that the infant had completed enough trials during the first session. The mean number of valid trials completed by infants was 35.93 ($SD = 10.13$). The mean proportion of valid trials was .69 ($SD = .18$), i.e., on average slightly more than two thirds of the trials completed by each infant were valid. Given the simplicity of the stimuli and the fact that central and peripheral stimuli were matched, this ratio was expected.

Infants rarely stayed fixated on the central stimulus throughout target presentation. On only 0.6% of all trials did this occur. The mean proportion of no-saccade trials for each infant was .005 ($SD = .015$).

Table 4.2 presents the means of the median saccadic reaction time by target side and condition. In order to establish that the Gap paradigm used for this study replicated previously reported effects, a 2×2 repeated measures ANOVA was conducted with Target Side (left and right) and Condition (Gap and Overlap) as within-subjects variables. The dependent measure was the median SRT. The ANOVA

replicated a main effect of condition, $F(1, 103) = 83.32, p < .001, \eta_p^2 = .45$, indicating that infants looked faster to the target in the Gap condition than the Overlap condition. The main effect of Target Side was significant at trend level, $F(1, 103) = 3.53, p = .063, \eta_p^2 = .03$. Infants tended to be slightly faster when making a saccade to a target on the right side of the screen. Finally, the Condition x Target Side interaction was significant, $F(1, 103) = 4.91, p = .029, \eta_p^2 = .05$. Posthoc comparisons using Bonferroni correction for multiple comparisons indicated that the effect of Target Side was significant in the Overlap condition ($p < .05$), but not in the Gap condition ($p > .20$). This interaction shows that infants were significantly slower at initiating saccades to the left side in the Overlap trials, whereas no such effect was present in the Gap trials

Table 4.2. Mean median saccadic reaction time (in ms) by target side and condition in the Gap task.

	Median SRT	SD	Minimum	Maximum
Gap Right	326	83	210	880
Gap Left	327	76	190	760
Overlap Right	370	113	210	950
Overlap Left	397	112	250	840

These findings replicate previous findings that infants initiate saccades faster in the Gap condition than in the Overlap condition (Csibra et al., 1998; Hood & Atkinson, 1993). However, none of the previous studies found an effect of target side or a condition by target side interaction. Given the fact that the main effect of target side was only marginally significant and the effect size was small, it seems likely that there is a small effect of target side, especially in the Overlap condition, and that this effect can only be detected in a large sample of infants.

Individual difference measures. In order to look at individual differences in Gap task performance, median saccadic reaction time was calculated for each condition,

and a proportional index was calculated to assess the mean “Gap effect” for each infant. Overall median SRT (across conditions) was also calculated to assess possible effects of overall reaction time. Median SRTs were used instead of mean SRTs in order to reduce the effect of extreme scores, which are common in reaction time measures. The mean of the overall median SRT (across conditions) was 344 ms ($SD = 69$). The mean of the median SRT in the Gap condition was 328 ms ($SD = 70$), and the mean of the median SRT in the Overlap condition was 377 ms ($SD = 98$).

In order to calculate an index of each infant’s Gap effect, the median SRT for the Overlap condition was divided by the median SRT for the Gap condition. The two medians were divided, not subtracted, in order to cancel out as much of the overall effect of saccadic reaction time as possible. Furthermore, the Overlap median SRT was entered as the numerator in order to get a score that increases when the Gap effect increases. A score of 1 on the index indicates the lack of a Gap effect (i.e., the two median SRTs are exactly the same). A score above 1 indicates a proportional Gap effect – for example a score of 1.5 would indicate that the infant was 50% faster in the Gap condition compared to the Overlap condition. A score below zero indicates a reversed Gap effect, i.e., the infant is faster on Overlap trials compared to Gap trials. The mean Gap effect was 1.16 ($SD = .18$), showing that infants were faster in the Gap condition than in the Overlap condition. However, scores ranged from .66 to 1.73, indicating that some infants showed the reverse pattern.

The Anticipation task

Group results. All infants completed the Anticipation task at 4 months of age. However, data from one infant had to be excluded because of experimenter error.

(The first few minutes of the session were not recorded on video and therefore could not be analysed offline.)

Most infants completed one to three Anticipation task sessions. The variation in number of sessions was due to the Anticipation task being a free viewing paradigm, i.e., infants often looked away from the screen during parts of the session. If the infant was unattentive during a substantial part of the session, an additional session (or partial session) was administered to ensure that enough trials were obtained from each infant. The mean number of trials completed by infants was 104.09 ($SD = 39.60$). The mean number of valid trials completed by infants was 61.70 ($SD = 23.15$). The mean proportion of valid trials out of the total number of trials was .63 ($SD = .23$), i.e., on average slightly less than two thirds of the trials completed by infants were valid. This is roughly equivalent to the amount of off-task behaviour reported by Canfield, Wilken, Schmerl and Smith (1995); in that study infants looked away from the display on approximately 30% of trials.

In order to get an idea of the distribution of saccadic reaction times, the frequencies of different latencies were plotted in a histogram. The histogram can be seen in Figure 4.3. The distribution is clearly bimodal with a peak for anticipatory saccades around 200 ms before target onset and a peak for reactive saccades around 300 ms after target onset.

Figure 4.3. Histogram of saccadic reaction times in the Anticipation task.

Note: Bin width is 20 ms (1 half-frame).

In order to assess infants' anticipatory looking patterns, it is necessary to define a criterion for anticipatory saccades. If the saccade occurs simultaneously with the target onset or very soon thereafter, the saccade can still be considered anticipatory since it is likely to have been initiated before the onset of the target. However, it is important to have an appropriate cut-off point to separate anticipatory saccades from reactive saccades (for discussion, see Canfield et al., 1997). In the original study by Haith and colleagues (1988), the cut-off point for anticipatory saccades was set to 200

ms after target onset. Saccades after this point were considered reactive. However, this criterion has subsequently been shown to be too inclusive. Canfield et al. (1997) found that the minimum latency of a reactive saccade to the target is only 133 ms across the age range of 2 to 12 months in their infant sample. Similarly, Csibra et al. (2001) set a conservative criterion of 120 ms post target onset for anticipatory saccades.

Using the latter criterion (120 ms) on all the available trials ($N = 10721$), the average proportion of anticipatory eye movements in the current study was .26. If only valid trials ($n = 6351$) were considered, the average proportion of anticipatory eye movements was .43. The average of individual infants' mean proportion of anticipatory saccades was .28 ($SD = .17$) when all trials were included, and .41 ($SD = .20$) when only valid trials were included. Note that the overall proportion of anticipations in the current study (26-28%) is very similar to what has been found in previous studies. For example, Haith et al. (1988) found that 3.5-month-old infants anticipated on 22% of trials. Canfield et al. (1995) found that 4-month-old infants anticipated on 26.7% of all trials. Johnson et al. (1991) found that 4-month-olds anticipated on 29.2% of trials. Finally, Csibra et al. (2001) found that 4-month-old infants in their ERP study anticipated the target on 30.1% of trials.

Even though the overall level of anticipation is well-established, it is relevant to know when during the experiment infants start to anticipate. This is especially relevant for understanding individual differences in the ability to anticipate since good anticipatory performance can be conceptualised either as an overall high level of anticipation, or as an ability to learn the temporal relationship between the targets fast. Figure 4.4 illustrates the proportion of anticipatory saccades during the first test session (60 trials) across all infants. The graph shows that infants start to anticipate

surprisingly fast, with the main increase occurring between Trial 1 and Trial 8. After this steep increase, the proportion of anticipation increases gradually until approximately Trial 20, at which point the level of anticipatory saccades stabilises around 30%.

As can be seen in Figure 4.4, anticipatory saccades seem to occur in “bursts” lasting a few trials. This pattern is probably due to small variations in the attractiveness of the stimuli presented in the sequence. For example infants may be more likely to anticipate following a particularly engaging cartoon. Conversely, a less interesting cartoon may result in the infant looking away from the screen on the following trial, making the trial invalid (and therefore not anticipatory).

Figure 4.4. Mean proportion of anticipatory saccades across all infants on each trial during the first test session.

A previous report found that infants make more anticipatory saccades to targets appearing on the right than on the left (Csibra et al., 2001). To test whether there was a side bias in the current sample, the mean proportion of anticipations to the right and

left was calculated for each infant, and a related t test was carried out. The mean proportion of anticipations when the target appeared on the right was .29 ($SD = .21$). The mean proportion of anticipations when the target appeared on the left was .26 ($SD = .20$). Even though the difference in the proportion of anticipations to the two sides was consistent with the results reported by Csibra et al. (2001), i.e., more anticipations to the right than the left, the effect was not statistically significant, $t(102) = 1.32, p = .19$.

Individual difference measures. Two indices were calculated to assess Anticipation task performance. The first index was an overall measure of anticipation performance. This index was simply the proportion of anticipatory saccades out of all valid trials. The second index was a measure of anticipatory learning, and was therefore termed the Anticipatory Learning index. It is relevant to know how quickly each infant starts to anticipate in the Anticipation task because, conceptually, this learning component could be different from the overall proportion of anticipatory saccades. The learning index was based on the trial number of the first valid trial where the infant reached a criterion of at least 60% anticipatory saccades across 5 trials, the criterion trial being the last one of these 5 trials. On average infants reached this criterion on valid trial number 15 ($SD = 13$). Eleven infants did not reach this criterion. In order to make correlational analyses involving the Anticipatory Learning index easier to interpret, the index was reversed by subtracting the criterion trial number from 60 (no infants reached the criterion later than valid trial number 60). In this way a high score on the index indicated faster anticipatory learning.

It was decided not to include a measure of anticipation performance across all trials given the fact that infants varied considerably in terms of the number of invalid trials. This was to some extent due to the free-viewing nature of the task, but likely

also due to individual differences in interest and attentiveness. Including invalid trials in the performance indices would therefore tend to increase the noise level in the data. Since this is also an important confound to take into account, the effect of how many valid trials infants were exposed to was entered as a control variable in a set of partial correlations reported below.

The IBQ-R

IBQ-R data were available for 98 infants. Six questionnaires were either not filled in by the parent or were lost in the post. IBQ-R factor scores were calculated for each infant in accordance with the factor analysis carried out by Gartstein and Rothbart (2003).

The Surgency/Extraversion score was obtained by calculating the mean of the following scales: Approach; Vocal Reactivity; High Intensity Pleasure; Smiling and Laughter; Activity Level; and Perceptual Sensitivity. The mean Surgency/Extraversion score was 3.36 ($SD = 0.69$).

The Negative Affectivity score was obtained by calculating the mean of the following scales: Sadness; Distress to Limitations; Fear; and reversed-scored Falling Reactivity (see footnote in Chapter 3 for details on reversed scale scores). The mean Negative Affectivity score was 2.72 ($SD = 0.58$).

The Orienting/Regulation score was obtained by calculating the mean of the following scales: Low Intensity Pleasure; Cuddliness/Affiliation; Duration of Orienting; Soothability; and Smiling and Laughter. The mean Orienting/Regulation score was 4.40 ($SD = 0.61$).

Correlations

Correlations between measures taken at 4 months of age are presented in Table 4.3. Note that correlations involving the proportion of anticipatory saccades in the Anticipation task were controlled for the effect of the proportion of valid trials completed by the infant. The analysis was carried out this way because the proportion of anticipatory saccades was significantly correlated with the proportion of valid trials, $n = 103$, $r = .326$, $p = .001$. This finding is not surprising – the more trials the infant is exposed to, the better the chance of picking up the regularity of the sequence, thus enabling better anticipatory performance. Since the effect of infants' attention to the stimuli is not the relevant measure in the current analyses, this effect needed to be partialled out in order to get a purer measure of infants' anticipation performance.

Table 4.3. Correlations between measures taken at 4 months of age.

		Gap task			Anticipation task		IBQ-R		
		Gap SRT	Overlap SRT	Gap effect	Proportion Anticipation ^a	Anticipatory Learning	Surgency/ Extraversion	Negative Affectivity	Orienting/ Regulation
Gap task	SRT Overall	<i>r</i> = .889** <i>p</i> < .001 <i>n</i> = 104	<i>r</i> = .904** <i>p</i> < .001 <i>n</i> = 104	<i>r</i> = .241* <i>p</i> = .014 <i>n</i> = 104	<i>r</i> = -.135 <i>p</i> = .175 <i>df</i> = 100	<i>r</i> = .007 <i>p</i> = .946 <i>n</i> = 92	<i>r</i> = -.028 <i>p</i> = .783 <i>n</i> = 98	<i>r</i> = .055 <i>p</i> = .589 <i>n</i> = 98	<i>r</i> = .050 <i>p</i> = .626 <i>n</i> = 98
	SRT Gap		<i>r</i> = .724** <i>p</i> < .001 <i>n</i> = 104	<i>r</i> = -.115 <i>p</i> = .244 <i>n</i> = 104	<i>r</i> = -.122 <i>p</i> = .223 <i>df</i> = 100	<i>r</i> = .027 <i>p</i> = .798 <i>n</i> = 92	<i>r</i> = -.042 <i>p</i> = .679 <i>n</i> = 98	<i>r</i> = .055 <i>p</i> = .588 <i>n</i> = 98	<i>r</i> = .027 <i>p</i> = .791 <i>n</i> = 98
	SRT Overlap			<i>r</i> = .579** <i>p</i> < .001 <i>n</i> = 104	<i>r</i> = -.218* <i>p</i> = .027 <i>df</i> = 100	<i>r</i> = .070 <i>p</i> = .510 <i>n</i> = 92	<i>r</i> = -.014 <i>p</i> = .889 <i>n</i> = 98	<i>r</i> = .031 <i>p</i> = .764 <i>n</i> = 98	<i>r</i> = .020 <i>p</i> = .843 <i>n</i> = 98
	Gap effect				<i>r</i> = -.181† <i>p</i> = .069 <i>df</i> = 100	<i>r</i> = .105 <i>p</i> = .319 <i>n</i> = 92	<i>r</i> = .020 <i>p</i> = .849 <i>n</i> = 98	<i>r</i> = -.082 <i>p</i> = .421 <i>n</i> = 98	<i>r</i> = .016 <i>p</i> = .878 <i>n</i> = 98
Anticipation task	Proportion Anticipation ^a					<i>r</i> = .684** <i>p</i> = .001 <i>df</i> = 89	<i>r</i> = .023 <i>p</i> = .823 <i>df</i> = 94	<i>r</i> = -.123 <i>p</i> = .231 <i>df</i> = 94	<i>r</i> = .124 <i>p</i> = .229 <i>df</i> = 94
	Anticipatory Learning						<i>r</i> = .184† <i>p</i> = .090 <i>n</i> = 86	<i>r</i> = -.036 <i>p</i> = .739 <i>n</i> = 86	<i>r</i> = .211† <i>p</i> = .051 <i>n</i> = 86
IBQ-R	Surgency/Extraversion							<i>r</i> = -.055 <i>p</i> = .592 <i>n</i> = 98	<i>r</i> = .549** <i>p</i> < .001 <i>n</i> = 98
	Negative Affectivity								<i>r</i> = -.149 <i>p</i> = .144 <i>n</i> = 98

Note: ***p* < .01, **p* < .05, †*p* < .10. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity. D/AP correction was carried out separately for tests of the relationship between the Gap and Anticipation tasks (15 tests: $p_{a15} < .008$), and for tests of these tasks in relation to IBQ-R factor scores (18 tests: $p_{a18} < .003$).

^a Controlled for proportion of valid trials in the Anticipation task.

Correlation between performance on the Gap and Anticipation tasks. The main question of interest in the correlation analysis was whether performance on the Gap task was correlated with performance on the Anticipation task. It was predicted that infants who showed a high degree of anticipation in the Anticipation task would disengage faster in the Overlap trials and demonstrate a smaller Gap effect. Such effects would indicate that infants who can disengage their attention with ease, also find it easier to control their attention in the absence of external stimulation in the Anticipation task. Furthermore, it would indicate that performance on two tasks that have been associated with frontal cortex activity in infant ERP studies (Csibra et al., 1998, 2001) share some of the same functional mechanisms.

As can be seen from Table 4.3, there was a significant negative correlation between median SRT in the Overlap condition of the Gap task and the proportion of anticipation in the Anticipation task, $df = 100$, $r = -.218$, $p = .027$. This result indicates that, as expected, infants who took longer to make a saccade to the target during visual competition (the Overlap condition) in the Gap task, tended to anticipate less in the Anticipation task, and vice versa. In contrast, Anticipation task performance was not significantly related to median SRT in the Gap condition or to overall median SRT. Furthermore, when the overall median SRT was partialled out of the correlation, the relationship between Anticipation task performance and the Overlap condition became slightly stronger, $df = 99$, $r = -.226$, $p = .023$, whereas the non-significant relationship between Anticipation task performance and the Gap condition became even weaker, $df = 99$, $r = -.003$, $p = .975$. This strongly suggests that the correlation is specific to the Overlap condition. Finally, the proportion of anticipatory saccades in the Anticipation task was also negatively related to the magnitude of the Gap effect,

though this effect was weaker and only borderline significant, $df = 100$, $r = -.181$, $p = .069$.

Even though a significant association in the expected direction was found between performance on the Gap and Anticipation task, it is important to acknowledge that this relationship did not survive correction for multiplicity using the D/AP procedure. Only within-task measures survived the correction in the current analyses. The effect should therefore be interpreted with some caution (see Discussion).

Correlations between performance on the Gap and Anticipation tasks and IBQ-R temperament scores. The IBQ-R factor scores were not significantly related to Gap task performance or to the proportion of anticipatory saccades in the Anticipation task (see Table 4.3). Both the Surgency/Extraversion score and the Orienting/Regulation score were positively related to the Anticipatory Learning index, though these correlations were only significant at trend level (Surgency/Extraversion: $n = 86$, $r = .184$, $p = .090$; Orienting/Regulation: $n = 86$, $r = .211$, $p = .051$). This may indicate that infants who were rated as more extraverted and as demonstrating better self-regulation start anticipating earlier in the Anticipation task.

As mentioned in the introduction to this chapter, previous studies have found associations between performance on the Gap and Anticipation tasks and IBQ scales related to self-regulation. In an attempt to replicate these findings, an additional correlation analysis was carried out using the same IBQ-R⁴ scales as in previous studies. Johnson et al. (1991) found that Fear was positively correlated with saccadic reaction time in Overlap trials at 4 months (i.e., infants who had difficulty

⁴ The IBQ-R scales used here are largely equivalent to the original IBQ scales (see Gartstein & Rothbart, 2003).

disengaging from the central stimulus were more fearful), whereas McConnell and Bryson (2005) found that Fear was positively correlated with saccadic reaction time in the Gap trials (but not Overlap trials) at this age. The analysis of the current sample replicated the association between the Fear scale and saccadic reaction time in the Gap task. However, the strongest correlation was between Fear and the overall median SRT (across conditions), $n = 98$, $r = .254$, $p = .012$. The correlation between Fear and median Gap SRT was of a similar magnitude, $n = 98$, $r = .248$, $p = .014$, whereas the correlation between the median Overlap SRT was somewhat weaker, $n = 98$, $r = .194$, $p = .056$. This indicates that infants who were generally slower to initiate saccades in the Gap task were more fearful. None of the other associations previously found between Gap task performance and temperament (see the introduction to this chapter) were replicated in the current study.

As regards Anticipation performance, Johnson et al. (1991) found that more anticipation was associated with a lower level of Soothability but was unrelated to Fear and Distress. The current study contradicts these findings. In the current sample, the proportion of anticipatory saccades in the Anticipation task (with proportion of valid trials partialled out) was *positively* correlated with Soothability, $df = 93$, $r = .230$, $p = .025$, and *negatively* correlated with Distress, $df = 93$, $r = -.231$, $p = .024$. These findings suggest that more anticipation in the current study was in fact associated with better self-regulation.

Summary of results. In summary, the group results from the Gap task replicated most of the findings from previous studies (Csibra et al., 1997; Csibra et al., 1998; Hood & Atkinson, 1993). However, in contrast to previous studies, a small Condition $\times$ Target Side interaction was found, indicating that infants were particularly slow in the Overlap condition when the target was presented on the left. Group results from

the Anticipation task validated the current paradigm by showing that the proportion of invalid trials and the overall level of anticipation was equivalent to previous studies (Canfield et al., 1995; Csibra et al., 2001; Haith et al., 1988; Johnson et al., 1991). It was also found that the amount of anticipation was considerably larger when only valid trials were included (i.e., trials where infants were attending to the stimuli and made a saccade from the old to the new target).

As expected, Gap task performance was significantly related to the amount of anticipation in the Anticipation task. Median saccadic reaction time in the Overlap condition was most strongly associated with anticipation performance.

The analysis involving temperament was more complex. Gap and Anticipation task performance were generally not significantly related to IBQ-R factor scores, though the Orienting/Regulation and Surgency/Extraversion factors did show a weak positive relationship with the rate of learning in the Anticipation task. Follow-up analyses based on previous findings indicated that overall saccadic reaction time in the Gap task was associated with level of Fear. This finding roughly replicates previous studies (Johnson et al., 1991; McConnell & Bryson, 2005). In contrast to the study reported by Johnson et al. (1991), Anticipation performance in the current study was associated with a higher level of Soothability and less Distress.

Discussion

The study reported in this chapter was concerned with the question of whether performance on two tasks that have been associated with the frontal cortex in early infancy was correlated. Furthermore, the study investigated whether performance on these tasks was associated with temperament dimensions related to self-regulation.

Gap task group results

The results from the Gap task replicated the effect of condition found in previous studies (Csibra et al., 1997; Csibra et al., 1998; Hood & Atkinson, 1993). Infants were approximately 50 ms faster in the Gap condition than in the Overlap condition. This Gap effect is slightly smaller than that found in previous studies (Csibra et al., 1998; Hood & Atkinson, 1993). One reason for this difference may be that the current study used relatively conspicuous moving objects as fixation and target stimuli (Hood & Atkinson [1993] used black and white stimuli, and in Csibra et al. [1998] target presentation involved only non-moving objects). It is possible that infants were so engaged by the moving (and colourful) central stimulus that looking towards the target required a small amount of disengagement even in the Gap condition. On the other hand, the fact that the current study used identical moving stimuli in all stimulus locations demonstrates that the Gap effect is present even when stimuli are moving, multi-coloured, and, most importantly, when stimulus salience is matched. This means that individual differences in disengagement found in the current study cannot be due to the central stimulus being more attractive than the target and vice versa.

Results from the Gap task also indicated that infants tended to be faster when the target appeared on the right side compared to the left side, especially in the Overlap condition. These findings contrast with previous studies where no effects related to target side were found (Csibra et al., 1997; Hood & Atkinson, 1993). However, faster responses when targets appear on the right side have been found in studies using other infant visual paradigms (Csibra et al., 2001; Nakagawa & Sukigara, 2007). Even though it is interesting that the Gap effect is modulated by the target side, it is worth

noting that the effect of target side (as well as the condition by target side interaction) is very small ($\eta_p^2 = .03$) compared to the overall effect of condition ($\eta_p^2 = .45$).

Anticipation task group results

Group results from the Anticipation task largely replicated what has been found in previous studies (Csibra et al., 2001; Haith et al., 1988). When all trials were included in the analysis, infants anticipated the target on slightly less than 30% of trials. However, the proportion of anticipatory saccades grew to approximately 40% when only valid trials were included. This is an important point because free-viewing paradigms tend to result in more off-task behaviour and therefore fewer valid trials. For this reason the proportion of valid trials needs to be taken into account when individual performance differences are assessed.

It was also found that infants started anticipating quite early in the Anticipation task. There was a large increase in the overall proportion of anticipatory saccades over the first 8-10 trials, followed by a slower increase until approximately Trial 20, at which point the proportion of anticipatory saccades stabilised at around 30% (see Figure 4.4).

The relationship between the Gap and Anticipation tasks

In terms of individual performance differences, it was predicted that infants who could easily disengage in the Gap task, thus demonstrating good saccadic control, would also be better able to produce endogenously controlled anticipatory saccades in the Anticipation task. This prediction was confirmed. The Gap task measure associated most strongly with good anticipatory performance was the median SRT in the Overlap condition. This indicates that the longer the infant took to orient to the target during competition between the two stimuli in the Gap task, the less likely the

infant was to anticipate in the Anticipation task and vice versa. This effect was unlikely to be due to infants' general speed of initiating saccades since the overall median SRT and the median SRT in the Gap condition were not significantly related to Anticipation task performance. When overall SRT was controlled for, the negative relationship between Anticipation task performance and median SRT in the Overlap condition increased slightly in strength, whereas Anticipation task performance and median SRT in the Gap condition became virtually unrelated. Furthermore, though the effect was slightly weaker, the size of the Gap effect (median Overlap SRT divided by median Gap SRT) was also negatively related to Anticipation task performance.

Establishing whether performance on the Gap and Anticipation tasks was correlated involved several comparisons of similar measures from the two tasks. As can be seen in Table 4.3, only within-task correlations survived the D/AP correction for multiplicity. Even though this result indicates that the effect is relatively weak and should be interpreted with some caution, it is important to remember that the association is consistent with the predictions outlined earlier. Furthermore, the effect was specific to the Gap task measures hypothesised to be associated with Anticipation task performance (i.e., median SRT in the Overlap condition and the index of the Gap effect), whereas other Gap task measures were found to be largely unrelated to Anticipation task performance.

The correlation between the Gap and Anticipation tasks indicates that the two tasks measure some of the same functions. Given that overall reaction time does not seem to be the main mechanism underlying the correlation, it seems feasible to suggest that the common function involved in the two tasks is related to the control of eye movements, not general processing speed. Thus, infants who can easily disengage

from one stimulus in order to look at another stimulus may be better equipped to control their eye movements also when no stimuli are present. In the Anticipation task the infant has to move his or her eyes on the basis of an expectation and in the absence of external stimuli. The current study suggests that infants who can easily disengage their attention when presented with competing external stimuli, can also more easily control their eye movements on the basis of expectation. Conversely, it may be the case that infants who easily anticipate events find it easier to disengage from a stimulus when they expect some unsystematic event to occur (in the Gap task infants know that targets appear in the periphery, though they do not know where the next target will appear).

Either way, it is interesting that two saccadic control tasks that have been related to frontal cortex functioning in infant ERP studies are related also at the behavioural level. This finding is novel and contradicts one previous study investigating the relationship between the two tasks (Johnson et al., 1991). The study by Johnson et al. (1991) did not find that Gap and Anticipation task performance was related in any of their three age groups (2-, 3-, and 4-month-olds). There are several possible explanations for this discrepancy. Firstly, even though the current study found that performance on the two tasks was related, this relationship was relatively weak, with r -values around .2. Such modest effects are difficult to detect in small samples, whereas the current sample consisted of more than 100 infants. Furthermore, in contrast to Johnson et al.'s (1991) study, the current study controlled for the proportion of valid trials in the Anticipation task. Controlling for the infant's attention to the stimuli in a learning-based task such as the Anticipation task is important in order to obtain a clean measure of anticipation performance. Finally, Johnson et al.'s (1991) study differed from the current study in several respects. In Johnson et al.'s

(1991) study infants were first presented with 18 Gap trials where the target side was contingent on the characteristics of the central stimulus. Following this, different types of test trials were presented, including Overlap trials where the contingent relationship was preserved. Johnson et al.'s (1991) paradigm is therefore to some extent a combination of the Gap and Anticipation tasks more than a separate test of these abilities. This is important in light of evidence that infants start making faster saccades to a target when the sequence is predictable (Haith et al., 1988). It is therefore possible that the effect of consistent predictability in Johnson et al.'s (1991) task may have masked some of the individual differences in Gap task performance.

In light of these issues, it is only fair to mention that the current study tested infants on the same day with the same equipment. Even though the Gap task did not involve a predictable sequence of stimulus presentation, it did present peripheral stimuli in another task on the same monitor. The Anticipation task always followed the Gap task for the simple reason that infants found the Anticipation task much more engaging, thus allowing more data collection. However, following the Gap task infants might have come to expect that stimuli appear in peripheral locations of the screen. This might to a certain extent account for the very fast learning curve observed over the first 8-10 trials in the Anticipation task. Furthermore, infants occasionally anticipated the target on the first Anticipation task trial, possibly indicating that they were expecting things to appear at the two peripheral locations. Nevertheless, there was always a break between the two tasks and the layout and stimuli were quite different (see Figures 4.1 and 4.2). In addition, individual differences in both the rate of learning and the proportion of anticipatory saccades in the Anticipation task were large, indicating that not all infants performed well in the task after having been exposed to the Gap task stimuli.

Possible neural substrates of the Gap and Anticipation tasks

It is worth noting that the two ERP studies linking the Gap and Anticipation tasks to the frontal cortex (Csibra et al., 1998, 2001) did not find identical effects. In the Gap task a left frontal positivity around target onset was found in the Gap condition (Csibra et al., 1998), whereas a right frontal positivity was associated with anticipatory saccades in the Anticipation task (Csibra et al., 2001). These differences may be due to the infants being different ages, 6 and 4 months respectively, and to the relatively low spatial resolution of the ERP method. Furthermore, not much is known about the functional significance of these lateralisation effects. They may to a certain extent be developmental in nature, given that such lateralisation is attenuated in adults (Csibra et al., 1997). However, it is also feasible to conclude that the mechanisms underlying performance on the two tasks are similar, but not identical. This is emphasised by the fact that the correlation between the Gap and Anticipation task in the current study was modest, around .2. It seems likely that some components of performance on the two tasks share the same underlying mechanisms, such as the need to control eye movements, whereas other components differ due to the specific demands of each task. In order to investigate the neural correlates of these shared and differing mechanisms further neuroimaging studies are needed.

A related issue concerns the hypothesised development of cortical control over subcortical mechanisms outlined at the beginning of this chapter. As discussed earlier, several cortical areas have been implicated in this process, including the primary visual cortex (Bronson, 1974; Johnson, 1990), parietal cortex (Atkinson et al., 1992; Braddick & Atkinson, 1988; B. Hood & Atkinson, 1990) and frontal cortex (Atkinson, 1984a; Atkinson & Braddick, 2003; Atkinson et al., 1992; Johnson, 1990). The maturation of connections between these areas and subcortical structures (as

opposed to the cortical areas per se) have also been emphasised as important in this development (Braddick & Atkinson, 1988; Johnson, 1990). Even though the evidence suggests that the cortical structures necessary for the control of eye movements in the Gap and Anticipation tasks are in place by 4 months of age (Hood & Atkinson, 1993; Johnson, 1990), our knowledge about which specific structures are involved is very sparse. For example, despite the ERP evidence involving the frontal cortex in these tasks (Csibra et al., 1998, 2001), it is possible that attentional functions mediated by the parietal lobe interact with executive control systems in the frontal cortex to enable infants to disengage visual attention and anticipate visual events. Thus, further studies are needed in order to establish the specific roles of different parts of the developing cortical-subcortical networks mediating visual attention in early infancy.

Frontal cortex task performance at 4 months and infant temperament

Turning to the relationship between the two frontal cortex tasks and temperament, only a few relatively weak relationships were found using the IBQ-R factor scores, though analyses of specific scales used in previous studies did indicate some significant associations. Self-regulatory function as measured by the IBQ-R factor Orienting/Regulation was not significantly related to Gap task performance or to the proportion of anticipatory saccades in the Anticipation task. The former result is a bit surprising in light of previous findings that infants who disengage easily show better self-regulation and less negative affect (Johnson et al., 1991; McConnell & Bryson, 2005). However, the follow-up analyses based on findings from previous studies did indicate that slower saccadic reaction times in the Gap task were associated with a higher level of fear, replicating findings from Johnson et al. (1991) and McConnell and Bryson (2005), though this effect was not condition-specific. Furthermore, the

proportion of anticipatory saccades in the Anticipation task was associated with lower levels of Distress and higher levels of Soothability. This finding contrasts with the results reported by Johnson et al. (1991). The rate of learning in the Anticipation task also tended to correlate positively with infants' self-regulatory abilities. Infants who had higher Orienting/Regulation factor scores tended to start anticipating earlier in the Anticipation task.

Posner and Rothbart (1998) have suggested that self-regulation in infancy may be related to later frontally-mediated effortful control abilities (see also, Gartstein & Rothbart, 2003; Rothbart et al., 2003). Though this prediction remains to be tested, it is interesting to note that infants in the current sample who performed well in the Anticipation task tended to show less distress and better self-regulation. Performance on the Gap task, on the other hand, was only related to temperament in a general way, not to a specific disengagement difficulty.

Conclusions

In conclusion, the 4-month study presented in this chapter demonstrated a modest relationship between two tasks that have been associated with the frontal cortex in infant ERP work (Csibra et al., 1998, 2001). Though further neuroimaging work is needed to fully establish the link, as well as the exact neural substrate, this finding strengthens the evidence that both tasks tap into frontally mediated saccadic control mechanisms. Early self-regulatory functioning as assessed by parent report, and which may be related to frontal cortex functioning (Posner & Rothbart, 1998), showed some positive association with performance on the Anticipation task.

Chapter 5 will present data from the follow-up study, which was carried out at 9 months of age. This study was mainly an attempt to replicate the findings presented in

Chapter 3, but also addressed additional questions regarding the association between performance on infant frontal cortex tasks and general motor and cognitive development.

Summary of Chapter 4

- The study presented in this chapter investigated the relationship between two tasks that have been related to the frontal cortex in infant ERP studies, the Gap task and the Anticipation task, in a large cohort of infants (Cohort 2).
- The relationship between these two tasks and infant temperament was also investigated.
- Previous group findings on the Gap and Anticipation tasks were replicated.
- As predicted on the basis of the ERP evidence, the Gap and Anticipation tasks were correlated ($r \approx .2$), indicating that the two tasks share functional mechanisms.
- The shared functionality between the two tasks is likely to involve frontally mediated saccadic control processes, though, given the modest size of the correlation, other processes and cortical areas are also likely to be involved.
- As regards infant temperament, slower saccadic reaction times in the Gap task were associated with a higher level of fear. This is broadly consistent with previous findings. However, the effect was general, i.e., not specific to a particular condition.
- Performance on the Anticipation task was positively correlated with infants' self-regulatory abilities. This is in line with a theory by Posner and Rothbart (1998) proposing that such temperament characteristics are associated with early frontal cortex functioning.

CHAPTER 5

The relationship between frontal cortex tasks, temperament, and general development at 9 months

Introduction

The two previous chapters presented data from (1) an initial project investigating a new infant inhibition task, the Freeze-Frame task, and (2) from the first phase of a large-scale project investigating the relationship between a set of infant tasks thought to have a frontal cortex component. These two projects were carried out in separate infant cohorts, Cohort 1 and Cohort 2. For clarity, individual studies will be referred to as a Cohort 1 or Cohort 2 study in the following.

The current chapter extends the findings from previous chapters by presenting further data from Cohort 2. In the first Cohort 2 study, which was carried out when infants were 4 months old, the relationship between two tasks that have been related to frontal cortex functioning (the Gap and Anticipation tasks) was investigated, and it was found that performance on the two tasks was modestly correlated (Chapter 4). In the second Cohort 2 study reported here, the same group of infants were tested at 9 months of age. Infants were tested on the same measures as those used with Cohort 1 in Chapter 3, that is, the Freeze-Frame task and the A-not-B task. The Cohort 1 study presented in Chapter 3 showed that performance on the Freeze-Frame task was significantly correlated with performance on the A-not-B task at 9 months of age. In particular, early individual differences in distractibility in the Freeze-Frame task were related to how well infants performed on the A-not-B task. However, the results from the 24-month follow-up study of Cohort 1, also presented in Chapter 3, were

somewhat surprising in that both early differences in the Freeze-Frame task *and* performance on the A-not-B task tended to correlate negatively with later frontal cortex measures. In contrast, another Freeze-Frame measure, Selective Inhibitory Learning, was relatively unrelated to A-not-B performance, but positively related to frontal cortex task performance at 24 months of age.

Based on these findings, it seemed important to replicate the relationship between the Freeze-Frame task and the A-not-B task in Cohort 2. Cohort 1 was relatively small ($n = 24$), and significance levels of the results generally ranged between .01 and .05. A replication of the effects in the larger Cohort 2 ($n = 104$) would consolidate the findings from Cohort 1, allowing more firm conclusions to be made, at least at 9 months of age. Testing infants in Cohort 2 on the same tasks and at the same age as Cohort 1 also allowed the pooling of all the available data for the genotype analyses presented in Chapter 7. Such analyses require a large sample size.

In addition to the Freeze-Frame and A-not-B tasks, the follow-up study of Cohort 2 included two sets of relevant concurrent measures that were not included at 9 months in Cohort 1. These were measures of temperament and general development. Temperament was assessed using the Revised Infant Behavior Questionnaire (Gartstein & Rothbart, 2003) and general cognitive and motor development was assessed by the Mullen Scales of Early Learning, AGS Edition (Mullen, 1995). In Cohort 1 such measures were taken at 24 months of age, but not in infancy.

Investigating the relationship between temperament and frontal cortex task performance is important because some theorists have hypothesised that certain temperament factors, such as self-regulatory function and effortful control, are related to the development of the frontal cortex (Posner & Rothbart, 1998; Rothbart et al., 2003). Furthermore, there is growing experimental evidence that temperamental

factors are related to performance on frontal cortex tasks (Gerardi-Caulton, 2000; Rothbart et al., 2003), though, as the findings reported in Chapter 3 demonstrate, these effects are not always in the expected direction (Bell, 2005; Bell et al., 2007; Bell, Wolfe, Morasch, & Cardell, 2006; Holmboe et al., 2008).

Given the negative association found between the Freeze-Frame and A-not-B tasks and later Effortful Control in Cohort 1 (Chapter 3), it is relevant to know whether performance on these tasks is also related to concurrent temperamental factors. In relation to this question, it is worth mentioning that it is not entirely clear which of the infant temperament factors are equivalent to Effortful Control in toddlerhood. Posner and Rothbart (1998) have suggested that the Orienting/Regulation scale may be a precursor of Effortful Control, but there is no direct evidence for this claim. Therefore, factor scores on all three IBQ-R factors were included in the analyses of the current study. Where previous studies have indicated a relationship between a frontal cortex task and a specific temperament scale, the factor score analyses were supplemented by analyses attempting to replicate the previous findings.

Regarding the question of the relationship between infant frontal cortex tasks and general development, the results from Cohort 1 indicated that both Freeze-Frame and A-not-B performance was relatively independent of general development at 24 months of age. However, performance on one of the frontal cortex measures taken at 24 months in Cohort 1, the Spatial Conflict task, was related to general motor development, indicating that not all frontal cortex measures can be assumed to be entirely independent of general development. Furthermore, the Cohort 1 study did not include concurrent measures of general development at 9 months of age, leaving

unanswered the question of whether infant frontal cortex tasks are related to general developmental factors in *infancy*. This question is addressed in the present chapter.

To sum up, the study presented in this chapter reports on the second set of data collected in Cohort 2 (the 4-month study was reported in Chapter 4). Infants were 9 months old. The analyses presented here had three main aims: (1) to replicate the relationship between the Freeze-Frame task and the A-not-B task reported in Chapter 3, (2) to establish the relationship between the infant frontal cortex tasks at 9 months and concurrent temperamental factors, and (3) to establish the relationship between the infant frontal cortex tasks at 9 months and concurrent measures of general development. The question of longitudinal relationships between the 4- and 9-month measures collected in Cohort 2 is addressed in Chapter 6.

Method

General procedures

Ninety-four infants from the original sample of 104 infants returned for the follow-up study at 9 months. This constitutes a drop-out rate of 9.62%. The mean age on the day of the visit was 274.70 days ($SD = 6.74$). The age distribution was slightly wider at 9 months than at 4 months (SD at 4 months = 4.93) because more re-schedules were necessary at the later age, and every effort was made to retain as many infants in the sample as possible even if the infant was slightly out of the ± 1 -week age range.⁵

⁵ For a discussion of the scheduling and re-scheduling procedure, see Chapter 2.

The Freeze-Frame task

Apparatus and stimuli. The equipment setup for the Freeze-Frame task was identical to the setup described in Chapter 3. However, some minor changes were made to the visual and auditory stimuli presented in the task. Most importantly, the animations used for interesting trials were different from the animations used in the original version of the task. The new animations were slightly smaller (approx. minimum height: 2.2 cm [2.1°]; approx. maximum height: 5.3 cm [5.1°]; approx. minimum width: 1.3 cm [1.2°]; approx. maximum width: 5.1 cm [4.9°]); and were 3D-like in appearance. The reason for changing the animations was that the infants completed a simplified version of the original Freeze-Frame task at 6 months of age (not reported in this thesis, see Chapter 2), and thus infants would have been exposed to the same stimuli twice if the original stimuli had been used. Although any effects of multiple presentations were likely to be small, such effects could potentially confound individual differences in learning during the task. For example, some infants might have habituated faster to the stimuli than other infants during the second presentation of the task if they had been presented with the same stimuli before.

The boring animation remained the same as in the original version of the task, though it was made slightly smaller to match the size of the new set of interesting animations (2.8×2.8 cm [$2.67^\circ \times 2.67^\circ$]). The boring animation was not used in the 6-month version of the task and it therefore seemed reasonable to use this stimulus at 9 months. The distractors were identical to the ones used in the original version of the task. Since the distractors were only flashed briefly on the screen in order to elicit automatic saccades, they were kept the same.

The sound stimuli used in the new version of the task were also slightly different. In addition to the original attractor sounds, a set of particularly interesting high-

quality sound effects were used towards the end of the task when infants started to lose interest in the task. These additional sounds helped obtain more data from the infants.

Procedure. The procedure was generally the same as the procedure used in the original study. The stimulus sequence and presentation times were the same as described in Chapter 3. However, there was a slight change in the calibration procedure. If infants did not calibrate before the distractor duration reached 1200 ms, distractor duration was set to 1200 ms for the rest of the experiment. That is, the distractor duration was essentially calibrated despite the fact that the infant had not looked to the distractor on two consecutive trials. This was done in order to avoid very long distractor presentations, which tended to cause infants to look away from the screen. Note that this change had no effect on the procedure for the vast majority of infants who calibrated before distractor duration reached 1200 ms (84 out of the 86 infants who calibrated in the task).

The new calibration procedure also allowed the experimenter to calibrate the distractor duration manually. This was necessary in cases where the experimenter did not release the key quickly enough for the computer to detect one of two consecutive looks to the distractor. In these cases the experimenter could press a key on the keyboard to indicate to the computer that the infant had calibrated at the current distractor duration. This procedure minimised the number of calibration errors in the sample ($n = 3$).

Another slight change in the procedure was that more trials were administered in the new version of the task. In the original version, the aim was to obtain approximately 60 trials from each infant. In the current version, infants were encouraged to complete 80 trials.

Data analyses. The data were analysed as described in Chapter 3. That is, the session was divided into phases, invalid trials were excluded, and the proportion of looks to the distractors was calculated separately for boring and interesting trials in each phase. However, the additional data collection allowed an extra phase in the analyses. Thus, there were 4 phases of the experiment, containing 16 trials each (8 boring and 8 interesting).

Video recordings of each infant's behaviour were coded offline. The coding procedure was similar to the procedure reported in Chapter 3, but a few additional criteria were added to make the coding more accurate. The trial was considered invalid if the infant was not looking at the central stimulus at distractor onset. The trial was also considered invalid if the infant blinked (i.e., the pupils were fully covered) during distractor presentation. In addition, the trial was considered invalid if these behaviours occurred during the 1000 ms following distractor presentation. This criterion was added because in trials where the infant looks away immediately following distractor presentation, it is impossible to know whether the infant *would have* looked to the distractor if they had not looked away from the screen. On rare occasions, a trial was excluded because the infant's eyes were out of view (e.g., if the infant's hand was in front of his or her eyes); such trials were considered invalid if the eyes were out of view for more than 2 frames (80 ms) during distractor presentation or within the 1000 ms following distractor presentation. Finally, trials where a saccade to the distractor was initiated earlier than 3 frames (120 ms) after distractor onset were also considered invalid; such saccades were most likely anticipatory or random. Inter-coder reliability was good for both looking behaviour ($\kappa = .94$) and validity ($\kappa = .86$), based on data from 10 participants.

The A-not-B task

Apparatus and stimuli. The setup of the A-not-B task was identical to the setup described in Chapter 3. Some of the toys used were different from the ones used in the study reported in Chapter 3, but were of a similar size and design.

Procedure. The A-not-B task was administered using the procedure described in Chapter 3. The only difference was that infants were encouraged to complete slightly more trials than in the initial study. In the current study infants were encouraged to complete 40 A-not-B trials.

Data analyses. Since the initial study indicated that the maximum intended delay on change trials (collected by the computer during task administration) was highly correlated with the cumulative delay in terms of individual performance differences ($r = .933$, see Chapter 3 for details), it was decided to use the former measure in the current analyses. Infants in Cohort 2 did not differ from infants in Cohort 1 in their performance on the task using the maximum intended delay as the dependent measure, $t(110) = 1.12, p = .265$.

The IBQ-R

Questionnaires were sent to parents a week before the visit and were returned either at the visit or by post within a week of the visit. One questionnaire was lost in the post, and one parent did not fill in the questionnaire. Scores on the Extraversion/Surgency, Negative Affectivity, and Orienting/Regulation factors were calculated using the same method as described in Chapter 4.

The Mullen Scales of Early Learning, AGS Edition

The Mullen Scales of Early Learning, AGS Edition (MSEL-AGS) is an individually administered test battery developed to assess motor and cognitive abilities in infants and young children (Mullen, 1995). The MSEL-AGS consists of five scales: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. The latter four scales are defined as *cognitive scales* with the Visual Reception and Fine Motor scales being the input and output side of visual organisation skills, respectively. Likewise the Receptive and Expressive Language scales are considered to be the input and output side of linguistic skills. A measure combining the standardised scores from the four cognitive scales, the Early Learning Composite, is considered a measure of general intelligence (Mullen, 1995).

The MSEL-AGS was standardised on a sample of 1849 children from four different regions of the U.S. The age range was 2 days to 69 months. The instrument has good internal consistency (ranging from .69 to .94 across scales in the age range tested in the current study), test-retest reliability (all $r_s > .80$ for children aged 1 to 24 months), and interscorer reliability (all $r_s > .90$). The correlation between scales is around .5 for most scales for infants below 12 months of age, indicating that the scales share some of their variance, but also contain variance specific to each scale. A factor analysis of data from the standardisation sample showed that psychometric g loaded most heavily (.80) on the Receptive Language scale in the youngest age group (Mullen, 1995).

In contrast to the BSID-II, which was used in the study in Cohort 1 (Chapter 3), the MSEL-AGS uses continuous scales. That is, the test administrator starts the assessment at an age-specific item on each scale, and then continues testing until the

infant's *basal* (highest three consecutive items that the infant can pass) and *ceiling* (lowest three consecutive items that the infant fails) have been established. This method is faster than the administration of the BSID-II where a complete set of items has to be administered for each infant in order to establish the basal and ceiling. Thus, in order to limit the length of the test session, which was already long for this age group, the MSEL-AGS was the best option for the current study. A study presented in the MSEL-AGS manual found that the MSEL-AGS Gross Motor Scale was highly correlated with the BSID Motor Scale ($r = .76$), and that the MSEL-AGS Early Learning Composite was highly correlated with the BSID Mental Scale ($r = .70$) (Mullen, 1995). This indicates that despite differential administration methods, the two instruments measure similar constructs.

In the current study infants completed the MSEL-AGS after the Freeze-Frame and A-not-B tasks. The test was scored during the session, and scores on the five scales and the Early Learning composite were calculated after testing. The session was also recorded on video so that the experimenter could check specific ratings after the session.

In two cases testing had to be discontinued on the day of the visit, but was completed in another visit to the laboratory two days later. In another case, the parent had to leave early, and the whole MSEL-AGS session was administered in the laboratory 4 days after the first visit.

Results

The Freeze-Frame task

Group results. All 94 infants in the 9-month study completed the Freeze-Frame task. The duration of distractor presentation was calibrated in an average of 5.48 trials

($SD = 8.55$). The mean calibrated distractor duration was 320.93 ms ($SD = 183.15$), ranging from 200 to 1200 ms (the distractor duration could not increase beyond 1200 ms in this version of the task). Note that these calibration data are very similar to the calibration data reported in Chapter 3. Five infants did not calibrate, i.e., they never looked to the distractor on two consecutive trials, and were excluded from the analyses. A further 3 infants had to be excluded because of calibration error. In these three cases the offline coding revealed that the experimenter had calibrated the distractor duration more than 10 trials too late or too early, resulting in too short or too long distractor durations for these infants. In all other cases the experimenter calibrated the distractor duration within 1-2 trials of the actual calibration trial.

In accordance with the approach taken in Chapter 3, analysis of the session was carried out from two trials before the calibration trial. This was done in order to include trials from the earliest point at which infants started to look consistently to the distractors. The mean number of trials completed by infants from this point was 72.55 ($SD = 12.12$), and the mean number of valid trials was 59.29 ($SD = 13.70$). The average proportion of valid trials was .81 ($SD = .10$). This is a slightly lower proportion of valid trials than that found in Chapter 3. The small difference between studies is most likely due to the slightly stricter coding criteria used in the current study (see Method).

Data from the Freeze-Frame task were divided into phases in the same way as described in Chapter 3. However, because infants completed more trials in the current study than in the initial study, the test session was divided into 4 phases with 16 trials in each phase (compared to 3 phases in the initial study). As previously, the proportion of looks to the distractors was calculated for each trial type (boring and

interesting) within each phase.⁶ Table 5.1 presents an overview of how many infants completed each phase and the mean proportion of looks to the distractors within each phase and trial type.

Table 5.1. Freeze-Frame descriptive statistics for the proportion of looks to the distractors across phases and trial types.

	<i>n</i>	Mean	<i>SD</i>
Boring - Phase 1	86	0.70	0.22
Boring - Phase 2	82	0.43	0.29
Boring - Phase 3	74	0.40	0.25
Boring - Phase 4	70	0.39	0.25
Interesting - Phase 1	85	0.47	0.26
Interesting - Phase 2	82	0.20	0.19
Interesting - Phase 3	82	0.13	0.16
Interesting - Phase 4	76	0.11	0.15
All Phases - Boring	63	-	-
All Phases - Interesting	73	-	-
All Phases	59	-	-

Note: In the upper part of the table *n* indicates the number of infants who completed each phase, i.e., had at least 4 valid trials in that phase. However, the value of *n* for each phase does not imply that the same infants completed previous or subsequent phases. The lower part of the table shows how many infants had enough valid trials in all boring phases, all interesting phases, and all phases (across trial types) of the experiment.

As can be seen from Table 5.1, overall infants decreased their looking to the distractors during the test session. However, the major decrease in looking seemed to occur between Phase 1 and 2 in both trial types. Furthermore, infants looked less to the distractors in the interesting trials than in the boring trials throughout the test session.

As was the case in Cohort 1, some infants in Cohort 2 did not provide data from all phases. Table 5.1 shows that on average the number of infants completing each phase decreased from 86 to 70 in the boring trials and from 85 to 76 in the interesting trials. However, only 59 infants had complete data from all phases of the experiment.

⁶ In terms of individual performance measures, the indices used were identical to the ones used in Chapter 3 (see below for details).

For this reason two separate analyses were carried out on the data: a standard repeated measures ANOVA, and a Linear Mixed Model (LMM). The latter model includes data from participants with missing data points (see Chapter 3). These analyses were carried out to replicate the Freeze-Frame group findings reported in Chapter 3. Infants who did not have data from all phases were also included in the correlational analyses reported later in this chapter whenever the appropriate measure was available (for descriptive statistics on these measures see Table 5.2).

Both the ANOVA and the LMM had two within-subjects variables, Trial Type (2 levels) and Phase (4 levels). The results from the repeated measures ANOVA replicated the results from Chapter 3. There was a main effect of Trial Type, $F(1,58) = 101.94, p < .001, \eta_p^2 = .64$, and Phase, $F(3,174) = 89.59, p < .001, \eta_p^2 = .61$, but no significant interaction, $F(3,174) = 0.35, p = .789, \eta_p^2 = .006$. Post-hoc tests using a Bonferroni correction indicated that the proportion of looks to the distractors differed between Phase 1 and all other phases (all $ps < .001$), as well as between Phases 2 and 4 ($p = .017$).

The LMM used an unstructured covariance structure, thus making no assumptions about the data. This analysis also replicated the main effect of Trial Type, $F(1,84.00) = 113.70, p < .001$, and Phase, $F(3,75.75) = 72.38, p < .001$, as well as the lack of an interaction, $F(3,76.78) = 1.13, p = .34$. Post-hoc analyses using a Bonferroni correction confirmed that infants looked significantly more to the distractors in Phase 1 than in all other phases (all $ps < .001$) and significantly more in Phase 2 than in Phase 4 ($p = .007$), whereas other phases did not differ significantly ($ps > .10$).

Freeze-Frame performance measures. The individual performance indices from the Freeze-Frame task were the same as the indices used in Chapter 3. The inhibition indices were General Inhibitory Learning, Selective Inhibition, and Selective

Inhibitory Learning. In addition, three general measures of Freeze-Frame performance were included: General Distractibility, General Distractibility in boring trials, and General Distractibility in interesting trials. Details on how these measures were calculated can be found in Chapter 3. Note that even though four phases were included in the group analyses reported above for descriptive purposes, Freeze-Frame performance indices involving phases were calculated on the basis of the first three phases in order to be identical to the measures used in Chapter 3.

Table 5.2 presents descriptive statistics for the six Freeze-Frame indices. The mean scores on the inhibitory indices and on the General Distractibility indices are highly similar to the results reported in Chapter 3 (see Table 3.1).

Table 5.2. Descriptive statistics for the Freeze-Frame performance indices.

	<i>n</i>	Mean	<i>SD</i>	Minimum	Maximum
General Inhibitory Learning	84	0.33	0.23	-0.30	0.80
Selective Inhibition	85	0.23	0.27	-0.38	0.88
Selective Inhibitory Learning	73	0.01	0.32	-0.64	0.95
General Distractibility, All Trials	86	0.34	0.12	0.09	0.70
General Distractibility, Boring	86	0.47	0.17	0.11	0.83
General Distractibility, Interesting	86	0.22	0.14	0.03	0.64

The A-not-B task

The A-not-B task was administered to all infants tested at 9 months ($n = 94$). However, a few infants refused to do the task; generally this meant that they fussed when the toy was hidden in the well, or simply did not reach to the wells. Therefore, infants who completed less than 10 trials ($n = 5$) were excluded from the analyses. The mean number of trials completed in the A-not-B task was 34.07 ($SD = 9.09$) before exclusion of these 5 infants. After the exclusion, the mean number of trials completed was 35.67 ($SD = 6.18$).

Infants performed at a similar level to that reported in Chapter 3 with most infants sustaining a 0-2 s intended delay on change trials. The overall proportion of correct responses across delays and trial types was .58. Figure 5.1 presents the frequencies of the maximum intended delays sustained by infants on change trials. The score -1 was allocated to 2 infants who did not pass on any change trials. These infants were not included in the correlational analyses reported below.

Figure 5.1. Number of infants succeeding at different delays in the A-not-B task.

As can be seen from the figure, the majority of infants (38.2%) could only succeed on change trials when no delay was imposed. At a 1-s delay 16.9% succeeded, and slightly more than a quarter of infants (27%) succeeded at a 2-s delay. Only 15.7% of infants could sustain a delay that was longer than 2 s. The mean maximum intended delay on change trials, excluding the two infants who did not pass any change trials, was 1.34 ($SD = 1.45$). These results replicate previous studies, as well as findings from Chapter 3, indicating that the A-not-B task is relatively difficult for 8-9-month-old infants (Bell & Fox, 1992, 1997; Hofstadter & Reznick, 1996).

However, more than half of the infants were able to sustain a delay on change trials, and a small group of infants did exceptionally well in the task, suggesting clear performance differences.

The IBQ-R

IBQ-R data were available for 92 infants. Scale scores on the Surgency/Extraversion, Negative Affectivity and Orienting/Regulation factors were calculated in accordance with the factor analysis reported by Gartstein and Rothbart (2003). The mean scores on the Surgency/Extraversion, Negative Affectivity, and Orienting/Regulation composites were 5.00 ($SD = 0.57$), 3.00 ($SD = 0.68$), and 4.81 ($SD = 0.49$), respectively.

The MSEL-AGS

All infants ($n = 94$) were tested on the MSEL-AGS. Mean raw scores and standardised scores are reported in Table 5.3. An infant's raw score is the number of points received on a specific scale (usually 0-2 points per item). The T-score, however, is a standardised score, that is, the score relates the infant's performance to that of the standardisation sample. During the standardisation procedure of the MSEL-AGS, normalised T-scores were developed for each age group of the standardisation sample so that each scale had a mean of 50 and a standard deviation of 10. The Early Learning Composite, which combines the four cognitive scales (Visual Reception, Fine Motor, Receptive Language, and Expressive Language), has a mean of 100 and a standard deviation of 15 (Mullen, 1995).

Table 5.3. MSEL-AGS raw and scale scores in Cohort 2.

	MSEL-AGS Scale	Mean	SD
Raw Score	Visual Reception	13.37	1.38
	Fine Motor	13.76	1.27
	Receptive Language	10.07	1.48
	Expressive Language	10.28	1.64
	Gross Motor	12.38	1.59
T-Score	Visual Reception	58.44	7.16
	Fine Motor	63.73	7.40
	Receptive Language	47.78	8.35
	Expressive Language	53.77	9.00
	Gross Motor	49.44	9.58
Standard Score	Early Learning Composite	111.99	10.35

As can be seen from Table 5.3, infants in the current sample performed better on some of the scales than the standardisation sample. Specifically, infants performed almost 1 standard deviation above the standardisation sample on the Visual Reception scale, and more than 1 standard deviation above the standardisation sample on the Fine Motor scale. The current sample also performed three quarters of a standard deviation above the standardisation sample on the Early Learning Composite. These differences are most likely due to background differences between the current sample and the standardisation sample. The MSEL-AGS was standardised on a sample of U.S. children, balanced on variables such as socio-economic status, rural/urban residence and parents' educational background (Mullen, 1995). In comparison, the current sample (Cohort 2) was an opportunity sample of parents who volunteered to participate with their infants. Most parents of infants in Cohort 2 lived in a city area (London), had moderately high socio-economic status and a professional or university degree (see Chapter 4 for detailed demographics).

Previous studies have found that childhood IQ is significantly associated with socio-economic status, with children from lower SES backgrounds obtaining lower scores on IQ tests (for review, see McLoyd, 1998). Therefore, differences in socio-

economic status between the standardisation sample and Cohort 2 are likely to account for the higher scores on some of the MSEL-AGS scales in the current study. This conclusion is strengthened by the fact that the standard deviation of most standardised scale scores in Cohort 2 was less than 10 (and less than 15 for the composite). This indicates that the range of differences in ability between infants in Cohort 2 was smaller than that observed in the standardisation sample. The potential effect of this range restriction in the correlational analyses will be addressed in the Discussion.

Correlation analyses

Correlations between the Freeze-Frame task and the A-not-B task. One of the main aims of the study presented in this chapter was to replicate the correlation found between the Freeze-Frame Selective Inhibition index and the A-not-B task in Cohort 1 (Chapter 3). Table 5.4 presents correlations between all six Freeze-Frame indices and performance on the A-not-B task as measured by the maximum intended delay on change trials. Only infants who completed more than 10 A-not-B trials and passed at least one change trial were included in the analysis ($n = 87$).

Table 5.4. Correlations between the six Freeze-Frame indices and A-not-B performance.

Freeze-Frame Index	A-not-B Performance
General Inhibitory Learning	$r = -.118$ $p = .306$ $n = 77$
Selective Inhibition	$r = .327^{**}$ $p = .003$ $n = 78$
Selective Inhibitory Learning	$r = .027$ $p = .832$ $n = 66$
General Distractibility, All Trials	$r = -.189^{\dagger}$ $p = .096$ $n = 79$
General Distractibility, Boring Trials	$r = .106$ $p = .354$ $n = 79$
General Distractibility, Interesting Trials	$r = -.397^{**}$ $p < .001$ $n = 79$

Note: $**p < .01$, $^{\dagger}p < .10$. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity (6 tests: $p_{\text{adj}} < .011$).

The results replicate the finding of a positive correlation between the Freeze-Frame Selective Inhibition index and A-not-B performance, $n = 78$, $r = .327$, $p = .003$.

The correlation between the two measures is highly significant and survives correction for multiplicity using the D/AP procedure. Thus, the current study clearly confirms that infants who show a large difference in distractibility in the two trial types early in the Freeze-Frame task (i.e., look less to the distractors in the interesting trials than in the boring trials) sustain longer delays in the A-not-B task.

However, the current study also revealed a highly significant negative correlation between General Distractibility in the interesting trials and A-not-B performance, $n = 79$, $r = -.397$, $p < .001$. In the Cohort 1 study reported in Chapter 3, the relationship

between General Distractibility in the interesting trials and A-not-B performance as measured by the cumulative delay was also negative, but the result did not reach statistical significance ($n = 21$, $r = -.237$, $p = .300$). The new finding in Cohort 2, which is considerably larger than Cohort 1, indicates that infants who look less to the distractors in the interesting Freeze-Frame trials *overall* perform better on the A-not-B task. It is worth noting that A-not-B performance was not significantly related to General Distractibility in the boring trials, $n = 79$, $r = .106$, $p = .354$, which strongly suggests that the effect was specific to the interesting trials. Furthermore, General distractibility in the boring and interesting trials was not correlated, $n = 86$, $r = .080$, $p = .465$, supporting the conclusion that performance on the two trial types has different underlying mechanisms.

None of the other Freeze-Frame indices was significantly associated with A-not-B performance, though there was a weak trend for General Distractibility across all trials to correlate negatively with A-not-B performance, $n = 79$, $r = -.189$, $p = .096$. This association is probably due to the overlap in variance between General Distractibility across trial types and General Distractibility in the interesting trials only. As would be expected, the latter two indices were highly correlated, $n = 86$, $r = .714$, $p < .001$.

These results suggest that it is mainly variability in the proportion of looks in the interesting trials which is related to A-not-B performance. Furthermore, differences in distractibility in the two trial types already in the first phase of the experiment are also associated with A-not-B performance, a result replicating findings reported in Chapter 3.

In the following correlational analyses, only Freeze-Frame measures found to be significantly correlated with A-not-B performance will be included. This approach is

taken in order to limit the number of pairwise correlations to the most relevant measures (cf. the discussion of multiplicity in Chapter 2).

Correlations between frontal cortex tasks and temperament at 9 months. Previous work on the relationship between infant frontal cortex tasks and temperament have been contradictory, with some findings suggesting that better self-regulatory ability is associated with good performance on such tasks (e.g., Johnson et al., 1991; see also Chapter 4), and other findings suggesting the opposite (Bell, 2005; Bell et al., 2006; see also Chapter 3). Another main aim of the current chapter was to address this question in Cohort 2.

Correlations between the two frontal tasks administered at 9 months, the Freeze-Frame task and the A-not-B task, and factor scores on the IBQ-R are presented in Table 5.5.

Table 5.5. Correlations between frontal cortex task performance and IBQ-R factor scores at 9 months of age.

IBQ-R factor	A-not-B Performance	FF Selective Inhibition	FF General Distractibility, Interesting
Surgency/Extraversion	$r = .114$ $p = .301$ $n = 85$	$r = .103$ $p = .353$ $n = 83$	$r = -.052$ $p = .642$ $n = 84$
Negative Affectivity	$r = .105$ $p = .341$ $n = 85$	$r = .046$ $p = .678$ $n = 83$	$r = -.185^\dagger$ $p = .091$ $n = 84$
Orienting/Regulation	$r = -.031$ $p = .776$ $n = 85$	$r = .024$ $p = .830$ $n = 83$	$r = -.014$ $p = .896$ $n = 84$

Note: $^\dagger p < .10$. No correlations survived D/AP correction for multiplicity (12 tests: $p_{.05} < .006$).

Perhaps surprisingly, the frontal cortex tasks were largely unrelated to infant temperament as assessed by the IBQ-R factor scores. The only correlation that

approached significance was a negative correlation between the Freeze-Frame General Distractibility index for interesting trials and the IBQ-R Negative Affectivity score. This may indicate that good frontal cortex performance is in fact associated with a more difficult temperament as suggested by the findings reported in Chapter 3 and recent studies by Bell and colleagues (Bell, 2005; Bell et al., 2006). However, the weakness of the relationship and the fact that the result is only borderline significant in a large sample of infants indicates that this is a subtle effect that needs further investigation.

To follow up on the findings by Bell and colleagues of a positive correlation between scores on the IBQ Activity Level and Distress scales and frontal cortex performance as assessed by the A-not-B task (Bell, 2005; Bell et al., 2006), an analysis of these two specific scales in relation to the Freeze-Frame and A-not-B tasks was carried out. The results of this analysis are presented in Table 5.6.

Table 5.6. Correlations between frontal cortex task performance and the IBQ-R Activity Level and Distress scales at 9 months of age.

IBQ-R Scale	A-not-B Performance	FF Selective Inhibition	FF General Distractibility, Interesting
Activity Level	$r = .307^{**}$ $p = .004$ $n = 85$	$r = .090$ $p = .420$ $n = 83$	$r = -.204^{\dagger}$ $p = .062$ $n = 84$
Distress	$r = .175$ $p = .109$ $n = 85$	$r = .118$ $p = .290$ $n = 83$	$r = -.195^{\dagger}$ $p = .076$ $n = 84$

Note: $**p < .01$, $^{\dagger}p < .10$. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity (6 tests: $p_{.06} < .011$).

As in the factor score analyses, the relationship between frontal cortex task performance and negative affect, in this case Distress, was relatively weak (see Table 5.6) and needs further replication before any firm conclusions can be drawn.

However, a significant relationship between A-not-B performance and Activity Level ($n = 85, r = .307, p = .004$) survived the D/AP correction and clearly replicated the findings of Bell and colleagues (Bell, 2005; Bell et al., 2006). This result suggests that infants exhibiting a higher activity level perform better on the A-not-B task. There was a similar trend for the Freeze-Frame General Distractibility index for interesting trials, $n = 84, r = -.204, p = .063$.

Correlations between frontal cortex tasks and scores on the MSEL-AGS at 9 months. The final question addressed by the current chapter was whether performance on the two frontal cortex tasks at 9 months were associated with performance on a standardised test of general development, the MSEL-AGS (Mullen, 1995). In Chapter 3, Freeze-Frame and A-not-B performance at 9 months was found to be relatively unrelated to general cognitive development at 2 years of age. In the present study the relationship between these tasks and concurrent general development was established.

Table 5.7 presents the correlations between the Freeze-Frame and A-not-B tasks and performance on the five MSEL-AGS scales and the Early Learning Composite.

Table 5.7. Correlations between frontal cortex task performance and MSEL-AGS scores at 9 months of age.

MSEL-AGS standardised score	A-not-B performance	FF Selective Inhibition	FF General Distractibility, Interesting
Visual Reception	$r = .151$ $p = .162$ $n = 87$	$r = .039$ $p = .721$ $n = 85$	$r = -.174$ $p = .110$ $n = 86$
Fine Motor	$r = .017$ $p = .879$ $n = 87$	$r = .160$ $p = .142$ $n = 85$	$r = -.071$ $p = .514$ $n = 86$
Receptive Language	$r = .059$ $p = .586$ $n = 87$	$r = -.044$ $p = .687$ $n = 85$	$r = .024$ $p = .823$ $n = 86$
Expressive Language	$r = .335^{**}$ $p = .002$ $n = 87$	$r = .153$ $p = .163$ $n = 85$	$r = -.193^{\dagger}$ $p = .075$ $n = 86$
Gross Motor	$r = .151$ $p = .162$ $n = 87$	$r = .161$ $p = .142$ $n = 85$	$r = -.148$ $p = .174$ $n = 86$
Early Learning Composite	$r = .233^{*}$ $p = .030$ $n = 87$	$r = .124$ $p = .259$ $n = 85$	$r = -.163$ $p = .135$ $n = 86$

Note: $**p < .01$, $*p < .05$, $^{\dagger}p < .10$. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity ($p_{adj} < .004$).

The results from this analysis indicate that A-not-B performance was related to general cognitive ability as measured by the MSEL-AGS Early Learning Composite, $n = 87$, $r = .233$, $p = .030$. A-not-B performance was also significantly related to performance on the MSEL-AGS Expressive Language scale, $n = 87$, $r = .335$, $p = .002$. Thus, infants who were able to sustain longer delays in the A-not-B task tended to have better expressive language skills as well better cognitive skills in general.

The Freeze-Frame measures showed a much weaker relationship with general cognitive performance. There was a trend towards a negative correlation between the General Distractibility index for interesting trials and the MSEL-AGS Expressive Language scale, $n = 86$, $r = -.193$, $p = .075$. This relationship suggests that infants

who look less to the distractors in the interesting trials in the Freeze-Frame task may have better expressive language skills. Only the correlation between A-not-B performance and MSEL-AGS Expressive Language score survived the D/AP correction for multiplicity.

Given that both performance on the A-not-B task and the Freeze-Frame task were related to performance on the MSEL-AGS Expressive Language scale, it is worth investigating whether the relationship between Freeze-Frame and A-not-B performance remains significant when variance in expressive language skill is controlled for. To address this question, a partial correlation analysis was carried out. This analysis revealed that the association between the Freeze-Frame Selective Inhibition index and A-not-B performance remained significant even after MSEL-AGS Expressive Language skill was controlled for, $df = 75$, $r = .300$, $p = .008$. The association between the Freeze-Frame General Distractibility index for interesting trials and A-not-B performance also remained significant after MSEL-AGS Expressive Language skill was controlled for, $df = 76$, $r = -.362$, $p = .001$. Furthermore, these effects also remained highly significant when general cognitive ability, as measured by the MSEL-AGS Early Learning Composite, was controlled for (Freeze-Frame Selective Inhibition/A-not-B: $df = 75$, $r = .305$, $p = .007$; Freeze-Frame General Distractibility in interesting trials/A-not-B: $df = 76$, $r = -.381$, $p = .001$).

Thus, it is clear that even though cognitive ability, in particular expressive language ability, is modestly related to frontal cortex task performance at 9 months of age, the relationship between the Freeze-Frame task and the A-not-B task is still robust when these factors are controlled for.

Discussion

The current chapter was concerned with three main research questions relating to the follow-up study of Cohort 2 at 9 months of age. Firstly, the analyses attempted to replicate the Freeze-Frame and A-not-B findings reported in Chapter 3. Secondly, the chapter addressed the question of whether performance on frontal cortex tasks administered at 9 months of age was related to concurrent infant temperament. Finally, the potential relationship between infant frontal cortex tasks and general cognitive and motor development was investigated.

Replication of the correlation between the Freeze-Frame and A-not-B tasks

The group findings from the Freeze-Frame task replicated the initial study very well. The mean calibration trial and overall proportions of looks to the distractors in the different trial types and phases (Table 5.1) were similar to the results reported in Chapter 3. Furthermore, the results of both the repeated measures ANOVA and the Linear Mixed Model replicated the initial Freeze-Frame group findings in that there were significant main effects of Trial Type and Phase, but no interaction. These results establish that, despite minor differences in stimuli between the current and original version of the task (see Method), the Freeze-Frame paradigm produces the same overall performance pattern. This also indicates that the Freeze-Frame task is robust to such minor changes, making the findings more generalisable.

As expected, the correlational analyses replicated the relationship between the Freeze-Frame Selective Inhibition index and A-not-B performance. This replication greatly strengthens the reliability of the finding, and strongly suggests that early differences in distractibility in the two Freeze-Frame trial types are related to how

well infants perform on the A-not-B task. Specifically, infants who look less to the distractors in the interesting trials compared to the boring trials in the first 16 trials of the Freeze-Frame task tend to sustain longer delays in the A-not-B task.

However, the current study also provided a new finding in terms of the relationship between the two tasks. It turned out that the overall proportion of looks to the distractors in the interesting trials, i.e., the General Distractibility index for interesting trials, was negatively related to A-not-B performance. That is, infants who looked less to the distractors in the interesting trials *overall*, tended to sustain longer delays in the A-not-B task. This finding extends the relationship between Freeze-Frame and A-not-B performance beyond the first phase of the Freeze-Frame task. It indicates that infants who are less distractible when the central stimulus is engaging find it easier to sustain a delay in the A-not-B task. Whether this effect is due to the infant actively inhibiting looks to the distractors in the interesting trials or infants simply being less distractible when engaged is difficult to establish because the measure does not index learning effects. However, the fact that General Distractibility in the boring trials did not correlate with A-not-B performance strongly suggests that the effect cannot be accounted for by distractibility level in any situation – only distractibility level when the infant is engaged is related to inhibitory performance in the A-not-B task.

Frontal cortex task performance at 9 months and infant temperament

The second aim of the current study was to establish whether the two 9-month frontal cortex tasks, the Freeze-Frame task and the A-not-B task, were related to infant temperament. Posner and Rothbart (1998) have suggested that infant self-regulatory ability is a precursor of later, frontally mediated effortful control

mechanisms. However, the literature in this area is somewhat contradictory. Studies investigating the association between frontal cortex task performance and temperament in toddlers and young children do tend to find that children who are better able to regulate their behaviour and who express less negative affect perform better on frontal cortex tasks (Gerardi-Caulton, 2000; Rothbart et al., 2003).

Furthermore, a few studies have found that 4- to 6-month-old infants who can easily disengage their attention in the Gap task, a task that has been linked to the frontal cortex (see Chapter 4), tend to be more soothable and show less distress (Johnson et al., 1991; McConnell & Bryson, 2005). Finally, results presented in Chapter 4 indicated that infants who performed well on another potential frontal cortex task, the Anticipation task, were more soothable and showed less distress.

On the other hand, recent studies from Bell and colleagues (Bell, 2005; Bell et al., 2006), as well as the results presented in Chapter 3, suggest that infants who perform well on frontal cortex tasks at a slightly older age (9-10 months) are more active, display more negative affect, and have poorer self-regulatory abilities. Furthermore, the results from Chapter 4 did not replicate the association between Gap task performance and distress and soothability found in previous studies (Johnson et al., 1991; McConnell & Bryson, 2005). It is therefore unclear whether good frontal cortex task performance is associated with better self-regulatory abilities as suggested by Posner and Rothbart (1998) or whether the opposite is in fact the case.

The study presented in Chapter 3 found that a high score on both the Freeze-Frame Selective Inhibition index and the A-not-B task at 9 months was associated with *less* Effortful Control at 24 months. The current study addressed the question of whether the Freeze-Frame and A-not-B tasks are also associated with concurrent infant temperament. Perhaps surprisingly, the frontal cortex tasks administered at 9

months showed little association with concurrent infant temperament as assessed by IBQ-R factor scores. There was a weak negative correlation between the Freeze-Frame General Distractibility index for interesting trials and score on the Negative Affectivity factor. Even though this effect was small and needs further replication, it does seem to contradict the theory that good frontal cortex performance is associated with a profile of mild temperament (Posner & Rothbart, 1998).

A follow-up analysis testing directly the findings by Bell and colleagues (Bell, 2005; Bell et al., 2006) confirmed a positive association between A-not-B performance and Activity Level. However, the association between performance on frontal cortex tasks (Freeze-Frame and A-not-B) and Distress was relatively weak and only approached statistical significance.

Frontal cortex task performance at 9 months and general development

The final question of the current chapter was whether performance on the two 9-month frontal cortex tasks was associated with general cognitive and motor ability. In the Cohort 1 study reported in Chapter 3, Freeze-Frame and A-not-B performance at 9 months was generally not found to be related to measures of general development at 24 months. However, in that study, one of the 24-month frontal cortex measures, the Spatial Conflict task, was found to be associated with motor development at the same age. It is therefore possible that frontal cortex task performance at 9 months of age is associated with concurrent cognitive and motor abilities.

The results from the correlational analyses in the current (Cohort 2) study indicated that A-not-B performance was moderately correlated with concurrent expressive language skill and to a lesser extent with general cognitive ability, as assessed by the MSEL-AGS. This means that children who performed well in the A-

not-B task tended to be more verbally expressive and to perform slightly better on a range of different tasks. Therefore, performance on the A-not-B task cannot be considered entirely independent of the infant's general cognitive ability. The Freeze-Frame task was associated with cognitive development to a lesser extent. There was a weak association between the General Distractibility index for interesting trials and expressive language skills as assessed by the MSEL-AGS, possibly indicating that infants who were less distractible when the central stimulus was engaging tended to be slightly more verbally expressive.

These results indicate that frontal cortex task performance at 9 months of age is at least weakly related to the infant's general cognitive abilities. This fits fairly well with the adult literature on the relationship between the frontal cortex and general intelligence. Patients with frontal lobe damage tend to have relatively spared general intelligence as assessed by traditional IQ tests such as the Wechsler Adult Intelligence Scale (Duncan et al., 1995; Warrington, James, & Maciejewski, 1986), which has led to the traditional view that the functioning of the frontal cortex is largely unrelated to general intelligence (Gazzaniga et al., 2002; Shallice & Burgess, 1991). More recently this view has started to change. For example, Obonsawin and colleagues (2002) found that, at least in healthy individuals, performance on most adult frontal cortex tasks is correlated with scores on the WAIS-R. Nevertheless, this study also found that some frontal cortex tasks were correlated with each other even when IQ was partialled out, and that one of the frontal cortex measures (a version of the WCST) was entirely independent of IQ (Obonsawin et al., 2002). The latter result indicates that at least some frontal cortex tasks may tap into specific cognitive functions which are relatively independent of general cognitive functioning.

Less is known about the relationship between the frontal cortex and general intelligence in children. Diamond and colleagues (1997) reported a large-scale study investigating frontal cortex functioning in children treated early and continuously for phenylketonuria (PKU), a genetically determined metabolic disorder. In untreated PKU, children develop severe cognitive impairment due to extensive brain damage. This brain damage is caused by high blood levels of an amino acid called phenylalanine (Diamond et al., 1997). In their study, Diamond and colleagues (1997) successfully demonstrated that through a biochemical mechanism impacting specifically on the dopamine system in the frontal cortex, children with early and continuously treated PKU were particularly prone to impairment on frontal cortex tasks. However, within this group, children with higher blood-levels of phenylalanine also scored lower on an IQ test, and, when IQ was controlled for, the difference between the PKU group and various control groups in terms of frontal cortex task performance was reduced. This finding supports the conclusion that at least some variability in frontal cortex task performance overlaps with variability in general cognitive functioning.

The findings from the current study mirror those of Diamond and colleagues (1997) in that concurrent measures of cognitive functioning were weakly, but significantly, related to performance on infant frontal cortex tasks. However, performance on the two frontal cortex tasks used in the current study, the Freeze-Frame task and the A-not-B task, was correlated even when the variance from the relevant MSEL-AGS scores was controlled for. Thus, the two frontal tasks do seem to measure a shared functional mechanism over and above general intelligence.

Finally, it is worth mentioning that scores on the MSEL-AGS had a relatively limited range. As pointed out in the Results section this is likely due to the infants

being from a relatively socially advantaged opportunity sample. In Chapter 2 the potential effects of such range restriction were briefly discussed. It was concluded that correlations between tasks might not be detected if infants did not differ enough on one of the measures, either because of sample characteristics or a lack of task sensitivity. However, despite the slightly restricted range in MSEL-AGS scores, the results clearly indicated that differences in these scores correlated with other measures, in particular performance on the A-not-B task⁷. It therefore seems reasonable to conclude that there was enough variability in the MSEL-AGS data to detect significant relationships in the current sample. Nevertheless, the findings can only be generalised to infants from a relatively high socio-economic background. In a less advantaged sample, general development might play a larger or smaller role in relation to frontal cortex task performance.

Conclusions

In conclusion, the present study replicated the relationship between the Freeze-Frame task and the A-not-B task found in Cohort 1 at 9 months of age (Chapter 3). Performance on the two frontal cortex tasks was only weakly related to concurrent temperament. In contrast to the findings reported in Chapter 4 (when infants were 4 months old), associations between frontal cortex tasks and temperament at 9 months tended to suggest that more active and possibly more frustratable infants performed better on these tasks. Frontal cortex performance was also to some extent associated

⁷ Note that the same conclusions apply to performance on the A-not-B task. In this task approximately 40% of infants could only pass the task at the most basic level (0-s delay on a change trial), somewhat reducing variability. However, there was clearly enough variability in the data to detect the highly significant relationship between the A-not-B and Freeze-Frame tasks found in both Cohort 1 and 2.

with aspects of general cognitive development, though the relationship between the Freeze-Frame and A-not-B tasks remained after controlling for these factors.

The current and previous chapter have investigated the relationship between frontal cortex tasks, temperament and general development separately at 4 and 9 months of age. However, as discussed in Chapter 1, one of the most powerful ways of providing converging evidence for frontal cortex functioning in infancy is the establishment of longitudinal relationships between purported frontal cortex tasks. Such relationships would be further evidence that the frontal cortex is functional from an early age and that such functioning is relatively stable during early development. Based on data from Cohort 2, Chapter 6 will address the question of longitudinal stability in frontal cortex task performance as well as the longitudinal relationship between these tasks and infant temperament and general development.

Summary of Chapter 5

- The study presented in this chapter was an attempt to replicate the finding of a correlation between the Freeze-Frame and A-not-B tasks at 9 months (cf. Chapter 3) in Cohort 2.
- The relationship between these two presumed frontal cortex tasks and concurrent temperament and general development was also investigated.
- The relationship between the two 9-month frontal cortex tasks was replicated in the larger cohort and can therefore be considered a fairly robust finding.
- The relationship between the two frontal cortex tasks and temperament at 9 months was relatively weak, but rather than suggesting that infants with good self-regulatory skills performed better on these tasks, the trend was in the opposite direction. Infants who were more active and showed more distress tended to perform slightly better on the frontal cortex tasks.
- Performance on the Freeze-Frame and A-not-B tasks was also weakly associated with measures of general cognitive development, especially expressive language ability, but the relationship between the two frontal cortex tasks remained highly significant even after controlling for these variables.

CHAPTER 6

The longitudinal relationship between frontal cortex tasks, temperament, and general development between 4 and 9 months of age

Introduction

The two previous chapters investigated the relationship between frontal cortex tasks in the same group of children (Cohort 2) at 4 and 9 months of age as well as the relationship between these tasks and measures of temperament and general development. It was found that at each age performance on each of the frontal cortex tasks was significantly related to performance on the other frontal cortex task. The association between frontal cortex tasks was stronger at 9 months ($r_s \approx .35$) than at 4 months ($r_s \approx .2$). Furthermore, performance on these tasks was related to infant temperament, though these associations tended to be relatively weak unless specific scales investigated in previous studies were used (as opposed to factor scores). Finally, the results reported in Chapter 5 indicated that general cognitive development was related to performance on the frontal cortex tasks administered at 9 months of age. However, the significant association between the two frontal cortex tasks (Freeze-Frame and A-not-B) remained strong even after controlling for cognitive ability.

The current chapter addresses the question of whether the frontal cortex tasks administered at 4 months of age measure similar functions to the frontal cortex tasks administered at 9 month of age. This is a key question in this thesis. Even though the cross-sectional data indicate that within a specific age frontal cortex tasks measure some of the same functions, these results do not address whether performance at one

point in time is related to performance at another point in time. In Chapter 3, a study in a smaller sample of children (Cohort 1) showed that frontal cortex measures taken at 9 months of age were related to frontal cortex measures at 2 years of age. However, the results of that study were complex, and correlations were not always in the expected direction.

Establishing whether longitudinal correlations exist between early frontal cortex tasks is essential for addressing the question of stability in frontal cortex functioning. It is important to recognise that a lack of such correlations does not necessarily mean that different tasks at the same age do not measure similar frontal cortex functioning; it may simply be that individual differences in frontal cortex functioning change across development, for instance because children develop at different rates. However, if correlations are found between frontal cortex tasks administered at different points in time, this is a clear indication that some stability exists in the functions measured by the tasks.

The hypothesis that these functions are mediated by the frontal cortex rests on both theoretical assumptions and converging evidence from previous studies, given the lack of direct evidence for frontal cortex involvement in most infant tasks. However, even though ultimately neuroimaging techniques are needed to address the latter issue, establishing whether cross-age correlations exist between these tasks is an important step towards establishing whether the tasks share at least some common functionality.

The main question of this chapter is therefore the potential longitudinal stability in frontal cortex task performance between 4 and 9 months of age. There is ample evidence to support frontal cortex involvement in the A-not-B task at 9 months of age (Baird et al., 2002; Bell & Fox, 1992; Diamond, 2002; Diamond & Goldman-Rakic,

1989). However, the evidence for frontal cortex involvement in the 4-month tasks is somewhat weaker given the low spatial resolution of the ERP methodology used to establish the link (Csibra et al., 1998, 2001), and given the fact that other cortical areas are also likely to be involved in the development of saccadic control (e.g., Braddick & Atkinson, 1988; Bronson, 1974; B. Hood & Atkinson, 1990; see Chapter 4 for discussion). Finding a relationship between the 4- and 9-month frontal cortex tasks would indicate that the 4-month tasks do measure some of the same functions as the Freeze-Frame and A-not-B tasks at 9 months of age, and that individual performance differences in these tasks are relatively stable over time. This would also be important evidence that the frontal cortex is at least partially functional before 8-9 months of age, a question which only a few studies have addressed (Csibra et al., 1998, 2001; Gilmore & Johnson, 1995; Johnson, 1995).

Two additional questions are addressed in the current chapter. Firstly, given the fact that temperament was found to be related to performance on some of the frontal cortex tasks in previous chapters, it seems relevant to address the question of whether these temperament characteristics are stable over time, and whether their relationship with frontal cortex performance is stable during development. Furthermore, given the generally contradictory findings in the literature regarding the relationship between frontal cortex functioning and temperament (see Chapters 3 and 5), it seems relevant to investigate the question of whether *changes* in temperament across development are related to frontal cortex task performance. The current study allowed this kind of analysis because temperament was measured at two points in time (at 4 and 9 months).

Finally, results from the 9-month study in Cohort 2 (Chapter 5) indicated that performance on the frontal cortex tasks was weakly but significantly related to general

cognitive ability as measured by the MSEL-AGS. It is therefore relevant to know whether frontal cortex tasks at 4 months are also related to general cognitive development at 9 months (the only age where this was assessed), and if so, how this impacts on the potential relationship between the 4- and 9-month frontal cortex tasks.

Methods

Methods for the tasks and instruments used in this chapter have been described in previous chapters. The Gap task, Anticipation task and the IBQ-R were described in Chapter 4. The Freeze-Frame task and the A-not-B task were described in Chapters 3 and 5. The MSEL-AGS was described in Chapter 5.

Results

Three sets of correlational analyses were carried out to establish (1) the relationship between the 4- and 9-month frontal cortex tasks, (2) the relationship between temperament and frontal cortex tasks across age, and (3) the relationship between general development and frontal cortex tasks across age. The results of these analyses are summarised in Figure 6.1 at the end of this Results section. As in Chapter 5, only Freeze-Frame measures found to be significantly correlated with A-not-B performance are included in the correlation analyses in order to limit the number of pairwise comparisons.

Longitudinal correlations between the 4- and 9-month frontal cortex tasks. The main question addressed in this chapter is whether performance on the 4-month frontal cortex tasks, the Gap task and the Anticipation task, is related to performance on the 9-month frontal cortex tasks, the Freeze-Frame task and the A-not-B task. Table 6.1 presents the correlations between these tasks.

Table 6.1. Cross-age correlations between key measures from the 4- and 9-month frontal cortex tasks.

		9-month measures		
		A-not-B Performance	FF Selective Inhibition	FF General Distractibility, Interesting
4-month measures	Gap Task: SRT Overlap Condition	$r = -.140$ $p = .196$ $n = 87$	$r = -.017$ $p = .876$ $n = 85$	$r = -.003$ $p = .976$ $n = 86$
	Gap Task: Gap Effect	$r = -.173$ $p = .108$ $n = 87$	$r = -.017$ $p = .879$ $n = 85$	$r = .019$ $p = .862$ $n = 86$
	Proportion Anticipation ^a	$r = .080$ $p = .469$ $df = 83$	$r = .189^\dagger$ $p = .086$ $df = 81$	$r = -.057$ $p = .608$ $df = 82$
	Anticipatory Learning	$r = .146$ $p = .204$ $n = 77$	$r = .122$ $p = .289$ $n = 77$	$r = -.099$ $p = .390$ $n = 78$

Note: $^\dagger p < .10$. No correlations survived D/AP correction for multiplicity (12 tests: $p_{a12} < .005$).

^a Controlled for proportion of valid trials in the Anticipation task.

As can be seen from the table, the 4- and 9-month frontal cortex measures were generally very weakly correlated. Only one cross-age correlation approached statistical significance. This was a positive correlation between the proportion of anticipatory saccades in the Anticipation task at 4 months (controlled for proportion of valid trials) and the Freeze-Frame Selective Inhibition index at 9 months, $df = 81$, $r = .189$, $p = .086$. Though this association must be considered quite weak, the correlation may indicate that the level of anticipation at 4 months of age is associated with early differences in distractibility in the two Freeze-Frame trial types at 9 months of age.

Longitudinal correlations between frontal cortex tasks and temperament. The second question addressed in this chapter is whether temperament characteristics as measured by the IBQ-R were stable between 4 and 9 months of age, and whether

temperament characteristics at each age, as well as changes in these characteristics, were longitudinally related to frontal cortex task performance. The cross-age correlations between the IBQ-R factor scores are shown in Table 6.2.

Table 6.2. IBQ-R cross-age correlations.

	IBQ-R 9 months: Surgency/ Extraversion	IBQ-R 9 months: Negative Affectivity	IBQ-R 9 months: Orienting/ Regulation
IBQ-R 4 months: Surgency/ Extraversion	$r = .374^{***}$ $p < .001$ $n = 90$	$r = .038$ $p = .722$ $n = 90$	$r = .290^{**}$ $p = .006$ $n = 90$
IBQ-R 4 months: Negative Affectivity	$r = -.115$ $p = .279$ $n = 90$	$r = .584^{***}$ $p < .001$ $n = 90$	$r = -.140$ $p = .187$ $n = 90$
IBQ-R 4 months: Orienting/ Regulation	$r = .223^*$ $p = .034$ $n = 90$	$r = -.141$ $p = .185$ $n = 90$	$r = .477^{***}$ $p < .001$ $n = 90$

Note: *** $p < .001$, ** $p < .01$, * $p < .05$. Bold typeface indicates correlations that are significant after D/AP correction for multiplicity (9 tests: $p_{adj} < .009$).

The three factor scores at 4 months were significantly correlated with the equivalent factor scores at 9 months, and these correlations survived the D/AP correction. This indicates that parental ratings of infant temperament were fairly consistent over time. It is also worth noting that the Negative Affectivity factor was the most stable over time. Furthermore, this factor was not significantly correlated with the two other factor scores across age, whereas the Surgency/Extraversion and Orienting/Regulation factors were correlated with each other both within (Table 4.3) and across age (Table 6.2).

Longitudinal correlations between temperament and the frontal cortex measures are presented in Tables 6.3 and 6.4. Table 6.3 presents correlations between the 4-month frontal cortex tasks and the 9-month IBQ-R factor scores, and Table 6.4

presents correlations between the 4-month IBQ-R factor scores and the 9-month frontal cortex tasks.

Table 6.3. Correlations between frontal cortex task performance at 4 months and IBQ-R factor scores at 9 months.

		4-month frontal cortex tasks			
		Gap Task: SRT Overlap Condition	Gap Task: Gap Effect	Proportion Anticipation ^a	Anticipatory Learning
9-month IBQ-R factor scores	Surgency/ Extraversion	$r = -.026$ $p = .805$ $n = 92$	$r = -.046$ $p = .662$ $n = 92$	$r = -.122$ $p = .253$ $n = 88$	$r = .026$ $p = .819$ $n = 82$
	Negative Affectivity	$r = .060$ $p = .573$ $n = 92$	$r = .017$ $p = .872$ $n = 92$	$r = -.197^\dagger$ $p = .063$ $n = 88$	$r = .019$ $p = .867$ $n = 82$
	Orienting/ Regulation	$r = .044$ $p = .676$ $n = 92$	$r = .055$ $p = .602$ $n = 92$	$r = -.047$ $p = .659$ $n = 88$	$r = -.050$ $p = .654$ $n = 82$

Note: $^\dagger p < .10$. No correlations survived D/AP correction for multiplicity (12 tests: $p_{a12} < .004$).

^a Controlled for proportion of valid trials in the Anticipation task.

Table 6.4. Correlations between frontal cortex task performance at 9 months and IBQ-R factor scores at 4 months.

		9-month frontal cortex tasks		
		A-not-B Performance	FF Selective Inhibition	FF General Distractibility, Interesting
4-month IBQ-R factor scores	Surgency/ Extraversion	$r = .244^*$ $p = .025$ $n = 84$	$r = .166$ $p = .135$ $n = 82$	$r = -.080$ $p = .474$ $n = 82$
	Negative Affectivity	$r = .226^*$ $p = .038$ $n = 84$	$r = -.103$ $p = .335$ $n = 82$	$r = -.134$ $p = .231$ $n = 82$
	Orienting/ Regulation	$r = .120$ $p = .277$ $n = 84$	$r = .158$ $p = .157$ $n = 82$	$r = -.163$ $p = .143$ $n = 82$

Note: $*p < .05$. No correlations survived D/AP correction for multiplicity (9 tests: $p_{a9} < .007$).

As can be seen from Table 6.3, only one correlation between a frontal cortex task administered at 4 months and temperament at 9 months approached significance. This was a negative correlation between the proportion of anticipations at 4 months (controlled for proportion of valid trials) and Negative Affectivity at 9 months of age, $df = 88$, $r = -.197$, $p = .063$. Conversely, as can be seen from Table 6.4, 4-month temperament was a stronger predictor of frontal cortex task performance at 9 months of age. Both Surgency/Extraversion and Negative Affectivity at 4 months were significantly correlated with performance on the A-not-B task at 9 months (Surgency/Extraversion: $n = 84$, $r = .244$, $p = .025$; Negative Affectivity: $n = 84$, $r = .226$, $p = .038$). However, these associations were not strong enough to survive the D/AP correction for multiplicity. The two Freeze-Frame indices identified as being associated with A-not-B performance at 9 months (Selective Inhibition and General Distractibility in interesting trials) were not significantly correlated with temperament at 4 months ($ps > .10$).

In order to establish potential associations between performance on the frontal cortex tasks at the two ages and *changes* in temperament during the first year of life, a difference measure was calculated. The score on each temperament factor from each infant at 4 months of age was subtracted from the equivalent factor score at 9 months of age. The order of subtraction of these scores is somewhat arbitrary, but the resulting score is a measure of the change in each factor between 4 and 9 months. A positive score indicates an increase in the factor score between 4 and 9 months, and a negative score indicates a decrease in the factor score between 4 and 9 months. Table 6.5 presents average changes in IBQ-R factor scores.

Table 6.5. Average change in IBQ-R factor scores between 4 and 9 months.

	Mean	SD	Minimum	Maximum
Surgency/Extraversion	1.66	0.68	-0.51	3.39
Negative Affectivity	0.22	0.56	-1.11	1.63
Orienting/Regulation	0.41	0.57	-0.83	1.92

As can be seen from Table 6.5, all three mean factor scores increased from 4 to 9 months. This result is broadly similar to the results reported by Gartstein and Rothbart (2003) in their cross-sectional validation study of the IBQ-R. Gartstein and Rothbart also found that older infants had higher levels of Surgency/Extraversion and Negative Affectivity. However, in contrast to the current study, they found that older infants had slightly lower levels of Orienting/Regulation than younger infants.

Table 6.5 also shows that the Negative Affectivity factor score was the most stable; scores on this factor only increased slightly between the two ages. However, the mean Surgency/Extraversion score increased by more than 1.5 scale points, indicating that on average infants were rated as being considerably more extraverted with age. Change scores on the Surgency/Extraversion and Orienting/Regulation factors were highly correlated, $n = 90$, $r = .529$, $p < .001$, whereas change scores on the Negative Affectivity factor was unrelated to change on the two other factors (all $ps > .80$). This indicates that change on the Negative Affectivity factor was independent of the correlated changes on the Surgency/Extraversion and Orienting/Regulation factors.

Given the contradictory findings regarding the relationship between infant temperament and frontal cortex task performance (see Chapters 3 and 5), it is relevant to know whether performance on frontal cortex tasks is related to changes in temperament over time as well as to concurrent temperament. Table 6.6 presents

correlations between the 4- and 9-month frontal cortex tasks and the IBQ-R change scores.

Table 6.6. Correlations between IBQ-R factor change scores and frontal cortex tasks at 4 and 9 months.

	IBQ-R Change in Surgency/ Extraversion	IBQ-R Change in Negative Affectivity	IBQ-R Change in Orienting/ Regulation
4-month Gap task: SRT Overlap Condition	$r = -.045$ $p = .673$ $n = 90$	$r = .078$ $p = .463$ $n = 90$	$r = -.009$ $p = .931$ $n = 90$
4-month Gap task: Gap Effect	$r = -.041$ $p = .699$ $n = 90$	$r = .187^\dagger$ $p = .078$ $n = 90$	$r = .028$ $p = .791$ $n = 90$
4-month Proportion Anticipation ^a	$r = -.069$ $p = .525$ $df = 86$	$r = -.128$ $p = .235$ $df = 86$	$r = -.154$ $p = .152$ $df = 86$
4-month Anticipatory Learning	$r = -.119$ $p = .292$ $n = 80$	$r = .057$ $p = .613$ $n = 80$	$r = -.261^*$ $p = .019$ $n = 80$
9-month A-not-B Performance	$r = -.136$ $p = .216$ $n = 84$	$r = -.111$ $p = .316$ $n = 84$	$r = -.149$ $p = .177$ $n = 84$
9-month FF Selective Inhibition	$r = -.074$ $p = .511$ $n = 82$	$r = .149$ $p = .183$ $n = 82$	$r = -.149$ $p = .183$ $n = 82$
9-month FF General Distractibility, Interesting	$r = .033$ $p = .767$ $n = 82$	$r = -.052$ $p = .640$ $n = 82$	$r = .152$ $p = .174$ $n = 82$

Note: $*p < .05$, $^\dagger p < .10$. No correlations survived D/AP correction for multiplicity (21 tests: $p_{a21} < .003$).

^a Controlled for proportion of valid trials in the Anticipation task.

As in the previous analyses, there were only a few relatively weak associations between the temperament measures and the frontal cortex measures. Infants who demonstrated fast anticipatory learning at 4 months tended to show a comparatively smaller increase, or a slight decrease, in Orienting/Regulation scores between 4 and 9 months compared to infants who were slow anticipatory learners, $n = 80$, $r = -.261$, p

= .019. There was also a tendency for infants who had a large Gap effect at 4 months of age to show a larger increase in Negative Affectivity between 4 and 9 months of age, but this relationship was only marginally significant, $n = 90$, $r = .187$, $p = .078$.

Longitudinal correlations between frontal cortex tasks and general development.

The final question in this chapter regarded the relationship between general development and frontal cortex task performance across age. In Chapter 5, it was found that the A-not-B task, and to a lesser extent the Freeze-Frame task, were associated with expressive language ability and general cognitive ability as assessed by the MSEL-AGS. However, the relationship between the two 9-month frontal cortex tasks remained significant after controlling for these factors. Given these findings, it is relevant to investigate whether (1) the 4-month frontal cortex measures were also related to general cognitive functioning at 9 months, and (2) whether any relationships between the 4-month and 9-month frontal cortex tasks existed after controlling for general cognitive functioning at 9 months.

Table 6.7 presents correlations between the 4-month frontal cortex tasks and MSEL-AGS scores at 9 months of age. The table shows that the size of the Gap effect at 4 months was negatively related to the two MSEL-AGS visual organisation scales at 9 months, Visual Reception ($n = 94$, $r = -.242$, $p = .019$) and Fine Motor ($n = 94$, $r = -.223$, $p = .031$). Median SRT in the Overlap trials was also negatively correlated with performance on the Fine Motor scale, $n = 94$, $r = -.285$, $p = .005$. In addition, both the size of the Gap effect and the median SRT in the Overlap trials were marginally correlated with the MSEL-AGS Early Learning Composite at 9 months (Gap effect: $n = 94$, $r = -.194$, $p = .061$; median Overlap SRT: $n = 94$, $r = -.189$, $p = .069$). These results were relatively, though not entirely, specific to the measures involving disengagement (i.e., the Gap effect and the median SRT in the Overlap

condition). Median SRT in the Gap condition did show a weak negative correlation with the MSEL-AGS Fine Motor scale, $n = 94$, $r = -.194$, $p = .060$, but was not significantly associated with the MSEL-AGS Visual Reception scale or the Early Learning Composite ($ps > .2$). These results indicate that having difficulty disengaging from stimuli at 4 months of age predicted poorer performance on visually guided cognitive tasks and possibly overall cognitive performance at 9 months of age.

Performance on the Anticipation task at 4 months was related to MSEL-AGS scores at 9 months to a lesser extent. Both measures from the Anticipation task were modestly related to performance on the Receptive Language scale, though this effect was only significant for the Anticipatory Learning index ($n = 84$, $r = .222$, $p = .043$). This indicates that infants who demonstrated faster learning in the Anticipation task at 4 months had better Receptive Language skills at 9 months of age. No other MSEL-AGS scales, including the Early Learning Composite, were significantly related to Anticipation task performance.

None of the relationships between the 4-month frontal cortex tasks and MSEL-AGS scores at 9 months survived the D/AP correction for multiplicity. This is mainly due to the large number of pairwise correlations involved when analysing all six MSEL-AGS scores (see Table 6.7).

Table 6.7. Correlations between 4-month frontal cortex tasks and MSEL-AGS scale scores at 9 months.

		9-month MSEL-AGS Scales					
		Visual Reception	Fine Motor	Receptive Language	Expressive Language	Gross Motor	Early Learning Composite
4-month frontal cortex measures	Gap Task: SRT Overlap Condition	$r = -.129$ $p = .216$ $n = 94$	$r = -.285^{**}$ $p = .005$ $n = 94$	$r = -.148$ $p = .155$ $n = 94$	$r = .037$ $p = .725$ $n = 94$	$r = -.003$ $p = .974$ $n = 94$	$r = -.189^{\dagger}$ $p = .069$ $n = 94$
	Gap Task: Gap Effect	$r = -.242^{*}$ $p = .019$ $n = 94$	$r = -.223^{*}$ $p = .031$ $n = 94$	$r = -.010$ $p = .927$ $n = 94$	$r = -.057$ $p = .585$ $n = 94$	$r = .064$ $p = .538$ $n = 94$	$r = -.194^{\dagger}$ $p = .061$ $n = 94$
	Proportion Anticipation ^a	$r = .053$ $p = .618$ $df = 90$	$r = .026$ $p = .809$ $df = 90$	$r = .177^{\dagger}$ $p = .091$ $df = 90$	$r = .007$ $p = .950$ $df = 90$	$r = .006$ $p = .953$ $df = 90$	$r = .101$ $p = .336$ $df = 90$
	Anticipatory Learning	$r = .077$ $p = .486$ $n = 84$	$r = -.012$ $p = .914$ $n = 84$	$r = .222^{*}$ $p = .043$ $n = 84$	$r = .047$ $p = .670$ $n = 84$	$r = .155$ $p = .160$ $n = 84$	$r = .130$ $p = .240$ $n = 84$

Note: $^{**}p < .01$, $^{*}p < .05$, $^{\dagger}p < .10$. No correlations survived D/AP correction for multiplicity (24 tests: $p_{a21} < .002$).

^a Controlled for proportion of valid trials in the Anticipation task.

Finally, it was investigated whether any cross-age correlations between the frontal cortex tasks were mediated primarily by differences in general cognitive functioning. Only one borderline significant correlation was found between frontal cortex tasks at different ages in the analyses above. This was a weak correlation between the proportion of anticipations in the Anticipation task (controlled for the proportion of valid trials) at 4 months of age and the Freeze-Frame Selective Inhibition index at 9 months of age ($df = 81, r = .189, p = .086$). When controlling for the MSEL-AGS Early Learning Composite score at 9 months this association was slightly reduced and no longer approached significance, $df = 80, r = .178, p = .110$. Nevertheless, the weak association between Anticipation performance at 4 months and performance on the Freeze-Frame Selective Inhibition index at 9 months did not seem to be mediated primarily by differences in general cognitive functioning.

Summary of results. Figure 6.1 presents a simplified summary of the relationship between frontal cortex tasks, temperament, and general cognitive development across the two age points. In order to maintain an adequate overview of the main results, only relationships between frontal cortex tasks and measures of temperament and general cognitive functioning are presented. That is, any associations between temperament factors and general cognitive development have been omitted from the figure; the reason being that the relationship between these variables was not the main focus of the analyses presented here. Furthermore, only the most relevant measures from each task are included in the figure. From the Gap task, the index of the Gap effect was included because this is a measure of the infant's ability to disengage that is relatively independent of overall reaction time. From the Anticipation task, the proportion of anticipations and the Anticipatory Learning index were included because these two measures estimate overall anticipatory performance and the speed

of anticipatory learning, respectively. From the Freeze-Frame task, the Selective Inhibition index and the General Distractibility index for interesting trials were included because these measures were most strongly associated with the other frontal cortex task at 9 months, the A-not-B task (Chapters 3 and 5). Finally, the Early Learning Composite from the MSEL-AGS was included because overall cognitive performance was the main variable of interest in relation to the frontal cortex tasks (overall motor performance was not significantly related to any of the frontal cortex measures in the analyses presented above).

Figure 6.1. Graphical summary of the relationship between frontal cortex tasks, temperament and general cognitive ability at 4 and 9 months of age (see text for details).

Note: Correlations indicated by a thick line are significant at $p < .01$, correlations indicated by a thin line are significant at $p < .05$, and correlations indicated by a dotted line are marginally significant, $p < .10$.

^a Controlled for proportion of valid trials in the Anticipation task.

In summary, despite significant correlations between frontal cortex tasks within age, only one frontal task cross-age correlation approached significance. This was a weak positive correlation between the amount of anticipation in the Anticipation task at 4 months and the Freeze-Frame Selective Inhibition index at 9 months.

Temperament was related to frontal cortex task performance across age. In particular, higher levels of Surgency/Extraversion and Negative Affectivity at 4 months predicted better performance on the A-not-B task at 9 months. There was also a weak tendency for infants who anticipated more in the Anticipation task at 4 months to score lower on the Negative Affectivity factor at 9 months. In terms of change in temperament over time, fast Anticipatory learning at 4 months predicted a comparatively smaller increase and occasionally a decrease in the Orienting/Regulation factor score between 4 and 9 months. Furthermore, there was a tendency for large Gap effects at 4 months to predict increasing Negative Affectivity scores between 4 and 9 months of age.

Finally, in terms of the longitudinal relationship between frontal cortex task performance and general development, it was mainly performance on the Gap task at 4 months which showed an association with performance on the MSEL-AGS at 9 months. The size of infants' Gap effect at 4 months correlated negatively with the MSEL-AGS visual organisation scales and tended to correlate negatively with the MSEL-AGS Early Learning Composite at 9 months.

Discussion

The main aim of the current chapter was to investigate whether performance on frontal cortex tasks administered at 4 and 9 months of age was correlated. As argued

in the introduction to this chapter, establishing stability in frontal cortex functioning constitutes important converging evidence for frontal cortex functioning in infancy.

However, the results indicated that the relationship between the frontal cortex tasks used here was fairly weak across age. There was a weak relationship between performance on the Anticipation task at 4 months and performance on the Freeze-Frame task at 9 months, indicating at least some shared variance between these two tasks. However, generally the relationship between frontal tasks within the same age was stronger than across age, particularly at 9 months. Thus, the relationship between the Freeze-Frame task and the A-not-B task was the strongest across all the frontal cortex tasks used in Cohort 2.

The weak relationship between the 4- and 9-month frontal cortex tasks

The weak relationship between frontal cortex tasks across the first year of life can be interpreted in several different ways. It is possible that individual differences in frontal cortex task performance are simply discontinuous during early development, that is, infants who perform well at one age do not necessarily perform well at another age. This pattern could be due to some significant development occurring between the two testing points. For example, Diamond has suggested that important changes in the frontal cortex during the second half of the first year of life underlie behavioural changes in A-not-B performance (e.g., Diamond, 1991; Diamond, 2002).

A related interpretation could be that at a very early age, such as 4 months, the frontal cortex is only just beginning to play a functional role. It should be borne in mind that even though infants can perform both the Gap and Anticipation tasks at 4 months, just a couple of months earlier they have great difficulty controlling their eye movements in such tasks (Hood & Atkinson, 1993; Johnson et al., 1991).

Performance differences in these tasks at 4 months of age may therefore be relatively weak indicators of frontal cortex functioning because this cortical area, and the functions associated with it, is still developing. Furthermore, 4-month-olds have a shorter attention span as evidenced by a larger number of invalid trials in the tasks administered at this age. This likely leads to more noisy data at 4 months compared to data collected at 9 months. These factors might also help explain why considerably stronger correlations were found between the 9-month frontal cortex tasks (Chapter 5) than between the 4-month frontal cortex tasks (Chapter 4).

Another possibility is that the weak associations across age indicate a lack of common functional mechanisms in the tasks. It may be that the 4-month frontal cortex tasks measure different aspects of frontal cortex functioning than the 9-months frontal cortex tasks. The 4-month frontal cortex tasks involve relatively basic saccadic control, whereas the 9-month frontal cortex tasks involve more active inhibition of response tendencies, and in the case of the A-not-B task, the maintenance of representations in memory. It is therefore possible that the processes engaged by the tasks involve different areas of the frontal cortex as well as other regions of the brain (see Chapter 4 for a discussion of other cortical areas involved in saccadic control and visual attention). This possibility will need to be further investigated in neuroimaging research.

Lack of longitudinal association between the Gap task and the Freeze-Frame task

One 4-month frontal cortex task that appeared to be particularly weakly associated with later frontal cortex tasks was the Gap task. Despite the finding of a modest correlation between the Gap and Anticipation tasks at 4 months of age, the Gap task was almost entirely unrelated to performance on the 9-month frontal cortex

measures. This shows that variation in visual disengagement ability at 4 months *does not* predict performance on the Freeze-Frame and A-not-B tasks at 9 months. Even though care must be taken when interpreting negative results, this finding is of interest given the interpretation of early differences in the Freeze-Frame task presented in Chapter 3. In Chapter 3 it was suggested that infants who had a large Selective Inhibition score in the Freeze-Frame task (i.e., they looked a lot less to the distractors in the interesting trials than in the boring trials in the beginning of the experiment) might experience some difficulty disengaging their attention when the central stimulus was attractive. However, the lack of correlation between Gap task performance at 4 months and the Freeze-Frame Selective Inhibition index at 9 months in Cohort 2 indicates that differences in disengagement *earlier* in infancy are unrelated to later differences in Freeze-Frame performance. Nevertheless, the possibility remains that concurrent disengagement ability is related to performance on the Freeze-Frame Selective Inhibition index at 9 months of age.

Gap task performance at 4 months and cognitive development at 9 months

One possible reason for the lack of association between the Gap task and the later frontal cortex measures could be that performance on this task is more predictive of general cognitive abilities than of abilities specifically related to the frontal cortex. Thus, in contrast to the lack of correlations with later frontal cortex tasks, the current study found that Gap task performance at 4 months was moderately predictive of general cognitive abilities at 9 months, in particular visual organisation skills as assessed by the MSEL-AGS.

Previous studies have found that some neurodevelopmental disorders such as autism, fragile X syndrome, and Turner syndrome are associated with difficulties in

disengagement as assessed by the Gap task (Landry & Bryson, 2004; Lasker, Mazzocco, & Zee, 2007; Zwaigenbaum et al., 2005). These disorders are often associated with developmental delay and other cognitive deficits. Furthermore, very premature infants, who often suffer varying degrees of neurological damage, and infants with neonatal brain damage perform poorly on Atkinson et al.'s (1992) fixation shift task (Atkinson & Braddick, 2007; Mercuri et al., 1997a, 1997b; Mercuri et al., 1996), and this impairment is associated with more general cognitive deficits later in childhood (Atkinson & Braddick, 2007; Mercuri et al., 1999). However, disengagement difficulty is not always linked to developmental problems since children with Down syndrome have better performance on the Overlap condition of the Gap task than normal controls (Landry & Bryson, 2004).

The association between performance on the Gap task and developmental delay has generally been observed in older infants and young children (Landry & Bryson, 2004; Lasker et al., 2007; Zwaigenbaum et al., 2005) and in longitudinal studies of paediatric populations (Atkinson & Braddick, 2007; Mercuri et al., 1999). The current study adds to this evidence by suggesting that performance on the Gap task at the age where the ability to disengage attention has just developed (Hood & Atkinson, 1993; Johnson et al., 1991) predicts cognitive ability later in the first year of life in typically developing infants.

Possible longitudinal association between the Anticipation task and the Freeze-Frame task

In contrast to the Gap task, the Anticipation task may be related to later Freeze-Frame performance, though, as mentioned above, the relationship between these two tasks was relatively weak and needs to be further established. Nevertheless, there was

some indication that infants who anticipated more in the Anticipation task at 4 months tended to show more Selective Inhibition in the Freeze-Frame task at 9 months. This result could be interpreted in two ways. It is possible that performing well in the Anticipation task at 4 months demonstrates that the infant has a high level of focused attention or concentration, enabling the infant to anticipate targets more often. This ability would be expressed in the Freeze-Frame task in that the infant would be less distracted when the stimulus was interesting. An alternative interpretation could be that infants who anticipate a lot at 4 months of age quickly learn to inhibit saccades to the distractors in the interesting Freeze-Frame trials at 9 months of age. Since the Freeze-Frame task alternates between interesting and boring trials, a learning/anticipation effect cannot be ruled out, but the infant would still have to inhibit looks to the distractors in the interesting trials in order to obtain a large Selective Inhibition score.

Admittedly, the relationship between the Anticipation task and the Freeze-Frame task was relatively weak, and caution is warranted in interpreting a common functional mechanism in the two tasks. If such a mechanism does exist, it only accounts for a small proportion of the variance in task performance. Nevertheless, further analyses indicated that at least general cognitive ability as assessed by the MSEL-AGS was not the main contributor to the relationship.

Longitudinal associations between frontal cortex tasks and temperament between 4 and 9 months of age

Turning to the relationship between temperament and frontal cortex task performance across age, the association between early temperament and A-not-B performance was the most noticeable. Both the level of Surgency/Extraversion and

Negative Affectivity at 4 months was positively related to A-not-B performance at 9 months. This finding is interesting given the fact that A-not-B performance was not significantly related to concurrent temperament factors (see Chapter 5). Gartstein and Rothbart (2003) occasionally refer to the Surgency/Extraversion factor as a measure of Positive Emotionality. Thus, it seems to be the case that infants who are more *reactive*, both in terms of negative and positive emotion, early in infancy (4 months) perform better on the A-not-B task later in infancy (9 months). This finding, like findings reported in Chapters 3 and 5, again seems to contradict the proposal by Posner and Rothbart (1998) that infants who demonstrate better self-regulatory abilities are also the ones who have a higher level of emerging frontal cortex functioning.

As discussed in Chapter 5, similar findings on the relationship between the A-not-B task and temperament have been reported by Bell and colleagues. This group found that infants who performed well on the A-not-B task were also more active and became more easily distressed (Bell, 2005; Bell et al., 2006). Bell, Wolfe and Adkins (2007) explain this relationship in terms of parent-infant interaction. They suggest that more reactive infants cause parents to support their attentional skills thereby leading to better development of these skills. The fact that infant temperament at a younger age predicted frontal cortex performance at a later age in the current study does suggest that a reactive developmental profile early in infancy somehow enhances frontal cortex functioning with age. However, this development is not necessarily mediated by parental behaviour. It is also possible that infants who are more reactive from an early age are simply more persistent and motivated to obtain rewards or desirable objects, enabling them to gain essential experience necessary for later frontal cortex skills.

To investigate the relationship between frontal cortex performance and temperament further, an analysis was carried out on *change* in temperament factor scores between 4 and 9 months of age. This analysis revealed that fast Anticipatory Learning at 4 months predicted a comparatively smaller increase or a slight decrease in scores on the Orienting/Regulation factor between 4 and 9 months of age. This result may seem to indicate that good frontal cortex functioning at an early age is associated with poorer regulatory functioning over time. However, results reported in Chapter 4 indicated that infants who learn quickly in the Anticipation task tended to have higher levels of Orienting/Regulation already at 4 months of age (Table 4.3). It is therefore more likely that slow learners in the Anticipation task catch up with the fast learners in terms of Orienting/Regulation between 4 and 9 months of age.

There was also a tendency for infants who showed a large proportion of anticipatory saccades overall at 4 months (not in terms of learning) to display less negative affect at 9 months. Thus, in contrast to the 9-month frontal cortex tasks, good performance on the Anticipation task did tend to be associated with better self-regulatory functioning both at the time of testing and later in infancy.

Conclusions

In conclusion, the associations between frontal cortex tasks across the first year of life were relatively weak compared to the within-age correlations, especially at 9 months of age. A potential relationship between Anticipation task performance at 4 months and the Freeze-Frame Selective Inhibition index at 9 months was found. This relationship was not primarily mediated by general cognitive functioning, but only accounted for a small proportion of the variance, and therefore needs further investigation.

Perhaps surprisingly, performance on the Gap task at 4 months was not related to performance on the frontal cortex tasks at 9 months. Instead, performance on this task was predictive of general cognitive development, in particular visual organisation skills, later in infancy.

Analyses investigating the longitudinal relationship between temperament and frontal cortex task performance provided mixed results. Analyses comparing early frontal cortex task performance to later temperament indicated that, consistent with Posner and Rothbart's (1998) proposal, one of the tasks, the Anticipation task, was associated with the early attainment of regulatory functioning and less negative affect later in development. However, in terms of the relationship between early temperament and later frontal cortex performance, it was clear that infants who were more reactive early in infancy, both in terms of positive and negative emotion, demonstrated better frontal cortex task performance at 9 months of age. The latter result is consistent with findings reported in Chapters 3 and 5 and suggests that good frontal cortex task performance in late infancy is associated with a reactive and sometimes difficult temperament rather than a calm and well-regulated temperament.

Summary of Chapter 6

- This chapter investigated the longitudinal relationship between frontal cortex tasks administered at 4 and 9 months of age in Cohort 2. (Within-age correlations were investigated in Chapters 4 and 5.)
- Longitudinal associations between frontal cortex performance, temperament and general development were also investigated.
- Longitudinal associations between the frontal cortex tasks were generally weak, and only one correlation approached significance.
- Performance on the Gap task at 4 months was primarily predictive of general cognitive development in the visual domain at 9 months, not performance on the frontal cortex tasks at that age.
- Consistent with the predictions of Posner and Rothbart (1998), good frontal cortex performance as assessed by the Anticipation task at 4 months was associated with a high level of self-regulatory functioning from an early age and less negative affect later in infancy.
- In contrast (and consistent with other findings in this thesis), good frontal cortex performance later in infancy as assessed by the A-not-B task (at 9 months) was associated with a high level of both positive and negative affect earlier in infancy (at 4 months).

CHAPTER 7

Frontal cortex task performance in infancy and variation in the Catechol-*O*-Methyltransferase gene

Introduction

The previous four chapters have presented data, from two cohorts of infants, investigating the relationship between a set of frontal cortex tasks in infancy. The overall picture of the results is that there are clear associations between the frontal cortex tasks administered at 9 months of age (Freeze-Frame and A-not-B). There is also some evidence that these measures predict performance on frontal cortex measures at 2 years of age, though these findings are preliminary given the small sample size of Cohort 1 (Chapter 3). A relationship between the 4-month frontal cortex tasks (Gap and Anticipation) was present, but modest. The longitudinal correlations between frontal cortex tasks were generally very weak, with only one of the 4-months frontal cortex tasks (the Anticipation task) showing some cross-age association with performance on one of the 9-month frontal cortex tasks (the Freeze-Frame task).

Even though these results indicate that at least some of the administered tasks share a significant proportion of their variance, this is not definitive evidence that the tasks are indeed related to the functioning of the frontal cortex. This issue has been partially addressed in the preceding chapters by controlling for the most likely confounds, such as general cognitive functioning. However, a direct relationship between performance on the administered tasks and biological markers of the frontal cortex would more definitively establish that the tasks tap into frontal cortex

functions. This chapter attempts to establish such a relationship by investigating the effect on task performance of a specific variation in the Catechol-*O*-Methyltransferase (COMT) gene, which impacts on the dopamine system in the frontal cortex.

Background

Variation is common in the human genome. A small sequence variation in the human genome that is present in at least 1% of the population is called a *polymorphism* (Lewis, 2008). An individual has two copies of a specific gene, one from the father and one from the mother. These copies are called *alleles*. An individual can inherit a different allele from each parent. Individuals with two copies of the same allele (one from each parent) are called homozygotes, whereas individuals with two different alleles (one from each parent) are called heterozygotes (Lewis, 2008).

The most common type of polymorphism in the human genome is the single nucleotide polymorphism or SNP (pronounced “snip”). A SNP consists of a single base-pair substitution in the DNA sequence, for example a substitution of C (cytosine) for T (thymine) (Lewis, 2008). An example of a C/T SNP is given in Figure 7.1.

Figure 7.1. A C/T single nucleotide polymorphism⁸.

The National Centre for Biotechnology Information database of SNPs and other small polymorphisms (dbSNP) presently contains more than 12 million entries (Wheeler et al., 2007). SNPs are present throughout the genome, with a SNP occurring in approximately 1 out of every 1,200 bases. However, not all of these SNPs are located in the approximately 20,600 protein-encoding genes in the human genome, and even when they are located in these regions, they do not always alter the resulting protein in any important way (Lewis, 2008).

Of course, choosing randomly from millions of SNPs, which may or may not play a functional role, is not the ideal way to find genetic mechanisms of psychological traits, or *phenotypes*. Quantitative methods have now been developed to look at clusters of SNPs in relation to traits and performance (e.g., Lewis, 2008; Plomin, DeFries, McClearn, & McGuffin, 2001). However, these methods are expensive and require very large sample sizes. An alternative approach has been to identify alleles that are likely to have a specific impact on the protein produced by the gene, which in turn influences behaviour. In the case of brain systems, the most relevant proteins are

⁸ Source: David Hall (http://en.wikipedia.org/wiki/Single_nucleotide_polymorphism).

the ones functioning as receptors, transporters, or enzymes catabolising neurotransmitters in the brain.

It is worth mentioning that many initial findings of an association between a candidate gene and a particular trait have subsequently failed to be replicated (Hutchison, Stallings, McGeary, & Bryan, 2004; Munafò, 2006). Therefore three important considerations need to be taken into account when adopting the candidate gene approach. Firstly, the investigated polymorphism should have a well-established biological mechanism that can potentially explain the relationship between the genotype and the behaviour of interest (e.g., Diamond et al., 2004; Hutchison et al., 2004). Secondly, any study involving the candidate gene approach should be replicated, and it should be established whether the effect of the polymorphism is the same in different populations (the frequency of most polymorphic alleles varies between populations) (Munafò, 2006). Finally, since the effects of just one gene are likely to be small, a large sample size is needed to detect an effect (Munafò, 2006).

The COMT gene

A polymorphism that conforms relatively well to these criteria is the Val¹⁵⁸Met SNP in the Catechol-*O*-Methyltransferase (COMT) gene. This is the polymorphism investigated in the current study. The COMT gene is located on chromosome 22, band q11.2. It is the only gene coding for the COMT enzyme, which metabolises catecholamines such as dopamine and noradrenaline by transferring a methyl group to a hydroxyl group of the catechol substrate (Chen et al., 2004; Männistö & Kaakkola, 1999; Tunbridge, Harrison, & Weinberger, 2006). Thus, the main biological function of COMT is the elimination of active and toxic catechols and their metabolites throughout the human body, including the brain (Männistö & Kaakkola, 1999).

The role of COMT in catabolising (breaking down) dopamine in the frontal cortex is particularly important due to the relative lack of dopamine transporters, and the positioning of these transporters at a distance from synaptic release sites, in this cortical region (Sesack, Hawrylak, Matus, Guido, & Levey, 1998). Thus, COMT accounts for more than 60% of the metabolic degradation of dopamine in the frontal cortex (Karoum, Chrapusta, & Egan, 1994). In contrast COMT catabolism only plays a minor role in the striatum, where the dopamine transporter is abundant and better situated for dopamine reuptake (Karoum et al., 1994; for review see Tunbridge et al., 2006).

The Val¹⁵⁸Met polymorphism in the COMT gene affects the activity level of the COMT enzyme. The polymorphism is an evolutionarily recent G (guanine) to A (adenine) missense mutation at codon 108/158, resulting in a substitution of methionine (Met) for valine (Val) in the COMT enzyme (Chen et al., 2004; Lachman et al., 1996; Tunbridge et al., 2006; Tunbridge et al., 2007). The Met variant of the enzyme is less stable at body temperature (Chen et al., 2004; Lotta et al., 1995), resulting in 3 to 4 times less COMT enzyme activity in the human liver and red blood cells (Männistö & Kaakkola, 1999). In the human brain this difference is smaller, but still considerable, with Met homozygotes having approximately 40% less COMT activity than Val homozygotes in the prefrontal cortex (Chen et al., 2004). The alleles are codominant, resulting in heterozygotes having an intermediate level of COMT expression (Egan et al., 2001; Männistö & Kaakkola, 1999; Tunbridge et al., 2006). This evidence strongly suggests that Met homozygotes have the *highest* baseline level of dopamine available in the prefrontal cortex (because less dopamine is catabolised) with heterozygotes having an intermediate level and Val homozygotes having the lowest level of prefrontal dopamine (Tunbridge et al., 2006; Tunbridge et al., 2007).

It is important to mention that the relationship between dopamine level and performance on frontal cortex tasks is most likely non-linear. Dopamine function in the prefrontal cortex has been hypothesised to have an ‘inverted-U’ relationship to frontal cortex performance, particularly working memory performance. This model was originally proposed by Goldman-Rakic et al. (2000) who demonstrated that an intermediate level of dopamine D₁ receptor activation in the frontal cortex resulted in optimal working memory performance in non-human primates. According to this model, too low levels of D₁ receptor activation, as seen in Parkinson’s disease and normal aging, leads to lower working memory performance. Too high levels of D₁ receptor activation is also detrimental, as is seen in stress and after amphetamine administration (Goldman-Rakic et al., 2000).

Recently, the inverted-U model has been shown to work at the level of individual frontal cortex neurons in non-human primates. Thus, neurons that are less tuned to a specific spatial location during the oculomotor delayed-response paradigm (see Chapter 1) show increased spatial tuning after low levels of D₁ receptor agonist administration, whereas a high level of D₁ receptor agonist administration erodes the spatial tuning of the neuron (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007).

Importantly, carriers of the Met/Met variant of the COMT gene are thought to have a near-optimal level of dopamine on the inverted-U function, whereas the Val/Val variant is thought to result in a slightly sub-optimal level of dopamine in the prefrontal cortex (Mattay et al., 2003; Tunbridge et al., 2006). This theory has been supported by an fMRI study by Mattay et al. (2003) investigating the effect of amphetamine, which increases prefrontal dopamine levels, on frontal cortex task performance as a function of the Val¹⁵⁸Met polymorphism. The results indicated that

after amphetamine administration Val/Val individuals improved on frontal cortex tasks in terms of both performance and neural processing efficiency, whereas the performance and neural efficiency of Met/Met individuals deteriorated at high task loads. This genotype effect was specific to the frontal cortex (Mattay et al., 2003). Similarly, Apud et al. (2007) found that tolcapone, a CNS penetrant specific COMT inhibitor, resulted in improved performance in Val homozygotes and worsened performance in Met homozygotes on some measures of frontal cortex function.

Several other studies have also demonstrated a relationship between the COMT Val¹⁵⁸Met polymorphism and performance on tasks associated with the frontal cortex. For example Egan et al. (2001) found that the COMT Val¹⁵⁸Met polymorphism affected performance on the Wisconsin Card Sorting Test. Val homozygotes performed significantly worse than Met homozygotes and heterozygotes (Egan et al., 2001). Furthermore, Met allele load significantly predicted neural efficiency in the frontal cortex during an fMRI task, the N-back task. In this task all genotype groups performed at the same level, but Val homozygotes showed significantly greater activation (indicating lower neural efficiency) in the frontal cortex than heterozygotes, and heterozygotes showed a significantly greater response in the frontal cortex than Met homozygotes. The association between COMT Val¹⁵⁸Met genotype and performance on the WCST in typical populations has been extensively replicated (e.g., Malhotra et al., 2002; Mattay et al., 2003) and confirmed in a recent meta-analysis by Barnett et al. (2007).

Other frontal cortex tasks have also been associated with the Val¹⁵⁸Met polymorphism. For example Stefanis and colleagues have shown that Met allele load, i.e., the number of Met alleles carried by an individual (0, 1 or 2), is associated with decreased response variability in the Continuous Performance Test but not overall

performance level (Stefanis et al., 2005). Goldberg and colleagues found an effect of COMT Val¹⁵⁸Met genotype on performance on the N-back working memory task in a sample of schizophrenic patients, their siblings, and control participants (Goldberg et al., 2003). Finally, Diamond and colleagues replicated the association between the COMT Val¹⁵⁸Met polymorphism and frontal cortex function in 6- to 14-year-old children (Diamond et al., 2004). This study found that children carrying the Met/Met variant performed significantly better than children carrying the Val/Val variant on a task requiring working memory and inhibition (the dots-mixed task), which Diamond and colleagues propose is specifically related to dopamine function in the prefrontal cortex (Diamond, 2001; Diamond et al., 2004). Only performance on this specific task was related to the COMT Val¹⁵⁸Met polymorphism, i.e., tasks related to the functioning of other areas of the brain, as well as a frontal cortex task shown to be unaffected by frontal dopamine level (the self-ordered pointing task), were not related to the COMT Val¹⁵⁸Met polymorphism (Diamond et al., 2004).

The present study

The study reported in this chapter investigated whether performance on the frontal cortex tasks administered at 4 (Cohort 2) and 9 months (Cohorts 1 and 2 combined) were associated with the COMT Val¹⁵⁸Met polymorphism. Based on previous research with adults and children, the expectation was that better performance on the frontal cortex tasks would be associated with Met load, that is, the more copies of the Met allele, the better the infant's performance.

Methods

Collection of buccal swabs

Tubes and labels for the samples were prepared by the experimenter before testing. Rubber gloves were worn during the preparation of the samples. Two tubes for collection of the samples were prepared for each infant. The tubes containing the samples were labelled “A” and “B”. The samples were identified by the infant’s participant number and date of birth.

Buccal (cheek) swabs were collected at 3.5 years of age in Cohort 1 as part of a follow-up study (not reported in this thesis), and at 4 months in Cohort 2. Both the experimenter and the parent wore rubber gloves during collection of the samples to avoid genetic contamination. The buccal swab was collected with an ordinary cotton bud by the parent. The parent was asked to rub the cotton bud on the inside of the infant’s cheeks for approximately 5-10 seconds in each cheek. The cotton bud was then put in the sample tube by the experimenter. Four such swabs were collected, two in each tube (Samples A and B). The samples were placed in a standard freezer immediately after collection and frozen at a temperature of -21°C.

Samples A and B were sent in an insulated container via courier in separate batches to collaborators at Semmelweis University in Budapest who carried out the DNA extraction and genotyping procedures.

DNA extraction and genotyping

DNA was isolated from buccal cells using a DNA-purification kit obtained from Gentra (Minneapolis, US). The COMT Val¹⁵⁸Met polymorphism was genotyped by a single-step allele-specific amplification procedure using two allele-specific and two

outer primers (comt-a: TGG TGG ATT TCG CTG GCA; comt-g: CAC ACC TTG TCC TTC AC; comt-k2: ACA CCC ATA CAA GCA TTC ATC; comt-k1: TGC TCA CCT CTC CTC CGT). The HotStar Taq DNA-polymerase kit (Qiagen, Valencia, US) was used, applying 0.125 U enzyme in a total volume of 10 μ L, in the presence of 1 x buffer and 1 x Q solution supplied, 1 μ M primers, 200 μ M of dATP, dCTP, dTTP, and 100 μ M of dGTP and dITP. The thermocycling was initiated by a 15 min 95°C denaturation step; it was followed by 40 cycles of 94°C for 1 min, 56°C for 30 sec, 72°C for 1 min; and a final extension at 72°C for 10 min.

Results

Allele distribution

Genotype data were available for 19 out of the 24 infants in Cohort 1. Collection of buccal swabs from the remaining 5 participants was not possible due to drop out between the 9-month testing session and swab collection at 3.5 years. Of the remaining participants 7 (36.8%) had the Met/Met genotype, 10 (52.6%) had the Val/Met genotype, and 2 (10.5%) had the Val/Val genotype. In Cohort 2 genotype data were available for 102 out of the 104 infants. Buccal swabs were not collected from two infants who did not have the swab taken at the first visit due to equipment problems, and dropped out before the next visit to the lab. Of the infants in Cohort 2, 25 (24.5%) had the Met/Met genotype, 49 (48%) had the Val/Met genotype, and 28 (27.5%) had the Val/Val genotype. When the two cohorts were pooled, genotype data were available for 121 infants. Of the combined sample 32 (26.4%) had the Met/Met genotype, 59 (48.8%) had the Val/Met genotype, and 30 (24.8%) had the Val/Val genotype. The allele distribution of the combined sample conformed to the Hardy-

Weinberg equilibrium, $\chi^2 = 0.073$, $df = 2$, $p = .964$, indicating that the sample was taken from a homogenous population.

COMT Val¹⁵⁸Met genotype and frontal cortex performance at 4 months

Only data from Cohort 2 were available at 4 months. A univariate ANOVA indicated that there was no effect of COMT Val¹⁵⁸Met genotype on Gap task performance as assessed by the Median SRT in the Overlap condition, $F(2,99) = 0.78$, $p = .459$, $\eta_p^2 = .016$, or as assessed by the Gap effect, $F(2,99) = 0.63$, $p = .533$, $\eta_p^2 = .013$. There was also no effect of genotype on Anticipation task performance in terms of proportion of anticipations (with proportion of valid trials entered as a covariate), $F(2,97) = 0.79$, $p = .457$, $\eta_p^2 = .016$, or in terms of anticipatory learning, $F(2,87) = 1.65$, $p = .199$, $\eta_p^2 = .036$.

Thus, performance on the two frontal cortex tasks administered at 4 months of age was not significantly related to COMT Val¹⁵⁸Met genotype in the present sample.

COMT Val¹⁵⁸Met genotype and frontal cortex performance at 9 months

Data from both cohorts were combined in the analyses of the relationship between COMT Val¹⁵⁸Met genotype and frontal cortex task performance at 9 months. Pooling the data from the A-not-B task was straightforward since the task was exactly the same in the two cohorts. However, a few minor parameters of the Freeze-Frame task differed between the two cohorts (see Chapter 5). Therefore, the repeated-measures ANOVA, demonstrating main effects of Phase and Trial Type but no interaction in the Freeze-Frame task (see Chapters 3 and 5), was repeated with Cohort as a between-subjects variable. This analysis replicated the main effects of Trial Type, $F(1,83) = 64.27$, $p < .001$, $\eta_p^2 = .44$, Phase, $F(2,166) = 61.10$, $p < .001$, $\eta_p^2 = .42$, and the lack of

interaction between Phase and Trial Type, $F(2,166) = 0.20, p = .82, \eta_p^2 = .002$.

Importantly, there was no significant main effect of or interactions involving Cohort (all $ps > .20$). These results were confirmed by a Linear Mixed Model as described in Chapters 3 and 5 (Trial Type: $F(1,113.53) = 96.10, p < .001$; Phase: $F(4,84) = 49.18, p < .001$; Phase $\times$ Trial Type: $F(4,75.37) = 1.03, p = .398$). This analysis, which included infants with incomplete Freeze-Frame data, also did not indicate a significant main effect of Cohort or any significant interactions involving Cohort (all $ps > .20$). Given the lack of significant performance differences between the two cohorts, it was deemed appropriate to combine the Freeze-Frame data for the genotype analyses.

A univariate ANOVA indicated that there was no effect of COMT Val¹⁵⁸Met genotype on A-not-B performance, $F(2,101) = 1.06, p = .351, \eta_p^2 = .021$. There was also no effect of COMT Val¹⁵⁸Met genotype on performance on the Freeze-Frame General Inhibitory Learning index, $F(2,97) = 0.34, p = .713, \eta_p^2 = .007$, the Freeze-Frame Selective Inhibition index, $F(2,98) = 0.05, p = .949, \eta_p^2 = .001$, or the Freeze-Frame Selective Inhibitory Learning Index, $F(2,83) = 1.35, p = .266, \eta_p^2 = .031$. However, COMT Val¹⁵⁸Met genotype was significantly associated with performance on the Freeze-Frame General Distractibility index for interesting trials, $F(2,100) = 3.38, p = .038, \eta_p^2 = .063$. Descriptive statistics for this index for each genotype group are presented in Table 7.1, and scatter and box plots showing the distribution of scores by genotype are presented in Figure 7.2.

Post-hoc tests using a Bonferroni correction for multiple comparisons indicated that infants with the Met/Met genotype scored significantly lower on the Freeze-Frame General Distractibility index for interesting trials (i.e., they looked less to the distractors on the interesting trials overall) than infants with the Val/Val genotype (p

= .035). Infants with the Val/Met genotype did not differ significantly from the two other groups (both $ps > .20$), although the mean score for this group was in between that of the two homozygous groups (Table 7.1). The Freeze-Frame General Distractibility index across both trial types was also marginally associated with COMT Val¹⁵⁸Met genotype, $F(2,100) = 2.90, p = .060, \eta_p^2 = .055$, again indicating a difference between the Met/Met and Val/Val genotypes ($p = .054$). However, the effect seemed to be relatively specific to variability in performance on the interesting trials since no significant association between COMT Val¹⁵⁸Met genotype and performance on the Freeze-Frame General Distractibility index for boring trials was found, $F(2,100) = .473, p = .625, \eta_p^2 = .009$.

Table 7.1. Descriptive statistics for the Freeze-Frame General Distractibility index for interesting trials (proportion of looks to the distractors in the interesting trials) by COMT Val¹⁵⁸Met genotype.

	Mean	<i>SD</i>	<i>n</i>
Met/Met	0.17	0.11	29
Val/Met	0.22	0.15	47
Val/Val	0.26	0.15	27
Total	0.22	0.14	103

Figure 7.2. (a) Scatter plot of the relationship between COMT Val¹⁵⁸Met genotype and the Freeze-Frame General Distractibility index for interesting trials. (b) Box plot showing the distribution of scores on the Freeze-Frame General Distractibility index for interesting trials in the three COMT Val¹⁵⁸Met genotype groups.

This pattern of results suggests that the COMT Val¹⁵⁸Met polymorphism modulates infants' looking behaviour in the interesting trials of the Freeze-Frame task. However, these differences were not observed for the Freeze-Frame Selective Inhibition index, suggesting that the effect is not due to early differential distractibility in the two trial types (in contrast to the relationship between the Freeze-Frame task and the A-not-B task reported in previous chapters). To further clarify the relationship between COMT Val¹⁵⁸Met genotype and the two Freeze-Frame trial types during the test session, the proportion of looks to the distractors in each phase was plotted as a function of genotype in two separate plots, one for each trial type. These plots are presented in Figure 7.3, and the equivalent descriptive statistics are presented in Table 7.2.

Figure 7.3. Mean proportion of looks to the distractors for each genotype as a function of phase and trial type in the Freeze-Frame task.

Table 7.2. Descriptive statistics for the proportion of looks to the distractors as a function of Freeze-Frame phase and trial type in each of the three COMT Val¹⁵⁸Met genotype groups.

	Genotype	<i>n</i>	Mean	<i>SD</i>
Boring - Phase 1	Met/Met	28	0.65	0.24
	Val/Met	47	0.70	0.21
	Val/Val	27	0.73	0.21
	Total	102	0.69	0.22
Boring - Phase 2	Met/Met	26	0.42	0.28
	Val/Met	47	0.39	0.30
	Val/Val	25	0.49	0.26
	Total	98	0.42	0.28
Boring - Phase 3	Met/Met	24	0.45	0.27
	Val/Met	39	0.38	0.23
	Val/Val	24	0.41	0.24
	Total	87	0.40	0.24
Boring - Phase 4	Met/Met	18	0.40	0.26
	Val/Met	33	0.39	0.25
	Val/Val	19	0.37	0.25
	Total	70	0.39	0.25
Interesting - Phase 1	Met/Met	28	0.39	0.23
	Val/Met	46	0.46	0.26
	Val/Val	27	0.48	0.26
	Total	101	0.45	0.25
Interesting - Phase 2	Met/Met	25	0.14	0.14
	Val/Met	47	0.21	0.19
	Val/Val	25	0.22	0.21
	Total	97	0.19	0.19
Interesting - Phase 3	Met/Met	25	0.09	0.12
	Val/Met	46	0.13	0.15
	Val/Val	25	0.18	0.18
	Total	96	0.13	0.16
Interesting - Phase 4	Met/Met	19	0.08	0.15
	Val/Met	37	0.09	0.14
	Val/Val	20	0.18	0.17
	Total	76	0.11	0.15
Overall – Boring	Met/Met	29	0.46	0.18
	Val/Met	47	0.47	0.17
	Val/Val	27	0.50	0.16
	Total	103	0.48	0.17
Overall – Interesting	Met/Met	29	0.17	0.11
	Val/Met	47	0.22	0.15
	Val/Val	27	0.26*	0.15
	Total	103	0.22	0.14
Overall	Met/Met	29	0.30	0.11
	Val/Met	47	0.34	0.13
	Val/Val	27	0.38	0.11
	Total	103	0.34	0.12

As can be seen from Figure 7.3 and Table 7.2, the relationship between COMT Val¹⁵⁸Met genotype and Freeze-Frame performance was fairly systematic in the interesting trials. Infants carrying the Met/Met genotype looked less to the distractors than the Val/Val group throughout the test session. All three genotype groups showed a sharp decrease between Phases 1 and 2. Between Phases 2 and 4 the Met/Met group and the Val/Val group showed a further slight decrease (maintaining an 8-10% difference in looks to the distractors), whereas the Val/Met group showed a slightly sharper decrease resulting in a mean proportion of looks to the distractors similar to the Met/Met group in Phase 4. It is notable that the Val/Met group starts out at the same point as the Val/Val group, but looks more similar to the Met/Met group at the end of the test session.

In the boring trials no systematic linear difference between genotype groups was discernible. The Met/Met group did not consistently look less to the distractors than the Val/Val group. In fact, the Met/Met group looked slightly more to the distractors in the boring trials than the two other groups towards the end of the test session. The Val/Met group seemed to plateau after Phase 2, whereas the Val/Val group showed a decrease throughout the session.

No pairwise comparisons between genotype groups involving individual phases of the Freeze-Frame task survived Bonferroni correction (see Table 7.2 for means within each phase and trial type). Only the overall proportion of looks to the distractors in the interesting trials (equivalent to the Freeze-Frame General Distractibility index for interesting trials) survived correction for multiple comparisons. Thus, this detailed analysis confirmed that the COMT Val¹⁵⁸Met genotype effect was specific to the overall rate of looking in the interesting trials, whereas no significant relationship was found between COMT Val¹⁵⁸Met genotype

and the proportion of looks to the distractors in the boring trials. The association between the COMT Val¹⁵⁸Met polymorphism and performance in the interesting trials was not found to be specific to any one phase.

Discussion

This chapter investigated whether there is an association between the COMT Val¹⁵⁸Met polymorphism and performance on a set of infant frontal cortex tasks. The COMT gene codes for the COMT enzyme which plays an important role in breaking down dopamine in the prefrontal cortex (Chen et al., 2004; Karoum et al., 1994; Sesack et al., 1998; Tunbridge et al., 2006). Because the presence of the Met allele reduces COMT's enzymatic efficiency, Met load is likely to be associated with a higher level of dopamine in the prefrontal cortex (Chen et al., 2004; Lotta et al., 1995; Männistö & Kaakkola, 1999; Tunbridge et al., 2006; Tunbridge et al., 2007).

The relationship between dopamine and performance on frontal cortex tasks does not appear to be linear. Instead, the evidence suggests that the optimal level of dopamine in the frontal cortex conforms to an inverted U-shape, with both too high and too low levels of dopamine leading to performance decrements (Goldman-Rakic et al., 2000; Mattay et al., 2003; Vijayraghavan et al., 2007). Evidence using well-established frontal cortex tasks in adults suggests that carriers of two Met alleles (the Met/Met genotype) have optimal performance. Carriers of two Val alleles (the Val/Val genotype) tend to perform significantly worse than Met/Met carriers on such tasks (Barnett et al., 2007; Egan et al., 2001; Goldberg et al., 2003; Malhotra et al., 2002; Mattay et al., 2003). One study has found an effect of the COMT Val¹⁵⁸Met polymorphism on frontal cortex task performance in children (Diamond et al., 2004).

Given these findings, the current study investigated whether performance on infant frontal cortex tasks was associated with COMT Val¹⁵⁸Met genotype. Such associations would strengthen the evidence that these tasks are related to the functioning of the frontal cortex. Based on previous research (e.g., Egan et al., 2001; Mattay et al., 2003), it was expected that infants with the Met/Met genotype would perform significantly better than infants with the Val/Val genotype, and that the Val/Met genotype would demonstrate intermediate performance levels.

Association between the COMT Val¹⁵⁸Met polymorphism and the Freeze-Frame task

Despite these predictions, only one of the infant frontal cortex tasks used in the current study showed a significant association with genotype. The COMT Val¹⁵⁸Met polymorphism was significantly associated with performance on the Freeze-Frame General Distractibility index for interesting trials. Only the difference in score between the Val/Val and Met/Met groups reached statistical significance, though as expected the Val/Met group had an intermediate mean score. This finding indicates that infants with the Met/Met genotype looked significantly less to the distractors in the interesting trials than infants with the Val/Val genotype. No such relationship was found between the COMT Val¹⁵⁸Met polymorphism and distractibility in the boring trials, indicating that the effect was specific to trials where the central stimulus was engaging. Furthermore, a detailed analysis of the Freeze-Frame data confirmed that the difference between the Met/Met and Val/Val groups was present in the interesting trials throughout the test session (see Figure 7.3 and Table 7.2).

The COMT Val¹⁵⁸Met polymorphism and other infant frontal cortex tasks

The general lack of association between performance on the infant frontal cortex tasks and COMT Val¹⁵⁸Met genotype is not entirely surprising. The effect of a single

gene is likely to be small even when the biological mechanism underlying the relationship is relatively well understood (Hutchison et al., 2004; Munafò, 2006). In addition to this issue, infant performance measures are generally noisier than adult performance measures, decreasing power further. These may be some of the reasons why the two frontal cortex tasks administered at 4 months were found to be unrelated to COMT Val¹⁵⁸Met genotype.

The lack of an association between COMT Val¹⁵⁸Met genotype and A-not-B performance is perhaps more surprising given the extensive evidence for frontal cortex involvement in this task (Baird et al., 2002; Bell, 2001; Bell & Fox, 1992, 1997; Diamond, 2002; Diamond & Goldman-Rakic, 1986, 1989; Freedman & Oscar-Berman, 1986; Verin et al., 1993). It is possible that the sample was simply too small to detect a subtle effect of the COMT Val¹⁵⁸Met genotype. Another limitation of the A-not-B task is the limited range of scores, which in the current study was 0 to 6 s. Given that about 40% of infants could only succeed at the 0-s delay, there may simply have been too little variability in the measure to detect a small genetic effect (for further discussion of the effect of limited range, see Chapters 2 and 5).

On the basis of these results, it is tempting to conclude that any effect of the COMT Val¹⁵⁸Met polymorphism is too small to affect performance on frontal cortex tasks in infancy in any significant way. Tunbridge and colleagues (2007) found that COMT expression in the prefrontal cortex in infancy was about half the amount of that found in adulthood, a finding that would seem to support the latter conclusion. However, the fact that the current study did find an association between COMT Val¹⁵⁸Met genotype and performance on the other frontal cortex task administered at 9 months, the Freeze-Frame task, contradicts this interpretation.

Genotype association with the overall level of distractibility in the interesting Freeze-Frame trials

The finding of an association between the COMT Val¹⁵⁸Met polymorphism and the Freeze-Frame General Distractibility index for interesting trials suggests that at least some variability in performance on an infant frontal cortex tasks can be explained by genetic variation impacting on dopamine modulation in the frontal cortex. This is encouraging in terms of demonstrating a more direct link between the biology of the frontal cortex and performance on tasks developed to assess frontal cortex functioning in infancy. However, the fact that it was the *overall* proportion of looks to the distractors in the interesting trials which was associated with the COMT Val¹⁵⁸Met genotype needs to be further considered.

Based on the findings from Chapters 3 and 5, as well as general evidence demonstrating the importance of the frontal cortex for inhibitory functioning (e.g., Diamond, 2001), it would seem more likely that COMT Val¹⁵⁸Met genotype would predict performance on the specific inhibitory indices from the Freeze-Frame task, i.e. the Selective Inhibitory Learning index and the Selective Inhibition index. The former index was found to be positively associated with performance on a frontal cortex task at 2 years of age (Chapter 3), and the latter index was found to be consistently associated with performance on the A-not-B task (Chapters 3 and 5). Given that performance on the A-not-B task was not associated with COMT Val¹⁵⁸Met genotype, it is perhaps not so surprising that the Freeze-Frame Selective Inhibition index showed no sign of a relationship with genotype. The Freeze-Frame Selective Inhibitory Learning index was also not significantly associated with genotype, though this measure was available for fewer infants (approximately 20 infants in the combined sample did not have enough data from Phase 3 and therefore could not

obtain a score on this index), resulting in less power in the statistical analysis. The Freeze-Frame General Distractibility index for interesting trials, however, was available for all infants, and the fact that the measure was based on a larger number of trials (i.e., all interesting trials as compared to only trials from specific phases of the task) may have enabled the detection of the small effect of the COMT Val¹⁵⁸Met polymorphism.

Because COMT Val¹⁵⁸Met genotype was not related to the selective inhibitory indices of Freeze-Frame performance, it could be argued that the effect was due to differences in the infants' general distractibility level. However, the fact that COMT Val¹⁵⁸Met genotype was unrelated to the equivalent measure for the boring Freeze-Frame trials indicates at least some specificity of the finding. In essence, the results suggest that COMT Val¹⁵⁸Met genotype did not play an important role in infants' distractibility level when they observed an uninteresting central stimulus, whereas engagement by an interesting central stimulus resulted in the Met/Met group being significantly less distracted by the peripheral distractors than infants in the Val/Val group.

The follow-up analyses presented in Figure 7.3 and Table 7.2 confirmed that this effect was present throughout the experiment. In the interesting Freeze-Frame trials the Met/Met group looked consistently less to the distractors than the Val/Val group in every phase of the experiment, with the Val/Met group moving from an average similar to the Val/Val group at the beginning of the test session to an average similar to the Met/Met group at the end of the test session (see Figure 7.3). This systematic picture was not present in the boring trials. It is important to point out that no single phase in the interesting trials produced a significant difference between genotype groups after correcting for multiple comparisons. However, the seemingly differential

patterns of decrease in looks to the distractors in the three genotype groups deserves further investigation, especially given the fact that less data were available for Phase 4 (Cohort 1 only completed 3 phases).

Genotype effects due to changes at the end of the Freeze-Frame session?

Given the discussion above, it is worth noting that performance on the A-not-B task was found to be negatively related to the Freeze-Frame General Distractibility index for interesting trials in Chapter 5. Because A-not-B performance was found to be unrelated to COMT Val¹⁵⁸Met genotype, this hints at the possibility that the variance shared by the Freeze-Frame General Distractibility index for interesting trials and COMT Val¹⁵⁸Met genotype is not the same the variance as that shared by the Freeze-Frame General Distractibility index for interesting trials and A-not-B performance. Given that the Freeze-Frame Selective Inhibition index, which is based on the first 16 trials of the task, was also found to be correlated with A-not-B performance, but unrelated to COMT Val¹⁵⁸Met genotype, it may very well be the case that it is changes in looks to the distractors in the interesting trials later in the test session that differs between genotypes. However, as mentioned above, the current study was not able to narrow the relationship down that precisely, most likely due to the subtlety of the genotype effect and the lower *n* in the later phases of the Freeze-Frame task.

The candidate gene approach in relation to the present study

In the introduction to this chapter, three important considerations for the candidate gene approach in behavioural research were outlined. The first of these considerations was that the investigated polymorphism should have a well-established biological mechanism that is likely to mediate the relationship between the genotype

and the behavioural measure of interest (Diamond et al., 2004; Hutchison et al., 2004). The current study investigated the effect of a polymorphism which has been consistently shown to have an impact on the dopamine-catabolising enzyme COMT, which plays a particularly important role in the frontal cortex (Chen et al., 2004; Lotta et al., 1995; Männistö & Kaakkola, 1999; Tunbridge et al., 2006; Tunbridge et al., 2007). In addition, the polymorphism has already been linked to performance on frontal cortex tasks in adults (Barnett et al., 2007; Egan et al., 2001; Goldberg et al., 2003; Malhotra et al., 2002; Mattay et al., 2003) and children (Diamond et al., 2004). Therefore, the current study meets the first consideration well.

The second consideration involves the need for replication of any candidate gene study and the establishment of the generality of an effect across different populations (Munafò, 2006). Since the current study, to the author's knowledge, is the first to investigate the effect of the COMT Val¹⁵⁸Met polymorphism on frontal cortex task performance in infancy, the single association found certainly needs replication. In addition, the association was found in a group of infants from a homogenous (see Methods), predominantly white sample, and cannot necessarily be generalised to other populations. Furthermore, as discussed earlier, replication in a larger sample may shed light on potential differential looking patterns across phases in the Freeze-Frame task resulting from variation in the COMT Val¹⁵⁸Met genotype. In this context, the Freeze-Frame task has the advantage that it is relatively quick to administer and analyse, and provides fine-grained individual performance indices.

Finally, the effect of a single polymorphism tends to be small (Munafò, 2006). Therefore, a large sample size is needed to detect an effect. The sample size of the current study was relatively large for an infant study ($n \approx 80$ -100 for most genotype analyses), but given the issues discussed above relating to noise in infant data as well

as potentially less COMT activity in the infant frontal cortex (Tunbridge et al., 2007), an even larger sample size may be needed to detect a COMT Val¹⁵⁸Met genotype effect, especially in younger infants. Nevertheless, the fact that an effect was found on Freeze-Frame performance at 9 months of age suggests that the candidate gene approach is viable in infant research.

Future directions

Even though the COMT Val¹⁵⁸Met polymorphism is currently the most thoroughly investigated polymorphism in relation to frontal cortex function, behavioural genetics is a rapidly developing field and other polymorphisms and genetic variations are of interest in relation to infant (and adult) frontal cortex functioning. In particular, polymorphisms affecting receptor levels and efficiency would be worth investigating in relation to the tasks presented in this thesis. For example, the dopamine D⁴ receptor appears to be more abundant in the prefrontal cortex (Oak, Oldenhof, & Van Tol, 2000), and the gene coding for this receptor contains a highly variable polymorphism in its coding region (the 48-bp VNTR in exon 3 of the DRD4 gene). This polymorphism has already been associated with selective attention and inhibitory function in older children (Cornish et al., 2005) and has also been associated with Attention Deficit Hyperactivity Disorder (Faraone et al., 2005; Li, Sham, Owen, & He, 2006).

Ultimately, more direct measures than genotype data are desirable in order to fully establish the impact of neurotransmitter systems on frontal cortex function. However, at present COMT expression in the brain can only be measured post mortem (e.g., Chen et al., 2004; Tunbridge et al., 2007). Receptor density in specific areas of the brain can be established using positron emission tomography (PET),

provided that appropriate radio-ligands are available (e.g., the dopamine D² receptor: Jönsson et al., 1999; Pohjalainen et al., 1998). Unfortunately, PET involves injection of radioactive compounds making this kind of research unsuitable for infants. Hopefully, future developments in these methods will allow more direct measurements of neurotransmitter levels in the infant frontal cortex.

Conclusions

In conclusion, though the COMT Val¹⁵⁸Met polymorphism showed little association with the infant frontal cortex tasks overall, the current study found evidence that distractibility when the infant was engaged, as assessed by the Freeze-Frame task, was related to COMT Val¹⁵⁸Met genotype. Infants with the Met/Met genotype were the least distractible and infants with the Val/Val genotype were the most distractible in this condition. Though this result is promising, the relatively small size of the effect ($r^2 = .062$) emphasises the need for a large sample as well as sensitive measures based on many trials to detect a genotype effect. In the future, it would be interesting to investigate potential interaction effects between COMT Val¹⁵⁸Met genotype and performance patterns during the course of the Freeze-Frame session, effects that the current study did not have sufficient power to detect. It would also be interesting to investigate the effect of other relevant candidate gene polymorphisms.

Summary of Chapter 7

- This chapter investigated the relationship between the frontal cortex tasks administered at 4 and 9 months (across cohorts) and a genetic variation which has been strongly implicated in frontal cortex function via its effect on dopaminergic neurotransmission, the COMT Val¹⁵⁸Met polymorphism.
- Previous studies have found that individuals with the Met/Met genotype perform better on frontal cortex tasks than individuals with the Val/Val genotype.
- Most of the frontal cortex tasks used in the current studies were not associated with COMT Val¹⁵⁸Met genotype, possibly due to the genotype effect being too small to detect in an infant sample.
- However, performance on the Freeze-Frame task did show a significant association with COMT Val¹⁵⁸Met genotype. Infants with the Met/Met genotype looked significantly less to the distractors in the interesting trials compared to infants with the Val/Val genotype.
- No genotype effects on looking patterns across the Freeze-Frame session reached significance. However, the effect of genotype was specific to the interesting trials since no such effect was found in the boring trials.
- These results indicate that the candidate gene approach constitutes a valuable way to address the question of frontal cortex functioning in infancy in future research.

CHAPTER 8

General Discussion

Introduction

The main aim of this thesis was to investigate frontal cortex functioning in infancy. This question was addressed using an individual differences approach and a longitudinal design. This approach has clear advantages because it allows the assessment of potential common mechanisms and stability in frontal cortex functioning as well as an investigation of component processes not easily revealed by group averages. Two cohorts of infants were tested on a set of tasks that have been related to frontal cortex functioning based on empirical evidence and/or theoretical argument. Associations between these tasks were established in an attempt to provide converging evidence for frontal cortex functioning in infancy.

Only a small number of potential frontal cortex tasks are available for infants, and even fewer have been reliably associated with the frontal cortex. Therefore, a second aim of the studies reported in this thesis was the development of a new task to assess frontal cortex functioning in infancy. The new task, termed the Freeze-Frame task, used eye movements as the dependent measure in order to obtain sensitive indices of individual performance differences at an early age.

A third aim of the work reported here was the investigation of the relationship between infants' performance on frontal cortex tasks and measures of temperament and general development. Temperament has been hypothesised to be associated with frontal cortex function. For example Posner and Rothbart (1998) have proposed that a high level of self-regulatory abilities in infancy are associated with the development

of frontally-mediated skills. However, recent evidence has contradicted the direction of this hypothesis. For example Bell and colleagues have found that infants who are less able to regulate distress tend to perform better on frontal cortex tasks (e.g., Bell, 2005; Bell et al., 2006).

The question of whether the frontal cortex is associated with general cognitive ability is controversial even in adults. Often patients with damage to the frontal cortex are not impaired on standard IQ tests (Duncan et al., 1995; Shallice & Burgess, 1991; Warrington et al., 1986). However, there is evidence to suggest that in typical adult populations performance on frontal cortex tasks is associated with general intelligence (Obonsawin et al., 2002). There is also evidence that frontal cortex functioning in early childhood is associated with other cognitive skills, such as language skills (Bell et al., 2007). It was therefore relevant for the current study to investigate how independent infants' performance on the frontal cortex tasks was of general cognitive development. Furthermore, collection of general developmental data enabled analyses of the association between frontal cortex tasks whilst controlling for one of the most likely confounds (i.e., general cognitive ability).

A final aim of the work presented here was an attempt to further validate the chosen frontal cortex tasks by investigating their relationship with a genetic polymorphism which has been strongly related to the neurobiology and function of the frontal cortex, the COMT Val¹⁵⁸Met polymorphism (Barnett et al., 2007; Chen et al., 2004; Diamond et al., 2004; Egan et al., 2001; Goldberg et al., 2003; Karoum et al., 1994; Lachman et al., 1996; Malhotra et al., 2002; Mattay et al., 2003; Sesack et al., 1998; Tunbridge et al., 2006; Tunbridge et al., 2007). Though the effect of a single gene is likely to be small, an association between the COMT gene and performance

on infant frontal cortex tasks would serve as further converging evidence for the proposed neural substrate of these tasks.

The following section will summarise the overall findings of the studies reported in this thesis. This section is followed by a discussion of the implications of these findings, limitations of the studies, and future directions.

Summary of results

The results reported in Chapter 3 confirmed that the new Freeze-Frame task was associated with performance on more established frontal cortex tasks both at 9 and 24 months of age. However, the findings were complex with some indices of Freeze-Frame task performance (e.g., the Selective Inhibitory Learning index) being associated with better performance on other frontal cortex tasks, and other indices (e.g., the Selective Inhibition index) being associated with poorer performance on some of the frontal cortex tasks. There was a positive correlation between the Freeze-Frame Selective Inhibition index and A-not-B performance, indicating that infants who initially were less distractible in the interesting trials compared to the boring trials tended to sustain longer delays in the A-not-B task.

Chapter 4 reported on the first results from a large-scale longitudinal study of the relationship between several frontal cortex tasks during the first year of life (Cohort 2). At the 4-month visit infants completed two tasks, the Gap task and the Anticipation task, that have been associated with the frontal cortex in infant ERP work (Csibra et al., 1998, 2001). This part of the study investigated whether performance on the two tasks was correlated and whether task performance was associated with differences in infant temperament. The results showed a weak association ($r \approx .2$) between Gap and Anticipation task performance. Even though the

association only accounts for a small proportion of the variance, this finding indicates at least some common mechanism in the two tasks. In terms of temperament, Gap task performance was related to fear, but this effect was non-specific, essentially indicating that infants with slower saccadic reaction times across conditions tended to be more fearful. Performance on the Anticipation task was associated with a higher Orienting/Regulation score, less distress, and a more soothable temperament, suggesting that good frontal cortex task performance at 4 months is related to better self-regulatory abilities.

Chapter 5 reported on the 9-month follow-up of Cohort 2. An important aim of this study was to replicate the correlation between the Freeze-Frame Selective Inhibition index and A-not-B performance found in Cohort 1 (Chapter 3). This association was successfully replicated, and can therefore be considered fairly robust. It was also found that A-not-B performance was negatively associated with the Freeze-Frame General Distractibility index for interesting trials, an association which was present, but not significant, in Cohort 1. Concurrent measures of temperament were generally not associated with performance on the frontal cortex tasks at 9 months of age. General motor development was also not associated with the frontal cortex tasks administered at 9 months of age. However, A-not-B performance was found to be associated with MSEL-AGS cognitive scores, in particular expressive language skills, suggesting that performance on this task is not entirely independent of cognitive ability. Performance on the Freeze-Frame task was relatively independent of general cognitive ability. Importantly, the moderate relationship between the Freeze-Frame Selective Inhibition index and A-not-B performance remained highly significant even after general cognitive ability was partialled out of the correlation.

The same was the case for the relationship between the Freeze-Frame General Distractibility index for interesting trials and A-not-B performance.

Chapter 6 explored the longitudinal correlations between the 4- and 9-month frontal cortex tasks, temperament, and general cognitive development in Cohort 2. The cross-age relationships between the frontal cortex tasks turned out to be fairly weak. There was a weak, marginally significant association between the Freeze-Frame Selective Inhibition index at 9 months and Anticipation task performance at 4 months, but caution is needed in interpreting this result since this was the only cross-age correlation found between frontal cortex tasks which approached significance. Overall, the results indicated that the within-age correlations between frontal cortex tasks were considerably stronger than the cross-age correlations. However, temperament measures did show some longitudinal association with performance on the frontal cortex tasks. Anticipation task performance at 4 months was associated with the early attainment of regulatory functioning and less negative affect at 9 months of age (though the latter association was marginal). This is consistent with the theory proposed by Posner & Rothbart (1998). However, infants who were more *surgent* and who showed more negative affect at 4 months tended to perform better on the A-not-B task at 9 months. This finding indicates that temperament differences in early infancy may be associated with better performance on frontal cortex tasks later in infancy. Note that concurrent temperament was not significantly associated with A-not-B performance at 9 months (Figure 6.1).

Chapter 7 investigated the relationship between performance on the frontal cortex tasks and variation in a gene which has been related to frontal cortex function, the COMT gene (e.g., Egan et al., 2001; Tunbridge et al., 2006). This study was conducted across both cohorts. The only significant finding was an association

between COMT Val¹⁵⁸Met genotype and the Freeze-Frame General Distractibility index for interesting trials, indicating that infants with more dopamine available in the frontal cortex were less distractible when they were engaged with an interesting visual stimulus.

Implications of results

Cross-sectional and longitudinal stability of frontal cortex task performance

Within-age correlations. The results of the studies presented were relatively promising in terms of within-age associations between frontal cortex tasks. At 4 months of age Gap task performance was related to Anticipation task performance. This finding indicates that infants who could more easily disengage their attention when presented with a competing external stimulus could also more easily produce endogenous (anticipatory) saccades when no stimuli were available to drive the saccade. Both tasks have been related to the frontal cortex in infant ERP studies (Csibra et al., 1998, 2001), so the finding of a correlation between the two tasks is promising in terms of establishing that these tasks have a frontal component related to the control of eye movements.

The 9-month frontal cortex tasks demonstrated a somewhat stronger association than the 4-month frontal cortex tasks. The relationship between the Freeze-Frame task and the A-not-B task was replicated in two independent studies (Chapter 3 and Chapter 5), and the association between the two tasks was the strongest observed across the frontal cortex tasks used in Cohort 2 ($r \approx .35$). This association was also the association which was most robust to the D/AP correction for multiplicity.

A measure that was consistently associated with A-not-B performance was the Freeze-Frame Selective Inhibition index. This index is a measure of the differential

distractibility in the two Freeze-Frame trial types in the first phase of the experiment, i.e., before any substantial learning has taken place. The interpretation of this index is complex because results reported in Chapter 3 indicated that the index may be associated with worse performance on frontal cortex measures at 24 months. Nevertheless, the association between the A-not-B task and the Freeze-Frame Selective Inhibition index is important because it indicates that the two tasks share a common functional mechanism.

The exact nature of the mechanism underlying the relationship between the Freeze-Frame Selective Inhibition index and A-not-B performance, as well as the relationship between General Distractibility in the interesting trials and A-not-B performance in Cohort 2, undoubtedly needs further investigation. However, working memory is an unlikely explanation of the association given the fact that the Freeze-Frame task has minimal working memory involvement. Learning is also a less likely mechanism, since differences *early* in the Freeze-Frame task (i.e., Phase 1) were correlated with A-not-B performance. It seems more plausible that attentional factors were at play. One interpretation of the result is that infants who were simply strongly engaged by, and therefore narrowly focused on, the interesting trials in the Freeze-Frame task were better able to maintain attentional focus on the relevant hiding location in the A-not-B task. Perhaps these infants performed better on the A-not-B tasks because they, despite the experimenter's distraction, were able to remain focused on the attractive toy in the well. Conversely, infants who were easily distracted in the interesting trials of the Freeze-Frame task may have lost focus on the toy hidden in the well during the delay⁹.

⁹ Anecdotally, there were clear differences in how eager infants were to obtain the hidden toy in the A-not-B task. Some infants sat quietly and observed the experimenter's distraction during the delay,

The relationship between the Freeze-Frame Selective Inhibition index and A-not-B performance emphasises the power of an individual differences approach. To a certain extent the finding of a correlation between the two tasks validates the Freeze-Frame task as a frontal cortex task because the A-not-B task has been extensively related to the functioning of the frontal cortex (Baird et al., 2002; Bell, 2001; Bell & Fox, 1992, 1997; Diamond, 2002; Diamond & Goldman-Rakic, 1989; Diamond et al., 1997). The validation is only partial because direct evidence using techniques such as neuroimaging is needed in order to definitively establish the involvement of the frontal cortex in Freeze-Frame performance.

However, the association also provides insight into aspects of A-not-B performance that may not have been considered in previous studies. For example, Diamond has suggested that working memory and inhibitory control are the key abilities underlying successful A-not-B performance (Diamond, 1985, 1991; Diamond et al., 1997). This proposal has been controversial with several theorists proposing alternative mechanisms underlying task performance (Clearfield et al., 2006; Munakata, 1998; Thelen et al., 2001). Mechanisms related to visual attention have not often been the focus of such debates. However, the finding of a robust association between differences early in the Freeze-Frame task and A-not-B performance points to the possibility that attentional mechanisms play a larger role in individual performance differences in the A-not-B task than previously assumed. How such attentional mechanisms relate to the frontal cortex needs to be further investigated.

whereas other infants struggled hard to get free from the parent in order to obtain the toy. However, such behaviours are difficult to quantify, and therefore the relationship between eagerness to obtain the toy and task performance was not assessed. Nevertheless, this would be an interesting question for future research.

Cross-age correlations. Whereas the within-age correlations between frontal cortex tasks indicated at least moderate associations, significant cross-age correlations between 4 and 9 months of age were rare (Chapter 6). There was a weak relationship between the proportion of anticipatory saccades in the Anticipation task at 4 months and the Freeze-Frame Selective Inhibition index at 9 months, possibly indicating that more anticipation early in infancy is associated with differences in distractibility at 9 months. However, this association needs to be replicated before any firm conclusions can be drawn given the weakness of the association and the lack of any other associations between the 4- and 9-month frontal cortex tasks.

The general lack of longitudinal stability during the first year of life may seem disappointing. The main conclusion which can be drawn from this finding is that, whereas different infant frontal cortex tasks are related to each other within the same age, individual differences in these functions are often not stable across ages. However, the smaller study reported in Chapter 3 did find several interesting longitudinal associations between frontal cortex tasks administered at 9 and 24 months of age.

It is possible that 4 months is simply too early to assess *stable* individual differences in frontal cortex functioning. Data from younger infants tend to be noisier than data from older infants. For example, in their longitudinal study of the Anticipation task, Canfield et al. (1997) found that variability in reaction time decreased over time and that individual differences in mean reaction time were only stable between 6 and 12 months of age. Such variability may obscure individual performance differences to a certain extent. In the current study, the relationship between tasks tended to be weaker at 4 months than later in development, perhaps indicating that performance was less reliable.

It is also possible that some important development of the frontal cortex takes place between 4 and 9 months of age, and that this development somehow consolidates individual differences in frontal cortex skills. This could explain why several significant associations between 9 and 24 months of age were found in Cohort 1 (Chapter 3), but not between 4 and 9 months in Cohort 2 (Chapter 6). Such developmental discontinuity has been suggested by Canfield et al. (1995) who found that anticipation level in the Anticipation task showed a lack of stability between 4 and 6 months of age. Similarly, Diamond (1991) has argued that developmental changes in the dorsolateral prefrontal cortex during the second half of the first year of life are essential in enabling infants to perform frontal cortex tasks, a conclusion which fits the neurophysiological data relatively well (e.g., Chugani et al., 1987; Franceschini et al., 2007; see Chapter 1).

A final possibility is that the 4- and 9-month tasks tap into different aspects of frontal cortex functioning. As pointed out in Chapter 1, this thesis uses the general term “frontal functions” because very little is known about the differentiation of function within the frontal cortex in infancy. It is certainly true that the 4-month frontal cortex tasks chosen for the presented studies are simpler than the 9-month frontal cortex tasks. Whereas the outcome measures of the 4-month tasks constitute relatively straightforward saccadic reaction time measures to index saccadic control, the later measures involve much more complex operations such as learning to inhibit an automatic response. Even though saccadic reaction time measures may be related to frontal cortex functioning in early infancy (see Chapter 4 for further discussion), the increased complexity of frontal cortex functioning later in development may not be directly related to these simpler processes.

Validation of the Freeze-Frame task

Another important aim of this thesis was the development of a new task to assess inhibitory functioning in infancy, a function that has been extensively related to the frontal cortex. In the Freeze-Frame task infants needed to inhibit looks to peripheral distractors in order to keep a central cartoon animated. The attractiveness of the central cartoon was manipulated on every trial in order to investigate the selectivity of infants' inhibitory responses. Thus, performance on the new task was based purely on saccadic eye movements, enabling the collection of sensitive individual performance data at an early age.

A few previous studies have demonstrated the applicability of using eye movement data in the study of infant frontal cortex functioning. Hofstadter and Reznick (1996) and Bell and Adams (1999) have validated looking versions of the A-not-B task, demonstrating the feasibility of such measures in infant frontal cortex research. Furthermore, Johnson (1995) found that infants as young as 4 months of age possess at least some ability to inhibit automatic saccades. Unfortunately, the latter study had to exclude a large proportion of infants because they failed on a pretest of cue sensitivity, making the task unsuitable for the study of individual differences.

The development of the Freeze-Frame task was an attempt to provide a new task sensitive to frontal cortex functioning, using eye movements as the dependent measure. The distractor duration was calibrated to each infant and a considerable number of trials were administered within a short period of time in order to obtain sensitive individual performance measures.

Group findings from the Freeze-Frame task indicated that 9-month-old infants stopped looking to the distractors during the course of the test session. They also

showed some selectivity right from the start of the test session in that they looked less to the distractors in the interesting trials than in the boring trials. However, as a group, 9-month-old infants were not able to selectively learn to inhibit looks to the distractors in the more engaging trials. Despite the lack of selective inhibitory learning in the group as a whole, performance indices from the Freeze-Frame task, including the Selective Inhibitory Learning index, turned out to be significantly associated with performance on other frontal cortex tasks. Performance on selective aspects of the task was associated with performance on the A-not-B task at 9 months, the Spatial Conflict task at 24, and perhaps even Anticipation task performance at 4 months. The Freeze-Frame task was also the only task found to be associated with the COMT Val¹⁵⁸Met polymorphism, which has been directly related to dopaminergic neurotransmission in the frontal cortex (Chen et al., 2004; Karoum et al., 1994; Männistö & Kaakkola, 1999). This indicates that the Freeze-Frame task has strong potential as a measure of infant frontal cortex functioning.

It is worth noting that the relationship between the different Freeze-Frame indices and performance on other frontal cortex tasks was complex. For example, the Freeze-Frame Selective Inhibition index was associated with poorer frontal cortex performance at 24 months in Cohort 1 (Chapter 3). Nonetheless, the Freeze-Frame task clearly taps into individual differences that relate to performance on infant frontal cortex tasks and perhaps even genetic factors impacting on the frontal cortex. This demonstrates the promise of using saccadic inhibition tasks in research on infant frontal cortex functioning.

Frontal cortex task performance and temperament

Temperament factors related to self-regulation have been suggested to be associated with the development of the frontal cortex (Bell et al., 2007; Posner & Rothbart, 1998). Therefore parent-report measures of infant temperament were included in the presented studies.

In the literature, initial studies with relatively young infants seemed to support the link between frontal cortex task performance and good self-regulatory skills. For example, Johnson et al. (1991) found that the ability to disengage easily from a central stimulus was associated with lower levels of fear and distress and higher levels of soothability in 4-month-old infants. Similar findings have been reported by McConnell and Bryson (2005) who found that 6-month-old infants who disengaged more easily were rated as exhibiting less distress and more positive emotion. These findings are consistent with Posner and Rothbart's (1998) suggestion that self-regulation is an early correlate of frontal cortex function.

However, more recent findings have contradicted the idea that good frontal cortex function is associated with better self-regulation, at least in older infants. Work by Bell and colleagues has indicated that infants who perform well on the A-not-B task have higher activity levels and show more distress to limitations (Bell, 2005; Bell et al., 2006).

Findings from the present studies partly replicate both sets of findings, but also diverge in important ways. At 4 months, Anticipation task performance was generally associated with better self-regulatory skills as assessed by the IBQ-R. There was also a tendency for infants who anticipated more at 4 months to show less negative affect at 9 months. These findings were slightly qualified by the analysis of IBQ-R factor

change scores which showed that infants who quickly learned to anticipate at 4 months showed a significantly smaller increase in Orienting/Regulation scores between 4 and 9 months of age¹⁰ (Chapter 6). This finding suggests that slow anticipatory learners at 4 months of age had caught up with the fast anticipatory learners in terms of self-regulation by 9 months of age. Nevertheless, the associations found at 4 months, and the marginal longitudinal correlation with negative affect at 9 months, confirms that fast learning and an overall high level of anticipatory responses in early infancy is associated with more developed self-regulatory skills.

Performance on the Gap task was not significantly associated with any of the three broad temperament factors. However, a follow-up analysis attempting to replicate the findings of Johnson et al. (1991) and McConnell and Bryson (2005) did indicate that Gap task performance was associated with the Fear scale of the IBQ-R. The associations between Gap task performance and the Distress and Soothability scales (Johnson et al., 1991; McConnell & Bryson, 2005) were not replicated. Furthermore, the Gap task measure most strongly associated with the Fear scale was the overall median SRT (across conditions), followed by the median Gap SRT. The association between the Fear scale and median SRT in the Overlap condition was only borderline significant. Whereas Johnson et al.'s (1991) study only included an Overlap condition, McConnell and Bryson (2005) found that it was in fact reaction time in the Gap condition that was associated with fear. Combined with the fact that the *Gap effect* (which partially controls for overall reaction time) in the present study was not significantly related to IBQ-R Fear, these findings strongly suggest that the effect is due to general mechanisms of saccadic control or processing speed, not to a particular difficulty in disengaging from a stimulus during visual competition.

¹⁰ The group results indicated that the average Orienting/Regulation factor score increased over time.

Even though the associations between Gap task performance and temperament in the current study challenge previous studies showing an association between negative emotion and difficulty of disengagement, there was a tendency for infants who demonstrated large Gap effects to show increasing Negative Affect between 4 and 9 months of age. It therefore seems reasonable to conclude that performance on the Gap task is related to some aspects of self-regulatory functioning, though a specific problem with disengagement may not always be the underlying factor.

Whereas the data from the 4-month frontal cortex tasks may be interpreted as at least partly consistent with the idea that good frontal cortex performance is associated with a higher level of self-regulatory skills, the 9-month data contradict this conclusion. Both the results presented in Chapter 3 and Chapter 6 suggest that good performance on frontal cortex tasks at 9 months are in fact associated with lower levels of self-regulation and higher levels of negative affect. This finding contradicts previous results from studies with both younger infants (Johnson et al., 1991; McConnell & Bryson, 2005) and young children (Gerardi-Caulton, 2000; Rothbart et al., 2003), but is consistent with the recent reports from Bell and colleagues (Bell, 2005; Bell et al., 2006).

In the Cohort 1 study reported in Chapter 3, frontal cortex task performance at 9 months was associated with lower levels of Effortful Control at 24 months. In the Cohort 2 study reported in Chapter 6, performance on the A-not-B task was associated with higher levels of Negative Affect and Surgency/Extraversion at an earlier age (4 months). It is interesting to note that performance on frontal cortex tasks at 9 months was not associated with concurrent temperament factors, though an analysis testing the effects found by Bell and colleagues (Bell, 2005; Bell et al., 2006) in Cohort 2 did replicate the association between A-not-B performance and IBQ-R Activity Level at

the same age. The relationship between A-not-B performance and Distress did not reach statistical significance, but was in the same direction as the results reported by Bell et al. (Bell, 2005; Bell et al., 2006). Similar results in terms of Activity Level and Distress were found for the Freeze-Frame General Distractibility index for interesting trials, though these associations were only marginally significant.

These results are interesting for several reasons. Firstly, they indicate that late in the first year of life good performance on frontal cortex tasks is not necessarily associated with an “easy” temperament. In fact, more active and frustratable infants tend to perform better on a well-established infant frontal cortex task, the A-not-B task. This could again point to attentional and motivational factors as discussed in the previous section. Such factors remain to be investigated systematically in relation to frontal cortex task performance, but the presented results indicate that mechanisms related to infant reactivity may play an important role in infant frontal cortex functioning.

Secondly, the longitudinal associations between infant temperament and performance on frontal cortex tasks at 9 months of age hint at potential developmental pathways underlying these relationships. If infant temperament at 4 months predicts frontal cortex performance at 9 months, it seems likely that early temperament may effect changes that improve the infant’s frontal cortex abilities. Such factors could be environmental as suggested by Bell and colleagues (Bell et al., 2007; see also, Wolfe & Bell, 2007a). These authors have proposed that parental intervention in terms of soothing and directing the frustratable infant’s attention may help the infant develop superior attention skills, allowing them to perform better on frontal cortex tasks. However, it is also possible that more active and frustratable infants attain better frontal cortex skills because they are more motivated to obtain objects and rewards

that require the development of such skills. Though these ideas are largely speculative at this point, they constitute a fruitful area of further investigation into how temperament mechanisms may affect the development of frontal cortex skills during infancy, and into a potential direct relationship between temperament and the functioning of the frontal cortex.

In conclusion, evidence from 4-month-old infants partially supports the idea that infants with better self-regulatory skills perform well on frontal cortex tasks. However, by 9 months of age the pattern has reversed. This shows that the relationship between temperament and frontal cortex functioning during early development is complex and depends on the age of the infant.

Frontal cortex task performance and general development

In the adult literature on frontal cortex function the relationship between the frontal cortex and general cognitive ability remains controversial. Most reports on the consequences of frontal lobe damage find that patients have intact intelligence as assessed by standardised tests (e.g., Shallice & Burgess, 1991; Warrington et al., 1986). However, recent evidence suggests that neuropsychological tests developed to assess frontal cortex functioning may not be independent of general intelligence, at least in normal populations (Obonsawin et al., 2002). Furthermore, Duncan and colleagues (1995) have shown that frontal cortex patients are impaired on tests of fluid intelligence, a measure closely related to the psychometric concept *g* (general intelligence as indicated by shared variance across a range of cognitive tasks). There is also recent evidence of a direct link between the developmental trajectory of frontal cortical thickness and IQ during childhood and adolescence (Shaw et al., 2006). Finally, associations between performance on frontal cortex tasks and other cognitive

abilities, such as language skills, have been found in children as young as 4 years of age (Wolfe & Bell, 2004, 2007b). It was therefore important to establish whether the infant frontal cortex tasks used in the current set of studies measured functioning which was relatively dependent on general abilities, or whether they measured at least partially independent functional domains.

In the work presented here, gross motor development during infancy was generally not found to be related to frontal cortex task performance. This finding is slightly contradictory to a study by Bell and Fox (1997) which found that infants with crawling experience performed better on the A-not-B task than infants who were not yet able to crawl. The reason for this slight discrepancy between studies may be that Bell and Fox's sample was one month younger than the sample in the present study, and therefore needed slightly more advanced motor skills in order to perform the task. Beyond infancy, however, motor skills did seem to make a difference. For example, poorer motor skills as assessed by the BSID-II (Bayley, 1993) were associated with worse performance on the Spatial Conflict task at 24 months of age (Chapter 3). These findings indicate that most infants had the necessary motor skills to perform the frontal cortex tasks administered at 4 and 9 months (e.g., reaching at 9 months), and that variation in these skills were not significantly associated with performance on the frontal cortex tasks. However, in toddlerhood motor skills may play a larger role in frontal cortex tasks which depend on a manual response.

The relationship between performance on frontal cortex tasks and general cognitive development tended to be stronger. At 9 months, A-not-B performance was significantly associated with general cognitive ability and expressive language skills as assessed by the MSEL-AGS (Mullen, 1995). That is, infants who could sustain longer delays in the A-not-B task performed slightly better on a range of cognitive

tasks and had particularly good expressive language skills. The Freeze-Frame measures found to be associated with A-not-B performance were only weakly, and not significantly, related to general cognitive and language abilities. Importantly, the relationship between the two 9-month frontal cortex tasks remained highly significant after controlling for general cognitive ability and expressive language skills. This result suggests that even though performance on frontal cortex tasks late in the first year of life is associated with general cognitive development, these tasks also measure unique variance. The mechanism underlying this unique variance needs to be further explored, but one interpretation is that this mechanism relates to attentional factors (see above).

It is important to remember that causality cannot be inferred in the relationship between frontal cortex task performance and general cognitive development. Infants with better general cognitive abilities may find it easier to cope with the demands of the A-not-B task, or infants with strong inhibitory and attentional skills may benefit from these abilities in a wide range of tasks. It is also perfectly possible that the relationship goes both ways.

No measures of general motor and cognitive development were obtained at 4 months of age in order to limit the protocol, which was already demanding at this early age. However, Chapter 6 reported on the relationship between frontal cortex task performance at 4 months and general cognitive development at 9 months. The results of these analyses indicated that Gap task performance at 4 months was related to several measures of cognitive development at 9 months, in particular scales assessing visual organisation skills. There was also some association between Gap task performance and general cognitive ability, with infants demonstrating large Gap effects and long saccadic reaction times in the Overlap condition tending to perform

less well on the Early Learning Composite of the MSEL-AGS at 9 months. These results were relatively specific to measures involving disengagement from a central stimulus (see Chapter 6). In contrast, performance on the Anticipation task was only related to one of the 9-month MSEL-AGS scales (Receptive Language).

Given that Gap task performance was relatively unrelated to performance on the 9-month frontal cortex tasks, but did show significant associations with measures of cognitive development later in infancy, this task appears to be the least “frontal” of the chosen tasks. Gap task performance seemed to tap into more general mechanisms of cognitive function, at least when assessed at 4 months of age. This is consistent with studies showing disengagement problems in children with developmental disorders (Landry & Bryson, 2004; Lasker et al., 2007; Zwaigenbaum et al., 2005) as well as in infants with early neurological damage associated with later cognitive deficits (Atkinson & Braddick, 2007; Mercuri et al., 1999). However, as discussed above, the Gap task did show some association with another frontal cortex task administered at the same age (the Anticipation task).

In conclusion, performance on infant and toddler frontal cortex tasks cannot be assumed to be independent of general motor and cognitive development, and these factors need to be taken into account when investigating frontal cortex functioning at an early age. Nevertheless, the association between frontal task performance and general abilities is far from omnipresent and most of the associations between frontal cortex tasks remained significant after controlling for general cognitive ability. Some tasks, such as the Gap task, may be more associated with general cognitive functioning than with specifically frontal cortex functions.

Frontal cortex task performance and COMT Val¹⁵⁸Met genotype

The main aim of investigating the relationship between infant frontal cortex task performance and the COMT Val¹⁵⁸Met genotype was to provide converging evidence for the frontal nature of these tasks. The results of these analyses are discussed in detail in Chapter 7. However, it is worth pointing out that the data did not provide overwhelmingly strong support for a direct link between the neurobiology of the frontal cortex and the tasks chosen for the current studies. This may in part be due to the relatively small effect that a single gene has on behaviour (Barnett et al., 2007; Munafò, 2006).

However, distractibility in the interesting trials of the Freeze-Frame task did show an association with COMT Val¹⁵⁸Met genotype, indicating that infants carrying two Met alleles (the combination associated with the most optimal level of dopamine in the frontal cortex) were less distractible when engaged by a dynamic animated stimulus. This effect was not observed in the boring trials of the Freeze-Frame task. The finding of a selective association between the COMT Val¹⁵⁸Met polymorphism and performance on the Freeze-Frame task is encouraging in terms of the use of genotype data to assess frontal involvement in infant tasks developed to assess such functioning. Surprisingly, this method has rarely been adopted in the study of frontal cortex functioning in infancy and early childhood (but see Diamond et al., 2004).

Limitations and future directions

Task validation

The studies presented in this thesis were a systematic, but essentially exploratory, attempt to establish the relationship between different infant tasks that have been related to the functioning of the frontal cortex. As might be expected, the overall pattern of results is not one of consistent unity. Whereas some frontal cortex tasks did seem to measure the same underlying functions, especially tasks administered at the same age, others were not related or had complex associations with each other (see Chapter 3). The following will discuss some of the possible reasons for this complex picture.

An important factor in the complexity of the results is the relatively weak validation status of many of the tasks. In the adult literature, years of reporting on patients with frontal lobe damage and the development of sensitive neuropsychological measures to assess and differentiate frontal cortex functions have lead to a relatively rich knowledge about frontal cortex function (e.g., Damasio, 1994; Fuster, 1997; Kramer & Quinlan, 2007; Stuss, 2007). Furthermore, neuroimaging studies have provided evidence for the neural substrate of component processes associated with the frontal cortex (e.g., D'Esposito & Postle, 2002; Gilbert et al., 2007; Kerns et al., 2004). In infants, however, the assessment of frontal cortex functions is far more challenging and is beset with methodological issues.

In her work during the 1980s and 1990s, Adele Diamond made the first systematic attempt at studying functions associated with the frontal cortex in infancy. She formalised Piaget's (1954) A-not-B task into an experimental paradigm and demonstrated that infant performance on the task was highly similar to that of non-

human primates with damage to the frontal cortex (Diamond, 1985; Diamond & Goldman-Rakic, 1989). Later she demonstrated that even small changes in the neurobiology of the frontal cortex could affect performance on the A-not-B task and other infant frontal cortex tasks in a population of children treated early and continuously for PKU (Diamond et al., 1997).

This work, along with similar work from other laboratories (e.g., Baird et al., 2002; Bell, 2001; Bell & Fox, 1992), demonstrated that assessment of frontal cortex functioning in infancy is possible, and that such capabilities are present at least in the second half of the first year of life. However, the A-not-B task is the only infant task which has been relatively consistently associated with the frontal cortex. The other tasks used in the work presented in this thesis have been less solidly associated with the frontal cortex. The Gap and Anticipation tasks have been associated with event-related potentials over the frontal scalp (Csibra et al., 1998, 2001), but this technique has a coarse spatial resolution. The Freeze-Frame task was developed specifically to be a more sensitive measure of inhibitory functioning in infancy, but still needs to be further validated in terms of its status as a frontal cortex task.

On this background, it is clear that the infant frontal cortex tasks investigated here are far from as well validated and standardised as adult frontal cortex tasks. Having a set of more established frontal cortex tasks would of course have been ideal, but such tasks are simply not available at present. Despite this limitation, the current studies constitute one of the first attempts to systematically investigate the relationship between some of the *most* established infant frontal cortex tasks available. This is a necessary step in conceptualising the functional status of the frontal cortex in infancy, as well as refining and assessing the tasks used to measure such functioning.

Thus, the work presented in this thesis has made an important start on the effort to establish whether tasks that have been *associated* with the frontal cortex do actually measure the same functions. The overall pattern of results suggests that from 9 months onwards frontal cortex tasks do measure some of the same processes, and that these processes are relatively independent of general cognitive functioning. The specifics of these processes, as well as their exact neural substrate, constitute an important area for future research.

At 4 months, the evidence for common underlying functions in frontal cortex tasks was less consistent. Though tasks administered at 4 months did share a proportion of their variance, they did not predict frontal cortex performance at 9 months, making further task validation and studies of developmental trajectories desirable. As discussed earlier, it is also possible that 4 months is simply too early to assess stable individual differences in frontal cortex functioning, despite some evidence that the frontal cortex starts to play a functional role already at this young age (Csibra et al., 1998, 2001; Dehaene-Lambertz et al., 2002; Nakano et al., 2008).

Issues relating to a correlational design

The studies presented in this thesis provide preliminary evidence for the relationship between frontal cortex tasks in infancy. As discussed above, the project was exploratory in nature given the relatively small amount of evidence linking the individual tasks to the frontal cortex in comparison to the evidence for adult frontal cortex tasks. Relatively few frontal cortex tasks developed for infants exist and these tasks have often only been investigated in a few key studies (e.g., Csibra et al., 1998, 2001; Gilmore & Johnson, 1995; Johnson, 1995; Johnson et al., 1991). The work presented here was an attempt to explore the relationship between some of these tasks

by looking at individual performance differences within and across age. An individual differences approach has clear advantages in that it allows the detection of shared variance between different tasks, and thus of common underlying processes. Furthermore, the addition of a longitudinal perspective allows the investigation of stability in such processes over time. However, this approach also has important limitations that need to be addressed.

One of the most important limitations of this approach is that it is purely correlational in nature. This is problematic because correlations between tasks can occur for the “wrong” reasons. That is to say, the shared variance and underlying mechanism may not be related to the functioning of the frontal cortex. For example, the consistent finding of a correlation between the Freeze-Frame task and the A-not-B task is encouraging because the A-not-B task has been consistently related to the functioning of the frontal cortex. This lends some indirect support to the Freeze-Frame task as a frontal cortex task. However, it is important to recognise that the shared variance between the tasks may not be due to differences in frontal cortex functioning. Controlling for general cognitive ability was an important step in ruling out one obvious alternative explanation for the relationship. However, other mechanisms could be at play that were not controlled for in the present studies, and these mechanisms may not necessarily be frontal in nature.

One solution to this problem would be to control for more potential confounding variables. However, given the fact that an indefinite number of variables can be controlled for in any study, eventually more direct measures of the neural substrate of task performance are needed. In this respect, neuroimaging evidence will be essential. Direct measurement of brain activity would solve many of the problems associated with the correlational method used here because it would enable the establishment of

a direct link between performance on the tasks and activity in the frontal cortex. This is therefore an important future direction of research on infant frontal cortex functioning.

Since the initiation of the work presented here, important developments in neuroimaging techniques suitable for use with young infants have taken place. In particular, the development of near-infrared spectroscopy (NIRS) now allows the direct measurement of blood oxygenation (a correlate of neural activity) in the cerebral cortex in response to experimental stimuli (e.g., Taga et al., 2003). Such methods have already been used to track baseline metabolic changes and functional development in the frontal cortex during the first year of life (Franceschini et al., 2007; Homae et al., 2007; Nakano et al., 2008).

Another way to address the issues associated with purely behavioural associations is the use of data relating frontal cortex task performance to variations in the neurobiology of the frontal cortex. This approach was adopted by Diamond and colleagues in their study of frontal cortex functioning in infants and children treated early and continuously for PKU (Diamond et al., 1997). However, this population is very rare (1 in 10,000 births), which limits the general practicality of such research. Alternatively, genetic data can be used to assess variability in the neurobiology of the frontal cortex. Collecting such data is relatively straightforward and can be carried out in typical populations of infants and children. This was the approach taken in Chapter 7 of this thesis. Even though the results should be regarded as preliminary, they did indicate that the genotype method can identify specific task measures that are associated with genetic factors impacting on the neurobiology of the frontal cortex.

Despite the promise of this method, it is important to bear in mind that effects are likely to be subtle and large sample sizes are required. Furthermore, the general issues

involved in behavioural research using genetic polymorphism data, such as using well-established candidate genes, non-stratified populations, and appropriate endophenotypes (i.e., behavioural or physiological measures likely to be associated with the genotype) (Diamond et al., 2004; Hutchison et al., 2004; Munafò, 2006) are just as important in an infant population as they are in an adult population.

Choice of measures

Another issue relating to the limited knowledge about the chosen infant frontal cortex tasks is the choice of measures from each task. The measures chosen from each task need to be the most informative, and a limited number of measures is preferable in order to avoid multiplicity. This is easier with more established frontal cortex tasks, such as the A-not-B task. However, several of the tasks used here provided measures that could potentially tap into different aspects of the infant's performance. For example, one measure of Anticipation task performance at 4 months involved the overall proportion of anticipatory versus reactive saccades, whereas another measure assessed the rate of anticipatory learning (i.e., how quickly the infant started to anticipate). Similarly, the Freeze-Frame task provided several performance indices. The results indicated that different measures from the same task did not always correlate with the same measures from other tasks. For example, the Freeze-Frame Selective Inhibition index was correlated with A-not-B performance at 9 months (Chapters 3 and 5), whereas the Freeze-Frame Selective Inhibitory Learning index was correlated with performance on the Spatial Conflict task at 24 months (Chapter 3).

Such findings indicate that different aspects of task performance may tap into different functional mechanisms. The exact nature and neural substrate of these

mechanisms need to be further investigated. Given the exploratory nature of the analyses, especially those involving less investigated tasks, it is difficult to establish which aspect of task performance is the best measure of frontal functioning at this point. Even though this is a clear limitation of the presented studies, the identification of diverse relationships between tasks is necessary to start exploring these mechanisms.

Assessment of temperament using parent-report questionnaires

In contrast to the frontal cortex tasks administered at each age, temperament was assessed using parent-report questionnaires. As discussed in Chapter 2, some theorists have criticised such questionnaires for being excessively prone to parental bias (Seifer, 2002; Vaughn et al., 2002). However, in contrast to the questionnaire most criticised by these authors (the Carey ITQ: Carey, 1970, 1973), more recent questionnaires, such as those developed by Rothbart and colleagues, have put an emphasis on asking very concrete, simple questions, thus avoiding abstract and comparative judgements as well as excessive memory load (see Chapter 2 for details). These questionnaires have now been extensively validated (Gartstein & Rothbart, 2003; Parade & Leerkes, in press; Putnam et al., 2001; Putnam et al., 2006) and were used for the studies in this thesis.

Nevertheless, studies investigating the correlation between parent-report questionnaires and directly observed temperament tend to find rather modest associations. For example, Parade and Leerkes (in press) found that maternal and paternal ratings of infant fear and approach behaviours were significantly associated with negative emotions elicited in a laboratory setting. However, the correlation between parent assessment and laboratory observation was influenced by parental

levels of depression such that only relatively non-depressed parents provided judgements consistent with the laboratory observations (Parade & Leerkes, in press). Similarly, Gartstein and Marmion (2008) found a significant correlation between parent-reported fear and fearful behaviour observed in the laboratory, but also that parents who rated themselves as higher in negative affect rated their infants as more fearful compared to the laboratory observations.

On the basis of such findings, it could be argued that direct observation and rating by a trained experimenter would have provided a more objective assessment of each infant's temperament. However, this was not feasible in the studies presented here. Firstly, testing infants using procedures eliciting strong negative emotions, such as fear and anger, could potentially have influenced infants' performance on the frontal cortex tasks if the temperament assessment was administered at the beginning of the test session. Conversely, infants were often tired at the end of the test session, and this could have influenced their emotional reactions in the temperament assessment. Thus, testing temperament on the same day as the frontal cortex tasks and general developmental assessments were administered would not have been ideal. Adding a session on a separate day was not practicable due to the intensive testing load already involved in the current studies.

Secondly, even though the correlation between parent report and laboratory measures of temperament tends to be weak, it is not fully established which measure constitutes the most accurate assessment of infant temperament. As discussed above, parent report is subject to bias, especially in high risk samples¹¹ (see also Chapter 2). However, laboratory-based observational methods often depend on engaging the

¹¹ Note that the infant cohorts studied in this thesis came from relatively affluent backgrounds (see Chapter 4 for details).

infant in one or two relatively emotion-provoking and rather unnatural situations, and no single laboratory observation protocol has yet been fully validated (Gartstein & Marmion, 2008). In contrast, parents see their infant's reactions in a range of different day-to-day situations. (For further discussion of these issues, see Gartstein & Marmion, 2008; Parade & Leerkes, in press.)

As Parade and Leerkes (in press) conclude, the optimal way to assess infant temperament would be to collect and code a set of observations of each temperament dimension in multiple contexts. This approach was beyond the scope of the current thesis, especially because the main focus of the research was on frontal cortex functioning in infancy, not on temperament. However, the associations found between frontal cortex task performance and parent-reported temperament should be considered with these caveats in mind, and ideally be confirmed in future studies linking infant frontal cortex performance to directly observed temperament dimensions. Furthermore, collection of data on parents' temperament and depression level in addition to the infant data would provide at least one way of controlling for parental bias.

The use of age-appropriate tasks

A final point which is worth considering is the use of different tasks at different ages. This approach was chosen in order to match tasks across age. In this way, the effect of age is controlled to a certain extent; 4-month-old infants are simply expected to make eye movements, whereas 9-month-old infants are encouraged to reach for a hidden object. The rationale for this approach is that in order to assess stability in frontal cortex functioning, tasks at each age need to be appropriate for the child's age, i.e., the child needs to possess the basic skills to perform the task (such as reaching at

9 months). However, this approach does not address how individual infants' performance on a specific task changes over time. Thus, other types of longitudinal study track the developmental progression on the same task across age.

The latter approach has provided interesting data within the area of infant attention, and constitutes a fruitful area of further investigation into infant frontal cortex functioning. For example, Canfield and colleagues (1997) investigated developmental growth curves in performance on the Visual Expectation Paradigm (referred to as the Anticipation task in this thesis), and found that two or three distinguishable developmental growth trajectories existed for one of the outcome measures (reaction time variability). Similarly, Colombo and colleagues (2004) identified two different developmental trajectories in a longitudinal study of habituation behaviour. The majority of infants showed a large decrease in look duration between early and late infancy, whereas about a quarter of infants showed the opposite pattern. Importantly, the former group subsequently performed better on tests of general cognitive ability and language, indicating that the *growth trajectory* in the same measure could be a strong predictor of later outcome (Colombo et al., 2004).

This approach has also been applied to the study of infant frontal cortex function in an early EEG study by Bell and Fox (1992). The study demonstrated that infants who could sustain increasingly longer delays on the A-not-B task between 7 and 12 months of age showed a distinct pattern of frontal EEG changes during this period of time compared to infants who could only sustain a short delay throughout the period (Bell & Fox, 1992).

The growth trajectory approach is valuable because not only does it allow the investigation of age-related changes in performance on frontal cortex tasks, it also allows the investigation of the relationship between these changes and other markers

of frontal cortex function. In the future, it would be interesting to investigate age related changes in performance on the frontal cortex tasks used here in order to establish developmental pathways and their correlates. In conjunction with methodologies such as neuroimaging and genetics this could potentially provide a more in depth understanding of frontal cortex functioning during the first few years of life.

Conclusion

This thesis has presented a set of studies investigating frontal cortex functioning during the first two years of life. Overall, performance on frontal cortex tasks was associated within the same age, but longitudinal stability was rare before 9 months of age. The relationships between frontal cortex tasks could generally not be accounted for by variation in general cognitive ability.

A new task, termed the Freeze-Frame task, was developed to assess inhibitory functioning in infancy using eye movements as the dependent measure. Performance on this task was related to performance on several other infant and toddler frontal cortex tasks and to genetic variation impacting on the neurobiology of the frontal cortex. The task therefore showed promise as a frontal cortex task.

Frontal cortex task performance was related to temperament factors, but contrary to one theory of the relationship between the frontal cortex and temperament (Posner & Rothbart, 1998), at the later ages more reactive infants performed better than infants who demonstrated good self-regulatory skills.

Analyses involving genetic polymorphism data generally did not indicate strong relationships between genetic variation affecting the neurobiology of the frontal cortex and the set of frontal cortex tasks used in the present studies. However, the

preliminary finding of a significant and selective relationship between performance on the new Freeze-Frame task and COMT Val¹⁵⁸Met genotype is encouraging in terms of future studies on the effect of genetic variation on infant frontal cortex task performance.

Future studies should focus on obtaining further neuroimaging and neurobiological evidence for frontal involvement in tasks developed to assess frontal cortex functioning in infancy. Furthermore, tracking performance on the same set of tasks in the same infants will enable a better understanding of developmental change and individual growth trajectories in frontal cortex functioning during infancy and early childhood.

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