UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE HEALTH AND LIFE SCIENCES

School of Psychology

Adolescent inattention/overactivity/impulsivity as an outcome of early institutional deprivation: The role of genetic factors

by

Suzanne Elizabeth Stevens

Thesis for the degree of Doctor of Philosophy

January 2009

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE HEALTH AND LIFE SCIENCES

Doctor of Philosophy

ADOLESCENT INATTENTION/OVERACTIVITY/IMPULSIVITY AS AN OUTCOME OF EARLY INSTITUTIONAL DEPRIVATION: THE ROLE OF GENETIC FACTORS

by Suzanne Elizabeth Stevens

This longitudinal study examined the association between early institutional deprivation and inattention/overactivity/impulsivity (IOI) in a sample of institution-reared children adopted from severely depriving conditions in Romania before the age of 43 months. The total sample comprised 144 institution-reared and 21 noninstitution-reared Romanian adoptees, and a comparison group of 52 nondeprived, U.K. born children, adopted between the ages of 0 and 6 months. Their development was assessed at ages 6, 11 and 15 years, with particular attention given to their outcome in mid-adolescence. The current study tested the hypothesis that the risk for IOI following early deprivation is moderated by individual genetic make-up, using a subsample of 129 children. Candidate genes were selected using two strategies (Moffitt et al., 2005): i) a phenotype-based strategy that employed genes implicated in the aetiology of ADHD (dopamine transporter and receptor genes); ii) a process-based strategy that used polymorphisms with functional significance in terms of individual's responsivity to early deprivation (glucocorticoid receptor gene).

The introductory section of the current thesis is organised into three sections: i) an overview of ERA study and the association between adversity and IOI; ii) a review of literature on the broader phenotype of ADHD; iii) a discussion of the role that genetic factors may play in the putative causal pathways to IOI and ADHD. The following section outlines the study sample, methods and instruments. The subsequent empirical section is divided into three chapters: the first presents the results on the persistence and presentation of IOI; the second and third chapters present the analysis of the role of genetic factors in the risk for IOI following early deprivation. There were three main study findings: First, institutional deprivation lasting around 6 months or more was associated with an increased risk for IOI impairment from childhood into midadolescence. Second, the phenotypic presentation of IOI shared several features in common with nondeprivation-related ADHD. Despite showing persistence and pervasiveness across settings, the effects of early deprivation were not deterministic, suggesting other risk factors may be moderating the association. The third main finding suggested that the adverse effects of institutional deprivation on IOI were moderated by specific polymorphisms within the dopamine transporter gene. The effects were seen over time and across measures of IOI. The results are then brought together in the final discussion chapter. When the GxE interaction findings are integrated with the observation of persistence of IOI impairment and the commonalities in phenotypic presentation with nondeprivation-related ADHD, the results provide support for the hypothesised gene-environment interaction model, whereby ADHD susceptibility genes moderate the risk for of IOI from prolonged institutional deprivation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

DECLARATION OF AUTHORSHIP

I, Suzanne Elizabeth Stevens, declare that the thesis entitled:

Adolescent inattention/overactivity/impulsivity as an outcome of early institutional

deprivation: The role of genetic factors

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published as:

Stevens, S., Sonuga-Barke, E., Asherson, P., Kreppner, J. & Rutter, M. (2006). A consideration of the potential role of genetic factors in individual differences in response to early institutional deprivation: The case of inattention/overactivity in the English and Romanian Adoptees study. *Occasional Paper: Association of Child and Adolescent Mental Health, 25*, 63-76.

Stevens, S. E., Sonuga-Barke, E. J. S., Kreppner, J. M., Beckett, C., Castle, J., Colvert, E. et al. (2008). Inattention/overactivity following early severe institutional deprivation: Presentation and associations in early adolescence. *Journal of Abnormal Child Psychology, 36,* 385-398.

Signed: ……..

Date:…….

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge the young people and their families who have taken part in the English and Romanian Adoptees (ERA) study. Without their remarkable commitment over the 10 years the study has been running this research would not have been possible. I would also like to acknowledge the financial support of the Economic and Social Research Council, UCB Pharma Ltd, the Department of Health, the Nuffield Foundation and the Jacobs Foundation. I would like to give my sincere thanks to my supervisors, Professor Edmund Sonuga-Barke and Dr Jana Kreppner. Edmund has given me expert supervision, inspiration, endless enthusiasm and helpful criticism over the course of my studentship. He provided me with the opportunity to undertake this degree and I sincerely thank him for all that he has taught me. Jana I would like to thank for giving such excellent advice, support and for helping me stay positive. I would like to thank Professor Sir Michael Rutter for his guidance and inspiring me think with a critical scientific mind. Next, I would like to thank the ERA team, to whom I am genuinely grateful for all their help, support and for giving me the confidence to keep going through difficult phases of the work: Celia Beckett, Jenny Castle, Emma Colvert, Amanda Hawkins, and Christine Groothues. Many others have provided me with helpful genotyping and technical support: Keeley Brookes, Ted Barker and Darko Turic. I am grateful to Robert Kumsta for his invaluable help with my research on the glucocorticoid system and to Christopher Bell and John Stevens for generously giving their time and proof reading skills.

I would also like to give my heartfelt thanks to my family in New Zealand: My parents, Beverley and John Stevens, and my sister, Michelle. Their faith in my abilities, the encouragement they have always given me to do the best I can and knowing that my achievements have made them proud gave me the confidence to embark on this doctorate and the strength to see it through to completion. I would also like to thank Charles Tatham for his generosity and support of my studies. Last but not least, I would like to thank my London family of friends. Thank you Dave, Victoria, Jason, Nicki, Tui, Filipa, Nathan and Anya, for keeping me sane and keeping me smiling, believing in me and reminding me to come up for air now and then.

DEFINITIONS AND ABBREVIATIONS

Defining inattention/overactivity/impulsivity and attention-deficit/ hyperactivity disorder

The central focus of the current thesis is the domain of impairment that encompasses the behaviours of inattention, overactivity and impulsivity as a specific outcome of early institutional deprivation. To provide some clarification about relevant terminology I have followed the approach suggested by Taylor (1998) in order to distinguish between hyperactivity, attention-deficit/hyperactivity disorder (ADHD) and hyperkinetic disorder (HKD). Furthermore a description of what is meant by inattention/overactivity/impulsivity (IOI) is provided along with an explanation of why an alternative label has been used for this pattern of behaviour in relation to the risk associated with early deprivation.

Hyperactivity

The term hyperactivity usually refers in the literature to the continuously distributed, heritable trait found in the normal population that consists of the core behaviours of overactivity (excessive motor activity, i.e. restless, cannot sit still for long, always fidgeting), impulsiveness (i.e. acting quickly without thinking) and inattention (i.e. easily distracted, concentration wanders). This term describes a disposition or syndrome rather than being a diagnostic term. ADHD and HKD, on the other hand, are diagnostic categories defined by similar sets of criteria.

Attention-deficit/hyperactivity disorder (ADHD)

ADHD, as defined by the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV-TR; 2000) classification criteria, is an early onset clinically heterogenous neuro-developmental disorder characterised by inattention, hyperactivity and impulsivity. In this context hyperactivity is analogue to specific overactive behaviours. There are several subtypes: inattentive; hyperactive-impulsive; and combined. For all subtypes a specific number of severe, maladaptive, impairing and developmentally inappropriate symptoms must be present. It is highly heritable and has a worldwide prevalence rate in children internationally of around 5% (Polanczyk et al., 2007). ADHD is associated with persistent negative outcomes in social, academic and occupational areas (e.g. low

self esteem, delinquency, conflict with parents) and a wide range of coexisting disorders (e.g. conduct problems, anxiety disorder, oppositional disorder). A more comprehensive description of the disorder is given in chapter two and is also available in Biederman & Faraone (2005). Multiple environmental and genetic risk factors are implicated in the aetiology of the disorder. Environmental risk factors are discussed in the following chapter, under heading 1.6, in relation early adversity and genetic risk is discussed in chapter 3.

Hyperkinetic disorder (HKD)

HKD is the diagnostic classification of the 'hyperactivity' syndrome from the International Classification of Diseases (ICD-10, World Health Organisation, 1992) used primarily in the U.K. and Europe. It too requires the presence of developmentally inappropriate inattentiveness, overactivity and impulsiveness that impairs social and academic functioning. HKD differs from ADHD in the organisation of the symptoms that constitute a diagnosis and in the level of pervasiveness required. The diagnosis of HKD is more stringent; it requires all three components to be present and that the child must meet the diagnostic criteria in both the home and school setting. Whereas for ADHD a child must meet all the criteria in one setting and only needs to show evidence of impairment from the symptoms in the other setting. HKD can not be diagnosed if other coexisting disorders such as conduct disorder or autism are present. Whereas ADHD can still be diagnosed in such circumstances, but only if the symptoms are not better described by the comorbid disorder.

Both the DSM and ICD diagnostic manuals are currently under revision and the formulation of ADHD and HKD are likely to be adjusted to bring them more up to date with advances in the science of the disorders. The new criteria for DSM and ICD are expected to be published in 2012 and 2015, respectively (Sonuga-Barke, 2008).

Inattention/overactivity/impulsivity (IOI)

In the current thesis the label of inattention/overactivity/impulsivity or IOI has been chosen. Although these behaviours form part of the constellation of characteristics that make up ADHD, HKD and hyperactivity, a distinction has been made because there has been some speculation as to whether the meaning of this outcome

pattern in response to institutional deprivation may be different from ordinary ADHD (Rutter, Roy & Kreppner, 2002). That is, institutional care may not be just another environmental risk factor for ADHD more generally, but rather IOI in this context may be better described as part of a 'deprivation-specific syndrome' (Kreppner et al., 2001). Further investigation of this proposition is currently underway by the ERA research team. The most compelling evidence comes from the overlap of IOI with a pattern of disinhibited attachment behaviour that may suggest the presence of some underlying common construct. Yet there has been relatively little systematic examination of the commonalities and differences of ADHD and deprivation-related IOI. Thus the presentation of IOI in an institutionally deprived sample and of ADHD in the general population are compared and contrasted as part of this thesis in terms of associated features and the possible underlying causal mechanisms.

Another point of clarification relates to previous ERA research papers that refer to the construct as simply inattention/overactivity, or I/O. This was due to the fact that at ages 6 and 11 years the domain of impairment being measured was based on the hyperactivity subscale on Rutter Scales. Impulsivity was not captured on this subscale and this was therefore acknowledged in the label. However, at age 15 the domain is assessed far more extensively using the parental Child and Adolescent Psychiatric Assessment (CAPA) interview (Lifetime Version developed for use in the English and Romanian Adoptees Study: Rutter M, Silberg J, Colvert E, Kreppner J., 2004; based on Angold et al., 1995) in addition to the hyperactivity subscale on the Strengths and Difficulties Questionnaire (Goodman, 1997), both of which tap impulsivity in their assessment items.

For ease of presentation, and to be consistent with the majority of the literature, in the current thesis ADHD will be used to refer to the diagnostic category in the general population and specifically to the symptoms measured by the CAPA interview in the ERA sample. The behavioural trait presented by children in the ERA study will be referred to as IOI. When comparisons are being made between IOI in the ERA sample and the corresponding behavioural trait in the general population the distinction will be made according to their deprivation status. That is, IOI in the ERA sample will be referred to as deprivation-related IOI, and IOI in the general population will be referred to as nondeprivation-related IOI.

Abbreviations

General abbreviations

ADHD - attention-deficit/hyperactivity disorder: Used to describe the disorder as seen in the nondeprived population; also in the context of symptoms scored using the CAPA interview in the ERA study.

CAPA - Child and Adolescent Psychiatric Assessment – ERA edition

DA - disinhibited attachment

DSM-IV-TR - Diagnostic and Statistical Manual of Mental Disorders, version IV, text revision (American Psychiatric Association, 2000)

ERA - English and Romanian Adoptees study

E'risk - environmental risk

GenERA - study of the role of genetic factors within the English and Romanian adoptees study

G'risk - genetic/haplotypic risk

HPA axis - Hypothalamic-pituitary-adrenal axis

ICD-10 - International Statistical Classification of Diseases and Related Health Problems, 10th Revision (World Health Organisation, 1992)

IOI - inattention/overactivity/impulsivity: Used to describe the cluster of behaviours observed in the ERA sample following early deprivation; also when referring to the behavioural trait in the nondeprived population (nondeprivation-related IOI)

IQ - intelligence quotient (used as a measure of cognitive functioning)

SDQ - Strengths and Difficulties Questionnaire

U.K. - United Kingdom

Genetic abbreviations

Bp - Base pair (within gene)

CHRNA4 - Cholinergic receptor, nicotinic, alpha 4 (gene)

DAT1 - dopamine transporter (gene)

DDC - dopa decarboxylase (gene)

DRD4 – dopamine (D4) receptor (gene)

GR - glucocorticoid receptor (gene)

FADS2 - fatty acid desaturase family of genes

MAOA - monoamine oxidase A (gene)

NET – norepinephrine transporter (gene)

SNP - single nucleotide polymorphism

SYP – synaptophysin (gene)

UTR untranslated region (of gene)

TPH2 - tryptophan hydroxylase 2 (gene)

VNTR: Variable number tandem repeat (genetic polymorphism)

Statistical abbreviations

ANOVA - Analysis of variance

β - Beta standardised regression coefficient

GxE - Gene-environment (interaction)

rGE - Gene-environment (correlation)

M - mean

n - number

n/s - nonsignificant (p-value)

p - observed significance level

r - correlation coefficient

R^2 - correlation coefficient squared

SD - standard deviation

S E - Standard error

t test - analysis of variance

χ^2 - chi square test

CHAPTER 1: INTRODUCTION

EARLY ADVERSITY AND THE RISK FOR INATTENTION/OVERACTIVITY/IMPULSIVITY

1.1 Introduction to the thesis

Over the last decade an increasing number of studies reported the lasting effects of early institutional deprivation on a number of areas of psychological functioning (Fisher et al., 1997; Gunnar & van Dulmen, 2007; Rutter et al., 2000; Rutter et al., 2007; Vorria et al., 2006; Zeanah et al., 2003). Early institutional deprivation refers to severe psycho-social, physical and nutritional deprivation experienced by some children reared during their early life in institutions. This research has made a substantial contribution to the wider literature concerning exposure to adverse environments in early life and the profound and persistent effects this can have on a child's subsequent capacity for normal behavioural and neurobiological functioning (Rutter, 1999; Taylor & Rogers, 2005). Reassuringly, the effects are not deterministic; with many children appearing to function normally and a large degree of heterogeneity in individual outcome despite their adverse early experience. However, to date it has not been possible to provide a sufficient explanation for these marked individual differences in response to this early deprivation. The current study is the first to examine the role of individual genetic makeup in explaining heterogeneity in outcome following early institutional deprivation. Specifically, the focus is on inattention/overactivity/impulsivity (IOI), the cluster of behaviours that form the diagnostic core of attention-deficit/hyperactivity disorder (ADHD), and which have been found to be elevated following early institutional deprivation.

IOI is an interesting candidate outcome to investigate for several reasons: First, IOI following early deprivation is one of a limited number of specific, negative sequelae associated with this environmental risk factor; second, ADHD in the nondeprived population is thought to have a strong genetic component to its aetiology; third, there is a high level of variability amongst individuals' long term behavioural response to institutional deprivation, suggesting that additional risk factors may influence vulnerability to the adverse effects of environmental risk.

The introductory section of the current thesis is organised into three chapters that aim to address the main subject areas of relevance to the investigation. In chapter 1, the focus is on the association between early adversity and IOI. The English and Romanian Adoptees (ERA) study will be introduced and a summary of the relevant findings from the study will be given. The current thesis forms a part of the main ERA study, and also incorporates an independent project looking at the role of genetic factors (GenERA study). In chapter 2, issues relating to our understanding of the broader phenotype of ADHD will be reviewed and examined in relation to IOI as an outcome of early deprivation. In chapter 3, a review of the role that genetic factors may play in the putative causal pathways to IOI and ADHD will be presented. The current GenERA study will be introduced, which investigates the interplay between genetic effects and institutional deprivation in relation to the risk for IOI.

The methodology section follows the introduction and is divided into two parts (chapters 4 and 5). In chapter 4, a description of the sample, the procedures and the measures used is provided. In chapter 5, the analytical methodology is described.

The subsequent empirical section is organised into three chapters (chapters 6, 7 and 8). In chapter 6, the results relating to the longitudinal persistence into the mid-adolescent period of IOI in the ERA sample are provided, alongside an analysis of the presentation of IOI in terms of its clinical significance and associated features. Chapters 7 and 8 present the analysis of the role of genetic factors in the risk for IOI following early deprivation. Specifically, chapter 7 examines the interaction between deprivation and genes in the dopamine system in relation to the risk for IOI. Chapter 8 investigates the role of the glucocorticoid receptor gene in this process. The final discussion chapter provides a summary and interpretation of the results as presented, together with a discussion of the wider implications of this research, the limitations of the current study and suggestions for future research.

1.2 Outline of chapter 1

The focus of the current chapter is on characterising IOI in the ERA sample within the context of early adversity and environmental risk for ADHD more generally. An

overview will be given in chapter 2 of the nature of nondeprivation-related ADHD as a psychiatric disorder; its presentation and associations, underlying pathophysiology, putative neuropsychological mechanisms, developmental course, and its treatment.

The present chapter is organised in several sections. First, an introduction to the English and Romanian Adoptees (ERA) study will be given. This includes (i) a description of the ERA project and the rearing conditions experienced by study participants in Romanian institutions, and (ii) a review of the specific sequelae of early institutional deprivation and the marked differences in behaviour between individuals experiencing similar levels of deprivation. Specific focus is on levels of inattention/overactivity/impulsivity (IOI). Second, an overview of previous ERA findings in relation to deprivation and IOI will be presented. Third, a review of the wider literature on the links between early adversity and IOI/ADHD will be given.

1.3 Background to the ERA study

The English and Romanian Adoptees (ERA) study was set up to follow the development of children who were adopted out of severely depriving institutions in Romania into the U.K. following the fall of the Ceauşescu regime in 1989. One of the study's objectives was to investigate the causal role that early adverse experiences associated with institutional deprivation play in determining developmental outcome (O'Connor et al., 2000; Rutter & English & Romanian Adoptees Study Team, 1998). It is one of a small number of studies that were set up to follow the development of internationally adopted children reared in their early years in Romanian institutions (Fisher et al., 1997; Marcovitch et al., 1997). The ERA study is the only study of its kind in the U.K., and the only study of its kind worldwide that has systematic and comprehensive longitudinal data with similar methods used at all four assessment waves (at ages 4, 6, 11 and 15 years). The study comprises a large representative sample of Romanian children (n=165) who were raised in severely depriving conditions in Romania during the late 1980s to early 1990s, prior to adoption, at varying ages (ranging from 0 to 43 months of age), by families living in the England. 144 of the children were reared in grossly depriving institutions, and the remaining 21 were adopted from impoverished home settings. Their development is compared with a sample of nondeprived children adopted within the U.K. before the age of 6 months.

1.3.1 Conditions in Romania

The conditions in the Romanian institutions during the time of the Ceauşescu regime ranged from poor to abysmal, and have been described in detail in several research reports (Castle et al., 1999; Johnson, 2001; Kaler & Freeman, 1994; Rutter & English & Romanian Adoptees Study Team, 1998). The infants were frequently confined to cots for long periods of time, often whole days and nights. Furthermore, if they were old enough to move around, they were frequently left tied to their cots. There were few, if any, toys and little stimulation. The staff to child ratio was very low: 20:1 or 30:1 in many of the institutions, and there was high rate of staff turnover. The care given was generally of a low quality, with no personalised caregiving and very little interaction between caregivers and children. In addition, feeding was often impersonal with infants fed using bottles that were left on the pillow or propped up above their heads. The food they received was of a very poor nutritional quality and insufficient quantity. The physical conditions were sometimes harsh with bathing often consisting of being sporadically hosed down with cold water. The grave nature of the situation in which the children were reared was apparent in their marked developmental delay and poor physical state at the time of entry to the U.K.: their mean weight, height and head circumference were more than 2 *SD* below U.K.-based age norms, and intellectual levels, were similarly depressed (O'Connor et al., 2000; Rutter & English & Romanian Adoptees Study Team, 1998).

The children enrolled in our study were predominantly placed in the institutions within the first two weeks of life. Although we do not have any systematic information on the reasons for individual's placement, evidence from surveys conducted at the time (Children's Health Care Collaborative Study Group, 1992) and the early age at which the children were admitted, indicated that this was due to the widespread economic hardship and strict social policy resulting in circumstances where families were too impoverished to care for their children. Admittance to institutions due to child impairment seems to have been less of an issue because the children would have been too young for developmental delay to be detectable. Moreover, there was an absence of any formal fostering system in Romania at the time and, as far as is known, no children were adopted from the institutions prior to 1989. Therefore, the subsequent timing of adoption out of the institutions was largely determined by political, rather than individual selection, factors brought about by the fall of the Ceauşescu regime, following which,

adoption became possible. This ruled out the possibility that older children had not been adopted at a younger age because of their individual impairment or other selection factors, such as returning to their family home. All of the above present the ERA study with significant methodological advantages over previous studies in the field. In particular, it allowed a more systematic examination of the effects of the time spent in extreme adversity.

The radical and easily timed change from one environment to another, namely from an early childhood spent in a grossly depriving institution to, in the majority of cases, an above average adoptive family rearing environment in the U.K., provided a unique opportunity to isolate the effects of early adverse environments from later experience, and to study their impact on later development.

1.3.2 Deprivation specific impairment: Overview of key findings from ERA study

Two key findings emerged following the assessments of the children during childhood. On the one hand, there was a striking degree of catch-up in both physical and intellectual domains demonstrated by a considerable number of children by the time they were 4-6 years of age. On the other hand, for a significant minority of children, residual deficits persisted (O'Connor et al., 2000; Rutter & English & Romanian Adoptees Study Team, 1998) and psychological dysfunction and psychiatric morbidity was common. The psychological deficits found were surprisingly specific and unusual in pattern and were associated with duration of time spent in the depriving conditions (Rutter et al., 2000; Rutter et al., 2001). Four specific domains of impairment were reported: Disinhibited attachment behaviours, quasi-autistic features, cognitive impairment and, most notably for the current investigation, inattention/overactivity/impulsivity. Each of these impairments was found to be more likely to be present if the child experienced over 6 months institutional deprivation and have, so far, persisted into adolescence (Kreppner et al., 2007; Rutter et al., 2007a; Rutter et al., 2007b; Stevens et al., 2008). In contrast, conduct problems, emotional difficulties and peer problems were not significantly elevated at age 6, nor were they associated with duration of deprivation (Rutter et al., 2001). By the time of the age 11 assessment, there was a significant increase in emotional problems in the

Romanian sample, found to be accounted for, in part, by previous deprivation-specific problems (Colvert et al., 2008). On the basis of these findings, there was a strong case for regarding these particular four outcomes as deprivation-specific (Kreppner et al., 2007; Rutter et al., 2001).

Despite the severity of deprivation, there was a marked degree of variability in the response of an individual to deprivation (Rutter et al., 2001). That is to say, poor outcome was not inevitable. This heterogeneity in outcome can be seen even among those children who experienced extended periods of institutional care. About one quarter of those adopted over the age of 2 years showed normal functioning at age 6 years. Moreover, at age 6 and age 11 the scatter of individual IOI scores was almost as large for the group who had experienced the longest 'dose' of deprivation as those who had experienced less than 6 months (see appendix 1). Such variability in outcome suggests that deprivation is not the only factor operating to influence the development of the ERA children. As such, the adverse developmental effects of institutional deprivation need to be viewed as probabilistic rather than deterministic. Thus, it is possible that other factors 'within' the adoptees themselves, or within their environments, act to moderate the effects of deprivation in a way that appears to protect some children while leaving others at risk. Attempts to explain this variability, which have focused on environmental factors such as the post adoption home environment, have not, so far, proved fruitful (Colvert et al., 2008). The aim of the current study is to examine the potential moderating role of genetic factors in determining the risk for IOI impairment. The rationale for this investigation is twofold. First, there is a growing body of evidence to suggest that gene-environment interactions in the context of early experience are influential on long-term outcome (e.g. Caspi et al., 2002; Laucht et al., 2007). It is plausible that specific genetic factors have placed some children at greater risk in relation to the adverse effects of environmental factors than others. Second, the genetic contribution to ADHD in the wider population has been established in the literature (Thapar et al., 2005). Taken together with the evidence of heterogeneity in outcome in the ERA study, it seems vital to explore the role of individual genetic makeup in the context of the risk for IOI following early deprivation. Are there genetic factors that either increase an individual's susceptibility, or alternatively, make them more resilient to the adverse effects associated with institutional deprivation? Is the risk for IOI moderated by factors similar to those relevant for attention-deficit/hyperactivity disorder in the wider

nondeprived population? These issues will be discussed in chapter 3 on the interplay between genetic and environmental risk factors.

1.4 IOI following severe early institutional deprivation: Evidence from the ERA study

1.4.1 Summary of ERA findings specifically relating to IOI

Elevated levels of IOI have been reported for Romanian institution-reared children (Rom IR) at age 6 (Kreppner et al., 2001) and at age 11 (Stevens et al., 2008). Moreover, IOI was significantly increased in the Rom IR sample compared with those adopted from impoverished home settings in Romania (Rom non-IR), suggesting that the adverse effect on IOI was specific to the institutional experience. Just as striking was the importance of prolonged institutional rearing for the development of IOI. Thus, the institution-reared Romanian children who were aged over 6 months (6 to 43 months) when they left the institutions and joined their respective U.K. families, were at particular risk for elevated levels of IOI compared with those adopted under the age of 6 months from Romanian institutions or from within the U.K. (Kreppner et al., 2001; Stevens et al., 2008).

The results indicated that extended institutional deprivation, 6 months or longer in duration, constituted a significant environmental risk for increased IOI in childhood and early adolescence, in particular. Moreover, by age 11 the risk associated with early deprivation could best be characterised by significant stepwise increases at around the 6 months of age mark, with little increase in risk after that point (Kreppner et al., 2007; Stevens et al., 2008). There was substantial individual continuity in impairment that indicated a persistent risk effect of institutional deprivation (Stevens et al., 2008). Moreover, analysis of the age 6 data showed that the effects of duration of deprivation on IOI were not accounted for by low birth weight (an index of prenatal risk) or malnutrition. Although IOI was correlated with cognitive level, IQ did not constitute a necessary mediator of the effects of deprivation on IOI (Kreppner et al., 2001)

Analysis of the age 4 and 6 year old data demonstrated that there were no consistent correlations between adoptive family demographic characteristics (parental age, parental education and SES) and the risk for IOI from institutional deprivation. There was little within-sample variation in the range of the

demographic variables, therefore, it was concluded that they were unlikely to influence the deprivation effects (Kreppner et al., 2001). Therefore, demographic data were not included in any further analyses (including the analyses performed in the present report).

Taken overall, the findings demonstrate that extended institutional deprivation, lasting 6 months or more, constitutes a significant and persistent risk for IOI impairment in childhood through to early adolescence. One of the goals of the present report is to examine whether institutional deprivation continues to represent a significant risk for IOI into mid-adolescence, when the children have spent a minimum of 11 ½ years in their adoptive homes.

1.4.2 Deprivation-related IOI presentation: Association with disinhibited attachment

Disinhibited attachment in relation to IOI represents, perhaps, the most obvious phenotypic area where deprivation-related IOI differs from that seen in the general population. Attachment disturbance of the type that corresponds to reactive attachment disorder, disinhibited subtype is a common feature noted across studies of institution-reared children (Chisholm, 1998; Roy et al., 2004; Rutter et al., 2007a; Zeanah et al., 2005). However, it is likely that the problems relate to deficits in reading of social cues and appreciating social boundaries, rather than a pattern of 'indiscriminate friendliness' (Roy et al., 2004). There is only a limited amount of research on the comorbidity of attachment disturbances and ADHD in noninstitution-reared samples, and where research has been conducted, it mainly focuses on secure/insecure or disorganised attachment relationships with parents, rather than disinhibition with strangers, and is often based on small clinical case studies (Clarke et al., 2002; Finzi-Dottan et al., 2006; Horvath & Markman, 2008; Stiefel, 1997). However, some insight into IOI in the ERA sample may be gained from this research. Early parent-child attachment security has been linked to attentional performance, and there is some evidence to suggest secure attachment may provide a level of protection against the cumulative risk associated with being male and exposure to early social and psychological adversity (Fearon & Belsky, 2004). Given that the children in the ERA study were not given the opportunity to form secure attachment relationships in the Romanian institutions, it follows that this may have influenced their future attentional skills.

Moreover, attachment theory may provide some insight into a possible mechanism to explain this link. Attachment theory holds that a secure and responsive early parent child relationship is an integral part of the development of effective self-regulation in the child and self-regulation is linked to impulse control, perseverance and behavioural inhibition, which make up important features of the nondeprivation-related IOI/ADHD phenotype. When considered together with the striking pattern of disinhibited attachment observed in our sample, and patterns of overlap noted by Kreppner et al. (2001), these studies highlight this as an important area of investigation when considering the phenotypic characteristics of IOI in adolescence. One goal of the present study is to explore the presentation of IOI in relation to disinhibited attachment and in terms of features commonly associated with ADHD in the wider nondeprived population.

1.5 Environmental adversity and risk for IOI/ADHD: evidence from the broader literature

In this section, a review of the broader literature on environmental risk for nondeprivation-related IOI and ADHD will be presented. Particular focus is given to the risk associated with early adverse experience on development and, specifically, institutional deprivation as a risk for IOI.

Studies have shown that stressful experience, such as maternal separation and institutional rearing or other traumatic experiences such as child abuse or neglect are associated with increased risk for persistent impairment and psychiatric disorder (Gunnar & van Dulmen, 2007; Kaufman et al., 2000; Kreppner et al., 2007). The evidence for multiple risk factors of small effect highlights the need to consider interacting influences and the context in which they operate. Emerging evidence on genetic moderation of the effect of exposure to environmental risk factors will be discussed in chapter 3.

1.5.1 Prenatal and perinatal risk factors

Prenatal factors such as maternal stress, smoking and alcohol use during pregnancy, and perinatal factors such as prematurity, have been linked to the risk for ADHD/nondeprivation-related IOI (Bhutta et al., 2002; Linnet et al., 2003).

The clearest evidence, perhaps, relates to prenatal exposure to maternal smoking, which has been associated with an increased risk for ADHD in population and clinical studies, possibly through a dose-response type relationship (Mick et al., 2002; Thapar et al., 2003). However, effect sizes are small and methodological flaws have been identified in this literature. It has been suggested that the link with ADHD may be confounded by the association between smoking and other risk factors such as social disadvantage and parental personality characteristics that may account for the impairment in offspring (Ramsay & Reynolds, 2000; Taylor & Rogers, 2005). Nonetheless, in the case-control study conducted by Mick et al. (2002) the twofold increase in rates of maternal smoking observed for children with ADHD remained after they adjusted for potential confounds, including those listed above. Maternal alcohol use during pregnancy has also been linked to IOI behaviour in offspring (Mick et al., 2002). However, the evidence is difficult to interpret with negative reports also found in the literature (Hill et al., 2000) and IOI behaviours forming a component of the Foetal Alcohol Syndrome phenotype. Whether these risks operate on a continuum, or whether a certain threshold needs to be met for effects to be significant, remains to be seen. Despite initial evidence that smoking during pregnancy may have a dose-response risk effect on offspring, the public health question of whether there is a 'safe' level of smoking or drinking during pregnancy has not been determined (Taylor & Rogers, 2005).

Maternal stress during pregnancy and the associated exposure of the foetus to increased levels of glucocorticoids have been implicated in childhood behavioural problems and ADHD (French et al., 2004; Kapoor et al., 2006; O'Connor et al., 2003b). This is supported by extensive research on prenatal stress using animal models that shows significant and long lasting behavioural effects and brain alterations in offspring (e.g. Weinstock, 2001). However, potential confounds include: That a mother who is stressed during pregnancy may well continue to be stressed during the child's upbringing, thus introducing further environmental adversity; additionally, stress during pregnancy can be a precursor to premature birth, which has risk effects of its own (for review, see Taylor & Rogers, 2005).

The adverse effects of prenatal exposure to toxins such as mercury or PCBs (polychlorinated biphenyls) have shown associations with impaired IOI

outcome, but they are also linked with broader neurodevelopmental problems (Banerjee et al., 2007).

There is some evidence to suggest that premature delivery, severe influenza attacks and neonatal seizures may also constitute significant perinatal risk factors for ADHD (Pineda et al., 2003). In a recent meta-analysis, there was an increased occurrence of ADHD, and substantial association with lowered IQ, in children who were born preterm compared with full-term controls (Bhutta et al., 2002). Preterm babies had over two times the relative risk of developing ADHD in 81% of the studies they examined. However, preterm babies are often underweight and low birth weight is associated with potentially confounding and influential factors including social disadvantage and poor antenatal care. Controversy remains over whether obstetric complications are cause, effect or epiphenomenon with respect to the development of behavioural disorders (Taylor & Rogers, 2005).

Overall, the evidence suggests there are multiple pre and perinatal risk factors of small effect. Further research is needed into the specificity of effects on outcome and the influence of genetic factors on: Exposure to environmental risks; the impact of the risk on development. Despite presence of significant associations, the putative environmental risk factors discussed here each account for a small amount of the overall variance in ADHD behaviours, suggesting that outcome is influenced by a variety of different risks.

1.5.2 Postnatal physical risk factors

The findings in relation to postnatal factors are even less straightforward. Although still controversial, dietary factors on the child have shown associations with inattentive/overactive/impulsive behaviour. A recent randomised, controlled study investigating the link between food additives and IOI found that administering artificial food colouring and/or sodium benzoate preservative lead to a significant increase in IOI behaviours (McCann et al., 2007). Specific food intolerances are often reported by parents and may influence IOI on an individual level (Aardoom et al., 1997). The association between lead and increased rates of ADHD is difficult to interpret, due to the link between exposure to lead, wider social disadvantage and more general neurodevelopmental problems (Levitt, 1999; Needleman, 1982).

There is some evidence to suggest a persistent increase in the frequency of attentional deficits among previously malnourished children compared to controls from a study of the long-term impact of early malnutrition on behavioural development (Galler & Ramsey, 1989). These findings have relevance for the ERA study because a large proportion of Romanian children in our sample were severely malnourished when they were adopted into the U.K. Although the period within which the children experienced malnutrition was longer for some cases in the ERA sample (up to 3 ½ years) compared with the Galler and Ramsey study (the first year of life), there are parallels in that the malnutrition was restricted to a finite period in infancy. Moreover, the association with attentional deficits extended into adolescence long after the exposure to the putative risk factor, and was detected in both a home and school setting (Galler & Ramsey, 1989). With respect to IOI in the ERA sample, there was some evidence that malnutrition was a contributing factor to the risk for IOI at age 6, particularly for teacher reports, but it did not completely explain the association with institutional care. Analysis revealed that duration of deprivation was the driving factor (Kreppner et al., 2001).

1.5.3 Postnatal social adversity as an environmental risk for IOI/ADHD: Evidence from the wider literature

Extreme adversity in early life represents, perhaps, the strongest socio-environmental indicator of ADHD-type problems. Some of the most compelling evidence probably comes from our own ERA study, but there is also evidence from other studies on early institutional rearing and exposure to early stress (Briscoe-Smith & Hinshaw, 2006; Gunnar & van Dulmen, 2007; Roy et al., 2000). The early psycho-social environment, the stimulation provided for a child and the responsiveness, availability and consistency of caregiving all play a fundamental role in child development and, specifically, for a child's capacity to self-regulate behaviour and emotions (Carlson et al., 2003). It follows that major disturbances to the early environment may impact on a child's capacity for normal development.

1.5.3.1 Institutional rearing and the risk for IOI

Inattention/overactivity/impulsivity, the cluster of behavioural problems that form the diagnostic core of ADHD, are common clinical characteristics of institutionally

reared children (Goldfarb, 1945; Fisher et al., 1997; Gunnar & van Dulmen, 2007; Roy et al., 2000; Roy et al., 2004; Tizard & Hodges, 1978). There is evidence to suggest that the increased rates of behavioural and emotional disturbance, in particular IOI, associated with institutional rearing could not be explained by biological background or preinstitutional experience (Roy et al., 2000) Moreover, it has been suggested that the increased levels of IOI were a function of the lack of individualised care, high staff turnover and formalised group rearing which are all characteristic of institutional rearing (Roy et al., 2000). This situation would result in caregiving that was less sensitive and responsive to individual children's needs and, therefore, limit the amount of 'response-contingent' stimulation (i.e. when a stimulus consistently follows the child's response) received. Gunnar and colleagues (2007) suggest that this form of stimulation is an integral part of normal postnatal brain development. Moreover, they suggest that the type of behaviours associated with institutional deprivation may be particularly affected by a lack of 'response-contingent' stimulation, with effects on the prefrontal cortex region which is important for attentional processes. However, teasing apart which aspects of early institutional deprivation are detrimental to development is problematic to investigate experimentally for obvious ethical reasons. For example, allocating children to different experimental groups where they are subjected to specific aspects of deprivation such as nutritional, psycho-social or physical deprivation, would not be ethically sound. A recent study attempted to provide some insight by using an experimental design that manipulated the number of staff providing care for each child in a Romanian institutional setting (Smyke et al., 2002). They reported that those children in 'standard care', who were looked after in large groups by around 20 rotating staff (usually 3 staff to 30 children per shift), had elevated rates of disordered attachment compared with a group of noninstitutionalised children and also compared with a group of children cared for in smaller 'pilot' units (10-12 children) by a reduced pool of consistent carers. Reducing the pool of prospective caregivers from 20 to around 4 staff, enabled increased opportunities for the children to form selective attachment (Smyke et al., 2002).

Gunnar et al.'s (2007) research on the behavioural problems of internationally adopted children reported that children reared in institutions prior to their adoption, particularly those from Russia/Eastern Europe, were at a significantly increased risk of attention problems than comparison children, raised

in foster care prior to adoption (Gunnar & van Dulmen, 2007). Their findings suggested that the early institutional experience was not associated with a generalised risk for behaviour problems, but related to a limited set of attentional and social problems. This highlights the need to focus on specific outcomes, and not measures of total behavioural problems when investigating the effects of early adversity.

A recent meta-analysis found internationally adopted children had higher rates of externalising problems than their non-adopted peers, with larger effect sizes for those children who had experienced preadoption adversity (Juffer & van IJzendoorn, 2005). Although both of these studies make an important contribution to the literature on early adversity and international adoption, they differ from the ERA study in important ways. The meta-analysis lacked the specific examination of the effects of institutional rearing (Juffer & van IJzendoorn, 2005), and the study by Gunnar et al. (2007) was cross-sectional and, therefore, lacked the capacity to examine intra-individual change. However, the available evidence does indicate that despite exposure to such adverse environments the majority of adoptees were remarkably well adjusted after being placed in their postadoption families (for review, see MacLean, 2003). The literature also suggests that older age at adoption was a strong predictor of later behavioural problems, and that behaviour problems are fairly stable and do not dissipate over time (Gunnar & van Dulmen, 2007).

Together, the findings from a range of studies across a range of samples and methodologies indicate that early adverse rearing experiences in institutional environments may be an especially potent postnatal risk factor for the development of ADHD-type problems, but the effects are probabilistic rather than deterministic.

1.5.3.2 Adverse family environment

Research on postnatal social adversity risk factors, such as abuse and neglect, experienced by some children in the wider population may hold some relevance for the developmental course of the ERA participants. A study of females with ADHD found that individuals with the disorder had a significantly increased likelihood of having a history of physical and/or sexual abuse (Briscoe-Smith &

Hinshaw, 2006). This subgroup of previously abused ADHD children displayed higher rates of aggressive behaviour than non-abused ADHD cases, suggesting that abuse may influence the developmental correlates of ADHD. Similarly, maternal depression in combination with ADHD disorder shows evidence of increasing the risk for the development of conduct problems (Chronis et al., 2007). Early childhood abuse has also been associated with increased activity levels in a separate study, however, the findings suggested that the link may be mediated by the presence of posttraumatic stress disorder (Glod & Teicher, 1996). These studies highlight the differential pattern and course of ADHD that may be a function of the specific environmental risks that were present, which has implications for the study of ADHD presentation and how aetiologies are viewed.

With respect to the effect of parenting on ADHD, the association with harsh discipline and parental sensitivity (Seipp & Johnston, 2005) appears to be mediated by child effects rather than directly driving the onset of the disorder (Belsky et al., 2007). Although unlikely to be primary causes, one can infer from the findings reported above that parenting and abuse history may be important in the developmental course of ADHD (Chronis et al., 2007; Sonuga-Barke, 2008).

Research on the risk for ADHD associated with adverse experiences is limited. These factors are often overlooked, possibly due to the high heritability estimates for ADHD and the body of evidence suggesting a largely neurobiological aetiology (Sonuga-Barke, 2008). Moreover, the likelihood of multiple overlapping adversities and the complex pathways to disorders means that disentangling effects is difficult. For example the high level of familial ADHD is likely to impact on parenting styles and parental sensitivity, which in turn may influence the development of ADHD behaviour. Further study is needed to investigate: (i) the possible exacerbating role that early adversity may have in the risk for ADHD and, (ii) to examine how genetic factors may moderate the impact of adverse social environments. Such research should aim to provide insight into the neurobiological mechanisms by which these processes may operate. The current study seeks to contribute and advance the scientific knowledge in this area by looking at the role of genetic factors on the risk for IOI associated with early institutional deprivation. By discerning the separate and combined effects of genetic risk and early adversity it should contribute to our understanding of the risk pathways to the development of IOI and ADHD more broadly.

1.6 Summary of chapter 1

In summary, the current chapter highlights four main issues that correspond to the goals of the thesis:

The ERA study provides compelling evidence that extended institutional deprivation constitutes a significant environmental risk for the development of IOI behaviours in childhood that persist into early adolescence. Other studies of internationally adopted, early deprived and/or institution-reared samples of children corroborate these findings. The first goal of the present study is to determine if risk associated with deprivation persists to influence IOI outcome in mid-adolescence, using the data from the age 15 assessment wave.

The adverse effects of deprivation on outcome are not deterministic, suggesting other factors are influential in the development of IOI. The current thesis aims to investigate whether genetic factors could influence an individual's susceptibility to the adverse effects of early deprivation (potential mechanisms are discussed in chapter 3). Moreover, several early adversity risk factors have been implicated in wider literature on the aetiology of ADHD-type behaviours. However, the evidence suggests that there are multiple risk factors of small effect, with complex pathways from risk to disorder. This evidence highlights the need to consider the moderating effects of factors such as genetic makeup.

The overlap between IOI and disinhibited attachment represents an area where the phenotype of deprivation related IOI may differ from that seen in the wider population. The current thesis aims to extend previous analyses of this overlap by including the ERA data from the mid-adolescent assessment wave.

This chapter has touched upon the issue that IOI behaviours form part of the constellation of features that make up ADHD, but that they may have a different meaning in relation to early institutional deprivation. This thesis aims to investigate the presentation and associated features of the deprivation-related IOI phenotype in comparison with that seen in relation to ADHD. The rationale and background to this investigation is outlined in the subsequent chapter.

CHAPTER 2: INTRODUCTION

A GENERAL REVIEW OF THE INATTENTION/OVERACTIVITY/IMPULSIVITY PHENOTYPE AND ITS RELATION TO ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

2.1 Chapter outline

In the second introductory chapter a review the extensive literature on ADHD and IOI will be presented. It is necessary to define what is meant by ADHD (in the wider population) in order to compare and contrast its pattern to that of IOI as a specific outcome of early deprivation, given the differences in aetiological background. The previous chapter addressed the topic specifically in relation to IOI and early adversity. The current chapter addresses the presentation and underlying mechanisms more broadly and aims to provide the background for the subsequent systematic examination of the commonalities and differences of ADHD and deprivation-related IOI. Moreover, these analyses and the discussion presented in the current chapter set the framework for the investigation of the potential role that genetic factors may play in moderating the risk for IOI in the ERA sample. The hypothesised mechanisms underlying the genetic influence are derived from the literature on the pathophysiological and neuropsychological processes implicated in the literature on ADHD. Moreover, the selection of candidate genes for the current study research was largely based on the catecholamine model of dysfunction involved in the neurobiology of ADHD, outlined below in section 2.2.2 (Sonuga-Barke, 2008; Pliszka, 2005).

The chapter includes: First, a review of the literature on the nature of nondeprivation-related ADHD as a psychiatric disorder, its underlying pathophysiology, putative neuropsychological mechanisms, presentation and associations, developmental course, and its treatment. Second, the hypothesised mechanisms, grounded in the literature on ADHD, are presented in relation to current investigation of IOI following early institutional deprivation. Third, the specific aims of the first empirical chapter to characterise the IOI phenotype in terms of its associated features, its continuity and its persistence, will be set out.

2.2 Characterising inattention/overactivity/impulsivity and attention-deficit hyperactivity disorder

2.2.1 ADHD as a psychiatric disorder: Theoretical perspectives

ADHD as a diagnostic disorder has largely been considered as a stable and unitary neurological condition fitting within the classic disease model. This model assumes that ADHD is a categorical outcome, rather than being part of a continuum of behaviour, and that 'cases' are qualitatively different from 'normal' individuals. This has lead to much of the research and clinical practice being focused on the idea of a fixed, core neuropathological dysfunction as a defining aetiological feature operating through cognitive dysfunction to affect behavioural outcome (Sonuga-Barke, 2008). Although it is outside the scope of the current thesis to explore this in detail, the validity of ADHD as a unitary disorder and whether it is best conceptualised as a continuum or a category, has been the subject of much research in itself (Fergusson & Horwood, 1995; Hinshaw, 1987). Indeed, Meehl's work on "the taxonic question" is very informative in this regard (Meehl, 1992; Meehl, 2004). The assumption with a disorder category is that affected individuals differ from normal individuals by "kind rather than degree", and that one can make qualitative differentiations between "types" of disorder. However, Meehl highlights the need to consider both the underlying latent structure of a disorder (reflecting the interplay between genetic and environmental risk factors, neuropsychological pathology and impairment) as well as the manifest symptoms (i.e. inattention, overactivity and impulsivity in the case of ADHD).

As research in the field progresses the disorder is increasingly being recognised from a developmental lifespan perspective (Sonuga-Barke, 2008). This perspective acknowledges that although ADHD typically affects school age children, the disorder and its manifest symptoms have a heterogeneous developmental course. Moreover, ADHD has a dynamic pattern of psychiatric comorbidity, impairment and treatment response that spans from infancy into adult life, with complex underlying aetiological interactions between genetic and environmental risk factors (Taylor, 1998). This perspective fits into a bio-psycho-social model that considers ADHD as the extreme end of a continuum of normal variation in the core symptoms of inattention, overactivity and impulsivity (Sonuga-Barke, 2008). That is, the relationship between normality and ADHD pathology is better described by a dimension than a categorical distinction with fixed

boundaries. The putative aetiological mechanisms integrated into this model recognise the heterogeneous nature of ADHD deficits and associated features and, thus, encompass the possibility of multiple complex pathways from risk to disorder. This is in contrast to the traditional model of ADHD that is underpinned by the assumption of dysfunction within the individual and focuses on single core deficits, largely in executive function or motivational processes (Sonuga-Barke, 1994; 2005). Models of underlying dysfunction will be discussed in more detail below in the section on pathophysiology and neuropsychological mechanisms.

The assumption behind both the disease model and the bio-psycho-social model is that ADHD is a biological disorder (Sonuga-Barke, 2008). There has been considerable debate over whether ADHD should instead be seen as a cultural construct stemming from socio-cultural factors in western society (predominantly the U.S.A.), and the way symptoms are interpreted and valued within that context. However, evidence of ADHD, as it is currently conceptualised, has been found in countries outside of America and the Western world, suggesting that the disorder concept can be applied to different cultural contexts (Rohde et al., 2005; Faraone et al., 2003). Moreover, much of the controversy centres on the use of pharmaceuticals (for which the market is very lucrative) to treat “challenging” children. Although it is outside the scope of the current study to engage in this debate more fully, it does raise important points that should be acknowledged when considering the overall validity of the ADHD concept.

The current thesis employs the bio-psycho-social model of disorder and the lifespan developmental approach (Sonuga-Barke, 2008) with respect to deprivation-related IOI by looking at the longitudinal continuity in symptoms and impairment, its associated features, and the impact of the interplay between risk factors across the course of the study period.

2.2.2 Pathophysiology of ADHD and associated neuro-psychological mechanisms

Investigations into the underlying neuropathophysiology of ADHD have provided strong evidence of structural abnormalities and alterations in brain functioning associated with the disorder (e.g. Castellanos et al., 2002). Although the specific neurobiological mechanistic pathways to ADHD are still not completely

understood, alterations in the dopaminergic and noradrenergic systems are thought to be involved (Swanson et al., 2007; Pliszka, 2005). The dominant theoretical models of underlying neuropsychological deficits are centred around two separate domains of functioning; i) executive function deficits within the domain of cognitive processing; ii) delay aversion within the motivational and energetic domain of functioning (Sonuga-Barke et al., 2008; Sonuga-Barke, 2008).

2.2.2.1 Pathophysiology

Structural neuroimaging studies indicate that individuals with ADHD have significantly smaller brain volumes than age and sex matched controls (Castellanos et al., 2002). Total and regional grey matter volumes are reduced, with the most consistent findings relating to alterations within the dorsolateral prefrontal cortex, caudate, pallidum, corpus callosum (white matter tract), and cerebellum regions. However, further systematic study is needed to disentangle the potentially mediating influence of medication, gender, comorbid disorders, pre and perinatal factors and familial risk on the structural effects found (Seidman et al., 2005).

The neurotransmitters most widely linked to the underlying biochemistry of ADHD are dopamine and norepineprine, which belong to the catecholamine family (Pliszka, 2005). However, the picture is far from clear, and the neurochemical complexity of the disorder is acknowledged in the literature, with simple core deficits in either system unlikely to account for ADHD symptomatology (Pliszka, 2005). The dominant catecholamine dysfunction model in ADHD is based largely on the research into the pharmacological treatment of the disorder using dopamine and norepineprine agonists (e.g. stimulants such as methylphenidate and amphetamine) and their efficacy in reducing the symptoms of ADHD. Moreover, animal models show modulation by these neurotransmitters of executive functioning and neuropsychological processes implicated in ADHD (models discussed in more detail in the following chapter, section 3.3). The catecholamine hypothesis of dysfunction has driven much of the candidate genes approach to molecular genetic research in relation to ADHD. Much of the evidence on genetic susceptibility has arisen through association studies on the risk associated with candidate genes in the dopamine system (Faraone et al., 2005). The selection of

candidate genes in the current study of moderation of the risk for IOI following early deprivation was grounded in this hypothesis.

2.2.2.2 Neuropsychological mechanisms

The underlying neuropsychological deficits linked to ADHD pathophysiology are based on the assumption of dysfunction. Much of the research has been influenced by the classic disease model, and the idea of a common, fixed core deficit (Sonuga-Barke, 2008). In contrast, the bio-psycho-social model allows for multiple causal and developmental pathways to disorder (Sonuga-Barke, 2005). The two most widely studied neuropsychological markers of underlying pathophysiology fall under two broad (simplified) headings; i) executive function deficits in cognitive processes; ii) motivational dysfunction in the form of delay aversion (Sonuga-Barke et al., 2008; Sonuga-Barke, 2008).

Executive function deficits in cognitive processes

The concept of executive function refers to higher-order neurocognitive processes that maintain and manage appropriate information and problem solving sets in order to achieve a future cognitive goal (Welsh & Pennington, 1988). Fronto-striatal brain circuits have been implicated in this regard (Dickstein et al., 2006). The model of underlying cognitive dysfunction in relation to ADHD is based on the hypothesis that the operative causal pathway to disorder symptoms is through a primary deficit in either a specific domain of cognitive control, such as response inhibition, working memory, or more generalised problems (Willcutt et al., 2005; Barkley, 1997). Significant associations have been found between ADHD and impairment at a group level on a range of tasks thought to tap executive function processes, including response inhibition, vigilance, working memory, and planning (see Willcutt et al., 2005 for meta-analysis). However, questions have been raised about the specificity of effects for several reasons and these have been reviewed in a recent paper by Sonuga-Barke and colleagues (2008): First, executive function deficits are not a necessary feature of ADHD, as many diagnosed individuals do not show weaknesses in cognitive control and some children without disorder exhibit deficits; second, the substantial association between ADHD and lowered IQ may indicate that the relationship with executive dysfunction might be better described by impairment in more basic cognitive processes; third, deficits in executive function are observed in relation to other disorders, such as conduct

problems and high-functioning autism which also exhibit high comorbidity with ADHD (Geurts et al., 2004). This neuropsychological heterogeneity lead Sonuga-Barke and colleagues (2008) to suggest that executive function deficits most likely interact with other risk factors in the causal pathways to ADHD, and raise the possibility that executive dysfunction may be a gateway problem that exposes children to the risk for multiple disorders.

Deficits in motivational processes: delay aversion

The underlying neuropsychological deficits of ADHD have not been fully explained by executive dysfunction, therefore, attention has been given to alternative or dual pathway models (Sonuga-Barke, 2005). Motivational dysfunction has been proposed as a possible domain of deficit, with the delay aversion hypothesis showing the most promise (Sonuga-Barke et al., 1992). Delay aversion has been described as part of a broad-based motivation framework, one expression of which is the preference of children with ADHD for immediate, over delayed, rewards (Sonuga-Barke et al., 2008). Several processes have been suggested in models of motivational dysfunction, including deficits in signalling future rewards and strong negative affect associated with delay which, therefore, motivates children with ADHD to avoid it where possible and exhibit a preference for immediacy over delay. Empirical support for the delay aversion hypothesis has been found in several areas. For example, individuals with ADHD show frustration at unexpected delay during tasks (Bitsakou et al., 2006); lower completion rate for long, challenging tasks due to premature disengagement (Scime & Norvilitis, 2006); increased activity during waiting period of a task (Antrop et al., 2000), although, the preference for immediacy over delay was reduced by including stimulation during the delay phase (Antrop et al., 2006).

The research outlined above in support of the delay aversion model alongside the evidence of intra-individual executive function variation, has highlighted the context dependent nature of ADHD associated impairment (Sonuga-Barke, 2008). It has lead to increased recognition that deficits in these domains are not a fixed or necessary feature of ADHD and to the development of more integrated models of causal mechanisms (Castellanos et al., 2006; Sonuga-Barke, 2005). These integrative frameworks emphasise complex neuropsychological and developmental pathways to disorder, and the need to

consider a dynamic pattern of interplay between causal factors and mediating and moderating processes in the risk for impaired outcome.

2.2.3 ADHD: Presentation and associations

This section will focus on the presentation of ADHD in the nondeprived population and its associated features and impairments. Emphasis is on four prominent features, typically associated with ADHD: i) the developmental link and overlap with conduct problems; ii) low IQ; iii) executive function deficits; iv) the gender discrepancy/prevalence amongst males. These features were chosen because they have been the subject of a substantial amount of empirical testing in relation to ADHD in the literature, and are arguably the most characteristic features of the ADHD phenotype. Moreover, the association with these features was investigated in our recently published paper (Stevens et al., 2008), which showed promising but somewhat inconclusive results, requiring further investigation. It is important to note that these four associated features are analysed in the empirical section (chapter 6) in relation to IOI in the ERA sample, in order to examine the similarities and differences between deprivation and nondeprivation related IOI. Other salient features of the ADHD phenotype will be also discussed below, albeit only briefly, as it is beyond the scope of the current thesis to cover all aspects in detail.

2.2.3.1 Heterogeneity

ADHD is a disorder with considerable heterogeneity and individuals may vary in terms of their severity, symptomatology and/or comorbidities. Multiple causal pathways and aetiological heterogeneity have also been implicated with respect to ADHD (Sonuga-Barke, 2008). Such heterogeneity raises questions about the internal validity of the disorder, and where the boundaries should be drawn between ADHD and other co-existing disorders and also between ADHD pathology and normality. As noted above, the DSM-IV-TR and ICD-10 diagnostic frameworks are currently being revised and aim to address some of the issues surrounding variation in presentation and association.

2.2.3.2 Comorbidity overview

Comorbidity with other psychiatric disorders represents a key clinical feature of ADHD. In childhood, the range of co-existing disorders include: Oppositional defiant and conduct externalising disorders; mood disorders; anxiety and depression; learning and developmental disorders including autism; motor disorders, such as Tourette syndrome (Pliszka, 1998; Spencer et al., 1998). The most commonly reported comorbidity relates to the externalising disorders, conduct and oppositional defiance, and this overlap will be considered in detail in the current thesis.

Conduct problems

Nondeprivation-related ADHD/IOI and conduct problems often co-occur, with highly correlated symptomatologies. Studies of clinic and population-derived samples of children and adolescents have found a high rate of ADHD cases comorbid with conduct disorder (CD) or oppositional defiant disorder (ODD); in the region of 40-90% (Jensen et al., 1997). This pattern of comorbidity is a common and pervasive long-term adverse outcome with strong homotypic continuity over time (Willcutt et al., 1999; Burke et al., 2005). A topic of considerable debate is whether ADHD comorbid with CD may represent a distinct familial subtype, characterised by more severe ADHD symptoms (Christiansen et al., 2008). However, despite evidence for a shared set of genetic risk factors, research has supported the distinction of these two domains of dysfunction (Thapar et al., 2001).

Developmental studies have suggested that the presence of early ADHD may predict the occurrence of ODD and subsequent CD, but ODD does not predict the later emergence of ADHD (Burke et al., 2005; Taylor et al., 1996). In addition to genetic influences, it is plausible that the similar set of environmental risk factors such as pre and perinatal adversity, and psychosocial/family risk, associated with ADHD and conduct/oppositional problems could help to account for the progression from one condition to the other (Thapar et al., 2006). The findings from the ERA study at ages 6 and 11 suggested that conduct problems were not a specific outcome of the deprivation experience (i.e. related to dose of deprivation) (Colvert et al., 2008), but the literature on comorbidity with IOI in deprived samples is limited. There has been mixed evidence from other samples

of postinstitutionalised children as to whether increases in levels of conduct and oppositional problems are observed (Gunnar & van Dulmen, 2007). In the recently published ERA study paper considerable contemporaneous overlap between deprivation-related IOI and conduct problems at ages 6 and 11 was reported, in addition to a complicated reciprocal pattern of developmental trajectories between the two outcome domains (Stevens et al., 2008). That is, in line with the literature on IOI in nondeprived groups of children (Burke et al., 2005), IOI in the Romanian institution reared sample was found to be a developmental precursor to later conduct problems, according to parent, but not teacher, reports. However, there was also some evidence from parent reports that early conduct problems lead to later IOI, a finding not supported in the developmental literature on ADHD. Teacher reports showed no developmental pathway from early IOI to later conduct problems. The current study aimed to build on this research by examining a more complete developmental picture using data from three assessment waves spanning from childhood to mid-adolescence. Moreover, the analysis presented in the empirical section will focus solely on the group of children who experienced at least 6 months of institutional rearing, with the aim of providing more clarity on the overlap of the two domains in relation to extended deprivation experience. This group of children was chosen based on the reported stepwise increase in risk associated with institutional deprivation at this level of exposure (Stevens et al., 2008; Kreppner et al., 2007)

Comorbid developmental disorder: Autism

ADHD and autism spectrum disorder (ASD) represent distinct nosological diagnoses, but ADHD-type symptoms are frequently observed in individuals with a diagnosis of ASD, thus the two conditions frequently co-occur. This overlap may hold particular relevance for the ERA study where autistic-like patterns have featured as a specific domain of impairment associated with institutional deprivation experience (Rutter et al., 1999; Rutter et al., 2007b). This overlap will be an important area of future study, both phenotypically and genetically, but will not feature in the analyses of the current thesis. In order to provide a comprehensive and detailed analysis, a limit had to be imposed on the number of potentially associated features included in the current investigation. Owing to the considerable overlap between IOI and disinhibited attachment that has been previously reported in the ERA publications, this feature took precedence

alongside the association with other salient features of the ADHD phenotype found in the wider population.

Although there appear to be commonalities between ADHD and ASD in terms of executive function deficit the disorders are divergent in the form that it takes. Theory of mind deficits are a prominent feature of the autistic phenotype, whereas motivational abnormalities and inhibitory dysfunction characterise the executive dysfunction associated with ADHD (Banaschewski et al., 2005). Moreover, the associated structural brain alterations vary between disorders (Brieber et al., 2007). ADHD is associated with reductions in brain size, unlike the increased brain volume seen with autism (Ellison-Wright et al., 2008; Stanfield et al., 2008). Similar candidate genetic regions have been implicated in the aetiology of both disorders, but the meaning of common genetic influences is not yet understood (Faraone et al., 2005).

2.2.3.3 Cognitive impairment: Low IQ

The second prominent feature of ADHD to be discussed, and subsequently analysed, in relation to deprivation-related IOI in the current study is the negative association between IQ and ADHD symptoms consistently reported in the literature. There is typically a correlation of around -.3 between ADHD symptom scores or diagnosis and IQ (Kuntsi et al., 2004) representing a deficit of between 9 and 13 IQ points (Frazier et al., 2004; Rucklidge & Tannock, 2001; Crosbie & Schachar, 2001) compared with normal controls. The nature of this association is open to several interpretations, and it is unclear from the literature whether the cognitive deficit associated with ADHD corresponds to a mild global deficit, or impairment in multiple specific areas that affect different aspects of cognitive functioning (Frazier et al., 2004). Goodman et al. (1995) theorised that nondeprivation-related IOI behaviour may interfere with learning success or performance on IQ tests or, perhaps, that low IQ increases the risk for IOI via its association with reduced self-esteem. Low IQ and IOI could also be "markers" of some common, underlying risk factor or factors such as variations in brain development, individual genetic makeup or shared environmental adversity (Goodman et al., 1995; Kuntsi et al., 2004).

As noted in the previous chapter, the analysis of the age 4 and 6 year-old ERA study data demonstrated that IQ did not constitute a significant mediator of the association between early deprivation and IOI. Moreover, in the recent paper on the age 11 assessment wave (Stevens et al., 2008) it was reported that the institution-reared study group had substantially depressed IQ scores compared with population norms, irrespective of IOI impairment. This makes the examination of the overlap between IOI and IQ following extended institutional-rearing complex. It is likely that the persistent association between IQ and duration of deprivation will affect the current study's analysis of the age 15 IOI data as well.

2.2.3.4 Executive function deficits

One of the dominant models of the psychopathophysiology of ADHD has focused on the role of executive dysfunctions, involving multi-faceted deficits in higher-order neurocognitive processes, such as working memory, response inhibition and interference control, which maintain and manage appropriate information and problem solving sets in order to achieve a future cognitive goal (Castellanos et al., 2006). A recent meta-analysis by Willcutt and colleagues (2005) demonstrated significant case-control differences, with medium effect sizes (d=.4 to .6) in several key domains. These included response inhibition, vigilance, spatial working memory and some planning tasks. Noteably, the effects were independent of IQ, academic attainment or comorbid disorders. However, there is substantial variability within, and between, ADHD samples, suggesting that neuropsychologically, ADHD is a heterogeneous disorder and a broader definition of the domains of psychopathological impairment associated with the diagnosis of ADHD should be considered (Sonuga-Barke et al., 2008; Doyle, 2006).

With respect to the findings in the ERA study, at age 11 there was an association between deprivation-related IOI and executive dysfunction in relation to interference control, measured on the Stroop Color-Word Interference Test (Stroop, 1935; Stevens et al., 2008) There was some indication that working memory, measured using a backward digit span task, was also negatively associated with IOI impairment, although the association fell short of statistical significance (Stevens et al., 2008). Unfortunately, the Stroop Test was not administered during the age 15 assessment wave, hence our measure of executive functioning is limited to the backward digit span in the current analysis on the association with deprivation-related IOI, presented in chapter 6.

2.2.3.5 Gender discrepancy

Although the picture is far from clear regarding the causes of gender differences in ADHD, the discrepancy in prevalence rates is undisputed, with ratios of girls to boys reported to be between 1:2 and 1:9 (Youth in Mind, 2001; Biederman et al., 2002; Heptinstall & Taylor, 2002). Girls with ADHD appear to be less at risk for comorbid externalising problems than their male counterparts. This is likely to influence referral to services and, thus, the high rate of gender discrepancy seen in clinic referred samples, because coexisting disruptive behaviour often drives parents to seek help. The gender discrepancy may indicate that ADHD in females is under-recognised and, therefore, under-diagnosed and treated. While there may be a degree of rater bias, this cannot explain the phenomenon fully (Maniadaki et al., 2005). Girls may be more resilient in relation to risks for the development of ADHD and differences in cognitive impairment, comorbid behaviour problems and some discrepancies in symptomatology have been noted (Heptinstall & Taylor, 2002). It is certainly true that ADHD in girls is under-researched, as the majority of literature has focused on male samples, with studies of female ADHD emerging only fairly recently (Arnold, 1996; Gaub & Carlson, 1997; Newcorn et al., 2001).

In contrast to the gender discrepancy seen in population and clinical samples, in the ERA institution-reared (IR) sample at age 6 there was a fairly even distribution of deprivation-related IOI across boys and girls (Kreppner et al., 2001). One possible reason for this is that early institutional deprivation is a particularly potent risk factor for female IOI that combines with other risks in a way that pushes certain girls over their "risk threshold". However, by early adolescence a sex difference in the prevalence of IOI impairment emerged in the group who experienced at least 6 months institutional deprivation, in the same direction but of a smaller magnitude than that seen in clinical and population studies. The ratio of girls to boys with persistent, early onset IOI at age 11 years was 1:1.6 (Stevens et al., 2008). This perhaps reflected a developmental process, whereby the influence of more general risk factors for IOI, other than those specifically related to the deprivation experience, increases as the child moves further away in time from the institutional exposure. If this were the case, then one would expect the gender imbalance to have increased by mid-adolescence.

A significant strength of the present study is that the data from the age 15 assessment wave are incorporated which will allow for better analysis of the

developmental course of IOI in the ERA sample. In particular, this will allow for the examination of whether the shift towards a pattern of discrepancy between the sexes was a transient phenomenon, or if it indeed represented a more stable 'real' move towards a phenotype similar to that seen in epidemiological and clinical samples.

2.2.4 ADHD throughout the lifespan: Overview

As noted above, the presentation of ADHD symptoms and associated features may change over the life span, but the assumption here is that the construct remains valid (Sonuga-Barke, 2008). Research has been conducted into the existence and validity of the disorder not only in the school age period, where it is most widely recognised, but also in the preschool period and into adult life (e.g. Kooij et al., 2005; Lahey et al., 2006). Individuals of different ages may be affected equally by symptoms but it is not clear whether impulsive behaviours, for example, will have the a similar significance or impact on social functioning for an individual who is 4 years of age compared with an adult. Moreover, when considering ADHD across the lifespan, issues of continuity and discontinuity throughout development need to be addressed (Sonuga-Barke, 2008). Longitudinal studies have begun to deal with some of the issues in terms of the continuity in presence and expression from one developmental stage to the next (e.g. Taylor et al., 1996). However, further descriptive work is needed on the degree of continuity over the lifespan as a whole, underlying causal mechanisms across development, moderating risk factors and the association with broader developmental psychopathology (e.g. the relationship between disruptive behaviour and negative life events) (Sonuga-Barke, 2008). Furthermore, questions are raised regarding the accurate detection and diagnosis of problems across the lifespan using the current DSM criteria, which are designed primarily for use with school age children along with the appropriate treatment of symptoms. The following sections set out some of the issues relating to ADHD in the preschool and adulthood years.

2.2.4.1 ADHD in the preschool years

ADHD in its current diagnostic form is probably not applicable to children under the age of 3 years (Sonuga-Barke, 2008). However, early predictors of ADHD and

other externalising type disorders are an important research initiative given the later impairment and level of social and academic dysfunction associated with disorder and the potential for effective early therapeutic interventions. Further research and work is needed to facilitate a full developmental account of the early neuropsychological precursors to ADHD, and the pathways from early emotional regulation and reactivity to later self regulation (Nigg, 2005). However, the research that has been conducted in this regard has implicated several predictive factors including; neurodevelopmental immaturity and state organisation difficulties (Auerbach et al., 2005), mildly abnormal movement in the neonatal period, involving a lack of fluency (Hadders-Algra & Groothuis, 1999) and severe sleep disturbances (Thunstrom, 2002). Moreover, early child temperament and the quality of parent-child relationships and attachment may influence subsequent behavioural problems, such as ADHD (Burgess et al., 2003; Hirshfeld-Becker et al., 2002). The putative association between attachment and ADHD has relevance to the current study and was discussed in more detail in the section 1.4.2.

With respect to the nondeprived population, from around the age of 3-4 years the manifestation of symptoms reaches a stage that is more recognisable in terms of an ADHD-type profile. The ADHD diagnosis shows validity for boys and girls (Hartung et al., 2002) and patterns of comorbidity, like those seen at later ages, begin to emerge. This period often involves a transition from family settings into nursery and school environments that may prove to be more difficult for children with deficits in attention and impulse control. Preschool children with ADHD appear to show specific problems in inhibitory control (Sonuga-Barke et al., 2002). There is evidence of modest stability of symptoms into middle childhood, with attentional and inhibitory deficits showing significant predictive power (von Stauffenberg & Campbell, 2007; Lahey et al., 2006). It is possible to distinguish the symptoms from generalised difficulties in manageability, and distinctions can be drawn between ADHD-type symptoms and other behavioural problems such as poor social and emotional adjustment (Sonuga-Barke et al., 1997).

Research on the developmental context for preschool ADHD places a particular focus on family factors, including the parent–child communication and interaction, parental coping (managing child and dealing with problem behaviour) and the quality of the child-care environment, in terms of stimulating and sensitive care within the family and external child-care providers (Allhusen et al., 2005;

Keown & Woodward, 2002). These studies report evidence of early child rearing experiences and family interaction influencing the development of inattention and hyperactivity. Moreover, child attentional control may also mediate the relationship between early maternal sensitivity on later externalising problems, illustrating further the dynamic and complex causal mechanisms that are implicated in ADHD aetiology (Belsky et al., 2007).

2.2.4.2 ADHD in adulthood

ADHD is increasingly being recognised as a persistent domain of impairment that reaches, in some form, into adult life. It is being picked up in primary care and adult psychiatric services, but further research is needed into adolescent ADHD, the transition into adult life and how to manage individuals presenting with ADHD symptoms for the first time in adulthood, given that the disorder is largely thought to be one with an early childhood onset (Nutt et al., 2007). Cross sectional epidemiological studies put the prevalence rate of ADHD in adulthood at around 2-4% (Kessler et al., 2006; Kooij et al., 2005) and follow-up studies of children with ADHD indicate that the disorder persists into adulthood for 10-50% of cases (Weiss et al., 1985; Biederman et al., 1993). These follow-up studies highlight the drop off in prevalence, suggesting that a substantial proportion of childhood and adolescent ADHD cases must no longer meet the diagnostic cut-off for the disorder once they reach adulthood. Despite the age-dependent decline in symptoms and lower rates of diagnosed disorder, residual symptoms are frequently associated with clinically significant impairment (Biederman & Faraone, 2005). This provides further support for applying a developmental framework when conducting research on ADHD (Sonuga-Barke, 2008).

By taking a developmental lifespan perspective in the current study, one can build on the existing body of research by looking at the presentation and associations of IOI in the ERA sample over the longitudinal study period, and contrasting that with what has been described in the wider literature on nondeprivation-related IOI and ADHD. Examining these factors across samples and risk environments adds to the overall understanding of ADHD as a disorder concept with multiple risk pathways and a heterogenous presentation.

2.2.5 ADHD: Treatment

The most common forms of treatment for ADHD are behaviour therapy and/or medication. Psychostimulants (e.g. methylphenidate and dexamphetamine) are widely used, and have been found to be effective in reducing symptoms. However, careful attention to monitoring, dosage and managing adverse side-effects may be required. Stimulants are thought to act by blocking the reuptake of catecholamines (e.g. dopamine, norepinephrine) at the neuronal presynapse, thus preventing them from being broken down by monoamine oxidase (Spencer et al., 2000a). More recently, atomoxetine has been introduced as a pharmacological treatment for ADHD. This drug acts by inhibiting the norepinephrine transporter thereby raising the synaptic levels of both dopamine (in the PFC) and norepinephrine (Taylor & Sonuga-Barke, 2008). How broadly to identify, classify and treat such problems is open to interpretation, and for this reason the widespread use of pharmacotherapy to treat childhood behaviour problems, such as ADHD, has been the subject of much controversy. It is beyond the scope of the current thesis to examine the advantages and disadvantages of different treatments and how and when they should be applied. The work of the Multimodal Treatment study of ADHD (MTA) has been empirically investigating the relative and combined effects of different treatment programmes (Swanson et al., 2008a; Swanson et al., 2008b).

The aspect of pharmacotherapy that is of relevance to the current study is the link to molecular genetic research. Along with evidence from neuroimaging and animal research, the known response of ADHD symptoms to stimulants and other medications which act on specific neurotransmitter pathways, has helped to inform molecular genetic research as to likely candidate genes associated with ADHD in the population. It, therefore, seems important that advances in the various research domains should feed back into one another. For example, by advancing the neuroscience of ADHD it may enable specific neuropsychological treatment programmes to be developed, and by advancing the molecular genetics of ADHD this may, in turn, inform pharmacological research and the development of new drug treatments.

2.3 Underlying mechanisms

The literature suggests that nondeprivation-related IOI, and its diagnostic corollary, ADHD, are heterogenous, multifactorial conditions with complex causal pathways consisting of multiple risk factors of small effect (Coghill et al., 2005; Asherson et al., 2005). Genetic factors have been regarded as highly influential in the aetiology of ADHD and will be discussed in detail in the following chapter.

The evidence suggests there are multiple pre and perinatal risk factors of small effect. The process by which these factors influence developmental outcome is difficult to disentangle from the context in which they operate. That is to say, each factor operates within the wider context of maternal and paternal mental health and the rearing environment they provide, the family's socio-economic status, lifestyle and other potentially influential contextual factors. Determining the neurobiological mechanisms by which environmental risk factors operate should be a major goal of future research in the area. Recent research by Mill et al. (in press) has begun to address these issues with their work on the mediating role of epigenetics in environmental risk mechanisms.

Although it may be hard to disentangle the adverse effects of environmental factors from genetically influenced effects of correlated parental behaviour operating through gene-environment correlations and the potentially mediating effects of epigenetic processes, the available evidence suggests that early life environmental pathogens are still linked to the risk for ADHD (Jaffee & Price, 2007; Mill & Petronis, in press). Where environmental risk factors have been implicated, they are, for the most part, concerned with the pre and perinatal environment (Taylor & Rogers, 2005; Banerjee et al., 2007), rather than the risk associated with the extended psycho-social deprivation experienced by the children in the ERA study, or other postnatal social factors. A discussion of these factors can be found in the previous chapter on environmental adversity.

This raises the question as to whether institutional deprivation should be seen as one (uncommon) route to a common disorder (ADHD), or whether deprivation-related IOI would be better conceptualised as a qualitatively different clinical phenotype with a distinct pathophysiology. One strategy for addressing this question scientifically involves; first, exploring whether deprivation related IOI and ADHD as seen in the wider population share a common pattern of association

features and presentation; second, by hypothesising a plausible, neurobiological mechanism by which early institutional deprivation might lead to ADHD in its normal clinical expression. Research on the neurobiological consequences of early stress provides empirically based evidence that may help to elucidate the relevant mechanisms operating in the current risk context. Exposure to early stress can have neurobiological effects on several developmental processes including neurogenesis, the multiplication and subsequent pruning of synapses, and myelination during specific, sensitive periods (Teicher et al., 2003). Furthermore, structural changes to specific brain regions are implicated following early stress, including reduced volume of the corpus callosum, neocortex, hippocampus and amygdala, and down stream functional alterations to the prefrontal cortex resultant from stress activated effects on dopamine and glucocorticoid receptor projections in the region. The prefrontal cortex in turn exerts inhibitory effects within regions that respond to subsequent stressors, and acts to limit feedback within the hypothalamic-pituitary–adrenal axis (Teicher et al., 2003).

2.3.1 Mechanistic pathways to IOI following early deprivation

The established findings presented above lead to the development of a hypothesis[2], although speculative at this stage, that relates to the patho-physiological pathway from early adverse experience to later IOI moderated by genetic factors. That is, extreme early stress modifies the developmental trajectory of associated brain structure and function via an altered neuroendrocrine response and interacting genetic factors, which then impact on later behavioural outcome. The putative biological mechanism may involve long term negative down-stream effects on neuro-transmitter branches (e.g. dopamine and norepinephrine systems; Pani et al., 2000) and brain circuits (e.g. dorsal striatum, prefrontal cortex) implicated in the patho-physiology of ADHD (Sanchez et al., 2001) of early stress-related dysregulations of the hypothalamic-pituitary–adrenal axis (Kaufman & Charney, 2001). If this were the operative pathway, then one would predict that IOI would be a persistent domain of impairment and would share many similarities with ADHD at the patho-physiological level because of the involvement of common dopamine modulated brain networks. But importantly, from the relevant literature

[2] This hypothetical mechanism was suggested in our recent paper on the age 11 findings (Stevens et al., 2008) and is developed further in the current thesis.

and the heterogeneity observed in the ERA sample, it appears that not all individuals are affected in the same way by environmental pathogens. Recent research into gene-environmental interactions in relation to psychiatric outcomes have started to elucidate some of the complex issues surrounding such variability in response and outcome (Moffitt et al., 2005; Caspi et al., 2002; Kahn et al., 2003). The investigation of the interplay between genes and environments on behavioural development will be explored in more detail in the following chapter.

2.4 Empirical aims

Before questions about the role that genetic factors may play in susceptibility to deprivation-related IOI can be explored in any detail, it is necessary to examine the persistence of IOI in the ERA sample, and the similarities in presentation and associated features between deprivation-related IOI and ADHD in the nondeprived population. The aim of the first empirical chapter (chapter 6 of the current thesis) is to address these issues using the longitudinal and cross sectional data available on IOI from childhood to mid-adolescence, and to build on the work published in the recent ERA paper on the findings from the age 11 assessment wave (Stevens et al., 2008).

CHAPTER 3: INTRODUCTION

REVIEW OF THE ROLE OF PUTATIVE GENETIC FACTORS

3.1 Chapter outline

The previous chapters have highlighted that not all children are affected in the same ways by environmental insults. The current chapter complements the aetiology sections of the previous chapters by reviewing the literature on the risk for ADHD in the nondeprived population from genetic factors, and placing it within the context of the potential role that such factors may play in accounting for the variability seen in the ERA study. The chapter is set out into several sections: First, an overview of the role of genetic factors in the risk for ADHD in the wider general population is presented; second, models of genetic mediation and moderation are discussed and theoretically applied to the ERA study; third, the specific details and rationale for gene-environment interactions being tested for in the current thesis are given; fourth, the research questions to be tested in empirical chapters 6, 7 and 8 are listed.

3.2 Background to the study of role of genetic factors

Highlighting the role of adverse early social environments, such as early institutional deprivation, may be especially important in understanding the aetiology of IOI, and its diagnostic corollary ADHD. The reason being is that individual variation in the presentation of these forms of psychopathology in the nondeprived population is thought by many to be determined in considerable part by genetic factors. This view is supported by numerous family, twin and adoption studies (for review, see Thapar et al., 2005). Furthermore, molecular genetic studies have gone some way in identifying susceptibility genes for ADHD (Faraone et al., 2005). However, the picture is far from complete, with genetic variants identified to date explaining only a small proportion of the overall genetic influence on ADHD (Asherson & IMAGE Consortium, 2004). In addition, there have been inconsistencies in the pattern of results of the association between specific candidate genes and the risk for ADHD. There are several possible reasons for this: First, like in the molecular genetic literature in general, the samples used may not have enough power to detect very small genetic effects; second, there may be

heterogeneity between the samples used in different studies (genetic, environmental, or both) that may arise through country of origin or phenotype subtype differences or where there are multiple risk pathways leading to disorder. Moreover there may be heterogeneity within the sample in terms of environmental experiences of the participants, which indicates that environmental influences need to be considered in conjunction with genetic influences. One possible explanation is that genes interact with environmental factors to increase the risk for IOI and ADHD more generally by making some children more susceptible than others to the potential biological and psychosocial environmental risk factors implicated in the aetiology of these disorders. In the current study, this possibility will be explored using the data generated by the ERA study.

3.3 Genetic factors and the risk for ADHD

As discussed in the previous introductory chapters, the underlying aetiology and pathogenesis of ADHD remains unclear, with a complex pattern of genetic and environmental risk factors thought to be involved. However, twin and adoption studies and quantitative measures of symptoms have demonstrated that most of the variation in ADHD can be attributed to genetic factors with an estimated heritability of around 0.76 (Biederman & Faraone, 2005) This has lead to molecular genetic investigations which have sought to isolate specific susceptibility gene variants that are functionally associated with ADHD. This research has been largely driven by candidate gene approaches, using association methods (case-control and family based studies), and, by a much lesser degree, linkage approaches (Mick & Faraone, 2008). In psychiatric molecular genetic research in general, candidate gene approaches investigate the association between a specific genetic polymorphism and a psychiatric trait. Linkage studies attempt to localise genes influencing a trait, by studying cosegregation of the phenotype with genetic markers across the genome, using genetically related individuals (Lander & Schork, 1994). This approach does not have the capacity to detect genes with moderate or small effects, while association studies do have this capability. Linkage studies have not been overly successful in relation to ADHD (equally true for other complex disorders), given there has been very few successfully replicated studies. So far the best evidence of linkage has been found for chromosomal regions 5p and 17p (Mick & Faraone, 2008).

Studies of the genetic aetiology of ADHD have, until fairly recently, been concerned with linkage or association with defined disorder categories. However, the more recent quantitative trait locus (QTL) approach to gene mapping is based on the hypothesis that the same genetic variants that increase susceptibility for disorder also influence continuous measures of symptom scores across the population (Asherson & IMAGE Consortium, 2004). This approach fits in with the idea, discussed in the previous chapter, of ADHD symptomatology being better conceptualised as a dimension rather than as a dichotomous, categorical outcome within the classic disease model.

The majority of studies have focused on candidate genes that regulate the dopamine, norepinephrine and serotonin neurotransmitter systems, and have linked multiple genes of small effect to the liability for ADHD. The small effect sizes make it likely that gene-gene and gene-environment interactions play an influential role (Thapar et al., 2005; Asherson & IMAGE Consortium, 2004; Comings et al., 2000).

Recent meta-analyses of available evidence suggests small, but significant, genetic effects with odds ratios in the region of 1.1 to 1.5 (Faraone et al., 2005; Yang et al., 2007; Li et al., 2006). Genetic variants associated with ADHD in three or more studies include variants of the dopamine transporter (DAT1, OR = 1.13 – 1.17), the dopamine D4 receptor (DRD4, OR = 1.16 – 1.34), the dopamine D5 receptor (DRD5, OR = 1.24), the synaptosomal-association protein 25 (SNAP-25, OR = 1.19), the serotonin transporter (SLC6A4, OR = 1.31), the serotonin 1B receptor (HTR1B, OR = 1.44) and dopamine beta-hydroxylase (DBH, OR = 1.33) genes (Faraone et al., 2005). Assuming a simple additive effect, these findings explain only a small proportion of the overall heritability for ADHD. A recent large scale screen of 51 candidate genes found evidence for an association between 18 genes and ADHD, using a clinically homogenous phenotypic sample (Brookes et al., 2006a). The significant findings included DRD4, DAT1 and SNAP-25, plus 2 other genes (NET1, MAOA), with replicated reports of association with ADHD. In addition, 5 genes were identified that had been reported to be associated with ADHD once before in the literature (CHRNA4, TPH2, SYP, FADS2 and DDC) (Brookes et al., 2006a). By using a refined phenotypic subtype (DSM-IV combined subtype ADHD, excluding cases with possible autism), this study goes some way to disentangling the issues surrounding aetiological genetic heterogeneity.

Much of the molecular genetic research has focused on the role of dopamine genes in the susceptibility for ADHD. Dopamine has been a focus of research based on the rationale of the catecholamine model of dysfunction implicated in the pathophysiology of ADHD (Pliszka, 2005) and the action within the brain of psycho-stimulant medication, used to treat ADHD. Moreover, the dopamine system is involved in the regulation of mood and movement and has been isolated as a potential candidate on the basis of neuroimaging, neuropsychological, pharmacological and animal studies (Thapar et al., 2005). Stimulant medication in the form methylphenidate or dexamphetamine is a dopamine reuptake inhibitor, and has been widely used to effectively treat ADHD. It works by blocking the pre-synaptic reuptake of dopamine, thus inhibiting the function of the dopamine transporter and increasing the availability of extracellular dopamine in the synapse (Spencer et al., 2000b; Thapar et al., 2005).

Neuroimaging studies have shown dysregulations of dopamine tone and phase, for example, higher DAT1 density has been found in ADHD cases compared with controls (Dougherty et al., 1999), furthermore DRD4 is prevalent in the pathways of the frontal subcortical region implicated in the pathophysiology of ADHD (Faraone et al., 2005). ADHD animal models have used targeted alterations of the dopamine system to validate the presumed mechansims, for example, a study using DAT1 knock-out mice found pharmacological responses and behavioural features that were similar to those seen in human ADHD cases (Gainetdinov & Caron, 2001). Furthermore, DRD4 knock-out mice show elevated synthesis and clearance of dopamine in the dorsal striatum and altered motor behaviours (Rubinstein et al., 1997). Molecular genetic studies have built on this research and begun to unravel the genetic complexity of ADHD. However, the overall effect sizes are small and there is variability between studies in the level of association between genotype and ADHD, particularly with regards to DAT1 effects (Yang et al., 2007). This suggests not only that many susceptibility genes are yet to be indentified, but that interplay between genes and environmental risk factors may play an important role that needs to be considered and investigated further.

3.3.1 Gene-environment interplay and risk for IOI

In reviewing this literature on risk factors for IOI and ADHD more generally, what is perhaps most striking is the small size of the associations that have been identified for individual risks, whether they are genetic or environmental. This makes it highly likely that, in trying to understand the aetiology of IOI, we will need to consider the combined influence of multiple genetic and environmental factors, each of small effect. Furthermore, it is very likely that to account for a significant proportion of the variation in this trait, gene by gene, gene by environment and environment by environment interactions will need to be considered.

In line with this, recently published data provide the first evidence that genes might moderate the impact of an environmental risk associated with ADHD symptoms. Kahn, Khoury, Nichols, & Lamphear (2003) have led the way for such investigations in their study of the joint effects of a dopamine transporter (DAT1) gene polymorphism, associated with ADHD, and maternal pre-natal smoking on hyperactivity-impulsivity, inattentiveness, and oppositional behaviour. They found a significant interaction between genetic and environmental factors: Only those children who carried two 10-repeat (10R) 'risk' alleles for the DAT1 polymorphism and were exposed to pre-natal smoking showed increased hyperactive-impulsive and oppositional scores. Furthermore, neither pre-natal smoking exposure, nor DAT1 10R genotype, was found to be significantly associated with increased hyperactivity scores when analysed as separate, independent risk factors. Further evidence of genetic moderation of early risk factors has been reported recently in relation to mothers' use of alcohol during pregnancy (Brookes et al., 2006b) and early psychosocial risk (Laucht et al., 2007) and the DAT1 polymorphism on risk for ADHD. These findings suggest that both genetic and environmental factors should be considered when looking at the aetiology of IOI in the population, and leads one to consider whether similar mechanisms may be influential in the risk for IOI following early deprivation. These studies are discussed in more detail in section 3.5.2 in relation to the rationale for selection of candidate genes in the current study.

The above studies represent part of a small, but growing, literature on the role of gene-environment interactions (GxE) in psychopathology (e.g. Caspi et al., 2002; Caspi et al., 2003; Eley et al., 2004; Kendler et al., 2005). GxE interactions are increasingly being recognised as playing an influential role, not just in

psychiatric impairment, but also in the wider medical domain of complex diseases (e.g. lung disease: Kleeberger & Cho, 2008; cardiovascular heart disease: Tiret, 2002; breast cancer: Chia, 2008). The GxE interaction approach to psychiatric genetics differs from the direct gene to disorder/endophenotype 'main-effect approaches' discussed above, as information about exposure to environmental risks is taken into account (Caspi & Moffitt, 2006). Instead of assuming that genes 'cause' the outcome, the GxE interaction approach assumes that the specific environmental pathogen is causal, and that genetic makeup increases an individual's vulnerability to the adverse effects of that pathogen (Caspi & Moffitt, 2006). Taken together, this research highlights both the value and the feasibility of studying GxE interactions and provide us with a plausible mechanism to explain the lack of replication, and small effect sizes, found in molecular genetic research and the heterogeneity in response to adverse environmental factors. Furthermore, they also move us toward a model of causal mechanisms in which multiple genetic and environmental risks act in concert (either additively or multiplicatively) to produce a spectrum of liability for a disorder or condition.

From such a perspective, the study of the interaction between early institutional deprivation and other environmental and genetic risk factors becomes a top priority. This is especially true in light of the need to account for the variance in the response of individuals to early deprivation, as described in previous chapters, so that the combination of factors that appear to put certain children but not others at particular risk can be identified. Given this heterogeneity, it becomes crucial to understand the nature of the causal mechanisms involved in the pathway between the risk associated with institutional care and outcome, in this case IOI. What factors account for these individual differences in outcome? Is it possible that peri-natal factors, such as alcohol consumption, smoking and malnutrition, which are potential risk factors for ADHD, play an influential role here? Pre-adoption experiences and, indeed, post-adoption experiences could also be involved in causal processes. However, the main question of interest here is whether individual differences in normal genetic variation influence susceptibility to the deleterious effects of early institutional deprivation. If so, what is the nature of this role?

3.4 Genetic mediation and moderation models: Their potential role in determining the effects of institutional deprivation

In this section the ideas of genetic risk-disorder pathway mediators, moderators and markers will be introduced, along with a discussion on their potential influence on the outcome of institutional deprivation (Stevens et al., 2006). More specifically, the potential role of active gene by deprivation correlations (rGE) and interactions (GxE) may play in helping to explain the ERA findings will be discussed.

3.4.1 Can active or evocative gene-environment correlations help to account for deprivation-related IOI outcome?

An active gene-environment correlation (rGE) exists when genetic effects influence individual differences in child behaviour that, in turn, alter exposure to environmental factors and either increase or reduce their later impact. That is, the genetic effects are steering the association, and operating indirectly through selecting or shaping the environment to influence later outcome. An active rGE is a special case of a mediated relationship, with individual differences in the level of environmental exposure mediating a primarily genetic effect on the risk associated with an adverse environment. Put another way, the risk pathway is through environmental mediation, but the exposure and experience of individuals to environmental pathogens is influenced by their genes (Rutter, 2006) Figure 3.1 illustrates how a particular genotype influences a behavioural characteristic that then increases exposure to environmental adversity, in turn increasing the individual risk.

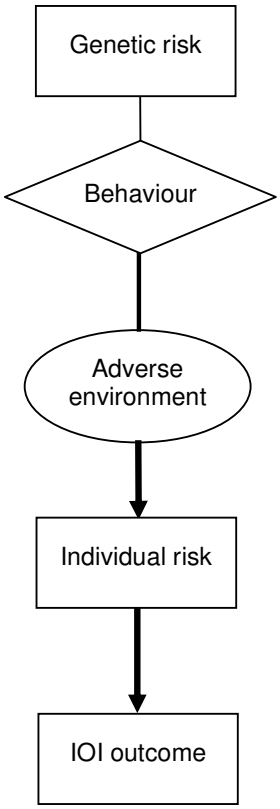


Figure 3.1

Path mediator model - active/evocative gene-environment correlation: genetic effects mediated by adverse environments

---

One way to operationalise an evocative rGE path mediator model in the context of the ERA study is to hypothesise that the heterogeneity found in IOI outcomes is the result of genetically-based differences in, for example, attractiveness, temperament or sheer tenacity that lead some children to elicit better care than others in the institutions themselves. If this were the case, then genetic effects would determine the level or impact of exposure to the noxious environment and so ameliorate the risk of the development of IOI. Unfortunately, the lack of direct measures of the way that child factors might have influenced the quality of institutional care means that we are unable to test this hypothesis in the ERA study. Furthermore, the strength of the association between levels of IOI and duration of institutional deprivation makes this an unlikely scenario. Therefore, this model will not be investigated further in the present study.

3.4.2 Could the level of exposure to institutional deprivation be acting as a marker of genetic risk?

Another possibility is that the presence of adverse environments is associated with genetic risk, although the environmental factors do not, in actuality, play a causal role in increasing the risk or determining the negative outcome. In this case, one might talk about the environment as marking the presence of the genetic risk rather than mediating it. This situation reflects some of the features of a passive rGE, although differing from the typical case where environments and genes affecting children are correlated because of their common origin in their biological parents. This sort of mechanism is illustrated in figure 3.2.

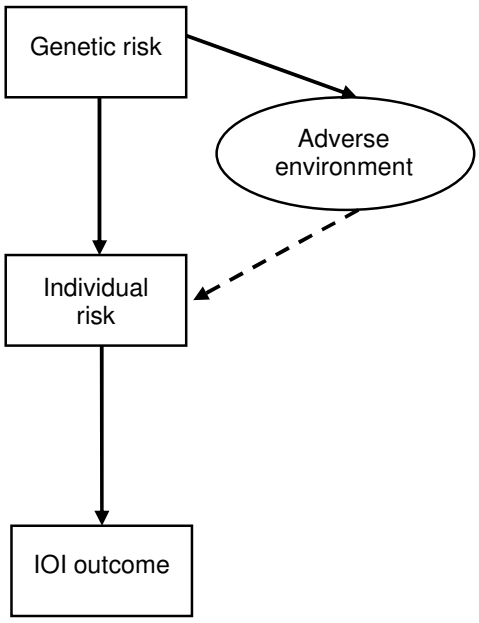


Figure 3.2
Path marker rGE model – genetic influence marked by environmental factors.

The notion of the 'path marker model' when applied to the ERA study leads to the hypothesis that individual differences in levels of IOI, especially those related to the duration of deprivation, comes about because those children who experienced longer periods of deprivation have greater genetic liability than earlier adopted children (i.e. is dose of deprivation associated with genetic risk?). Do later adopted children who are more at risk for IOI also carry more 'risk genes'? This raises

questions about the possible role selection processes, on the part of potential adoptive parents, may be playing in the relationship between genetic makeup and environmental risk. This hypothesis can be directly tested through genotyping for susceptibility genes for IOI and comparing the frequency of risk alleles between the adoptee environmental risk groups. If that were true then we would expect to find a higher percentage of children who posses the risk allele in the later placed adoptee group compared with those adopted out early. However, there is no reason on the face of it to predict such a relationship. Although previous studies of institution-reared children have been methodologically challenged by the fact that children who resided longer in institutions comprised those who had not been adopted sooner, possibly due to behavioural or developmental problems (which could be genetically influenced), this was not the case in the ERA study, as children could not be adopted until the fall of the Ceauşescu regime. It is also relevant to note that it is unlikely that selection into the institutions was due to existing child impairment. This is evidenced by the vast majority of children entering the institutions in the first few weeks of life for reasons of family poverty, due to the economic climate in Romania at the time. Moreover, if selection into the institutions had been due to parents being unfit to take care of the child (due to potentially genetically influenced reasons such as mental illness), this should have affected all the children in the sample equally, irrespective of age at adoption.

Furthermore, adoptive parents choosing older children are more likely to be able to select on the basis of vulnerability and existing (possibly genetically mediated) problems. While some parents might choose positively to adopt children at increased risk, or with more marked problems, out of a sense of altruism, this would be the minority, as the majority were motivated to adopt by infertility (Groothues, Beckett & O'Connor, 1998/1999). In the case of younger children, it would be harder to identify those children at risk and so choose those less vulnerable. On balance, the operation of these sorts of selection pressures would lead to the older adopted children being, if anything, at lower genetic risk than younger adopted children. This view is further supported if one reflects on the impact of mortality within the institutions, in as much as the vulnerable children would be less likely to survive to the time of a later adoption. In fact, an analysis of the presence of problems by the date at which children were adopted in the ERA study did not support the idea that these sorts of selective pressures were operating in either direction. Children of all ages were adopted into the U.K.

between February 1990 and September 1992 and, although there was a significant difference between the ages of those children adopted in the first and second year (those adopted in the first year, 1990: mean age = 13.31 months; those adopted in the second year, 1991: mean age = 18.74 months, t (129) = -2.81, p<.01), no significant association between year of adoption and marked IOI at age 6 years was found (χ^2 = 4.39; df = 2; p = .11).

It is an important exercise to discuss alternative potential mechanisms in order to explore what may be the most plausible model. Due to the evidence presented above, the path marker model does not appear to be a likely fit to the data. However, an analysis of genotype frequencies between the groups will be carried out and presented, so that the possibility that dose of deprivation is associated with genetic risk for IOI (i.e. rGE) can be ruled out.

3.4.3 Can gene-environment interaction effects help to account for deprivation-related IOI heterogeneity?

A further model worthy of consideration is one that takes into account the combined effects of genes and environment on outcome. An outcome may be dependent on multiple risk factors and the interplay between those factors. Genetic and environmental factors can combine to determine outcome in a number of different ways, e.g. additive co-action, multiplicative interaction (based on a logarithmic scale), or synergistic interaction (non logarithmic) (see Rutter, 2006; Rutter & Pickles, 1991). Moreover, Rutter (1983; 2008) makes a distinction between: i) Ronald Fisher's biometric concept of GxE interaction as a statistical phenomenon that needs to be removed in order to accurately partition genetic and environmental contributions to the variance in a behavioural trait, and ii) the notion of GxE interaction introduced by Lancelot Hogben that requires investigation of the processes be undertaken with an understanding of developmental biology. The current discussion will be limited to additive and synergistic interactions within the conceptualisation of GxE interaction as a developmental phenomenon, inferring underlying biological mechanisms, as distinct from the conceptualisation of GxE as a biometric interaction, defined as a purely statistical feature (Tabery, 2007; Rutter, 2008; Rutter, 1983).

3.4.3.1 Gene-environment additive effects

Additive effects represent the simpler of the two possibilities, referring to simple summing, or co-action of the effects of two or more risk factors, in this case institutional deprivation and genetic risk. This hypothesis can be tested directly through genotyping. This will be done in the ERA study by looking across the adoptee risk groups at the levels of IOI/ADHD for those with, and without, the identified risk allele. Using a multivariate statistical model, one would expect there to be significant main effects of both the risk factors, but no statistical 'interaction' effect (Rutter, 1983). Figure 3.3 presents a hypothetical representation of the results of a study examining the combined effects of exposure to early institutional deprivation (environmental risk factor) and particular risk alleles (genetic risk factor) on the expression of IOI, demonstrating additive effects.

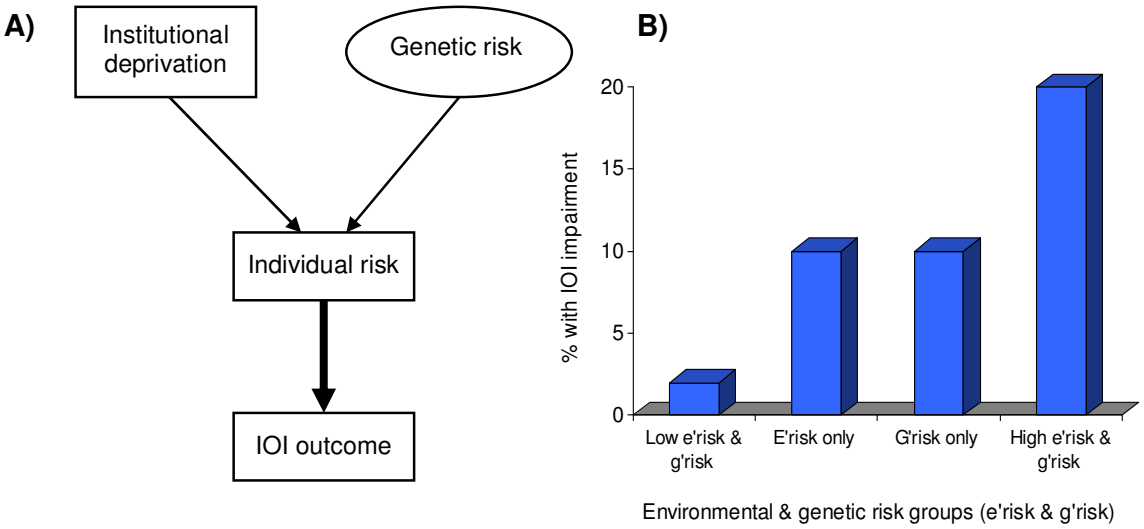


Figure 3.3
Model of additive effects of genetic vulnerability and institutional deprivation on the risk for IOI: pathway model (A) and hypothetical outcome model (B)

3.4.3.2 Gene-environment synergistic interactions

Synergistic interactions, on the other hand, suggest that the presence of one factor alters the expression of the other. That is, the effects of a risk factor are lessened or heightened by the presence or absence of another risk factor – in this case by

one factor moderating the effect of another factor on an outcome. Path moderators are conceptually distinct from mediators and markers in that a moderator variable influences the strength or nature of the relationship between a potential risk factor and the outcome of interest. In other words, the relationship changes as a function of the moderator, which determines the conditions for the causal effects of an independent or predictor variable on outcome (Baron & Kenny, 1986). A genetic moderator may make an individual more susceptible or vulnerable to the risk effects of the environment, or, alternatively, more resilient. This appears to be the case for several known examples of gene-environment interactions where the effects of known environmental risk factors are moderated by genetic effects that have no main effect on their own (Moffitt et al., 2005). In the ERA scenario the environmental factor, early institutional deprivation, is potentially a strong risk factor for later impairment in several psychological domains. However, certain candidate genes may moderate the impact of early institutional deprivation by increasing an individual's vulnerability to its effects. For example, an individual may possess a risk allele associated with ADHD that interacts with early deprivation to increase the risk created by the environmental pathogen during exposure, and thus increase an individual's susceptibility to later IOI. Alternatively, an individual may be more resilient to the risk posed by institutional deprivation because of the protective effect of specific genetic factors; for instance, it is plausible that a particular variation of a candidate gene involved in the regulation of the HPA axis may exert a protective effect against chronic early stress, such that if a child possesses this allele it may make him or her more resilient to the negative effects of early institutional deprivation and, thus, decrease the likelihood of later impairment.

The risk and protective models presented above prompt the question: Does normal genetic variation in individual children make them more or less susceptible to early institutional deprivation? To explore this possibility, synergistic interactions between environmental and genetic risk need to be tested for. Could the environmental risk, presented by duration of time exposed to early institutional deprivation, interact with individual genetic makeup to make individuals with a certain risk allele more vulnerable to its deleterious effects? Figure 3.4 set out what is perhaps the most likely hypothesis; that the presence of specific risk alleles moderates the impact of deprivation by creating vulnerability to the effects of the noxious environment associated with deprivation on later outcome. This

hypothesis could be tested directly through genotyping. Again, this will be done through genotyping for susceptibility genes and then applying the data to a multivariate analysis of variance model to investigate the levels of IOI across adoptee and genotype risk groups, to see whether the presence of both genetic and environmental risk significantly increases the risk for individual IOI impairment. Figure 3.4 illustrates hypothetically the sorts of results that would support the presence of a moderating effect of genetic factors within the ERA study.

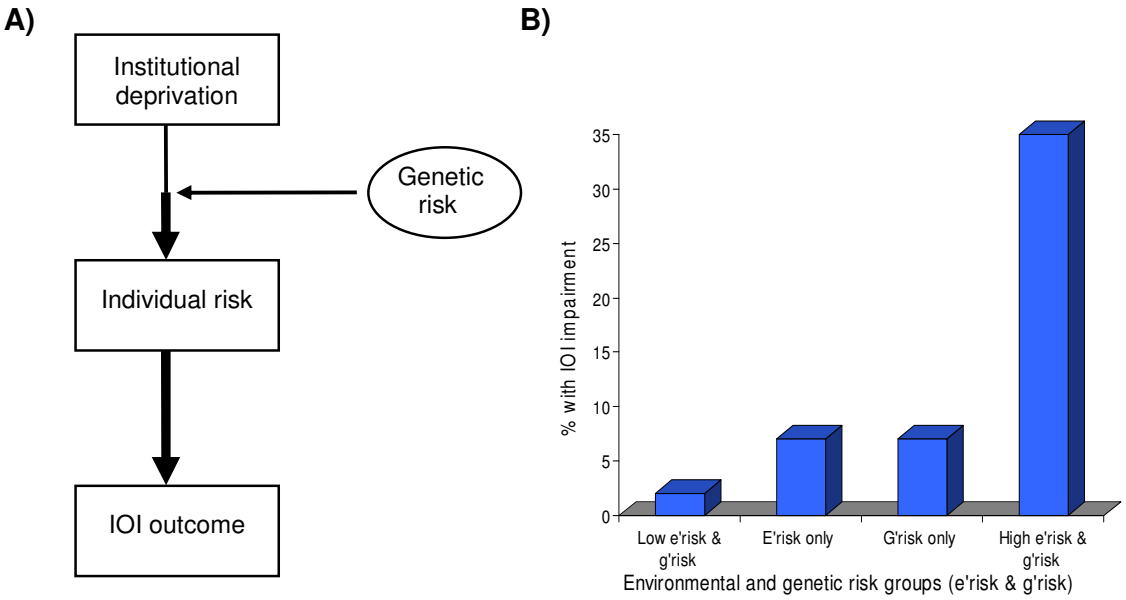


Figure 3.4
Model of interaction effects of genetic vulnerability and institutional deprivation on the risk for IOI: pathway (A) and hypothetical outcome (B)

3.4.3.3 Considerations when testing for gene-environment interactions

The above models of synergistic and additive interaction, which set out the combined effects of genes and environments, provide the most likely hypotheses of the causal pathway between the risk associated with institutional deprivation and variability in individual IOI outcome. Therefore, these interplay models will provide the theoretical and analytical focus for the main study.

There has been a series of important recent publications on the key issues and research strategies for GxE investigations and interplay between genes and behaviour more generally (Moffitt et al., 2005; Rutter, 2006; Caspi & Moffitt, 2006; Rutter et al., 2006). Moffitt, Caspi and Rutter (2005) outline seven key strategic

steps that should be considered for a sound investigation of GxE interaction in the field of psychopathology:

1. There should be evidence to suggest that GxE is likely from quantitative behavioural-genetic research on disorder being investigated. Information can be taken from heritability estimates that index not only direct effects between genes and disorder, but also interactions between genes and environmental factors. The evidence in relation to the heritability of ADHD supports this step.
2. A plausible candidate environmental pathogen needs to be identified using several criteria: i) heterogeneity in individuals' response to the environmental risk factor; ii) a plausible neurobiological pathway from the environmental pathogen to the disorder; iii) compelling evidence that the risk factor has environmentally mediated causal effects. With respect to the putative risk in the ERA study, institutional deprivation: i) there is definitely variability in individual's response; ii) a plausible mechanism of the pathway from risk to disorder was hypothesised in the previous chapter and iii) there has been some published evidence of the causal effects of the environmental risk factor on IOI, and it is a primary aim of the current thesis to examine the longitudinal evidence and the persistence of effects into mid-adolescence.
3. Optimise the environmental risk measurement by considering age-specific and cumulative risk effects, reliability of retrospective reports and proximity of the risk. The aim is to identify specific proximal risk factors and measure them as precisely as possible, which will increase the statistical power of the study and reduce the sample size needed to test effects.
4. Identify candidate susceptibility genes that have shown either direct gene to disorder association, or have functional significance in terms of an individual's reactivity to the environmental risk factor. These two selection strategies are discussed in more detail, below, in relation to the identification of candidate genes for the current investigation.
5. Test for the GxE interaction using appropriate conventional statistical techniques within epidemiological cohort studies, genetic association studies, longitudinal cohort studies or sample of individuals exposed to a known pathogen. The ERA study has the advantage of being both a longitudinal study with the obvious benefits that this entails (e.g. repeated assessments, analysis of the trajectories of cause and effect), and also

comprising a sample of children exposed to a measured and identified environmental pathogen.

6. Evaluate the generalisability to other sample of any GxE interaction effects found.
7. Replication and meta-analysis.

In summary, a careful well planned investigation of GxE interaction should consider measured genes and specific measured environments with selection based on empirical evidence, and operating via a biologically plausible mechanism. Moreover, the key logic of the judicious selection of genes and environments is to limit multiple testing and 'fishing' for interactions and, thus, the likelihood of uncovering a host of false positive results (Rutter, 2008). This kind of measured and hypothesis driven research is better placed to isolate specific GxE interaction effects than what Rutter (2008) has discussed as the quantitative 'black box' analyses undertaken by behavioural geneticists in the 1980's and early 90's that were testing for interactions between the "totality of anonymous genes and the totality of anonymous environments", and were, therefore, unlikely to find GxE interaction effects. Moreover, examining the combined effects of specific environmental risks and genetic liability on later psychological impairment is vital in order to help disentangle inconsistencies in molecular genetic findings.

3.4.3.4 Gene-environment interplay: 'mediation' via gene expression

It is important to note the interpretative difficulties inherent in field studies of GxE interplay, such as the current investigation. That is, in nonexperimental studies it is not possible to determine whether any putative GxE interaction that is detected may in fact be more accurately be defined as environmental effects on gene expression and downstream effects on behavioural functioning. That is, the effects of the adverse environment influence behavioural outcome, in this case IOI, through epigenetic gene expression processes (i.e. E→G→IOI rather than GxE→IOI).

Epigenetics refers to modifications in gene expression that are heritable, but reversible, that do not involve any change in DNA sequence (Henikoff & Matzke, 1997). The term was originally coined by Waddington in the 1940s and

referred to the causal interactions between genes and their products that bring the phenotype into being (as cited in Jablonka & Lamb, 2002). Current thinking about the concept includes the mechanism whereby gene expression is altered by extracellular signals (Rutter, 2006). More specifically, epigenetic mechanisms, such as DNA methylation and histone acetylation (chemical processes), have been implicated as mediators in the process by which environments can affect gene expression (Rutter, 2006).

It is beyond the scope of the current thesis to test these mechanisms directly, not least because gene expression is tissue specific and is therefore not possible in investigations of behaviour in humans, where brain tissue would be required. However, the interesting research by Meaney and colleagues (2001) using animal models may provide some insight into relevant processes in early risk and development. They reported that naturally occurring variations in maternal care (licking and grooming of rat pups) altered the expression of genes in offspring within brain regions that regulate behavioural and endocrine (HPA and metabolic/cardiovascular) responses to stress. Moreover, the effects established long term individual differences in the stress reactivity of the offspring (Meaney, 2001). A recent theoretical paper by Mill and Petronis (2008) related the process of epigenetic regulation to the relationship between early pre and perinatal risk and the development of ADHD. They hypothesise that epigenetic mechanisms mediate the link between early risk factors and long term alterations in ADHD outcome. This important paper highlights that by understanding these processes better it will further our understanding of how environmental pathogens influence psychopathology and how best to interpret the results of studies of GxE interplay.

3.5 Testing for GxE interaction in the ERA study: institutional deprivation, genetic risk and IOI outcome

In this section the GenERA study of the role of genetic effects within the ERA project will be introduced. This study focuses on the interaction between specific susceptibility genes and institutional deprivation on the risk for IOI. The analysis of these effects forms the second part of the empirical section of the current thesis (chapters 7 and 8). An overview of the phenotype of interest will be given, along with a description of candidate genes under investigation and the strategy used for their selection.

3.5.1 GenERA study: selecting the phenotype

The GenERA study was set up using the ERA study data to explore the potential role that normal genetic variation may have in influencing susceptibility to the risk effects of early institutional deprivation on later behavioural outcomes. The GenERA study aims to examine whether GxE interaction accounts for some of the heterogeneity in responses shown in the sample population. Specifically, the moderating role played by genetic variants that alter the regulation of dopamine and glucocorticoid systems will be examined in relation to impact of early institutional deprivation on the increased levels of IOI found in the ERA study.

As described in detail in the previous chapters, the outcome or phenotype of interest is inattention/overactivity/impulsivity. A particular strength of the study design is that it allows both between-subjects and within-subjects approaches to be used. Furthermore, longitudinal data on the full study sample are available over three assessment waves. Between-subjects data on IOI outcome are available from deprivation and genotype risk groups. This enables us to examine continuities and discontinuities in developmental patterns of impairment and GxE interaction over time. IOI at age 6 and 11 years was measured using parent and teacher reports of behaviour on the Revised Rutter Scales (Elander & Rutter, 1996; Hogg, Rutter, & Richman, 1997). At age 15, a more comprehensive measurement of IOI was obtained through parent and teacher ratings on parallel versions of the Strengths and Difficulties Questionnaire (Goodman, 1997) based on the Rutter Scales (Elander & Rutter, 1996), and a section on ADHD behaviours in the standardised Child and Adolescent Psychiatric Assessment (CAPA) interview (Angold et al., 1995; Angold & Costello, 2000). The interview was carried out with the adoptive parents and adapted for the purposes of the ERA study (Rutter, Silberg, Colvert & Kreppner, 2003; see chapter 4, section 4.3.2 for details).

3.5.2 GenERA study: selecting the genotype

Two approaches have been applied to select the specific candidate susceptibility genes for the current study: A phenotype-based strategy and a process-based strategy (Moffitt et al., 2005). The distinction is conceptual, and it is plausible the

candidate genes from both selection strategies may influence the hypothesised underlying mechanistic pathway from risk to disorder.

3.5.2.1 Phenotype-based selection strategy: dopamine genes

Molecular genetic research on genes associated with IOI and its diagnostic corollary, ADHD, provides the evidence to enable the selection of candidates for a phenotype-centred investigation of GxE interaction. In particular, research has centred on the risk for IOI/ADHD associated with two functional polymorphisms within genes that encode proteins involved in the dopaminergic system in the brain; the dopamine transporter (DAT1) gene, which codes for a protein that regulates the reuptake of dopamine at the presynaptic level; and the dopamine D4 receptor (DRD4) gene, in particular a polymorphism that codes for an amino acid chain in the third intra-cytoplasmic loop of the receptor involved with G-protein coupling (Li et al., 2006; DiMaio et al., 2003).

The studies of the human DAT1 gene (SLC6A3) have focused on a common 10-repeat (10R) high risk allele of a 40-base pair (bp) variable number tandem repeat (VNTR) polymorphism within the 3'untranslated region (UTR) of the gene, which varies in copies between 3 and 13 and is located on chromosome 5p15.3 (Giros et al., 1992; Cook et al., 1995; Yang et al., 2007). This polymorphism is referred to as DAT1 40-bp (3'UTR) in the current chapter.

The DRD4 studies have focused on the risk associated with the 7-repeat (7R) allele of a 48-bp VNTR in exon III of DRD4 located within chromosome 11p15.5, with the 4-, 7- and 2-repeat alleles being the most prevalent (DiMaio et al., 2003). The 7-repeat allele has been shown to produce a blunted response to dopamine (Faraone et al., 2005). The effects of DRD4 on the risk for ADHD are small (pooled OR=1.34), but generally robust and consistent across European populations and study methods (Li et al., 2006). The overall effects of DAT1 are even smaller and variable across populations and study methods, with a recent meta-analysis estimating a small but significant effect: OR=1.17, $\chi^2(1)=8.11$; $p=.004$ (Yang et al., 2007) and another meta-analysis reporting that the overall effects were either very weak or nonsignificant (Li et al., 2006).

There are two possible reasons for this increased variability in association: first, the 40-bp (3'UTR) VNTR polymorphism may be a marker for a linked alternative functional polymorphism elsewhere on the gene; Second, the functionality of this gene may be especially susceptible to moderation by

environmental risk factors and if the effects of the environment are not accounted for in the experimental design then associations may be missed (Brookes et al., 2006b). Evidence for a synergistic association between exposure to an environmental pathogen and a common DAT1 haplotype (comprised of the 10R allele of the 40-bp VNTR in 3'UTR and a 6 repeat (6R) allele of the 30-bp VNTR in intron 8) is consistent with these propositions (Laucht et al., 2007). The DAT1 30-bp VNTR (intron 8) is described in detail in Vandenbergh et al. (2000). The DAT1 30-bp polymorphism has recently been demonstrated as significantly associated with ADHD (p=.01), along with the combined haplotype of the two markers (p=.02) across samples (Asherson et al., 2007). Moreover, in a recent study, significantly elevated rates of ADHD were observed in group of children from a high risk community sample that possessed both the DAT1 10R-6R haplotype and had experienced psychosocial adversity, but no main effect of haplotype, or the genotypes separately were found (Laucht et al., 2007). An earlier study showed that the environmental risk for ADHD associated with maternal pre-natal smoking was also moderated by the DAT1 10R-6R haplotype (Brookes et al., 2006b).

3.5.2.2 Process-based selection strategy: glucocorticoid receptor gene

The current study also includes a process-based approach to candidate gene selection by investigating the possible influence of a specific candidate gene that has functional significance in terms of the effects of the environmental risk factor, rather than in relation to the disorder (Moffitt et al., 2005). Namely, one could select a candidate susceptibility gene that affects an individual's vulnerability, or reactivity, to the environmental pathogen's adverse effects, which, in the current study, is early institutional deprivation. It is necessary to have a biologically plausible pathway that incorporates the risk associated with institutional deprivation, genetic moderation of physiological responsiveness to that environmental pathogen and the outcome of interest, IOI. The vast majority of research into the interplay between genes and responsiveness to adverse environments has been carried out with animals, making research with humans an important, exciting and novel area of study. In the current study, genetic variation that influences an individual's stress response to the environmental risk factor, institutional deprivation, possibly through the hypothalamic-pituitary-adrenal (HPA) axis or the central nervous system (CNS) neurotransmitter functioning, provides the most obvious focus.

Biological processes

Empirical evidence is limited, however, one can speculate about the possible biological processes involved. Exposure to early stress in the form of early institutional deprivation influences various aspects of neurobiological functioning (Gunnar & Quevedo, 2007). This may have an impact on an individual's physiological stress response, and their ability to develop adequate regulation of the HPA axis functioning, following associated prolonged activation and elevations in cortisol levels. Although the physiological stress response is necessary for survival, repeated frequent activation, particularly during early brain development, has been linked to the increased risk for physical and psychological disorders (Gunnar & Quevedo, 2007). As discussed in section 2.3 of the previous chapter on the hypothesised mechanism operating with respect to the risk for IOI in the ERA sample, dysregulations of HPA axis activity, which are associated with chronic elevations of cortisol activity, can have long lasting and profound down-stream effects on brain development and functioning in the circuits and neurotransmitter systems implicated in the pathophysiology of ADHD.

Institutional deprivation as a model for early stress

When discussing the potential effects of early stress and stress system activation on development in the ERA study, it becomes important to address the issue of the meaning of "stress" in relation to institutional deprivation. Stress, in the context of the relevant research on the effects of early experience, can refer to adverse over-stimulation, abuse or animal models which involve exposure to harmful or dangerous stimuli. However, although physical and psychological deprivation refers to an absence or lack of nutrition, care, stimulation, or opportunity for attachment, this may also be considered to be a stressful experience. Thus, while there is a distinction between deprivation and stress per se, it is possible they occupy the same spectrum of experience. In spite of the differences, the research on early stress is significant in that it shows that variation in experience can interact with genetic makeup to affect physiological responsiveness and later behavioural outcome. Moreover, several of the animal studies in this area use maternal separation as the model for early stressful experience. This model holds

much relevance for the ERA study, as the children experienced maternal deprivation in the Romanian institutions.

Evidence from studies of early stress

There is some evidence to support the hypothesised link between early deprivation and HPA axis dysregulation from a study of salivary cortisol levels of Romanian adoptees who experienced prolonged institutional deprivation before being adopted into Canadian families (Gunnar et al., 2001). The study showed that these children had elevated ambulatory cortisol levels compared with those adopted out of the institutions early and the Canadian born control children. In addition, severe neglect may alter the diurnal cortisol rhythm, as illustrated by a study of 2-year-olds living in Romanian institutions which showed dysregulated cortisol activation compared with children reared in a family setting (Carlson & Earls, 1997; see Gunnar & Quevedo, 2007). Evidence from the wider literature on parent-child attachment may also hold some relevance for our study. Research has indicated that for insecurely attached children, stressors are capable of producing elevated adrenocortical activation (Spangler & Schieche, 1998), unlike in securely attached infants, or for those with a responsive caregiver (Nachmias et al., 1996; Gunnar et al., 1992).

Animal models also provide support for the effects of early stress on HPA axis functioning. For example, adult animals who were exposed to repeated periods of maternal separation showed significantly increased HPA responses to stress, and associated reduced glucocorticoid receptor binding was exhibited in the frontal cortex, hippocampus and hypothalamus, which resulted in reduced negative feedback sensitivity (Plotsky & Meaney, 1993; Liu et al., 2000; as reviewed in: Meaney, 2001).

Functional link between early deprivation, glucocorticoids and dopaminergic system

Animal models have also shown stress system activation associated with early prolonged maternal separation that impacted on the development of the mesocorticolimbic dopamine system via the associated effects of the HPA axis (Meaney et al., 2002). That is, there appears to be functional connections between the HPA axis and the dopaminergic system, a key system in the aetiology

of ADHD. The majority of midbrain dopamine neurones express glucocorticoid receptors (Harfstrand et al., 1986), and the regulation of stress induced dopamine release is dependent on glucocorticoid levels (Piazza & LeMoal, 1996). Meaney et al (2002) demonstrate that there are specific effects of maternal separation on several aspects of the dopamine systems via nonspecific effects on the development of the HPA axis that influence stress reactivity and behavioral sensitivity to cocaine. Specifically, maternal separation resulted in increased reactivity of the HPA axis to stress, associated elevation of adrenal glucocorticoid release during stress and regulation of the mesolimbic dopamine system by the circulating glucocorticoids.

Genes that have functional significance for stress physiology therefore provide good candidates for the current investigation of the process-based hypothesis of GxE. Furthermore, we can indirectly test whether the speculative hypothesis that HPA axis dysregulations associated with hypothesised chronic cortisol activation are influential in the causal pathways to IOI symptomatology. Cortisol, a glucocorticoid agent, is secreted upon activation of the HPA axis, and exerts its effects in the central nervous system mainly via the glucocorticoid receptor (GR).

Glucocorticoid Receptor gene (NR3C1)

Recent research has provided evidence for the functional significance of several single nucleotide polymorphisms (SNPs) of the GR on glucocorticoid sensitivity (Wust et al., 2004; van Rossum et al., 2003; van Rossum & Lamberts, 2004). Moreover, these specific GR genes have been shown to moderate individuals' physiological and psychological responses to stress (Kumsta et al., 2007; Wust et al., 2004; Ising et al., 2008). A primary function of the GR is to exert negative feedback in the HPA axis circuitry, which results in the termination of the stress response (de Kloet et al., 2005). Four GR polymorphisms have been shown to significantly influence sensitivity to glucocorticoids (reviewed in: van Rossum & Lamberts, 2004), but only two, the intronic BclI (rs41423247) and the A/G SNP in exon 9beta (rs6198), show sufficiently large frequencies to be included in the current analyses with the ERA sample. These subtle variations in sensitivity to glucocorticoids are likely to moderate the influence of cortisol on the development of other neuronal systems. Studies have shown associations between these two

GR polymorphisms and altered HPA axis response to psychosocial stress, as well as GC sensitivity (Kumsta et al., 2007, 2008; Wust et al., 2004).

The *Bcl*I SNP is a common G/C variant located within intron B of the GR gene (Fleury et al., 2003; van Rossum et al., 2003). It has been shown in numerous studies to be related to indices of body composition, metabolic parameters and glucocorticoid sensitivity (for review, see: van Rossum & Lamberts, 2004; Wust et al., 2004). Moreover, homozygous carriers of the G allele have exhibited diminished HPA axis stress hormone responses to a psychosocial stressor (Kumsta et al., 2007; Wust et al., 2004; Ising et al., 2008), and were found to be at an increased risk of developing major depression (van Rossum et al., 2006).

The common 9beta polymorphism is an A/G variant in exon 9beta that also shows evidence of associations with glucocorticoid sensitivity and altered HPA axis response following exposure to stress. Moreover, available molecular evidence suggests that this polymorphism on is likely to have functional effects glucocorticoid sensitivity (Derijk et al., 2001; Kumsta et al., 2007; see Kumsta et al., 2007). Kumsta et al (2007) reported that male carriers of the GR 9beta AG genotype showed elevated cortisol and adrenocorticotropic hormone responses to psychosocial stress.

These studies indicate that specific variations of the GR gene regulate the HPA axis stress response and may, in turn, influence an individual's vulnerability to later psychopathology. The mechanism hypothesised to be influencing the outcome of the young people in the ERA study involves moderation, by specific GR polymorphisms, of stress induced dysfunction of the HPA axis that influences the development of neurotransmitter systems implicated in the pathophysiology of ADHD. That is to say, the adverse experience of prolonged early deprivation and the stress induced hyperactivation of the HPA axis with associated cortisol secretion may influence the course of neurobiological development. Cortisol influences the brain primarily via the GR, and so subtle differences in glucocorticoid sensitivity and HPA feedback mechanisms that are influenced by GR gene polymorphisms may affect the pathogenesis of IOI in the ERA sample via their downstream effects on the dopamine system.

The genetic mechanisms involved in these processes are not well understood, particularly in relation to the genetic moderation of the effects of early stress on hyperactivity, and research in this area is therefore of a priority.

3.6 Chapter summary

The findings from the ERA study have provided strong evidence for the impact of early environmental influence on later psychopathology. Specifically, the length of time spent in depriving conditions in Romanian institutions has been found to be strongly associated with specific forms of psychological sequelae; namely, IOI, quasi autistic features, cognitive impairment and disinhibited attachment. However, given the variation, or spread, of responses seen in the sample of children in the ERA study, an important question as to what accounts for this heterogeneity in outcome is raised. Despite strong environmental causal evidence, genes may still potentially play a role in this process, either additively or through interaction with 'dose' of institutional deprivation. It is plausible that normal genetic variation may act as a moderator of the effects of deprivation, possibly through a process-based or phenotype-based mechanism. In other words, genetic factors may influence an individual's susceptibility or resilience to early stress, or, alternatively, genes known to be associated with ADHD may act in conjunction with the environmental risk factor to increase an individual's liability to later IOI. The direct testing of the possible genetic and biological processes (e.g. biological programming, gene expression and neurobiological dysfunction) is outside the scope of the current GenERA study, but the study will test for the genetic moderation processes by building on the behavioural framework set down by the first empirical chapter 6.

As discussed above, recently there has been a considerable amount of research evidence for GxE interactions with respect to a range of childhood, adolescent and adult psychiatric disorders in addition to ADHD and across a different populations and settings (e.g. Caspi et al., 2002; Caspi et al., 2003; Eley et al., 2004; O'Connor et al., 2003a). However, there is a notable absence in the literature with regards to the longitudinal investigation of the developmental pathways of GxE interaction, and the patterns of continuity of effects. By exploring GxE interaction effects over time, the current study will make a significant contribution to the research area by testing the robustness of the GxE theory, and

provide insight into the developmental mechanisms that could help to explain the presence of such effects. With respect to the ERA study, one could hypothesise that the experience of early institutional deprivation, during sensitive periods of development, leads to alterations in gene expression or neurobiological dysfunction in carriers of specific 'risk' alleles that moderate the detrimental impact of the environmental pathogen on long-term behavioural development. One would expect fundamental alterations in neurobiological development as a function of early experience expressed as GxE interaction effects to be detectable early on and persistent over time. This lead to the prediction that children who possessed the dopamine or GR risk genotypes would have been particularly susceptible to the adverse effects of early institutional deprivation, and were at particular risk for the development of early onset, persistent IOI/ADHD type symptoms. This, in turn, may help to explain some of the observed heterogeneity in outcome. It is possible that gene by gene and environment by environment interactions help account for the heterogeneity in outcome that is found, but investigation of these possibilities is outside the scope of the current thesis. This thesis' novel approach to the longitudinal study of combined effect of genetic and environmental risk factors aims to provide valuable insight into the causal and developmental pathways leading to disorder following early risk exposure, and enables us to explore whether the GxE interaction hypothesis represents a mechanism for understanding heterogeneity in outcome over time. Longitudinal data spanning approximately 10 years of childhood development is utilised, with the aim of investigating the extent to which DRD4, DAT1 and GR genotypes interact with early institutional deprivation in a synergistic manner to increase the risk for IOI at ages 6, 11 and 15 years.

3.7 Thesis research questions

3.7.1 Early deprivation and IOI: Characterising the risk and examining associated features

The first empirical chapter (6) sets out to investigate the developmental and aetiological pathways between early institutional deprivation and IOI in the ERA sample, and to examine the associated features of the deprivation-related IOI phenotype. The following questions address these aims:

1. Does the risk for IOI associated with severe early institutional deprivation persist to age 15 years?

2. If so, what effect does duration of deprivation have on IOI at this age?

3. Are the rates of deprivation-related IOI/ADHD found in the adolescent Romanian high risk sample clinically significant?

4. Is there individual continuity in IOI behaviour over time?

5. Is deprivation-related IOI phenotypically similar to IOI/ADHD as seen in the nondeprived population in terms of:

    a. The developmental link and overlap with conduct problems?

    b. The association with low IQ?

    c. The association with executive dysfunction?

    d. The gender discrepancy/prevalence amongst males?

6. Is the deprivation-related phenotype characterised by particular underlying ADHD subtype symptoms?

7. Is there overlap between IOI and disinhibited attachment in mid-adolescence?

3.7.2 Early deprivation and IOI: moderation of risk by genetic factors

The second part of the empirical section of the current thesis aims to examine the moderation of the risk associated with early deprivation by investigating specific genetic polymorphisms. The prediction is that children who possess the specific dopamine or glucocorticoid receptor genotypes, described above in section 3.5.2, are particularly susceptible to the adverse effects of early institutional deprivation and at particular risk for the development of early onset, persistent IOI/ADHD type symptoms.

The results are presented in two separate chapters; one relating to moderation of the deprivation risk by dopamine genes (chapter 7) and the second relating to moderation by glucocorticoid receptor genotypes (chapter 8).

Accordingly, the following questions were used to test the predicted genetic moderation of risk

3.7.2.1 Dopamine gene research questions

1. Are there gene-environment correlations between the dopamine transporter (DAT1) genotypes/haplotype and institutional deprivation?

2. Is there a gene-environment correlation between dopamine receptor (DRD4) genotype and institutional deprivation?

3. Does DAT1 genotype interact with early deprivation to increase the risk for IOI?
    a. DAT1 40-bp VNTR located in the 3'UTR (10 repeat = risk genotype)
    b. DAT1 30-bp VNTR located in intron 8 (6 repeat = risk genotype)

4. Does DAT1 10R-6R haplotype to interact with early deprivation to increase the risk for IOI?

5. Does DRD4 (exon III) genotype interact with early deprivation to increase the risk for IOI.

6. Does DAT1 genotype/haplotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?
    c. DAT1 40-bp VNTR located in the 3'UTR (10 repeat = risk genotype)
    d. DAT1 30-bp VNTR located in intron 8 (6 repeat = risk genotype)
    e. DAT1 haplotype (10R-6R = risk haplotype)

7. Does DRD4 (exon III) genotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

3.7.2.2 Glucocorticoid receptor gene research questions

1. Are there gene-environment correlations between the glucocorticoid receptor (GR) genotypes/haplotype and institutional deprivation?
2. Is there specific GR 9beta-*Bcl*I haplotype associated with IOI in the GenERA sample as a whole?

The aim of the preliminary haplotype analysis is to isolate a specific SNP for the GxE interaction analysis may confer increased risk for or protections from IOI in the GenERA sample.

3. Does GR *Bcl*I or 9beta genotype interact with early deprivation to increase the risk for IOI?

4. Does GR *Bcl*I or 9beta genotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

CHAPTER 4: METHODOLOGY

SAMPLE, PROCEDURE & INSTRUMENTS

4.1 Sample

4.1.1 Selection of ERA sample

The current PhD project utilises the sample (n=217) from the larger ERA longitudinal study (see Rutter & English & Romanian Adoptees Study Team, 1998). The ERA study comprises a large sample of Romanian children (n=165) who were reared during infancy in deprived conditions before being adopted into families in the U.K. A comparison sample of 52 within country (U.K.) adoptees was selected, who were all aged below 6 months when adopted. Four assessment waves have been completed with the children and their adoptive parents being seen when the children were aged 4, 6, 11 and 15 years.

4.1.1.1 Romanian sample

The Romanian children in the sample were drawn from the 324 children processed through the U.K. Home Office and/or the Department of Health between February 1990 and September 1992 and adopted into U.K. families. The children were all aged below 43 months at time of entry to the U.K. The sample comprises 144 children who spent the majority of their early life (minimum of two weeks) in severely depriving conditions in Romanian institutions during the late 1980s to early 1990s, before they were adopted by families living in the U.K. In addition, there were 21 Romanian children who were adopted from family settings, and their ages at adoption were spread throughout the age range. This group of non-institutionalized children provided an additional useful comparison subsample in that they experienced the general hardship and poverty suffered by under privileged Romanian families at the time, but they were not subject to the experience of institutional rearing and its associated risks. Of the families approached to participate in the ERA project, 81% agreed to take part in the initial assessment waves at age 4 and 6 years. A stratified sampling strategy was employed, so that approximately equal numbers of children were obtained for the age band groupings (<6 months, 6 to <24 months, 24 – 43 months) and random selection was then used within the bands. However, as there were lower numbers of children adopted over the age of 2 years, resulting in numbers falling below

target levels, all available children in the older age band were included. The final sample consisted of 58 children placed between 0 and 6 months, 59 placed between 6 and 24 months and 48 placed between 24 and 43 months. A particular strength of the design of the ERA study is the stratified nature of the risk and duration of early institutional deprivation.

4.1.1.2 U.K. sample

The comparison sample of 52 within-U.K. adoptees were obtained through a range of voluntary adoption agencies and Social-Service departments. The children were all below 6 months of age when adopted. Because the families were approached via the adoption agencies, it was not possible to calculate the exact proportion who agreed to participate in the study, however, the participation rate is estimated to be around 50%. The comparison sample was specifically chosen in order to control for the experience of adoption and of being brought up, post adoption, in an above average rearing environment, but to vary in terms of the experience of early severe psycho-social and nutritional deprivation.

4.1.1.3 Gender

The male to female ratio was balanced within the sample as a whole and within adoptee groups were possible. However, gender distribution could not be controlled in the U.K. group, as it was a volunteer sample, or the eldest Romanian adoptee group, as all available families who adopted children over the age of 24 months were approached to participate. This resulted in an overrepresentation of females in the Romanian 24 – 43 month group, and boys in the U.K. group. Table 1 sets out the distribution of children across gender split and adoptee groups.

Table 4.1

Distribution of U.K. & Romanian adoptees by gender and adoptee age group

	Adoptee group				
	U.K.	Rom <6	Rom 6-<24	Rom 24-43	Total
Both sexes	52	58	59	48	217
male	34 (65%)	31 (53%)	26 (44%)	17 (35%)	108 (50%)
female	18 (35%)	27 (47%)	33 (56%)	31 (65%)	109 (50%)

4.1.2 PhD study sample

4.1.2.1 Sample for analysis of IOI phenotype

For part one of the empirical analysis of the current PhD, which investigates the prevalence and presentation of IOI in the ERA sample, all available data on IOI/ADHD were utilised from the age 6, 11 and 15-year-old assessment waves. The age 4 data were not included in the present analyses, because a full data set was not available from this assessment wave; the children aged 24 months or over were already too old at the start of data collection to be included at that stage. The Romanian non-institution (Rom non-IR) reared children were kept separate from the Romanian institution reared (Rom-IR) groups, as previous ERA study analyses suggested that the risk for IOI was specific to the institutional rearing experience (Kreppner et al., 2001). Table 2 sets out the number of children for which IOI data was available across adoptee groups, gender, assessment wave and assessment tools. Assessment tools are described below under heading 4.3.

Table 4.2

Sample size across institutional deprivation adoptee groups, assessment wave, gender and IOI assessment method

		Adoptee group						
			Rom	Rom-IR	Rom-IR	Rom-IR		% of total ERA
IOI/ADHD measure		U.K.	non-IR	<6	6-<24	24-43	Total	sample
Parent report								
Age 6	Both sexes	52	21	44	50	43	210	97%
(Rutter Scales)	*male*	*34*	*11*	*21*	*22*	*17*	*105*	97%
	female	*18*	*10*	*23*	*28*	*26*	*105*	96%
Age 11	Both sexes	48	20	42	49	40	199	92%
(Rutter Scales)	*male*	*32*	*11*	*22*	*22*	*14*	*101*	94%
	female	*16*	*9*	*20*	*27*	*26*	*98*	90%
Age 15	Both sexes	46	17	42	38	41	184	85%
(SDQ)	*male*	*30*	*8*	*22*	*18*	*15*	*93*	86%
	female	*16*	*9*	*20*	*20*	*26*	*91*	83%
Age 15	Both sexes	47	17	44	44	43	195	90%
(CAPA interview)	*male*	*31*	*8*	*23*	*22*	*16*	*100*	93%
	female	*16*	*9*	*21*	*22*	*27*	*95*	87%
Teacher report								
Age 6	Both sexes	47	18	43	44	40	192	88%
(Rutter Scales)	*male*	*33*	*9*	*20*	*18*	*16*	*96*	89%
	female	*14*	*9*	*23*	*26*	*24*	*96*	88%
Age 11	Both sexes	50	19	35	44	40	188	87%
(Rutter Scales)	*male*	*33*	*11*	*17*	*18*	*15*	*94*	87%
	female	*17*	*8*	*18*	*26*	*25*	*94*	86%
Age 15	Both sexes	45	14	33	36	36	164	76%
(SDQ)	*male*	*29*	*5*	*15*	*19*	*15*	*83*	77%
	female	*16*	*9*	*18*	*17*	*21*	*81*	74%

Overall, the ERA families have been very committed to the research throughout the duration of the study, and we have seen a very low rate of attrition in the sample. Data on IOI were available from parents and/or teachers on 214 out of 217 children at age 6 (99%), 210 children at age 11 (97%). Interview and/or questionnaire data on IOI at age 15 were available on 210 cases (97%).

4.1.2.2 Sample for analysis of the role of genetic factors

The second part of the empirical analysis examining the role of specific candidate susceptibility genes on the risk for IOI, utilised DNA taken from cells located on the inside of the participants' mouths using buccal swabs, following the protocol outlined in Freeman et al. (2003). Twenty three ERA study families could not be contacted for various reasons including; withdrawal from the study; contact lost with the family; no response from childcare authorities; firm refusal to participate in 15 year old assessment wave and difficult family circumstances, which made contacting them for DNA inappropriate. In addition, DNA was not able to be collected from three participants due to the severity of their behavioural, cognitive and/or physical impairment. DNA data was collected from a total of 129 of the possible 191 ERA study participants (68%). DNA was received from 97 participants out of the 142 (68%) Romanian adoptees, and 32 out of the 49 (65%) U.K. adoptees who were approached for DNA collection.

Every effort was made to collect as many samples as possible (see procedure section 4.2.2), but owing to the sensitive nature of genetic data collection, particularly for participants coming from an at-risk adopted sample, we had a higher than anticipated refusal/non-return rate.

The distribution of cases across the adoptee groups and gender that were included in the genetic analyses is outlined below in table 3.

Table 4.3

Sample size for genetic analyses across institutional deprivation adoptee groups and gender

	Adoptee group							
	U.K.	Rom non-IR	Rom IR <6	Rom IR 6-<24	Rom IR 24-43	Total	ERA sample contacted	% of ERA sample
Both sexes	32	13	32	29	23	129	191	68%
male	22	6	18	17	10	73	99	74%
female	10	7	14	12	13	56	92	61%

For the analysis of genetic effects the sample was split into high and low environmental risk groups (described in the following chapter, section 5.1). DNA

was received from 77 of the 111 (69%) participants in the low environmental risk group who were approached for data collection. In the high environmental risk group DNA samples were received from 52 out of the possible 80 cases approached (65%).

Systematic analyses conducted on the total ERA to compare the group whom we received DNA from (n=129) and the group we did not receive DNA from (n=88) revealed no significant differences between the groups. *T* tests were performed to compare the groups on the following relevant background variables: Age at adoption (*p*=.12); weight at adoption (index of subnutrition; Romanian sample only: *p*=.96); developmental level at adoption assessed retrospectively using the Denver questionnaire (Frankenburg, van Doornick, Liddell & Dick, N., 1986) (Romanian sample only[3]: *p*=.67). A comparison was also made with respect to IOI outcome, revealing no significant differences between the DNA cases and the missing cases (age 6: *p*=.37, *p*=.56; age 11; *p*=.84, *p*=.69; age 15: *p*=.72, *p*=.67, for parent and teacher reports, respectively). Moreover, the groups did not differ in terms of their overall impairment assessed using a composite measure (used in Kreppner et al., 2007) that incorporated assessments of disinhibited attachment, IOI, cognitive impairment, autistic features, emotional problems, peer problems and conduct problems (age 6: *p*=.12; age 11: *p*=.92). Five children (one U.K., four Rom IR) were excluded from the analysis of multiple impairment, on the basis of the severity of their impairment which made the standard battery of cognitive assessment unsuitable.

4.1.3 Family demographics and adoptee background

Adoptive family background and pre-adoption experience have been investigated and reported fully in previous ERA publications. Below is a summary of these accounts (see Rutter & English & Romanian Adoptees Study Team, 1998; O'Connor et al., 2000; Castle et al., 1999; Stevens et al., 2008)

4.1.3.1 Adoptive family demographics

In the sample as a whole the adoptive parents of the Romanian and U.K. adoptees did not differ from one another in terms of their educational or occupational status. Both were generally middle-class and were slightly better educated than the

[3] Only the children in the over 6 month group were included in this analysis as the scaling properties of the Denver made the results from the children <6 months on arrival meaningless.

general U.K. population. Slight differences were apparent in parental age and family composition between the adopters of Romanian versus U.K. children, but these differences reflected domestic adoption policy in place at the time (for full review see O'Connor et al., 2000). The main difference was that the parents of the Romanian adoptees were slightly older than those who adopted within the U.K. For example, at the time of the 6-year-old assessment wave, the mean ages for fathers were 42 and 44 years, for the U.K. and Romanian samples, respectively ($t(205)= -2.76$, $p<.01$). For mothers the mean ages were 40 and 42, for U.K. and Romanian samples, respectively ($t(214) = -1.98$, $p<.05$) (see O'Connor et al., 2000). There were also some differences with respect to family composition between the families with U.K. and Romanian adoptees. The parents of Romanian adoptees were more likely to have had biological children prior to adopting: 33% versus 2%, respectively ($\chi^2(1, n = 217) = 27.89$, $p < .01$); and a lower proportion had adopted prior to the adoption included in the ERA study: 4% versus 48%, respectively ($\chi^2(1, n=217)= 54.43$, $p<.01$). These data suggested that adoption policy at the time gave preference to prospective within-U.K. adoptive parents who do not have biological children, and that adoption of Romanian children was not primarily motivated by infertility (O'Connor et al., 2000). There were no differences in adoptive family characteristics within the group of Romanian participants with respect to the age at which they were adopted. Previous ERA analyses showed that these demographic variables did not effect behavioural or cognitive outcome, so were dropped from further consideration (Kreppner et al., 2001; O'Connor et al., 2000).

4.1.3.2 Background of Romanian participants prior to adoption

The Romanian children who were adopted into the U.K. in early infancy did not differ from those adopted at an older age with respect to the age at which they were placed in institutions (admittance usually occurred during the first weeks of life), or their level of developmental delay and their physical condition at adoption (O'Connor et al., 2000). The majority of children were in very poor health and were severely malnourished (Rutter & English & Romanian Adoptees Study Team, 1998). Most children were adopted from institutions (144/165, 87%) and the remainder were adopted from impoverished family settings (not necessarily their birth family). Although information on the children's pre-adoption experiences

were not systematically available, the largely anecdotal evidence clearly suggests very gross global deprivation across the sample. This was apparent in the poor physical condition and developmental delay of the children when they reached the U.K. (Rutter & English & Romanian Adoptees Study Team, 1998; Castle et al., 1999), and supported further by the evidence from reports of the conditions for children who remained in the institution (Kaler & Freeman, 1994). The individual reasons for admittance to institutions were, again, not systematically recorded but it is reasonable to assume that extreme economic adversity was the driving factor, rather than existing child impairment or developmental delay. This is evidenced largely by the early age at which the majority of children were placed in the institutions, the harsh economic conditions at the time, anecdotal reports and the degree of catch-up following removal from the depriving environment (O'Connor et al., 2000; Rutter & English & Romanian Adoptees Study Team, 1998).

The adoptive parents did not always know the ethnicity of the children they adopted, therefore, the collection of systematic data on ethnicity was not possible. However, in Romania as a whole, the ethnic makeup of the population is over 90% Romanian, defined by a common language, and includes a substantial minority of Roma people (estimates vary between 5–10% of the population), with additional minorities of people from neighbouring countries (e.g. Hungary and Russia). At the time of entry to the U.K., only a small minority of children possessed even the most basic language skills. Of the children aged 18 months or over (the age by which in a normal population the vast majority of children would be attempting to reproduce words) only 13 out of 57 were using 3 recognisable words, and none had even minimal fluency in spoken Romanian language, despite the age range of the children reaching 3.5 years. Language development throughout the sample was tested at age 6, and all assessments were carried out in English (Croft et al., 2007).

4.2 Procedures

4.2.1 Family visits: Interview and questionnaires

Study families were visited in their homes around the time of the adoptive child's 6th 11th and 15th birthdays. The primary caregiver (usually the mother) was interviewed in their home by experienced researchers. Information was collected on demographics, service use, child's social and behavioural functioning and

family characteristics. Moreover, a range of questionnaires were completed by the adoptive mother and father in order to provide additional measures of their child's functioning and relationships within the family. At ages 6 and 11 years, data on IOI were collected from both parents (where appropriate) using the hyperactivity subscale of a standardised questionnaire measure of behavioural functioning; the parent version of the Revised Rutter Scales (Hogg, Rutter & Richman, 1997). At age 15 the hyperactivity subscale on the Strength and Difficulties Questionnaire was used to measure IOI (SDQ; Goodman, 1997). Assessment of conduct problems was also made using the relevant subscales of these questionnaire measures. At age 6 the questionnaires were completed during the course of the visit (when possible), but at ages 11 and 15 they were usually returned by post, using a stamped addressed envelope supplied by the ERA project. The change in collection procedure may help to account for the slight attrition in completion rate we observed from age 6 to the later waves (see table 2). The semi-structured parental interviews with mothers usually lasted around 2.5 hours and were audio-taped and subsequently coded by the trained researchers. The format of the visits varied slightly across the assessment waves, with the most significant difference being the addition of the Child and Adolescent Psychiatric Assessment (CAPA) interview at the age 15 visit, which increased the duration of the visit (Lifetime Parent Version developed for use in the English and Romanian Adoptees Study: Rutter, Silberg, Colvert & Kreppner , 2004; based on Angold et al., 1995). The ADHD section of the CAPA interview was used as an additional, and more in depth, measure of symptomatology in mid-adolescence.

The children were assessed by trained researchers using a comprehensive test battery comprising a combination of semi-structured interviews, questionnaires and standardised measures of cognitive, neuropsychological, behavioural, social and emotional functioning. The assessment usually took place over two visits lasting approximately 2 - 2.5 hours each, and were largely video and audio-taped for subsequent coding. Following each visit, the developmental researchers completed observational ratings of the child's behaviour during the visit. The child version of the CAPA interview, carried out with the children when they were aged 15, did not include a section on ADHD (the main study was designed before the thesis presented here was conceived) (Lifetime Child Version developed for use in the English and Romanian Adoptees Study: Rutter, Silberg, Colvert & Kreppner, 2003; based on Angold et al., 1995). The aspects of child

assessment used in the current study related to the investigation of features associated with IOI, namely cognitive impairment, executive functioning and disinhibited attachment. The instruments used to tests these domains are described in detail in section 4.3, below.

Consent was sought during the parental visit to contact the child's teacher to complete a questionnaire on behaviour at school. At ages 6 and 11, teachers were sent the teacher version of the Revised Rutter Scales (Hogg, Rutter & Richman, 1997). At the age 15 assessment wave, teachers were sent the SDQ (Goodman, 1997). In summary, assessment of IOI/ADHD was made from reports collected via multiple informants (mother, father and teacher) and a range of methods (questionnaire and interview instruments).

4.2.2 DNA data collection

A separate ethics application was completed to cover the collection of DNA data for the GenERA study (see ethical approval section 4.4, below). DNA was collected using buccal swabs taken from the inside of participants' mouths as part of the age 15 assessment protocol. However, data collection was already underway for the main study when the research in the current thesis was commenced. This meant that DNA samples were collected by post from families that had already been seen, or in person from those yet to be seen for the main 15 year old assessment. Because the collection of genetic material for research is a sensitive area, particularly for an adoptive sample, and not well understood by the general population, special care was given to ensure that comprehensive information was provided and any questions or concerns were answered with clarity and sensitivity. If the main ERA study interviews had not yet taken place, then an information/cover letter and consent form was given to the mother during the parental interview for her initial consent. A sheet with frequently asked questions and answers was also provided at this stage for both the parent and the child to examine. In the situation where the family had questions or concerns about DNA collection, this would be communicated to the author by the researcher and, additionally, an option was provided on the consent form if the family wanted further information before giving consent. The families were then followed-up with a phone call or met in person by the author. An information sheet containing information about the whole developmental assessment with the child was sent

out in advance of the visit to ensure informed consent was obtained. The specific information/consent form for collecting a DNA sample was shown to the participant along with the general consent forms (copies of all the information and consent forms can be found in appendix 2).

In the situation that both the parent and the young person agreed for a DNA sample to be provided, then the interviewer left the mouth cell collection pack with the parent or participant and collected the sample when they returned for the second visit. If all the interviews and tests were done in one session (i.e. there was only one visit with the 15-year-old), then the interviewer attempted to get the sample during the session, ensuring that the mouth was free from food before doing so. Alternatively, a stamped addressed envelope was provided for sample return. Each mouth cell collection pack comprised an instruction sheet and one tube containing storage fluid, 10 cotton buds and labelled with the participant's ERA study identification number. The young person could perform the mouth swab themselves, or ask their parent to assist. The collection of mouth cells took approximately 5 minutes to complete.

For those families who had already been seen as part of the main ERA study when the current project commenced, a pack was sent out containing: A cover letter/information sheet; consent forms for both parent and adolescent; a frequently asked questions and answers sheet; one tube with storage liquid and 10 cotton buds; an instruction sheet for collecting the mouth cells. The pack also included a stamped addressed envelope for returning the tube, mouth swab sample and consent forms. If samples or notification of refusal to participate were not received, then follow-up phone calls were made and additional packs sent out when necessary. Visits to family homes were carried out in person specifically with the purpose of collecting DNA samples in order to maximise the number of samples obtained.

The collected samples were then stored in a secure facility in the laboratory at the Social, Genetic and Development Psychiatry (SGDP) Centre, where they remain for the time being until they are destroyed in accordance with the conditions of ethical approval. The DNA extraction of the buccal swab samples was completed in the SGDP laboratory by an experienced geneticist, Dr Keeley Brookes, following standard procedures outlined by Freeman and colleagues (2003).

4.2.3 Genotyping procedure

The specific genotypes used in the current analysis are described below in the sections on instruments and data analysis. In brief, two polymorphisms in the dopamine transporter (DAT1) gene, one in the dopamine receptor (DRD4) gene and two in the glucocorticoid receptor (GR) gene were studied in the current analysis. The genotyping of the DAT1 and DRD4 variable number tandem repeat (VNTR) markers was completed by Dr Keeley Brookes in the SGDP laboratory followed standard genotyping protocols, using 30 cycles of annealing 64ºC (DAT1 intron 8); 60 ºC (DAT1 3'UTR) or 55 ºC (DRD4 exon III) for 1 minute and extension at 72 ºC for 1 minute. Polymerase chain reaction products were genotyped on 2% agarose gel, checked and repeated whenever the band pattern was not clear (Brookes et al., 2006b; Brookes et al., 2005). The genotyping of the GR 9beta polymorphism was also completed by Dr Keeley Brookes at the SGDP laboratory using standard TaqMan SNP genotyping protocols (for more details, see *http://www.appliedbiosystems.com*). The genotyping of the GR *Bcl*1 polymorphism was carried out by using KASPar technology by KBiosciences (*http://www.kbioscience.co.u.k.*).

4.2.3.1 Genetic risk

Assessment of an individual's genetic risk was determined by how many risk alleles they carried of specific polymorphisms within the dopamine transporter, dopamine receptor and glucocorticoid receptor genes. Selection of the candidate genes was determined by the literature on the molecular genetics of ADHD, and early stress paradigms. The full rationale for selection and further details about the genotypes can be found in the introductory section 3.5.2.

Dopamine genotypes/haplotype

With respect to the dopamine genes, three variable number tandem repeat (VNTR) polymorphisms were selected; two within the dopamine transporter (DAT1) gene and one within the dopamine receptor (DRD4) gene. The studies of the human DAT1 gene (SLC6A3) in relation to ADHD have focused on a common 10-repeat (10R) putative high risk allele of a 40-base pair (bp) VNTR polymorphism within the 3' untranslated region (UTR) of the gene, which varies number of copies (between 3 and 13) and is located on chromosome 5p15.3

(Yang et al., 2007; DiMaio et al., 2003). This polymorphism is referred to as DAT1 40-bp (3'UTR) in the current chapter. There is also evidence for a synergistic association between exposure to an environmental pathogen and a common DAT1 haplotype (comprised of the 10R allele of the 40-bp VNTR in 3'UTR and a 6 repeat (6R) allele of the 30-bp VNTR in intron 8) (Laucht et al., 2007; Brookes et al., 2006b). The DAT1 30-bp VNTR (intron 8) is described in detail in Vandenbergh et al. (2000). The putative risk associated with the DAT1 40-bp (3'UTR) and the DAT1 30-bp (intron 8) genotypes will be analysed in relation to the ERA sample along with a haplotype analysis combining the two genotypes (for details, see data analysis section below).

The DRD4 studies have focused on the putative risk associated with the 7-repeat (7R) allele of a 48-bp VNTR in exon III of DRD4, located within chromosome 11p15.5, with the 4-, 7- and 2-repeat alleles being the most prevalent (DiMaio et al., 2003). The 7-repeat allele has been shown to produce a blunted response to dopamine (Faraone et al., 2005). This DRD4 genotype will also be used in the subsequent genetic analyses in the current study.

Glucocorticoid receptor (NR3C1) genotypes

The glucocorticoid receptor gene (GR) was selected because specific genotypes have been shown to moderate an individual's physiological and psychological response to stress (Wust et al., 2004; Kumsta et al., 2007). In the current study, the association with IOI was initially assessed in relation to a 4 genotype model made up of haplotypes from two single nucleotide polymorphisms (SNP) in the GR gene termed Bcl1 and 9beta (see appendix 6; adapted by R. Kumsta from Kumsta et al., 2007). The Bcl1 (rs41423247), a common polymorphism of the GR, is a SNP identified as a C to G nucleotide change in intron B, 646-bp downstream of the 3'end of exon 2 (Fleury et al., 2003; van Rossum et al., 2003). The 9beta (rs6198) is another common SNP of the GR and represents an A to G change at position 3669 in the 3'UTR at the end of exon 9beta (Kumsta et al., 2007). The Bcl1 C-G polymorphism is isolated for further examination in the moderation analyses of the current study.

4.3 Instruments

Table 4.4

Overview of all measures used in the current study

Feature	Assessment age	Measure
Institutional deprivation	entry to U.K.	Duration of deprivation expressed as: adoptee group status and environmental risk group status
Genetic risk	15 - 16 years	Dopamine transporter (DAT1) genes DAT1 40-bp VNTR (3'UTR) 10R = risk DAT1 30-bp VNTR (intron 8) 6R = risk Dopamine receptor (DRD4) gene DRD4 48-bp VNTR (exon III) 7R = risk Glucocorticoid receptor (GR) genes GR *Bcl*1 SNP (intron B) C:G GR 9beta SNP (exon 9beta) A:G
IOI	6 & 11 years	Parent report Rutter Scales: hyperactivity/inattention subscale Teacher report Rutter Scales: hyperactivity/inattention subscale
	15 years	Parent report SDQ: hyperactivity subscale CAPA: ADHD section Teacher report SDQ: hyperactivity subscale
Disinhibited attachment	6 years	Latent variable combining: parental interview items observer ratings: physical contact during tasks
	11 & 15 years	Latent variable combining: parental interview items observer ratings: behaviour during assessment
Conduct Problems	6 & 11 years	Parent report Rutter Scales: Conduct difficulties subscale Teacher report Rutter Scales: Conduct difficulties subscale
	15 years	Parent report SDQ: Conduct problems subscale Teacher report SDQ: Conduct problems subscale
Cognitive functioning	6 years 11 & 15 years	McCarthy Scales of Children's Abilities WISC: Wechsler Intelligence Scales for Children (short form)
Executive functioning	15 years	Backward digit span (WISC subtest)

The current study's outcome of interest, IOI, was assessed longitudinally using questionnaire measures. The cross sectional analyses using data from the age 15 assessment wave also utilised the CAPA interview. IOI behaviour was hypothesised to relate to duration of deprivation and genetic makeup. Associated features of the IOI phenotype were also assessed. An overview of all measures used in the current study is summarised in table 4.4, and described in the sections below.

4.3.2 IOI assessment using questionnaires

4.3.2.1 IOI assessment at age 6 and 11 years: Rutter Scales

The Revised Rutter Parent (A2) and Teacher (B2) Scales for school-age children (Elander & Rutter, 1996), with supplementary questions from Behar and Stringfield (1974) and described in Hogg, Rutter and Richman (1997), were administered at ages 6 and 11 years. The scales are widely used research measures of emotional and behavioural problems in school-age children. A factor analysis of this particular version has not been performed, but the psychometric properties of the original and revised scales have been extensively evaluated with positive results (for review, see Elander & Rutter, 1996). The reported re-test reliability was high: 0.89 for teacher ratings made three months apart by the same teachers and 0.79 for ratings made by different teachers. Agreement between mothers' and fathers' ratings on the parents' scales was 0.64, and mothers' re-test reliability after three months was 0.74. Comparisons with other similar instruments are reported in Elander & Rutter (1996).

The questionnaires were completed at both assessment waves by mothers, fathers and teachers. Parents were asked to complete the questionnaire on the basis of their child's behaviour in the last 3 months. Teachers were asked about behaviour during the current school year. At the age 11 assessment, the questionnaires were completed by each child's primary school main class teacher, (i.e. before they matriculated to secondary education). The scales comprise sets of items describing different behaviours. Each item, or statement, is scored on a 3 point scale of 0 – 2: 0 for *doesn't apply,* 1 for *applies somewhat,* 2 for *certainly applies.* The hyperactivity/inattention subscale (3 items) plus the supplementary questions (1 item on the parent scales; 3 items on the teacher scales) of the questionnaire were used. The items for mothers and fathers are identical, but teacher scales include additional items to the parent scales. The internal

consistency of the subscale using Cronbach's alpha was calculated for the ERA sample as a whole for mother, father and teacher ratings: mother: α= .84, .87; father: α= .83, 86; teacher: α= .92, .91, at ages 6 and 11, respectively. A complete set of items for the parent and teacher scales is available in appendix 3, listed alongside the equivalent questions from the SDQ used at age 15 (see below).

4.3.2.1 IOI assessment at age 15 years: SDQ

For the 15 year old assessment of IOI the Strengths and Difficulties Questionnaire was used (SDQ; Goodman, 1997). Full details of the SDQ are available on the website: *www.sdqinfo.com*. The SDQ is a brief measure designed to assess pro-social behaviour and psychopathology in children and adolescents. Informant ratings were again obtained from mothers, fathers and teachers. The questionnaire comprises sets of behaviour descriptor items based largely on the Rutter Scales. The Rutter Scales and the SDQ correlate very highly; for the total difficulties score a correlation of r= .88 and .92, for the hyperactivity/inattention subscale a correlation of r= .82 and .90, for parent and teacher reports, respectively (Goodman, 1997). The hyperactivity/inattention subscale of the SDQ was used for the current analysis of IOI in the ERA sample. Items are scored on a similar 3 point Likert scale to the Rutter Scales: 0 for *not true,* 1 for *somewhat true* and 2 for *certainly true.* Codings were reversed for the 'strengths' or positive items on the SDQ (i.e. 0 for *certainly true,* 1 for *somewhat true* and 2 for *not true*). The items on the parent and teacher questionnaires are identical and can be found in appendix 3. The psychometric properties of the SDQ have been evaluated and the measure shows good reliability and validity (Goodman, 2001). Factor analysis of the questionnaire items confirmed that the subscales describe and distinguish the behavioural domains well. The retest reliability after 4-6 months was .72 and .82, for parents and teachers, respectively. The internal consistency of the hyperactivity subscale is similarly satisfactory: parent: α = .77; teacher: α= .88 (Goodman, 2001). The ERA sample shows a similar pattern of high internal consistency for the subscale, tested using Cronbach's alpha: α= 86, .83 and .88, for mother, father and teacher reports, respectively.

4.3.3 IOI assessment using parental interview: CAPA

At age 15 parents were interviewed using the Child and Adolescent Psychiatric Assessment (CAPA) interview. The psychometric properties of the CAPA have been evaluated and suggest good validity and reliability for the interview (Angold & Costello, 2000). The ADHD section of the parent CAPA interview was used as an additional, and more comprehensive, measure of ADHD-type symptoms in the ERA sample in mid-adolescence (for list of symptoms, see appendix 4, table A2). The version of the CAPA interview used in the current study is the Lifetime Parent Version modified for use in the ERA study (Rutter, Silberg, Colvert and Kreppner, 2004). The ERA version is based closely on the original CAPA interview (Angold et al., 1995), but streamlined and adapted for the purposes of the ERA study by Professor Michael Rutter, one of the original authors of the CAPA, and the ERA team. A comprehensive rationale of the ERA version was provided by Professor Rutter and described below (M. Rutter, personal communication, February 22nd 2004).

The CAPA interview is an investigator-based structured interview, designed to obtain detailed descriptions of behaviour or emotions. For each behavioural item in the interview schedule, there is a brief description of the concept of the item and explicit coding instructions. Specific instructions are provided for the mandatory questions, and possible supplementary questions are listed to facilitate accurate coding of behaviours. For each item, the interviewer asked first about behaviour since the child was 11 years of age. In the event that the parent's answer indicated that the child's behaviour within that time had been problematic, then a further question was asked about behaviour over the preceding 3 months. The order of questions about the timing of disorder represents one of the significant alterations to the original CAPA interview schedule. The original CAPA was designed primarily to obtain details of current disorder (i.e. in the last 3 months), whereas, the ERA study was concerned with behaviour since age 11, and the codes from this period are used in the current analyses. Accounts of actual behaviours on actual occasions, rather than generalised descriptions, were required for coding, in order to help avoid biases created in overall perceptions and to trigger memories of specific behaviours.

The three behavioural domains of ADHD; inattention, overactivity and impulsivity, were covered in the hyperactivity/ADHD section of the CAPA. There

were a total of 11 symptom items, which are listed in appendix 4, table A2. The intensity of each behavioural item was coded on a 3-point scale: 0 for *not present;* 2 for *present in at least 2 activities and at least sometimes uncontrollable by the child or by admonition;* and 3 for *present in most activities and almost never controllable by the child or by admonition*. Reliability testing using the ERA study data showed a high level of internal consistency within the ADHD section of the CAPA when tested using Cronbach's alpha: $\alpha = 91$.

The schedule was set up as a diagnostic interview and, therefore, details about age of onset, disorder across settings and overall incapacity were obtained. The age of onset was noted for each of the 11 symptom items that were coded as present in the ratings of intensity. Separate codes were given for the overall presence of inattention and impulsivity at home, school and elsewhere. Overactivity across settings was rated on two separate items relating to 'fidgetiness' or 'restlessness'. Disorder in each setting was coded as: 0 for *absent* or 2 for *present.* The overall incapacity caused by all ADHD symptoms was rated on a 3-point scale: 0 for *no;* 1 for *yes, maybe*; and 2 for *yes, definitely*. Parents were asked about whether ADHD behaviour interfered with family, school or other activities.

ERA interviewers held meetings once to twice a year during data collection to ensure that all researchers were applying the same criteria for coding. More frequent meetings were held between the parental interviewers. Inter-rater reliability was carried out on approximately 10% of the sample's interviews. There was very high agreement between the ratings of the parental interviewers: $\kappa = .97$. The particularly high reliability statistic may be due in part to the large proportion of zeros coded on most of the interview schedules.

4.3.4 Assessment of associated features

The association between several specific features linked to ADHD in the wider population were analysed in relation to deprivation-related IOI/ADHD in the ERA sample. IQ (cognitive functioning) was also entered as covariates in the model used to test the interaction between institutional deprivation and genetic risk on the risk for IOI/ADHD. Accordingly, the following domains were assessed and measured as follows:

4.3.4.1 Assessment of cognitive functioning at age 6

At age 6 the children were assessed on the McCarthy Scales of Children's Abilities (McCarthy, 1972). The children were tested on four of the five McCarthy subscales: verbal, perceptual, quantitative and memory skills. The motor skills subscale was not included in the 6-year-old assessment. The McCarthy Scales were standardised in the USA in the early 1970's, and found to be highly correlated with other tests of IQ, including the Weschler Preschool and Primary Scale of Intelligence (WPPSI): r=.62-.71 with the 3 WPPSI IQs (McCarthy, 1972). The current study uses adjusted McCarthy scores that account for changes in norms from 1972 (for details see: Beckett et al., 2006). The McCarthy General Cognitive Index has a mean of 100 and a standard deviation of 10. IQ data was collected on 126 of the 129 children (98%) available for the analysis of genetic moderation of risk associated with institutional deprivation for IOI at age 6, in which, this variable was entered as a covariate.

4.3.4.2 Assessment of cognitive functioning at age 11 and 15

Cognitive function was tested using a short form of the Wechsler Intelligence Scales for Children at the age 11 and 15 assessment waves (WISC III$^{U.K.}$; Wechsler, 1992). The WISC is a widely used standardised measure of intelligence with established reliability and internal validity (Wechsler, 1992; Sattler, 2002). The WISC scales have a mean score of 100 and a standard deviation of 15 points. Four subscales of the WISC were administered as part of the ERA assessment battery: two measuring performance abilities – vocabulary and similarities; two measuring performance abilities – block design and object assembly. These four subtests were selected to provide a good estimate of full scale IQ (reliability coefficient = .94; Sattler, 2002), and were pro-rated to form a full scale IQ score. There were three Romanian IR children who have been excluded from the analysis of the association between IOI, IQ and executive function as the severity of their cognitive impairment was to such a degree, that these aspects of the assessment battery were not suitable and, therefore, not administered (Beckett et al., 2006). These were the same three children from whom DNA was not able to be collected due to the severity of their impairment, and, thus, were not included in the genetic analyses either.

4.3.4.3 Assessment of executive functioning at age 15

The concept of executive function covers a broad range of cognitive processes. In this study, only one aspect of executive functioning was assessed at age 15, verbal working memory. This was tested using the backwards digit span subtest on the WISC III$^{U.K.}$ (Wechsler, 1992), and used in the analysis of features associated with IOI. In the test, participants orally repeated a series of digits in the reverse order to which they were orally presented. The score used in the current analysis is the raw score of successfully completed trials.

4.3.4.4 Disinhibited attachment

Because there was no established protocol to assess attachment features in children aged 4 to 6 years when the study was set up, these features were assessed using a combination of interview items and investigator ratings. The ratings from the two sources were combined to form a single latent variable for each assessment wave (ages 6, 11 and 15). The details on the construction of this variable are included in the data analysis section. As the interview and investigator measures of disinhibited attachment were constructed for the purposes of the ERA study there were no established reliability or validity estimates. However, in order to increase the validity of the measures they were adapted each age point by the lead child psychiatrist on the study, Professor Michael Rutter, in order to make the questions more developmentally appropriate. Moreover, fit indices from the construction of the latent variable were used to ensure that the items 'fitted' together well, in terms of tapping the same underlying construct (see heading 5.4.2), thereby providing an indication of the validity of the measure.

Parental interview

A section in the investigator-based, semi-structured parental interview was designed to assess variations within children's (attachment) behaviour towards the parent and strange adults in novel and familiar settings, and was conducted with parents (usually mothers) when the children were 4, 6, 11 and 15 years of age. The data gathered from when the children were age 4 were not used in the current analysis as a full data set from this assessment wave is not available. The assessment of disinhibited attachment was made according to parental responses to questions about essential components of disinhibited behaviour. Each item was

rated on a three point scale (0/1/2): a score of '1' was given if there was some or mild evidence of disinhibited behaviour; a score of '2' was given if the behaviour was marked or pervasive. At age 6 the items were: definite lack of differentiation among adults with respect to the child's social response to them; clear indication that the child would readily go off with a stranger; definite lack of checking back with the parent in anxiety-provoking situations. At ages 11 and 15 the items were adjusted to make them more developmentally appropriate and a question regarding the making of personal comments to strangers was added. At age 11, the items were: social boundaries/physical closeness with strangers; approach/too friendly with strangers; failure to check back/wandering off; personal comments. At age 15 parents were asked only about behaviour since age 11. The items were: approach/too friendly with strangers; personal comments; physical contact.

Investigator ratings at age 6

Independent observational ratings of the child's interaction with the investigator (a stranger) were also made at each assessment wave. This was assessed in a different way at age 6 than at ages 11 and 15. At age 6 the child's behaviour towards the investigator was rated over the course of three tasks: puppets, Bus Story (Renfrew, 1991) and balloons. A rating was made on a three point scale (0/1/2) with respect to the extent to which the child made use of socially inappropriate physical contact in these three situations. Marked inappropriate contact was defined as multiple instances of holding the experimenter's hand or staying exceptionally close; child often had a hand on the experimenter; child cuddled in; child eager to sit on the experimenter's lap. The inter-rater reliability on 15 cases as measured by weighted kappa was .80 ($p < .001$) (Rutter et al., 2007a).

Investigator ratings at age 11 and 15

At age 11 and 15, more detailed ratings were made by the investigator with respect to children's interactions with the investigator over the course of the assessment session. A total of 6 items were included, which were available at both assessment waves, and were each scored on a three point scale (0/1/2). A rating of '1' corresponded to *some* and '2' corresponded to *clear* evidence of disinhibited behaviour. Most of the children were seen twice by the investigators at age 11 and

at age 15, so a mean score of the ratings made at the two visits was calculated. The items included were: unsolicited physical contact; verbal violation of boundaries; social violation of boundaries; amount of spontaneous comments; overall relationship with examiner; general disinhibition (lack of social reserve). A correlation matrix at age 11 showed substantial and significant correlations between the items, in the range of .15 to .76, but with most in the range of .35 to .76. At age 15, a correlation matrix showed a similar pattern of substantial and significant correlations between the items, in the range of .16 to .74. There was one non-significant correlation between 'spontaneous comments' and 'relationship with examiner' (r=.10, *p*=.18).

4.3.4.5 Assessment of conduct problems at age 6 and 11

Conduct problems, like IOI, were assessed age 6 and 11 using the Revised Rutter Parent (A2) and Teacher (B2) Scales for school-age children (Elander & Rutter, 1996) with supplementary questions from Behar and Stringfield (1974) and described in Hogg, Rutter and Richman (1997). The evaluation of the psychometric properties of the Rutter Scales described above in relation to IOI applies here, also (Elander & Rutter, 1996). The conduct difficulties subscale (5 items on the parent scales; 6 items on the teacher scales), plus the supplementary questions (3 on the parent scales; 4 on the teacher scales). Mothers, fathers and teachers completed the questionnaires on behaviour in the last 3 months (for parents) or during the last school term (for teachers). Again, the items were scored on a 3 point scale of 0 – 2: 0 for *doesn't apply,* 1 for *applies somewhat,* 2 for *certainly applies.* The items for mothers and fathers are identical, however, teachers rated conduct problems on several additional items. A complete set of the items is available in appendix 5, listed alongside the questions from the SDQ used at age 15.

4.3.4.6 Assessment of conduct problems at age 15

At age 15 conduct problems were assessed by mothers, fathers and teachers using the corresponding subscale of the SDQ (Goodman, 1997). The evaluation of the reliability and validity of the SDQ, reported above in the section on the assessment of IOI, applies here too (Goodman, 2001). The correlation between the Rutter conduct difficulties subscale and the SDQ conduct problems subscale

is: r=.88 and .91, for the parent and teacher reports, respectively (see Goodman, 1999). Items were again scored on a similar 3 point scale to the Rutter Scales: 0 for *not true,* 1 for *somewhat true* and 2 for *certainly true.* Codings were reversed for the 'strengths' or positive items on the SDQ (i.e. 0 for *certainly true,* 1 for *somewhat true* and 2 for *not true*). The conduct problems scale on the SDQ includes items relating to both conduct problems and oppositional-defiant type behaviours. Therefore, a broader definition of conduct disturbances can be applied to the relevant analysis in the current study of problems in mid-adolescence. The items on the parent and teacher questionnaires are identical and can be found in appendix 5.

4.3.5 Assessment of predictor variables: Duration of institutional deprivation

The age in months at which individual children entered the U.K. following their adoption from Romania was taken as an index of their duration of deprivation. No such data on age at adoption were available on the U.K. sample, however, it is known that they were all adopted under the age of 6 months. The vast majority of the Romanian children entered residential institutions within the first weeks of life and remained there until they were adopted. Their age at entry to the U.K. was, therefore, equal to the time spent in grossly depriving institutional environments for the majority of the children. Age at entry was treated as a categorical variable and described in detail below in the section on data analysis. The non-institution reared Romanians showed a different behavioural response in terms of IOI impairment from those adopted from institutions, irrespective of their age at entry. Therefore, they were kept separate in the initial analyses, and then later included in the low environmental risk group (see details on data analysis below).

4.4 Ethical approval

Ethical approval was sought in 1992 for the entire study from which the present study forms a part. Ethical approval was obtained from the Institute of Psychiatry and the Bethlem and Maudsley NHS Trust (reference number 59/92). The ERA study was granted updated ethical approval in 2003 for the mid-adolescent follow-up study. A separate ethics approval application for the genetics study was made. Ethical approval was granted in 2005 by the South London and the Maudsley NHS Trust (Bethlem and Maudsley Hospitals/Institute of Psychiatry) Research Ethics

Committee (REC reference number: 05/Q0706/174; IOP reference number 107/05). Copies of the ethical approval are attached in appendix 8.

4.5 Statement of personal share in the investigation

This PhD study is formed as part of the wider ERA project and also as an independent study of the role of genetic factors (GenERA). Data from three assessment waves of the main ERA study are utilised in the current thesis: age 6, 11 and 15. The author joined the project soon after data collection had begun for the mid-adolescent (15-year-old) wave, and has been involved in the data preparation and analysis of the 11 year old IOI data and the data collection, coding and analysis, in addition to the administration and ongoing direction of the 15-year-old assessment wave. Additionally, the author has set up and managed the GenERA study, in conjunction with Professor Edmund Sonuga-Barke.

In terms of the GenERA study, the author has been closely involved, in collaboration with Professor Sonuga-Barke, in the design, set up, management and analysis. This has involved formulating the information and consent forms, and completing the ethical approval (with guidance from Philip Asherson), designing the DNA data collection protocol and managing the collection process. The author has collected the majority of samples (either by post or in person) and organised the collection of DNA samples carried out by other members of the research team during family visits, arranged for the DNA extraction and genotyping, and, in discussion with Professor Sonuga-Barke, Dr Philip Asherson and Dr Keeley Brookes determined which markers were to be genotyped. Finally, the author has managed and analysed the genotyping data received.

In terms of the author's involvement with the main ERA study, data collection at age 15 was shared between several researchers, including the author. The ERA study employed six researchers in total: Four developmental researchers, who assessed the children and two parental interviewers. The author was a member of the developmental group and was responsible for one quarter of the child assessments (n=30 participants: 25 completed; 5 participants refused). Each researcher collected data on a range of child development measures and each participant was visited twice by the same interviewer (usually on separate days). Therefore, the author collected data on social, behavioural, cognitive and neuropsychological functioning, including the comprehensive child CAPA

interview, although much of these data are not presented in the current study. Furthermore, the author coded the collected data, was involved in discussions and decisions about coding schemes and completed part of the reliability analysis.

The information from parents on IOI behaviour gathered using questionnaires was collected as part of the parental assessment, which the author was not involved with. The teacher questionnaires were sent out by the team's administrator, a role the author performed for 9 months prior to the commencement of the PhD studentship. The task was completed by the administrator successor. Towards the end of the age 15 wave the author ascertained which cases were missing (i.e. the questionnaires that had not been posted back by parents or teachers). This was followed up by the administrator. The author was responsible for the data entry and data management of these questionnaires and analysis of the resultant data.

The information on ADHD symptomatology obtained from the CAPA interview was collected by the parental interviewing team, of which the author was not a part. However, the author managed and analysed these data. All of the analyses presented in the current thesis were conducted by the author.

Finally, the author has published two papers in conjunction with Professor Sonuga-Barke and colleagues on the ERA team. One on the age 11 IOI findings (Stevens et al., 2008) and a theoretical paper on the potential role of genetic factors in relation to IOI in the ERA study (Stevens et al., 2006).

CHAPTER 5: METHODOLOGY

DATA ANALYSIS

All statistical analyses were conducted using SPSS version 14.0 or 15.0 (SPSS Inc, 2005), any exceptions to this are noted where relevant. Effect sizes were calculated using an online calculator found at: *http://web.uccs.edu/lbecker/Psy590/escalc3.htm* (Becker, 1998/1999). An alpha level of .05 was used throughout the analyses.

5.1 Defining the study group variable

The analyses in the subsequent empirical chapters are presented in several stages which necessitate the sample being split in different ways to optimise the power so that it is possible to address the specific questions being asked, and the analyses being conducted. First, in the initial analysis of the overall effect of institutional deprivation on IOI, the study sample is divided into 3 adoptee groups: U.K., Romanian non-institution-reared (Rom non-IR) and Romanian institution-reared (Rom-IR). Second, the effect of duration of deprivation is investigated, and for these analyses the sample is split into 5 groups: U.K., Rom non-IR, Rom IR <6months, Rom IR 6 to <24 months and Rom IR 24 to ≤43 months. Third, for the examination of the rates of abnormal IOI/ADHD, the features associated with IOI/ADHD and the genetic analyses, the sample was split dichotomously into high and low risk environmental groups. The low environmental risk (e'risk) group comprises the U.K., Rom non-IR and Rom-IR <6 month subgroups. The high e'risk group comprises the Romanian children who experienced 6 months or more of institutional deprivation (i.e. Rom-IR 6 to <24 and Rom IR 24 to 43 month subgroups).

5.2 Analysis of behavioural data: Rutter Scales and SDQ

For analyses using the Rutter Scales and the SDQ, parent and teacher composite scores were created for both the IOI and conduct problems subscales. A combined parent score was calculated by taking separate mean mother and mean father z-scores across questionnaire items of each informant, and then calculating a mean of the two. This combined mean was then standardised to enable

comparison across raters and assessment waves. In order to maximise the sample size, children who had obtained ratings from only one parent were also included. The correlations between mother and father reports of IOI behaviours on the Rutter Scales (at 6 and 11 years), and the SDQ (at age 15), were high for the sample as a whole at each assessment wave, respectively: $r(175) = .74$, $p<.001$; $r(174) = .78$, $p<.001$; $r(159) = .81$, $p<.001$. Teacher scores were calculated by taking the mean z-score across the items for each behavioural domain.

In order to examine markedly abnormal IOI behaviour, its persistence over time, and to compare with prevalence rates and associated features found in the population, cut-offs were calculated by transforming the continuous outcome measure of IOI into categorical data. There were no established cut-off criteria for the Rutter subscales and, therefore, the following strategy was developed based on the procedure used for assessing behaviour rated on the SDQ (Goodman, 1997). The strategy used was the same as in the paper published on the age 11 findings (Stevens et al., 2008). In the current study, it enabled the longitudinal analysis of rates of abnormal behaviour within the ERA sample over the whole study period, and allowed us to compare rates of problem behaviour with those from a population sample using normative data on the SDQ. The equivalence of the two scales in terms of correlation between scales, items included in the subscales and rating structure, justified the application of a cut-off from one scale being applied to the other (see 'instruments' section 4.3.1, in the previous chapter).

The cut-off was calculated for the Rutter Scales according to the procedure for determining behaviour in the abnormal range, as outlined on the official SDQ-info website (Youth in Mind, *http://www.sdqinfo.com/ScoreSheets/e1.pdf*; Goodman, 1997). For the hyperactivity subscale on the SDQ a score of 7 or above on the summed composite (score of 0, 1 or 2 per item on a five question subscale; making a possible total score of 10) was considered in the abnormal range. The abnormal cut-off was transformed for the Rutter Scales by taking the lower limit of the abnormal banding on the SDQ and dividing by the number of items on the SDQ hyperactivity scale to obtain an average score per item, at or above which would be considered abnormal:

Equation: 7 (lower limit) ÷ 5 (items) = 1.4

This cut-off was then applied to the mean scores of parents and teachers on the Rutter Scales. At age 15 the SDQ cut-off of a total score of 7 or greater over the 5 subscale items was applied directly to the data.

5.3 Analysis of ADHD behavioural data: CAPA interview

The data on ADHD symptoms gathered using the CAPA interview is utilised in two ways. First, it is treated as a continuous variable using individual's total symptom score across the 11 symptom items (for list of symptoms, see appendix 4, table A2). Second, a research diagnosis of ADHD was generated by applying a modified version of the DSM-IV criteria and this was used as a categorical variable (see below). To do this, data was collapsed from a 3-point scale (0/2/3) to a dichotomous 0/1 variable (symptom present/absent). Owing to the very small number of '3's coded and the criteria for a code of '2' requiring a high level of impairment these two codes were collapsed into a single category and recoded as a '1'. The continuous CAPA ADHD symptom variable therefore ranged from 0 to 11. The total scores were standardised to allow comparisons across measures. The distribution was positively skewed, but performing a log or square root transformation did not alleviate the skew, owing to the high number of zeros in the original distribution. However, the standardised residuals generated in a regression analysis using the CAPA z-scores that co-varied for confounding factors (the same covariates used in subsequent ANOVA analyses) showed a sufficiently normally shaped distribution (see appendix 7).

The formulation of the criteria for the categorical cut-off was performed in consultation with a qualified and experienced child psychiatrist who is an author of the CAPA interview, Professor Michael Rutter. The diagnostic algorithm was designed to correspond as closely as possible to that set out by the DSM-IV-TR criteria (American Psychiatric Association, 2000), and to make use of the breadth of data available from the CAPA interview. The algorithm was based on 4 main criteria: symptom count, age of onset, presence of symptoms across settings and clinically significant impairment. These criteria correspond broadly to those required for a diagnosis of ADHD using the DSM-IV-TR and all four criteria had to be met in order for a research diagnosis of ADHD to be assigned. First, the symptom count criterion specified that four out of a possible seven overactive/impulsive symptoms and/or three out of a possible four inattentive symptoms had to be coded as present. The symptoms were divided in this way to

fit with the subtype classifications of the DSM-IV-TR. A DSM-IV-TR diagnosis requires that at least six out of nine overactive/impulsive symptoms and/or six out of nine inattention symptoms are present (for list of DSM-IV-TR symptoms sees appendix 4, table A3). Second, all symptoms were coded with an age of onset on the CAPA. The DSM-IV-TR criterion states that some hyperactive/impulsive or inattentive symptoms that caused impairment must be present before age 7 years. The CAPA criterion specified that 5 or more symptoms had to be present before the age of 7 years. Third, the presence of symptoms across home, school or 'other' settings was assessed for each symptom domain. The DSM-IV-TR criterion requires that some impairment from symptoms is present in two or more settings. The CAPA diagnostic criterion specified that symptoms must be present in more than two settings for at least one of the three ADHD domains (i.e. overactivity and/or impulsivity and/or inattention). The fourth, and final, criterion related to clinically significant impairment. This was assessed using the rating of overall impairment from ADHD symptoms given on the CAPA. The DSM-IV-TR criterion states that there is clear evidence of clinically significant impairment in social, academic or occupational functioning. The CAPA condition was that a 'definite yes' answer was given to overall impairment rating of ADHD behaviour interfering with family, school or other activities.

5.4 Analysis using associated features

For the longitudinal gene-environment interaction analyses (empirical chapters 7 and 8) where IQ was added as a covariate in the repeated measures AVOVA models, a mean score was used, which averaged individuals' scores from the age 6, 11 and 15 assessment waves. That is, a continuous mean IQ score variable was used that was calculated by averaging participants' age 6 McCarthy score and their age 11 and 15 WISC scores.

5.4.1 Gender discrepancy

Gender discrepancy in the rates of deprivation-related IOI was investigated by comparing the rates between males and females in the high e'risk sample with the prevalence rates found in the normal population. Normative data from a large representative British survey of child and adolescent mental health, which used the SDQ questionnaire, was exploited (Department of Health & Office for National

Statistics: Meltzer, Gatward, Goodman & Ford, 2000). The sample included 10,438 individuals aged between 5 and 15 years. Complete SDQ information was obtained from 10,298 parents (99% of sample), 8,208 teachers (79% of sample) and 4,228 11-15 year olds (93% of this age band) (Youth in Mind, *http://www.sdqinfo.com/bb1.html*).

5.4.2 Disinhibited attachment

To optimise the data available across informants and also to provide an index of the validity of the items to tap the same underlying construct, confirmatory factor analyses (CFA) were performed on the parent interview and investigator rating items. A one-factor model was used to create a comprehensive latent variable factor score underlying the item sets measuring disinhibited attachment at each assessment wave. Analyses were conducted in collaboration with Dr Ted Barker, a statistician at the SGDP Centre, using Mplus Version 4.1 (Muthén & Muthén, 1998-2006), with a Robust Weighted Least Squares estimator, to suit the categorical nature of the data. The model chi-square, the comparative fit index (CFI, critical value ≥ .90) (Bentler & Bonett, 1980), the Tucker Lewis Index (TLI, critical value ≥ .90)(Little et al., 2003) and the root mean squared estimate of approximation (RMSEA, critical value ≤ .08) (Browne & Cudeck, 1993) were used to determine model fit. The overall fit statistics of the final model for each assessment wave were high (CFI = .96 - .97; TFI = .94 - .96), even though the RMSEA fit indices were above the generally advisable level (RMSEA=.13 - .17). However, it has been suggested that categorical data may not be well described by these fit indices (Yu & Muthén, 2002), and, thus, it considered logical to choose the CFI and TLI as indicators of adequate fit for each CFA model.

5.5 Genotyping: Frequencies and data analysis

As noted above, the ERA sample was split dichotomously for the gene-environment interaction analyses into low and high e'risk groups. Table 5.1 sets out the subsample sizes for each of the genotypes and the haplotypes examined.

Table 5.1

Sample sizes across environmental risk groups for genotypes/haplotypes analyses

	Environmental risk groups		
Genotypes/ haplotypes	Low e'risk	High e'risk	total
DAT1 40-bp (3'UTR)	76 (60%)	51 (40%)	127
DAT1 30-bp (intron 8)	75 (59%)	52 (41%)	127
DAT1 10R-6R haplotype	74 (59%)	51 (41%)	125
DRD4 48-bp (exon III)	75 (60%)	51 (40%)	126
GR *Bcl*1	74 (62%)	46 (38%)	120
GR 9beta	77 (61%)	50 (39%)	127
GR Bcl1-9beta haplotype	74 (62%)	46 (38%)	120

5.5.1 Genotyping success

Genotyping of the VNTR markers was successful for over 95% of samples (DAT1 40-bp (3'UTR): 98.4%; DAT1 30-bp (intron 8): 98.4%; DRD4: 97.7%) and each marker was in Hardy Weinberg equilibrium (DAT1 40-bp (3'UTR): p=.72; DAT1 30-bp (intron 8): p=.96; DRD4: p=.94). Genotyping of the GR SNPs was successful for 93% of samples and both markers were in Hardy Weinberg equilibrium (GR *Bcl*1: p=.93; GR 9beta: p=.97).

5.5.2 Genotype frequencies

DAT1 40-bp (3'UTR VNTR):

In the genotyped sample as a whole, 58% of cases (n=74) were homozygous for the 10 repeat allele (high risk genotype; high g'risk) and 42% (n=53) were either heterozygous for the 10 repeat allele or homozygous for the low risk genotype (i.e. carried two copies of the 9 repeat allele). Using a Pearson's chi-square test (throughout), no differences in genotype frequency between the adoptee groups were found ($\chi^2(1)$=.01, p=.92): 58% of the low e'risk sample (n=44) were 10R homozygotes, and 42% (n=32) carried at least one non 10 repeat allele. In the high e'risk sample 59% (n=30) were 10R homozygotes and 41% (n=21) were non 10R carriers.

DAT1 30-bp (intron 8) VNTR:

66% of the sample (n=84) were homozygous for the 6 repeat allele (high g'risk group), and 34% (n=43) were either homozygous for the low risk genotype (i.e. 5 repeat) or were 6R heterozygotes. There were no differences in genotype frequencies between the e'risk groups ($\chi^2(1)=.83$, $p=.36$). In the low e'risk group 69% (n=52) were 6R homozygotes, and 31% (n=23) carried at least one non 6 repeat allele. In the high e'risk group 62% (n=32) carried two copies of the 6R allele and 39% (n=20) carried at least one non 6R allele.

DAT1 10R-6R haplotype:

The DAT1 haplotype combining the 40-bp VNTR (3'UTR) and the 30-bp VNTR (intron 8) was constructed following the approach used by Brookes et al. (2006b). There were haplotype data available on 125 study participants. The high risk haplotype group comprised the individuals who were homozygous for both the 10R 40-bp VNTR and the 6R 30-bp VNTR (n=62, 49.6%). The low risk haplotype group comprised all other haplotype combinations (n=63, 50.4%). There were no detectable differences in frequency between adoptee groups ($\chi^2(1)=.70$, $p=.40$). In the low e'risk group 53% (n=39) possessed the high risk 10R-6R haplotype and 47% (n=35) carried one of the low risk haplotypes. In the high e'risk sample 45% (n=23) were high risk 10R-6R haplotype carriers and 55% (n=28) possessed one of the low risk haplotypes.

DRD4 (exon III) genotype:

The high g'risk group consisted of the children who possessed at least one 7 repeat allele of the 48-bp VNTR in exon III of DRD4 (n=30, 24%). The low g'risk groups consisted of those who possessed no 7-repeat alleles (n=96, 76%). No differences in frequencies were detected between the e'risk groups ($\chi^2(1)=.13$, $p=.72$). In the low e'risk group 23% (n=17) carried at least one 7R allele and 77% (n=58) carried no 7R alleles. In the high e'risk sample 25% (n=13) possessed at least one 7R allele and 75% (n=96) possessed no 7R alleles.

GR BclI genotype:

49% of the sample (n=59) were homozygous for the C allele and 51% (n=61) were either homozygous for the G allele or were G:C heterozygotes. Again, the chi-

square test was significant, suggesting there were differences between the e'risk groups in terms of there genotype frequencies ($\chi^2(1)=7.69$, $p<.01$). In the low e'risk group 39% (n=29) were C homozygotes and 61% (n=45) carried at least one G allele. In the high e'risk group 65% (n=30) carried two copies of the C allele and 35% (n=16) carried at least one G allele.

GR 9beta genotype:

63% of the sample (n=80) were homozygous for the A allele and 37% (n=47) possessed at least one G allele (i.e. G:G or A:G). The chi square test showed there was no association between e'risk group and genotype ($\chi^2(1)=2.86$, $p=.09$). In the low e'risk group 69% (n=53) were A homozygotes and 31% (n=24) carried at least one G allele. In the high e'risk group 54% (n=27) were homozygous for the A allele and 46% (n=23) carried at least one G allele.

GR Bcl1-9beta haplotypes:

The GR haplotypes were a combination of the two GR SNPs: *Bcl*I and 9beta and were constructed using an adaption by Robert Kumsta (2008) of the approach outlined in Kumsta et al. (2007). Three haplotypes were formulated: the most common haplotype (MCH); the *Bcl*I G; and 9beta G. The approach paired these haplotypes (or alleles), to yield genotypes which were divided into 4 groups: MCH homozygotes (n=32, 27%), *Bcl*I G (one or two *Bcl*I G haplotypes; n=43, 36%), 9beta (one or two 9beta G alleles; n=28, 23%) and the mixed group (*Bcl*I G and 9beta G haplotype; n=17, 14%). Diagrams of the approach are available in appendix 6. The Pearson's chi square test revealed a significant association between environmental risk group and haplotype group ($\chi^2(3)=10.86$, $p<.05$).

Table 5.2

Sample sizes across environmental risk groups and GR haplotype groups

	Environmental risk groups	
GR haplotype groups	Low e'risk	High e'risk
MCH	18 (24%)	14 (30%)
*Bcl*I G	32 (43%)	11 (24%)
9beta G	11 (15%)	17 (37%)
Mixed	13 (18%)	4 (9%)
Total	74 (100%)	46 (100%)

CHAPTER 6: RESULTS

EARLY DEPRIVATION AND THE RISK FOR IOI: CHARACTERISING THE PHENOTYPE

6.1 Chapter outline

The following chapter is organised into three sections. The first explores the association between IOI and institutional deprivation longitudinally, combining the mid-adolescent data with that collected at the age 6 and age 11 assessment waves and cross sectionally using just the age 15 data. This section extends previous work on IOI as a specific area of deficit associated with institutional rearing by examining the pervasiveness and persistence in the period since the children left the institutions in infancy into mid-adolescence. One of the major advancements of the age 15 assessment wave was the inclusion of the CAPA interview, which was used to collect data on ADHD symptomatology as well as a wide range of other psychiatric domains. The data, collected from interviews with the participants' primary caregivers, complemented and enhanced the questionnaire data available from the Rutter Scales and SDQ completed by parents and teachers. The aim of this section was to address important questions about the persistence of risk effects over a substantial time period and the developmental trajectory of these effects on IOI outcome. Additionally, this section aimed to address questions about the clinical significance of the rates of IOI/ADHD abnormality by examining longitudinal patterns within the sample and by making comparisons with rates of nondeprivation-related IOI/ADHD found in the general population.

The second section aimed to characterise the deprivation-related IOI phenotype by examining patterns of association with phenotypic features commonly linked to IOI/ADHD in the wider nondeprived population. In addition, the issue is explored as to whether the deprivation-related phenotype can be characterised by specific underlying ADHD subtype symptoms, i.e. hyperactive/impulsive or inattentive. The similarity or distinctiveness of the phenotype is relevant as different causal mechanisms may be implicated in the aetiology of IOI in deprived and nondeprived samples, which may in turn lead to

the presentation of the phenotype differing as a function of moderating and mediating factors associated with institutional deprivation.

The third section of this chapter explores the association between IOI and disinhibited attachment behaviour, another common feature seen in institution-reared groups of children, but not an area that has received much attention in the ADHD literature. This pattern of behaviour shares many of the features of reactive attachment disorder, disinhibited subtype, with the defining feature being an unusually friendly approach to strangers, and was observed in the ERA study by the parents of participants and by investigators alike. The overlap between the two domains has been noted in earlier papers on IOI at age 6 (Kreppner et al., 2001; Kreppner et al., 2001) and briefly explored using the age 11 data (Stevens et al., 2008).

This chapter provides the background for the subsequent analyses in chapters 7 and 8 that investigate the moderation of environmental effects by genetic factors and explore important issues about the interaction and nature of causal mechanisms over time.

6.2 Background to analyses

6.2.1 IOI and institutional deprivation: Cross sectional and longitudinal associations

IOI has been identified in previously published ERA study papers as a specific area of impairment in childhood and early adolescence with robust associations with duration of institutional rearing (Stevens et al., 2008; Kreppner et al., 2001). The aim of the first part of the current chapter was to investigate the persistence and continuity of IOI in the ERA sample from childhood into mid-adolescence, and in particular the specific association with extended periods of institutional deprivation. The association between institutional rearing and IOI will be examined by way of analysis of variance tests of within sample group differences. A longitudinal analysis of the developmental trajectory of IOI will be presented in terms of persistence and change, both on an individual and a group level. The chapter's structure is based largely on our recently published paper on the age 11 findings but also includes the age 15 data (Stevens et al., 2008). Therefore the current study will extend previous work by looking at behaviour cross sectionally in

mid-adolescence and also longitudinally over three assessment waves spanning close to a decade. Moreover, unlike at earlier assessment age points, in mid-adolescence we have in-depth data on ADHD symptomatology and diagnostic criteria from the CAPA interview with parents as well as the questionnaire data from the SDQ from parents and teachers. This complements and broadens earlier analyses by addressing questions about the clinical significance of the IOI/ADHD phenotype in the ERA study. This was examined in two ways. First, by employing the SDQ as a measurement tool in our study, questions about clinical significance can be addressed by carrying out a between sample comparison of rates of IOI behaviour in the ERA sample with population based norms produced as a result of a large scale national study of child and adolescent mental health in Britain that also utilised the SDQ (Youth in Mind: *http://www.sdqinfo.com/bb1.html*; Meltzer, Gatward, Goodman & Ford, 2000). Furthermore, as the CAPA interview is designed to be used as a research diagnostic tool the section covering ADHD symptomatology is particularly informative about the clinical significance of IOI behaviours previously identified by the Rutter Scales and the SDQ.

6.2.2 IOI and associated phenotypic features

The aim of the second section was to extend to age 15 years our analysis of the data from the age 11 assessment wave work on characterising the deprivation-related IOI phenotype (Stevens et al., 2008). The rationale and structure of the current analysis is similar to that outlined in the paper but by utilizing data from three assessment waves spanning childhood into mid-adolescence this chapter aimed to provide a broader analysis of the developmental commonalities and distinctiveness of deprivation-related IOI phenotype compared with that seen in the non deprived general population. Furthermore, as noted above, at age 15 in addition to the questionnaire measure we have far more comprehensive data on ADHD presentation and symptomatology from the CAPA interview. By employing the data we have available on a wide range of relevant ADHD symptoms as well as age of onset, presence of symptoms across settings and clinically significant impairment from symptoms, we were able to construct a research diagnosis that was more closely akin to the clinical diagnostic criteria for ADHD specified in the DSM-IV-TR. This allowed a more clinically informed comparison of the association between deprivation-related ADHD in the ERA sample and the phenotypic

features with ADHD seen in nondeprived samples. The motivation for this investigation was the proposition put forward in previous research in the area that IOI following institutional rearing may be qualitatively different from that seen in children on the ADHD spectrum in the wider nondeprived population (Roy et al., 2004). This possibility leads us to reflect on the role of different putative causal mechanisms associated on the one hand, with deprivation-related IOI and, on the other hand, the corresponding domain of impairment seen in the nondeprived population. By identifying IOI in the ERA sample as a deprivation-related disorder, it immediately sets its aetiology apart from nondeprivation related IOI, where susceptibility genes have been found to play an influential role; both independently and via interactions with environmental risk factors, such as maternal prenatal smoking. Furthermore, non deprivation-related IOI is highly heritable and where environmental factors have been implicated they are largely concerned with the pre- and perinatal environment (Taylor & Rogers, 2005), rather than the risk associated with the extended psycho-social deprivation experienced by the children in the ERA study, or other post-natal social factors. Moreover, recent research by Thapar and colleagues (2008) suggests that the risk associated with prenatal factors such as maternal smoking and alcohol use may be mediated by parental genes. Indeed, the possibility that prenatal and genetic factors play an influential role in the risk for IOI in the ERA sample cannot be ruled out. The subsequent chapters in which we explore moderation by specific susceptibility genes of environmental risk associated with institutional deprivation addressed these issues.

With respect to aims of the current chapter and the findings reported in the related published paper (Stevens et al., 2008) the different putative causal mechanisms outlined above and in the introductory chapters indicate that the phenotypic features and developmental pathways associated with nondeprivation-related IOI may not apply to the behavioural phenotype seen following early institutional deprivation. Moreover, the published paper went some way to address these issues, but no firm conclusions could be reached (Stevens et al., 2008). By combining these data with the data from the mid-adolescent assessment wave the current chapter aimed to provide a clearer developmental picture of deprivation related IOI and explore more fully the phenotypic features patterns of association, building on the findings from the age 11 assessment wave. Four areas consistently shown in the literature to be associated with nondeprivation-related

IOI were examined in relation to IOI in the ERA sample both longitudinally and cross sectionally: i) the developmental link and overlap with conduct problems; ii) low IQ; iii) executive function deficits and iv) the gender discrepancy/ prevalence amongst males. The association with these factors is discussed in detail in the chapter 1 of the introduction. In addition an analysis of the separate ADHD subtype symptoms is presented using the CAPA interview data.

6.2.3 IOI and disinhibited attachment

The third section of the current chapter investigates the association between IOI and disinhibited attachment behaviour in mid-adolescence. The analyses build on those presented in the paper published on the age 11 findings (Stevens et al., 2008). Disinhibited attachment in relation to IOI represents perhaps the most obvious phenotypic area where deprivation-related IOI differs from that seen in the general population. Attachment disturbance of the type that corresponds to reactive attachment disorder, indiscriminately friendly/disinhibited subtype is a common feature noted across studies of institution-reared children (Zeanah et al., 2005; Chisholm, 1998; Rutter et al., 2007a; Roy et al., 2004). There is only a limited amount of research on the comorbidity of attachment disturbances and ADHD in non institution-reared samples and where research has been conducted it mainly focuses on secure/insecure or disorganised attachment relationships with parents, rather than disinhibited approach to strangers, and is often based on small clinical case studies (Horvath & Markman, 2008; Finzi-Dottan et al., 2006; Stiefel, 1997; Clarke et al., 2002). However, attachment theory holds that a secure and responsive early parent child relationship is an integral part of the development of effective self-regulation in the child and self-regulation is linked to impulse control, perseverance and behavioural inhibition, which make up important features of the nondeprivation-related IOI/ADHD phenotype. In combination with the striking pattern of disinhibited attachment observed in our sample and pattern of overlap noted by Kreppner et al. (2001), these studies highlight this as an important area of investigation when considering the phenotypic characteristics of I/O in adolescence.

Our paper reporting the age 11 findings on the effects in early adolescence within the ERA sample supported the idea that the two domains were dissociable but overlapping constructs warranting further investigation of the nature of the

association. The current analyses sought to extend these analyses into mid-adolescence and thereby present a more complete developmental picture of the overlap between the two domains.

6.3 Research questions

The aim of this chapter was to extend the findings reported on the age 11 assessment wave by investigating IOI in relation to early deprivation cross sectionally in mid-adolescence and longitudinally between 6 and 15 years in order to gain a fuller picture of the developmental pathways and also to examine in detail the deprivation-related phenotype. The following research questions set out to achieve this:

1. Does the risk for IOI associated with severe early institutional deprivation persist to age 15 years?
2. If so, what effect does duration of deprivation have on IOI at this age?
3. Are the rates of deprivation-related IOI/ADHD found in the adolescent Romanian high risk sample clinically significant?
4. Is there individual continuity in IOI behaviour over time?
5. Is deprivation-related IOI phenotypically similar to IOI/ADHD as seen in the nondeprived population in terms of:

   a. The developmental link and overlap with conduct problems?

   b. The association with low IQ?

   c. The association with executive dysfunction?

   d. The gender discrepancy/prevalence amongst males?
6. Is the deprivation-related phenotype characterised by particular underlying ADHD subtype symptoms?
7. Is there overlap between IOI and disinhibited attachment in mid-adolescence?

6.4 Results section 1: IOI persistence and clinical significance

The analytical strategy used in the recent paper on the age 11 follow up was closely followed in the current chapter in order to examine continuity and change over time in firstly, the effect of institutional deprivation on outcome and secondly, the association between IOI and relevant phenotypic features (Stevens et al., 2008).

6.4.1 Does the risk for IOI associated with severe early institutional deprivation persist to age 15 years?

Early rearing in the extremely depriving conditions of the Romanian institutions constituted a significant risk factor for elevated levels of IOI in the ERA sample at ages 6 and 11 years (Kreppner et al., 2001; Stevens et al., 2008). A within-sample evaluation was carried out across the three main ERA institutional deprivation adoptee groups using data from two informants and multiple assessment waves to investigate continuity in IOI impairment on a group level. The focus of this initial question was to investigate whether the groups differed in their level of IOI over time and if the level was still raised in mid-adolescence, as it had been at ages 6 and 11 years, for the Romanian institution-reared (Rom IR) group as a whole in comparison with the U.K. and the non institution reared Romanian (Rom non-IR) children after the children had spent at least 11 ½ years in their adoptive homes.

6.4.1.3 IOI and institutional deprivation effects over time: Longitudinal analyses

To investigate the overall effect of institutional rearing on levels of IOI over time repeated measures ANOVA tests were carried out between the three main adoptee groups: U.K., Rom non-IR and Rom IR, with assessment age included as a within subjects factor. Data on IOI behaviour from the Rutter Scales at ages 6 and 11 were analysed in conjunction with the age 15 data from the SDQ. Parent and teacher reports on the questionnaires were analysed separately and the results are presented in table 6.1. The sphericity assumption of the model was not met and so the Huynh-Feldt correction was applied.

Table 6.1

The main effects and interaction of institutional deprivation and assessment age on inattention/overactivity/impulsivity over time

	Main effects		Interaction
IOI age 6-11-15	Institutional deprivation group	Assessment age	Institutional deprivation group x age
parent report	F(2,169)=5.91**	F(1.9,311.9)=.63, p=.52	F(3.7,311.9)=.47, p=.74
teacher report	F(2,129)=8.74***	F(1.9,246.3)=.28, p=.75	F(3.8,246.3)=.08, p=.98

*p<.05; **p<.01; ***p<.001

Parent report at ages 6, 11 and 15 years: Institutional deprivation effects

The longitudinal analysis of the parent report data on IOI behaviour at ages 6, 11 and 15 years showed there was a highly significant overall effect of institutional deprivation adoptee group, no effect of assessment age and no interaction between age and group (*p*=.003*, p*=.52*, p*=.74, for the three effects, respectively). This indicated that adoptee groups differed in their IOI behaviour consistently over time, suggesting that institutional deprivation had a significant and persistent influence on levels of IOI from childhood to mid-adolescence, but average group levels of IOI did not change over time.

Teacher report at ages 6, 11 and 15 years: Institutional deprivation effects

The results from the longitudinal analysis of the teacher reports of IOI behaviour at ages 6, 11 and 15 on the Rutter Scales and the SDQ mirrored the results from parent reports presented above. Again there was a highly significant main effect of institutional deprivation adoptee group over time, no effect of assessment age and no interaction between age and adoptee group (*p*<.001*, p*=.75*, p*=.99, for the three effects respectively). These results added support to the finding outlined above that the main adoptee groups differed in their IOI behaviour and the significant effect of institutional rearing on outcome was consistent over time.

6.4.1.4 IOI and institutional deprivation effects at age 15: Cross sectional analyses

The cross sectional association between institutional rearing and level of IOI behaviour was explored to investigate the specific influence in mid-adolescence, to

compare with that reported in the published papers on the earlier assessment ages and to examine if institutional deprivation continued to be a significant risk factor for elevated IOI scores. Analysis of variance tests between the three institutional deprivation adoptee groups (U.K., Rom non-IR, Rom IR) were carried out using the data from the SDQ on IOI collected from parents and teachers at the age 15 assessment wave. Additionally, the ADHD symptom scores from the parental CAPA interview, also carried out when their children were aged 15, have been used to investigate the main adoptee group differences.

Parent ratings of IOI at age 15 (SDQ): Institutional deprivation effects.

The pattern of results reported in the paper on the age 11 assessment wave (Stevens et al., 2008) continued into mid-adolescence. That is, a higher mean IOI z-score, as rated on the SDQ, was found for the Rom IR group compared with the mean z-score for both the U.K. comparison group and the Rom non-IR group (Rom IR: *M*=0.18, *SD*=1.03; U.K.:*M*= -0.37, *SD*=0.92; Rom non-IR: *M*= -0.30, *SD*=0.56). An ANOVA test showed that the difference between the groups was significant ($F(2,181)=6.29$, $p=.002$). This effect was supported by post hoc Tukey's HSD tests. There was a significant mean difference in scores between the Rom IR sample and U.K. group ($p=.003$). The tests also showed there was no appreciable difference between the U.K. and the Rom non-IR groups ($p=.96$), whereas the mean difference in scores between the Rom IR and non-IR groups was much larger and although it fell short of significance it was in the expected direction in terms of the detrimental effect of institutional deprivation ($p=.14$).

Parent ratings of ADHD symptoms at age 15 (CAPA): Institutional deprivation effects

Similarly, parents also rated the young people in the Rom IR sample as having a higher level of ADHD symptoms on the CAPA interview compared with the other two main adoptee groups. Again the ANOVA test showed there was a significant difference between the groups ($F(2,192)=3.84$, $p=.02$). The mean z-score for the Rom IR group was significantly higher than the mean of the U.K. group as tested by a post hoc Tukey's test (Rom IR: *M*=0.14, *SD*=1.06; U.K.: *M*=-0.29, *SD*=0.82; $p=.03$). Whereas the Rom non-IR sample had similar scores to the U.K. group with no significant difference between the two groups (Rom non-IR: *M*= -0.24,

SD=0.77; *p*=.98). The difference between the Rom IR group and non-IR group was in the expected direction but did not reach significance (*p*=.30).

Teacher ratings of IOI at age 15 (SDQ): Institutional deprivation effects

The findings reported above were supported by the data from the teacher reports of IOI behaviour at age 15 on the SDQ. The Rom IR sample had the highest mean IOI z-scores compared with both the U.K. comparison group and the Rom non-IR group (Rom IR: *M*=0.21, *SD*=0.99; U.K.: *M*= -0.40, *SD*=0.93 Rom non-IR: *M*= -0.33, *SD*=0.84). The difference in mean scores was borne out by a significant ANOVA test of group differences ($F(2,161)=7.22$, *p*=.001). Post hoc Tukey's tests showed that the Rom IR sample had significantly higher scores than the within U.K. group (*p*=.001) and the difference between the Rom IR and non-IR groups approached but did not reach significance (*p*=.12). There was no appreciable difference between the U.K. and Rom non-IR groups (*p*=.98).

6.4.1.5 Nonparametric analyses

A check was carried out to ensure that the findings held when the data was analysed using nonparametric analyses. The main assumptions of the parametric one-way ANOVA model are: Continuous dependent variable; independent sample groups; normal distribution and homogeneity of variance. In the current study the dependent variables, IOI/ADHD mean z-scores, were continuous and the sample groups being compared were independent (U.K., Rom non-IR and Rom IR). With regards to the normality of the distribution, for the sample as a whole the distribution of scores was moderately positively skewed for parent and teacher SDQ ratings of IOI. For the ADHD symptom scores on the CAPA there was a fairly strong positive skew (see appendix 7: Figure A4). However the departure from normality was not extreme, as measured by their kurtosis values. The Kurtosis values for the three outcome measures at age 15: Parent SDQ, teacher SDQ and CAPA, were: -0.58; -0.69 and 1.44, respectively. The values were all less than $|2|$ and thereby within the rule of thumb range for suggested kurtosis values needed to meet the ANOVA assumption requirements. With regards to the equality of variances, the most disparate variances were for IOI scores of the Rom IR and Rom non-IR groups rated by parents on the SDQ (δ^2=1.06; δ^2=0.31, for the two samples, respectively). The largest variance was less than 4 times the size of the smallest variance and therefore meeting the rule of thumb for keeping within

the ANOVA assumption for equal variances. The sizes of the within sample groups were somewhat unequal for the analysis above using the parent SDQ, teacher SDQ and CAPA scores (see table 6.2). However, as noted above, the group with the smaller sample size, Rom non-IR, was not associated with the larger variance so the likelihood of the test reporting nonexistent differences in the mean score was reduced. Furthermore, for the analyses in the subsequent section the Romanian IR sample was split into the duration of deprivation groups as per the original ERA experimental study design. This helped to even up the sample sizes. In the later analyses subsample groups were pooled into low and high environmental risk groups, again resulting in sample sizes that are more even than in these preliminary analyses.

Table 6.2

Mean ranks for inattention/overactivity/impulsivity and ADHD symptoms across institutional deprivation adoptee groups at age 15

	Adoptee groups						
	Mean ranks			Sample size			
IOI/ADHD measure	UK	Rom non-IR	Rom IR	UK	Rom non-IR	Rom IR	Kruskal Wallis test
Parent report							
Age 15 (SDQ)	72.43	78.50	101.10	46	17	121	$\chi^2(2)=11.64^{**}$
Age 15 (CAPA)	80.49	88.00	105.58	47	17	131	$\chi^2(2)=9.23^{**}$
Teacher report							
Age 15 (SDQ)	62.98	67.75	92.83	45	14	105	$\chi^2(2)=14.19^{***}$

*p<.05; **p<.01; ***p<.001

Although the ANOVA test is fairly robust against minor violations of the assumptions such as those presented above, the Kruskal-Wallis non parametric analysis of variance test, which does not assume the population has a normal distribution, was employed as more stringent test of between group differences. The mean rank for the Rom IR group was consistently the highest across the measures of IOI/ADHD, compared with the U.K. and Rom non-IR groups (table 6.2). The chi-square test showed highly significant group differences in line with the parametric ANOVA findings for both parent and teacher reports of IOI on the SDQ and also the CAPA ratings at age 15 (p=.003; p <.001; p =.010, for the three measures, respectively).

Given the equivalent findings when using parametric or non-parametric statistics for all measures of IOI/ADHD symptoms, the ANOVA test seems robust enough to withstand any minor violation of assumptions by the distribution IOI in our sample. Therefore, ANOVA tests in conjunction with *t* tests where appropriate have been used in the subsequent analysis of IOI as a continuous outcome. For the analyses of group differences using *t* test statistics, equal variances were only assumed if Levene's test for equality of variances was not significant.

6.4.1.6 IOI and institutional deprivation effects – summary

The research question asked whether the risk for IOI associated with severe early institutional deprivation persisted to age 15 years. The longitudinal analysis showed that the adoptee groups consistently differed in their IOI behaviour across both parent and teacher measures of IOI, providing evidence that institutional deprivation had a significant and persistent influence on levels of IOI from childhood to mid-adolescence, and average group levels of IOI did not change over time. The cross sectional analyses using the age 15 data showed that the Romanian IR group had a significantly higher mean level of IOI/ADHD than the U.K. and Rom non-IR groups. The difference in mean scores was supported by highly significant parametric and non parametric tests of between group differences. The findings were in line with those published on the age 6 and age 11 assessment waves.

6.4.2 What effect does duration of deprivation have on IOI?

The aim of the following analyses was to examine the effect of duration of time spent in the globally depriving Romanian institutions on IOI longitudinally over time and cross-sectionally, in mid-adolescence. Earlier ERA study research has found extended institutional deprivation conferred an increased risk for the development of elevated levels of IOI compared with short periods or no institution rearing. Table 6.3 shows the mean IOI z-scores, standard deviations, sample sizes and ANOVA results across adoptee groups, differing in terms of duration of deprivation, according to parent and teacher reports on the questionnaire measures at ages 6, 11 (Rutter scales) and 15 (SDQ) along with parental reports on the CAPA interview on ADHD symptomalogy at age 15. In the current chapter

the longitudinal analyses are presented first and aimed to examine whether there was continuity over time in the effect of duration of deprivation on levels of IOI at a group level. Second, the cross-sectional analyses investigate whether a) the specific association between duration of time spent in the institutions and IOI at age 15 and b) if the pattern of association displayed a step-wise increase in risk for IOI at around the 6 months of age at adoption point, as demonstrated by the reported findings from the age 11 assessment wave (Kreppner et al., 2007; Stevens et al., 2008). The following analysis is a between-group comparison of levels of IOI for individuals in the Romanian institution reared sub-sample, split into three evenly sized groups according to their age at entry to the U.K: < 6 months, 6 to <24 months and 24 to 43 months, alongside the U.K. and non-institution reared Romanians.

Table 6.3

Mean levels of inattention/overactivity/impulsivity and ADHD symptoms (and standard deviations) across institutional deprivation adoptee groups, assessment wave, gender and informant

		Adoptee groups											
		Mean z-scores (SD)					Sample size						
IOI/ADHD measure		UK	Rom non-IR	Rom-IR <6	Rom-IR 6-<24	Rom-IR 24-43	UK	Rom non-IR	Rom-IR <6	Rom-IR 6-<24	Rom-IR 24-43	Total	ANOVA results
Parent report													
Age 6	Both sexes	-.29 (.86)	-.31 (.93)	-.32 (.83)	.35 (1.01)	.42 (1.09)	52	21	44	50	43	210	F(4,205)=6.76***
(Rutter Scales)	*male*	*-.04 (.90)*	*-.37 (.88)*	*-.35 (.76)*	*.37 (1.15)*	*.58 (.99)*	*34*	*11*	*21*	*22*	*17*	*105*	*F(4,100)=3.49**
	female	*.75 (.54)*	*-.25 (1.05)*	*-.29 (.90)*	*.33 (.91)*	*.52 (1.16)*	*18*	*10*	*23*	*28*	*28*	*105*	*F(4,100)=5.10****
Age 11	Both sexes	-.30 (.82)	-.32 (.84)	-.21 (.83)	.39 (1.08)	.25 (1.14)	48	20	42	49	40	199	F(4,194)=4.84**
(Rutter Scales)	*male*	*-.17 (.86)*	*-.34 (.63)*	*-.10 (.90)*	*.48 (1.16)*	*.45 (1.31)*	*32*	*11*	*22*	*22*	*14*	*101*	*F(4,96)=2.51**
	female	*.58 (.69)*	*-.30 (1.08)*	*-.32 (.75)*	*.32 (1.02)*	*.14 (1.05)*	*16*	*9*	*20*	*27*	*28*	*98*	*F(4,93)=3.05**
Age 15	Both sexes	-.37 (.92)	-.30 (.56)	-.19 (.99)	.44 (1.11)	.33 (.90)	46	17	42	38	41	184	F(4,179)=5.81***
(SDQ)	*male*	*-.18 (.94)*	*-.26 (.36)*	*.01 (1.03)*	*.51 (1.22)*	*.49 (.85)*	*30*	*6*	*22*	*16*	*15*	*93*	*F(4,88)=2.37; p=.058*
	female	*-.71 (.77)*	*-.33 (.71)*	*-.41 (.93)*	*.37 (1.04)*	*.23 (.94)*	*16*	*9*	*20*	*20*	*26*	*91*	*F(4,86)=4.68***
Age 15	Both sexes	-.29 (.82)	-.24 (.77)	-.32 (.61)	.65 (1.22)	.07 (1.04)	47	17	44	44	43	195	F(4,190)=8.10***
(CAPA)	*male*	*-.15 (.97)*	*-.39 (.40)*	*-.27 (.73)*	*.70 (1.38)*	*.32 (1.07)*	*31*	*6*	*23*	*22*	*16*	*100*	*F(4,95)=3.72***
	female	*-.58 (.19)*	*-.11 (1.00)*	*-.37 (.44)*	*.60 (1.07)*	*-.07 (1.01)*	*16*	*9*	*21*	*22*	*27*	*95*	*F(4,90)=5.69****
Teacher report													
Age 6	Both sexes	-.32 (.77)	-.40 (.78)	-.39 (.91)	.39 (1.01)	.53 (1.05)	47	18	43	44	40	192	F(4,187)=9.24***
(Rutter Scales)	*male*	*-.17 (.79)*	*-.73 (.43)*	*-.36 (.84)*	*.59 (1.07)*	*.41 (1.06)*	*33*	*9*	*20*	*18*	*16*	*96*	*F(4,91)=5.49****
	female	*-.69 (.59)*	*-.08 (.95)*	*-.36 (.98)*	*.24 (.97)*	*.61 (1.05)*	*14*	*9*	*23*	*26*	*24*	*96*	*F(4,91)=5.69****
Age 11	Both sexes	-.37 (.94)	-.24 (.86)	-.27 (.78)	.51 (1.06)	.26 (.97)	50	19	35	44	40	188	F(4,183)=6.98***
(Rutter Scales)	*male*	*-.16 (1.07)*	*-.33 (.85)*	*-.12 (.78)*	*.76 (1.12)*	*.31 (.98)*	*33*	*11*	*17*	*18*	*15*	*94*	*F(4,89)=3.39**
	female	*-.79 (.37)*	*-.11 (.91)*	*-.42 (.77)*	*.33 (1.00)*	*.23 (.98)*	*17*	*8*	*18*	*26*	*25*	*94*	*F(4,89)=5.95****
Age 15	Both sexes	-.40 (.93)	-.33 (.84)	-.13 (.86)	.58 (1.07)	.16 (.91)	45	14	33	36	36	164	F(1,159)=6.29***
(SDQ)	*male*	*-.28 (.99)*	*-.38 (.92)*	*.17 (.99)*	*.54 (1.22)*	*.25 (.85)*	*29*	*5*	*15*	*19*	*15*	*83*	*F(4,78)=2.26; p=.07*
	female	*-.61 (.61)*	*-.31 (.85)*	*-.36 (.68)*	*.62 (.92)*	*.10 (.97)*	*16*	*9*	*18*	*17*	*21*	*81*	*F(4,76)=5.33****

*p<.05; **; p<.01; ***p<.001

6.4.2.1 IOI and duration of deprivation effects over time: Longitudinal analyses

Repeated measures ANOVAs were used to test the overall effect of duration of deprivation on IOI from childhood to mid-adolescence, using the data collected from parents and teachers on the questionnaire measures of IOI behaviour at the age 6,11 (Rutter Scales) and 15 (SDQ) assessment waves. Duration of deprivation adoptee group was entered as the between subjects factor (U.K., Rom non-IR, Rom IR <6, 6-<24 and 24-43) and assessment wave as a within subjects factor. The sphericity assumption of the model was not met so the Huynh-Feldt correction was applied. Parent and teacher reports are analysed separately and the results are presented in table 6.4.

Table 6.4

The main effects and interaction of duration of deprivation and assessment age on inattention/overactivity/impulsivity over time

	Main effects		Interaction
IOI age 6-11-15	Duration of deprivation group	Assessment age	Duration of deprivation group x age
parent report	F(4,167)=6.77***	F(1.9,312.2)=1.12; p=.32	F(7.5,312.2)=.66; p=.62
teacher report	F(4,127)=9.42***	F(1.9,246.6)=.30; p=.74	F(7.8,246.6)=.62; p=.76

*p<.05; **p<.01; ***p<.001

Parent report at ages 6, 11 and 15 years: Duration of deprivation effects

Longitudinally, according to parent reports of IOI on the Rutter scales and SDQ, there was a highly significant overall effect of adoptee group, no effect of assessment age and no interaction between age and group *(p*<.001*, p*=.32*, p*=.72, for the three effects, respectively). The strength of the effect provides evidence that the duration of deprivation groups differed in their level of IOI and that there was a continuous effect across assessment ages. Figure 6.1 shows graphically the effect of duration of deprivation over time on IOI scores, as rated by parents. The differentiation between the two groups who experienced 6 months or more institutional deprivation and the other three “low risk” groups (U.K., Rom non-IR and Rom IR <6) was quite striking, suggesting that extended

deprivation confers a substantial risk for elevated levels of IOI. Moreover, this effect seems to be stable over the 9 years of the assessment period and continued to be apparent after the children have spent at least 11 ½ years in their adoptive homes.

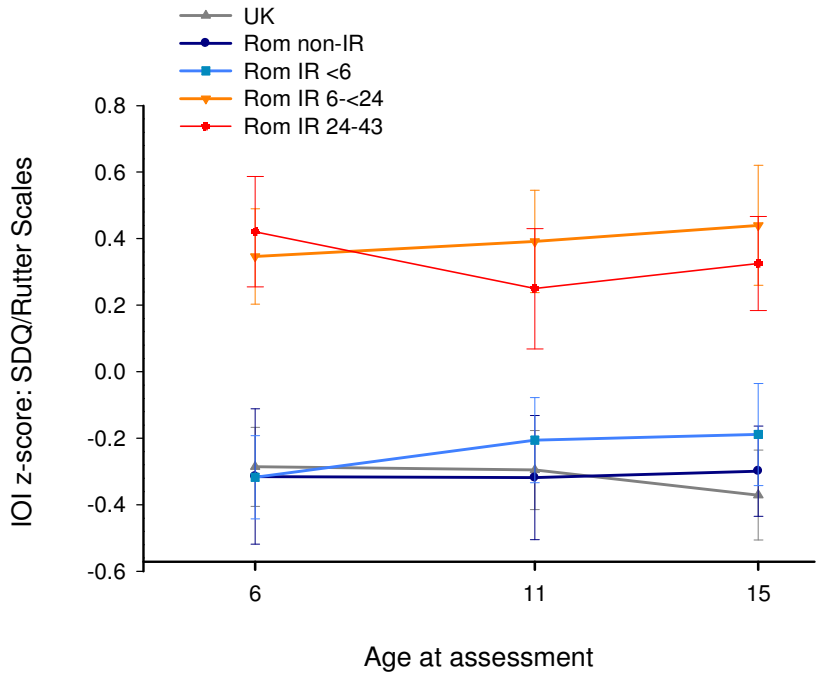


Figure 6.1
IOI over time: The effect of duration of deprivation (parent report)

Teacher report at ages 6, 11 and 15 years: Duration of deprivation effects.
The teacher report data showed the same pattern of longitudinal results as the parent report data presented above. That is, there was an overall effect of adoptee group, no effect of assessment age and no interaction between group and age over time ($p<.001$, $p=.74$, $p=.76$, for the three effects, respectively). Again, this indicated that the adoptee groups, as defined by duration of deprivation experience, had significantly different levels of IOI and this effect was not influenced by assessment age. Figure 6.2 displays the IOI scores given by teachers over time and across the duration of deprivation adoptee groups. The results were similar to the parent reports in that the two later placed Rom IR groups were consistently rated as having the highest IOI scores, providing further

support for extended institutional deprivation as a potent risk factor for IOI impairment. The graph seems to suggest that by age 15 the groups appear to have changed in their pattern of association, i.e. the Rom IR 6-24 month group was the highest scorer and the groups are more evenly spaced in their levels of IOI. However, as noted above, this apparent change was not borne out as a significant interaction effect between adoptee group and assessment age. The specific association between duration of deprivation and IOI in mid-adolescence is explored in more depth in the following section.

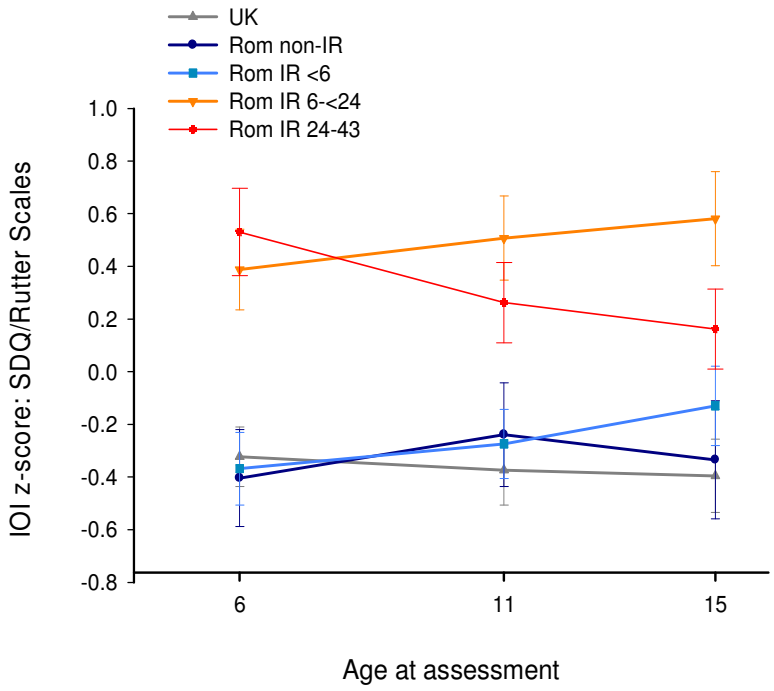


Figure 6.2
IOI over time: The effect of duration of deprivation (teacher report)

6.4.2.1 IOI and duration of deprivation effects at age 15: Cross sectional analysis

To investigate the effect of duration of deprivation on IOI in mid-adolescence within -sample comparisons of mean z-scores at age 15 were made across the sample groups, split according to duration of deprivation experienced, for the three measures of IOI/ADHD symptoms available (table 6.3). The focus was on whether the two later placed Rom-IR adoptee groups (6-<24 and 24-43 months) were at particular risk for IOI impairment.

Parent report age 15 (SDQ): Duration of deprivation effects

The ANOVA test showed that there was a highly significant overall difference between the adoptee groups with respect to their mean levels of IOI, as measured by the SDQ in mid-adolescence ($p<.001$). The young people who had experienced at least 6 months institutional rearing seemed at particular risk for elevated levels of IOI compared with those who experienced less than 6 months or no institutional care or were adopted from within the U.K.

The post hoc Tukey's HSD tests revealed no significant difference between the two late placed Rom-IR groups (6 to <24 and 24 to 43 months) in their level of IOI, as measured by parent reports on the SDQ *(*$p=.98$). Moreover, no differences were found between the U.K., Rom non-IR and Rom IR <6 months groups (U.K. vs. Rom non-IR*:* $p=.999$; U.K. vs. Rom IR <6*:* $p=.90$; Rom non-IR vs. Rom IR <6: $p=.99$).

The findings were in line with those found at the age 6 and age 11 assessment waves where the two late placed groups did not differ significantly in their level of IOI, rated by parents on the Rutter Scales (age 6*:* $p=.996$; age 11*:* $p=.96$) and nor did the U.K., Rom IR <6 months and Rom non-IR groups differ from one another (p's ≥ .99). Moreover, the post hoc Tukey's HSD tests at age 15 also revealed that the mean IOI scores of the Rom IR 6 to <24 month group were rated by parents as significantly elevated compared with the U.K., and Rom IR <6 months groups and a marginally significant difference in the same direction was found compared with the Rom non-IR group *(*$p=.001$, $p=.03$, $p=.06$ for the three group contrasts, respectively). Similarly for the Rom IR 24 to 43 months group, the level of IOI was rated by parents as being significantly higher than that in the U.K. group *(*$p=.01$).

A comparison was then made between the Rom-IR <6 months group and a pooled subsample consisting of the two later placed Rom IR groups (6 to <24 and 24 to 43). The *t* test showed that there was a significant difference between the two groups *(*$t(119)= -2.98$, $p=.004$). The mean scores indicated that the combined later placed subsample was rated as having a significantly higher level of IOI than the group of children who entered the U.K. before the age of 6 months (Rom IR 6 to 43: $M=0.38$, $SD=1.01$; Rom IR <6: $M= -0.19$, $SD=0.99$).

Parent report age 15 (CAPA): Duration of deprivation effects

The results from parental reports on the ADHD section of CAPA interview supported the findings from the SDQ (see table 6.3). The two later placed Rom-IR groups who had experienced 6 months or more institutional deprivation were rated as having higher symptom levels than the three 'low risk' groups (U.K., Rom non-IR, Rom IR <6). The ANOVA test showed there was a main effect of duration of deprivation group on ADHD symptom score at age 15 ($p<.001$). The post hoc Tukey's tests showed a similar but not identical pattern of results as those for the SDQ. Again there was no appreciable difference between the three low risk groups (U.K. vs. Rom non-IR*:* $p=1.000$; U.K. vs. Rom IR <6: $p=1.000$; Rom non-IR vs. Rom IR <6: $p=.99$). Furthermore, in line with the results above, the Rom IR 6 to<24 month group had significantly higher levels of IOI than the U.K., Rom non-IR and Rom-IR <6 months groups ($p<.001$, $p=.009$, $p<.001$ for the three group contrasts, respectively). However, the difference between the Rom-IR 6 to <24 and the 24 to 43 months groups also reached significance ($p=.04$). Nonetheless, these two later placed Rom IR groups still formed a homogenous subset according to Tukey's HSD test. Moreover, when a *t* test was performed comparing the Rom IR <6 months group with a pooled subsample of the two later placed groups (Rom IR 6 to <24 and 24 to 43 months) a significant difference in the level of ADHD symptoms was found between the two groups: $F(128.88)=-4.41$, $p<.001$ (note that equal variances were not assumed). The mean ADHD symptom score of the combined late placed subsample was significantly higher than that of the Rom IR <6 months group (Rom IR 6-43: $M=.37$, $SD=1.17$; Rom IR <6: $M= -.32$, $SD=.61$).

Teacher report age 15 (SDQ): Duration of deprivation effects

The findings from the teacher reports on the SDQ at age 15 lent strong support to the pattern of results presented above (see table 6.3). The analysis of variance test showed that the duration of deprivation adoptee groups differed significantly in their IOI scores ($p<.001$). Again, difference in mean scores suggested that the young people who had experienced at least 6 months institutional rearing seemed at particular risk for elevated levels of IOI compared with those who experienced less than 6 months or no institutional care or were adopted from within the U.K. Post hoc Tukey's tests supported this distinction. The two late

placed Rom IR groups did not differ from one another (p=.33), nor were there any differences between the three low risk groups (U.K. vs. Rom non-IR: p=1.000; U.K. vs. Rom IR <6: p=.73; Rom non-IR vs. Rom IR <6: p=.96). The Rom IR 6 to <24 month group was rated by teachers as having significantly higher IOI scores compared with the U.K., Rom non-IR and Rom IR <6 months groups (p<.001, p=.02, p=.02, for the three contrasts, respectively. The difference between the Rom IR 24 to 43 months group and the U.K. group was marginally significant (p=.07). When the two later placed Rom IR groups were combined and then compared with the Rom IR <6 months group a significant between group difference was found (t(103)= -2.46, p=.02). The combined late placed group had a substantially higher mean z-score (M=0.37, SD=1.01) compared with the early placed <6 months group (M= -0.13, SD=0.86).

Summary of duration of deprivation effects on IOI

As noted in tables 6.3 and 6.4 and illustrated by figures 6.1 and 6.2, the two later placed Romanian IR groups, who experienced at least 6 months institutional rearing, showed consistently elevated levels of IOI/ADHD across home and school settings and different measurement devices from childhood to mid-adolescence, relative to the U.K., Rom non-IR and Rom IR children adopted under the age of 6 months. These findings are in line with those outlined the Stevens et al. (2008) paper and provide strong evidence that institutional deprivation lasting for a duration of at least six months, confers a substantial, significant and persistent risk for IOI impairment, but that further risk is not incurred in a linear fashion as one moves to the ≥24 months group. By and large, there were no major differences in level of IOI/ADHD between the two later placed adoptee groups and the significant contrast throughout all the analyses was between this high risk cluster on the one hand and a low risk cluster consisting of the U.K., Rom non-IR and Rom IR <6 months groups on the other. This suggests that IOI in the Rom IR subsample aged 6 months or over at entry to the U.K. was related to the deprivation experience. Post hoc tests confirmed this distinction. Therefore, in the subsequent analyses investigating the clinical significance of deprivation-related IOI, individual continuity of IOI impairment and associated phenotypic features, the two late placed groups have been combined to form a high environmental risk (high e'risk) subsample. Where a within sample

comparison is required, the U.K., Rom non-IR and Rom IR <6 months groups have also been pooled to form a low environmental risk (low e'risk) subsample.

6.4.3 Are the rates of deprivation-related IOI/ADHD found in the adolescent Romanian high risk sample clinically significant?

In the previous section evidence was provided to show an elevated level of IOI in the high e'risk group of children, who had experienced at least six months institutional deprivation. This current section builds on these analyses and sets out to investigate whether this pattern of elevated scores translated into clinically significant rates of IOI impairment. This was done by classifying individuals into normal versus abnormal IOI cut-off groups using the guidelines for scoring the SDQ given on the sdqinfo website (*http://www.sdqinfo.com/b2.html*) and applying it to our questionnaire measures of IOI (SDQ and the Rutter scales), and for the CAPA data a 'research diagnosis' of ADHD was applied using a classification algorithm based on the DSM-IV-TR criteria. Details about the procedure and criteria for classification can be found in chapter 5 under headings 5.2 and 5.3 in the method section. Parent and teacher reports are kept separate for the analyses using the Rutter Scales/SDQ to examine whether the same pattern of impairment could be seen across settings. The analyses are split into two sections: The first is primarily descriptive and presents the within sample percentages of cases above cut-off across the high and low e'risk groups, assessment ages and informants, along with Pearson's chi-square tests of association. The second section focuses on the clinical significance of the rates in the high e'risk sample in mid-adolescence and at the age 6 and 11 assessment waves by comparing with the rates found in the general population. Population-based norms from a large scale national study that utilised the SDQ and investigated child and adolescent psychopathology in Britain were used to investigate this (*http://www.sdqinfo.com/bb1.html*; Meltzer, Gatward, Goodman & Ford, 2000).

6.4.3.1 Rates of abnormal IOI within the ERA sample

Table 6.5 sets out the percentages and numbers of cases in the abnormal range for IOI across e'risk groups, gender, informant and assessment waves. Pearson's chi-square is used to test whether the e'risk group is associated with, IOI

impairment (i.e. an abnormal range classification). Teacher reports follow those from parents. Within the results from each informant the findings from age 6 and age 11 are discussed first, then the results from the mid-adolescent assessment wave and finally the overall stability of effects over time is presented.

Parent report of rates of abnormal IOI at ages 6 and 11 years (Rutter Scales): Within sample analysis

The Pearson's chi-square tests revealed that there was a highly significant association between environmental risk group and IOI impairment at both the age 6 and age 11 assessment waves, according to parent reports on the Rutter Scales (age 6: p=.001; age 11: p<.001). The high e'risk group had significantly higher rates of children in the abnormal range for IOI than the low risk group, suggesting that deprivation lasting at least six months was a significant risk factor for clinically significant IOI impairment. This effect was apparent for both males and females, when the sexes were analysed separately (age 6_{male}: p=.02; age 6_{female}: p=.02; age 11_{male}: p<.001; age 11_{female}: p=.05). A more comprehensive discussion of gender effects is presented in a subsequent section 6.5.1.4.

Parent report of rates of abnormal IOI at age 15 (SDQ): Within sample analysis

The effect of environmental risk group on IOI impairment in childhood and early adolescence described above was mirrored in the results at age 15. Again, there was a highly significant association between risk group and IOI outcome, according to parent reports on the SDQ (p=.004). The proportion of children above cut-off in the high risk group (24%) was significantly higher than the proportion in the low risk group (9%). When the sexes were analysed separately the same pattern of effects was found, with a significant association in the male subsample and a marginally significant association in the female subsample (age 15_{male}: p=.01; age 15_{female}: p =.07).

Table 6.5

Percentages above inattention/overactivity/impulsivity and ADHD cut-offs across environmental risk adoptee groups, gender and informant

		Environmental risk groups ±				
		% above cut off		number of cases above cut off		
IOI/ADHD measure		Low e'risk	High e'risk	Low e'risk	High e'risk	chi-square results
Parent report						
Age 6	Both sexes	4%	18%	5	17	$\chi^2(1, N=210)=10.84^{**}$
(Rutter Scales)	*male*	*5%*	*18%*	*3*	*7*	$\chi^2(1, N=105)=5.11^{*}$
	female	*4%*	*19%*	*2*	*10*	$\chi^2(1, N=105)=5.52^{*}$
Age 11	Both sexes	6%	24%	6	21	$\chi^2(1, N=199)=13.81^{***}$
(Rutter Scales)	*male*	*6%*	*33%*	*4*	*12*	$\chi^2(1, N=101)=12.84^{***}$
	female	*4%*	*17%*	*2*	*9*	$\chi^2(1, N=98)=3.84,\ p=.05$
Age 15	Both sexes	9%	24%	9	19	$\chi^2(1, N=184)=8.37^{**}$
(SDQ)	*male*	*10%*	*30%*	*6*	*10*	$\chi^2(1, N=93)=6.16^{*}$
	female	*7%*	*20%*	*3*	*9*	$\chi^2(1, N=91)=3.31,\ p=.07$
Age 15	Both sexes	4%	16%	4	14	$\chi 2(1, N=195)=8.83^{**}$
(CAPA)	*male*	*5%*	*26%*	*3*	*10*	$\chi 2(1, N=100)=9.61^{**}$
	female	*2%*	*8%*	*1*	*4*	$\chi 2(1, N=95)=1.71,\ p=.19$
Teacher report						
Age 6	Both sexes	8%	32%	9	27	$\chi^2(1, N=192)=17.58^{***}$
(Rutter Scales)	*male*	*7%*	*29%*	*4*	*10*	$\chi^2(1, N=96)=9.29^{**}$
	female	*11%*	*34%*	*5*	*17*	$\chi^2(1, N=96)=7.26^{**}$
Age 11	Both sexes	8%	19%	8	16	$\chi^2(1, N=188)=5.38^{*}$
(Rutter Scales)	*male*	*10%*	*24%*	*6*	*8*	$\chi^2(1, N=94)=3.51;\ p=.06$
	female	*5%*	*16%*	*2*	*8*	$\chi^2(1, N=94)=2.99;\ p=.08$
Age 15	Both sexes	9%	29%	8	21	$\chi^2(1, N=164)=11.63^{**}$
(SDQ)	*male*	*12%*	*35%*	*6*	*12*	$\chi^2(1, N=83)=6.28^{*}$
	female	*5%*	*24%*	*2*	*9*	$\chi^2(1, N=81)=6.23^{*}$

*p<.05; **; p<.01; ***p<.001

± *Low e'risk: UK, Rom non-IR, Rom IR <6 months*
High e'risk: Rom IR 6 to <24 and 24 to 42 months

The consistency in the pattern of effects using parent reports of their children's behaviour from childhood to mid-adolescence suggests that at a group level the association between extended institutional deprivation and increased rates of IOI impairment is a developmentally stable effect within the ERA sample.

Parent report of rates of abnormal IOI at age 15 (CAPA): Within sample analysis.

The findings presented above from the questionnaire measures of IOI behaviour were corroborated by the results using the CAPA interview data on ADHD caseness at age 15. There was a much higher proportion of children in the high e'risk group that reached cut-off (16%) compared with that in the low e'risk group (4%). The Pearson's chi-square test confirmed that the association between e'risk group and ADHD research diagnosis was highly significant *(p*=.003). The results for the separate sexes showed a similar significant association between risk group and IOI impairment for the boys but association fell short of significance when the girls were analysed separately (age 15_{male}*: p* =.002; age 15_{female}*: p* =.19). Small cell sizes for the number of females above cut-off, particularly in the low e'risk group, may have influenced the *p* value (low e'risk: n=1; high e'risk: n=4). However, overall, these results provided further evidence of the significant effect of extended deprivation on IOI/ADHD outcome, an effect that was consistent across measurement tools.

Teacher reports of rates of abnormal IOI at ages 6 and 11 years (Rutter Scales): Within sample analysis

Teachers reported the same pattern of effects as parents. Significant chi-square statistics at both ages 6 and 11 indicated there was a significant association between e'risk group and IOI impairment, rated on the Rutter Scales (age 6*: p*<.001; age 11: *p*=.02). By and large, the association was consistent across the sexes, when analysed separately. The effect was highly significant at age 6 for both males and females (age 6_{male}*: p*=.002; age 6_{female}*: p*=.01). At age 11 the direction of effect was the same but levels of significance were marginal (age 11_{male}*: p*=.06; age 11_{female}*: p*=.08).

Teacher reports of rates of abnormal IOI at age 15 (SDQ): Within sample analysis

The result from the mid-adolescent phase mirrored those above with a significant chi-square test of association according to teacher reports on the SDQ *(p*=.001). The proportion of children that were classified by teachers as above the IOI impairment cut-off was significantly higher in the group who had experienced extended deprivation compared with those children in the ERA sample who had not (high e'risk=29%; low e'risk=9%). This pattern was seen in both the male and

female subsamples, with significant associations between group and outcome found for each sex (age 15_{male}*: p*=.01; age 15_{female}*: p*=.01). These results provide strong evidence that extended deprivation is a significant risk factor for IOI impairment in a school setting that is persistent, on a group level, from childhood to mid-adolescence.

Summary of effects: Rates of IOI/ADHD within the ERA sample

The findings were clear and developmentally stable, showing a substantial and significant effect of extended institutional deprivation that was persistent across settings, assessment ages, gender and different styles of measurement. The highly significant association between environmental risk group and IOI/ADHD impairment corroborated the duration of deprivation effects presented in the preceding section, providing further evidence that deprivation lasting six months or more constituted a significant risk factor for elevated levels and increased rates of IOI/ADHD impairment within the ERA sample.

6.4.3.2 Clinical significance of the rates of deprivation related IOI/ADHD in adolescence: Between sample analyses

This section deals with the clinical significance of the increased within-sample rate of deprivation-related IOI/ADHD impairment that was established in preceding section by comparing it with the rate in the general population. Only the data on ERA high e'risk sample (who experienced 6 months or more institutional deprivation) were used for the following analyses. First, the rates of abnormal IOI in early and mid-adolescence (using the Rutter Scales and SDQ data) are compared with normative data from a large representative British survey of child and adolescent mental health, which used the SDQ (*http://www.sdqinfo.com/bb1.html*). Details of the national survey and the relevant methodology can be found in the chapter 5 under heading 5.2. A full description of the population sample can be found in Meltzer, Gatward, Goodman & Ford (2000). The rate of the research diagnosis of ADHD in the ERA high e'risk sample, calculated using the CAPA interview with parents, is then discussed in relation to the rates of ADHD reported in the literature.

Rate of deprivation-related IOI versus population norms (Rutter Scales and SDQ)

We have established that in relation to the low e'risk ERA sample there were significantly elevated proportions of IOI in the high e'risk group, persistent from childhood to mid-adolescence. To answer the question about the clinical significance of the rates in early and mid-adolescence, a comparison with age appropriate population norms was necessary. The comparison between the ERA e'risk sample and population figures utilised normative SDQ data for 11 – 15 year olds (parent report: n=4443; teacher report: n=3407) and cut-offs for deprivation-related IOI impairment in the ERA sample were based on the frequency distribution for SDQ scores in the normal population (*http://www.sdqinfo.com/bba9.pdf*). Chi-square tests of independence were used to assess the association between group and IOI outcome. To allow direct comparison with the population norms, parent and teacher reports on IOI in the ERA sample are analysed separately. Figure 6.3 sets out the percentages above the abnormal cut-off in the high e'risk Romanian groups (≥6 months' institution-rearing) at ages 11 and 15 compared with the population norms.

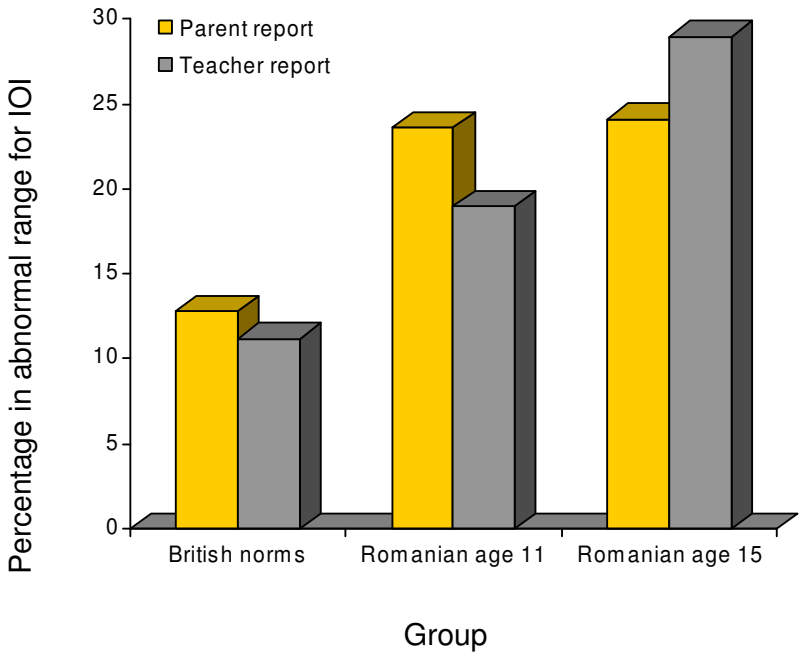


Figure 6.3

Percentages in abnormal range for IOI: British population norms and Romanian institution-reared high e'risk sample, aged 6-43 months at entry to U.K.

Figure 6.3 shows that compared with the age matched British population norms, the Romanian high e'risk group had close to twice the proportion of cases in the abnormal range for IOI at age 11 (1.8; 1.7) and age15 (1.9; 2.6), according to parent and teacher reports, respectively. Chi-square tests of independence were performed to examine statistically the association between sample group (Rom e'risk vs. British population) and IOI impairment. At age 11 the association between these variables was significant according to both parent and teacher reports (age 11_{parent}: $\chi^2(1, N=4532)=8.97$, $p<.01$; age $11_{teacher}$: $\chi^2(1, N=3491)=4.98$, $p<.05$). The same pattern of results was found in mid-adolescencewith a significant association between sample group and IOI outcome (age 15_{parent}: $\chi^2(1, N=4522)=8.68$, $p<.01$; age $15_{teacher}$: $\chi^2(1, N=3479)=22.19$, $p<.001$). Given the similar pattern of results across assessment ages and informants, the findings presented above provide strong evidence to suggest that children in the Romanian e'risk group were more likely to be rated as being in the abnormal range for IOI than would be expected from population norms. This indicates that the elevated rates of IOI impairment found in the Romanian e'risk group in adolescence are clinically significant and pervasive across home and school settings, with some suggestion that the effect got larger as the children reached mid-adolescence.

Clinical significance of ADHD: Research diagnosis

At age 15 years, 16% of the e'risk sample received a research diagnosis of ADHD (see table 6.5). That is, their symptom count was above the CAPA cut-off for the hyperactivity/impulsivity and/or the inattentive subtype and they met the criteria for age of onset, presence of symptoms across settings and significant impairment in activities. Population studies of the prevalence of ADHD estimate that the disorder affects around 5% of children worldwide (Polanczyk et al., 2007). The proportion of children in our ERA e'risk group assigned a research diagnosis of ADHD is over three times that seen in general population samples. It is prudent to note that although our diagnostic criteria are closely based on the DSM-IV-TR model and were formulated through consultation with an experienced child psychiatrist and one of the authors of the CAPA interview (Professor Sir Michael Rutter) the full range of symptoms used for the diagnosis of ADHD in the DSM-IV-TR were not included in the CAPA schedule (see appendix 4 for the CAPA and DSM-IV-TR symptom items. Because the samples included and the

methodologies employed were somewhat different in our study and those in the literature, this comparison is mainly for illustrative purposes. However, the substantial increase in rates of ADHD in the Romanian e'risk compared with that in the normal population provides additional validation for the Rutter Scale/SDQ findings presented above. By examining the rates of IOI/ADHD across different styles of measurement, the conclusion that deprivation-related IOI/ADHD is clinically significant domain of impairment is strengthened.

6.4.4 Is there individual continuity in IOI behaviour over time?

According to both parent and teacher reports, the overall mean z-score of the Romanian high e'risk group (aged 6 – 43 months at entry to the U.K.) remained fairly stable across assessment ages (age 6_{parent}: *M*=0.38; age 11 $_{parent}$: *M*=0.33; age 15_{parent}: *M*=0.38; age $6_{teacher}$: *M*=0.46; age $11_{teacher}$: *M*=0.39; age $15_{teacher}$: *M*=0.38). The overall mean level of IOI and the proportion of children above the cut-off for IOI impairment appeared to be relatively stable across assessment ages and informants and remained high into mid-adolescence. The level of individual continuity in behaviour can be tested in two ways: i) using correlations and; ii) using a threshold approach to examine whether it was the same or different individuals who were reaching cut-off across the three time points.

6.4.4.1 Correlational analysis of IOI continuity

To get a picture of overall continuity, or persistence, of IOI behaviour within the e'risk group bivariate, Pearson's correlations were carried out across the three assessment waves: Age 6 to age 11 and age 11 to age 15. There were highly significant correlations, according to both parent and teacher reports, between the questionnaire ratings of IOI at ages 6 and 11 years (parent report: r=.67, p<.001; teacher report: r= .43, p<.001) and between ages 11 and 15 years (parent report: r =.85, p<.001; teacher report: r =.51, p<.001).

6.4.4.2 Categorical analysis of IOI continuity

The analysis of whether it was the same or different children with IOI impairment at each assessment wave was explored using the data from the Rutter

Scales/SDQ. The same categorical approach used in the analysis in the preceding section on clinical significance to calculate the abnormal versus normal cut-off groups was applied here (see method section 5.2)

Figures 6.4 and 6.5 illustrate the continuity of IOI behaviour between ages 6 to 11 and 11 to 15 years for parent and teacher reports of normality and impairment. Note that data from all three assessment waves were required for a case to be included in the analysis. The McNemar change test was then used to statistically test categorical cut-off changes over time.

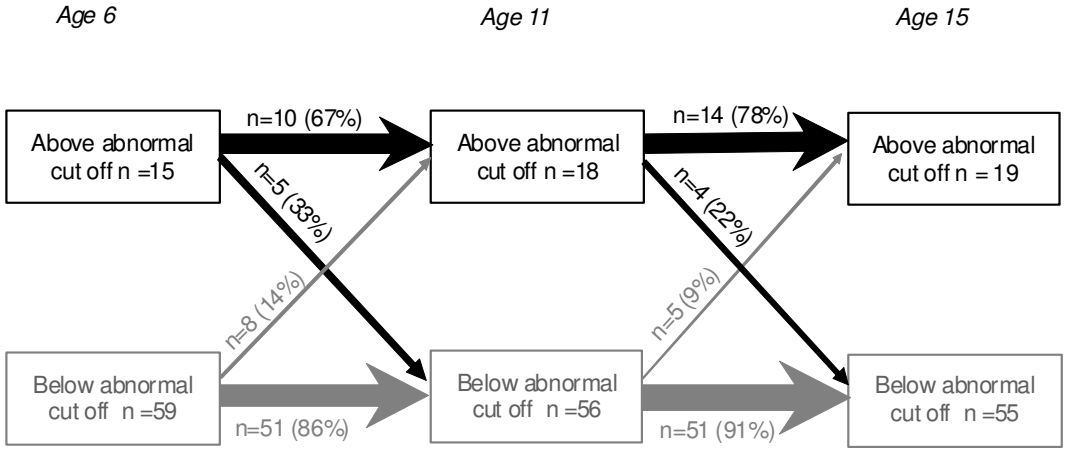
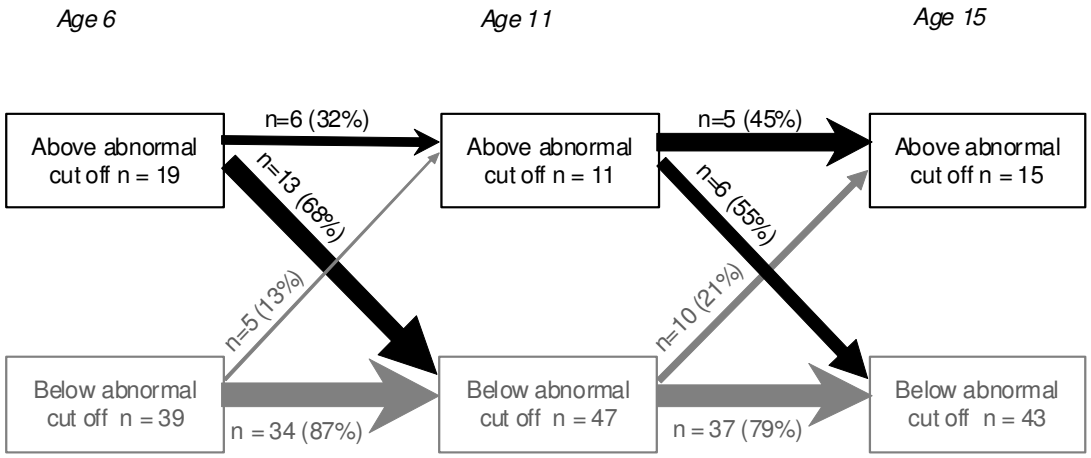


Figure 6.4
IOI continuity and change for individual children in the Romanian IR high e'risk sample aged 6 – 43 months at entry to the UK (parent report)

Parent reports of individual continuity: Age 6 - 11 - 15 years (Rutter Scales/ SDQ)
Figure 6.4 shows there was moderate to strong individual continuity for reports of IOI impairment and normality from childhood to mid-adolescence for the Romanian high e'risk sample. The vast majority of children in the normal range for IOI at age 6 remained below cut-off at age 11 (86%) with a similar proportion

staying below cut-off between ages 11 and 15 (91%). The continuity of impairment was also fairly strong from age 6 to age 11 with two thirds (67%) of children staying above cut-off. Persistence of impairment seemed to be slightly stronger from early to mid-adolescence with four fifths (78%) of those above cut-off at age 11 continuing to be in the abnormal range at age 15. The McNemar change test, used to assess categorical cut-off changes over time, showed there was no significant difference in the movement between IOI cut-off categories between ages 6 and 11 years (p=.45) or between ages 11 and 15 (p=1.00).



n=58 (41% of dataset missing)

Figure 6.5

IOI continuity and change for individual children in the Romanian IR high e'risk sample aged 6 – 43 months at entry to UK (teacher report)

Teacher reports of individual continuity: Ages 6-11-15 years (Rutter Scales/ SDQ).

Similar to the parent reports above, teachers reports showed that a high proportion of cases stayed in the normal range throughout the assessment period. 87% of cases below cut-off at age 6 remained in the normal range at age 11. Similarly in adolescence, 79% of children stay below cut-off from age 11 to

age 15. According to teachers, there seemed to be substantial proportion of individuals moving from the abnormal range to the normal range, from one assessment wave to the next. That is, only a third of the children above cut-off for IOI at age 6 remained in this category at age 11 (32%), and between ages 11 and 15 just under a half remained in the abnormal range (45%). However, the McNemar change test showed that the degree of change between categorical cut-offs over time was not significant between ages 6 and 11 (p=.09) or between ages 11 and 15 years (p=.24). Therefore, the proportion of individuals moving from impairment to normality was not significantly different from that moving from normality to impairment.

Summary of individual continuity of IOI over time

The results indicate moderate to high individual continuity in IOI behaviour from childhood to mid-adolescence, both in terms of mean scores and categorical impairment or normality. This was particularly clear with respect to reports of IOI behaviour from parents, but teacher reports showed a less consistent pattern of continuity. Nevertheless, the correlations between IOI scores at consecutive assessment waves were of medium strength according to teachers, and high according to parents. Given the inherent problems with cut-off analyses, correlational method may be more a powerful indicator of continuity than the categorical approach. For example, cases that hover around the cut-off and score above at some assessment ages and below at others would not be picked up as showing persistent levels of IOI in a categorical analysis. However in a correlational analysis such cases would accurately show high correlation across the ages.

By and large, the categorical analyses corroborated the correlational data with high levels of continuity for cases in the normal range and medium to high persistence of individual's IOI impairment; excepting the categorical analysis using teacher reports, which showed a substantial amount of drop-off in terms of cases falling below cut-off from one assessment wave to the next, particularly between ages 6 to 11 years.

In summary, if taken overall the findings provide evidence to show that IOI is a fairly stable domain of impairment in the Romanian high e'risk sample, on both an individual and a group level.

6.5 Results section 2: Presentation of the phenotype

6.5.1 Is deprivation-related IOI similar to IOI/ADHD as seen in the general, non-deprived population in terms of its associations?

This section aimed to characterise the deprivation-related IOI/ADHD phenotype by examining the relationship within the high-e'risk sample with certain features commonly associated with IOI/ADHD in the general population. This will be presented in relation to four features of the non deprivation-related IOI/ADHD phenotype: The developmental link and overlap with conduct problems; the association with low IQ; the association with executive function deficits; and the gender discrepancy/ prevalence amongst males. The aim was to examine whether the ERA deprivation-related phenotype was similar to, or distinct from, that attributed to 'common' IOI/ADHD found in the general population. This was done in two ways: First, the Romanian high e'risk sample was categorised according to whether they received a research diagnosis of ADHD using their data from the CAPA interview with parents. The CAPA diagnosis was used instead of the SDQ measure as it provided a more comprehensive assessment and is closely aligned with the DSM-IV-TR clinical diagnostic criteria for ADHD. The pattern of concurrent associations at age 15 between deprivation-related ADHD and the phenotypic features of interest are presented in table 6.6. *T* tests were used to compare the scores within the high e'risk sample of the two cut-off groups (above and below the diagnostic cut-off) across the measures of behavioural phenotypic features, i.e., conduct problems, IQ and executive function. The presence of a discrepancy between the genders was analysed by looking at the ratio of males to females with a research diagnosis of ADHD within the Romanian high e'risk sample and comparing with that reported in the literature on the ADHD in population and clinical samples.

The second part of the current section aimed to complement and extend these analyses by utilising the continuous questionnaire data available from all three assessment waves from the Rutter Scales/SDQ. The questionnaire data was used for within sample analyses of a) the mid-adolescent correlations between IOI and the phenotypic features of interest and b) the developmental pathways from and between IOI and conduct problems. Additionally, the cut-offs for deprivation-related IOI impairment using the SDQ/Rutter Scales data were

used to investigate the developmental pattern of sex differences. These were calculated using the guidelines for scoring the SDQ on the SDQinfo website (*http://www.sdqinfo.com/b2.html*). British population norms on non deprivation-related IOI in 5-10 year olds and 11-15 year olds were utilised for the analysis (*http://www.sdqinfo.com/bb1.html*).

Table 6.6

Pattern of associations at age 15 between ADHD & conduct problems, IQ, executive function, disinhibited attachment and gender in the Romanian high e'risk sample ±

		CAPA ADHD research diagnostic groups				
		Means (SD)		Sample size		
Phenotypic features		below cut off	above cut off	below cut off	above cut off	*t* test
Conduct problems *(parent report)*		.43 (.39)	.89 (.50)	64	12	$t(13.59)=-3.07^{**}$
Conduct problems *(teacher report)*		.23 (.31)	.54 (.42)	60	12	$t(13.62)=-2.41^{*}$
IQ		89.37 (15.24)	88.29 (15.19)	60	14	$t(19.59)=.24$; $p=.81$
Executive function		5.60 (1.90)	4.57 (1.99)	60	14	$t(18.92)=1.76$; $p=10$
Disinhibited attachment		.39 (.66)	.95 (.67)	73	14	$t(18.25)=-2.84^{*}$
Gender *(% in each group)*	male	74%	26%	28	10	*n/a*
	female	92%	8%	45	4	*n/a*

*p<.05; **p<.01; ***p<.001
± *Romanian institution-reared sample aged 6-42 months at entry to UK*

6.5.1.1 Is deprivation-related IOI phenotypically similar to ADHD in the general non-deprived population in terms of its developmental link and overlap with conduct problems?

IOI/ADHD and overlap with conduct problems

Table 6.6 (above) shows the mean conduct scores within the Romanian e'risk group for those above and below the research diagnosis cut-off for ADHD using the CAPA interview. According to both parent and teacher reports of conduct problems on the SDQ at age 15, the children with a research diagnosis of ADHD had significantly higher conduct problem scores than those below cut-off (parent*:* $p=.009$; teacher*:* $p=.03$)

The correlations between IOI and conduct problem scores (from the Rutter Scales/SDQ) reported in figure 6.6 show support for the analysis above using the CAPA interview data. There were significant bivariate correlations between the outcomes at all three assessment waves according to both parent and teacher reports on the Rutter Scales and SDQ (parent: $p=.02$; $p<.001$; $p<.001$; teacher: $p<.001$; $p<.001$; $p<.001$, for ages 6, 11 and 15 respectively. The results show there was a strong contemporaneous association between IOI and conduct problems with the high e'risk sample, which was persistent over time, pervasive across settings and particularly apparent from early adolescence onwards.

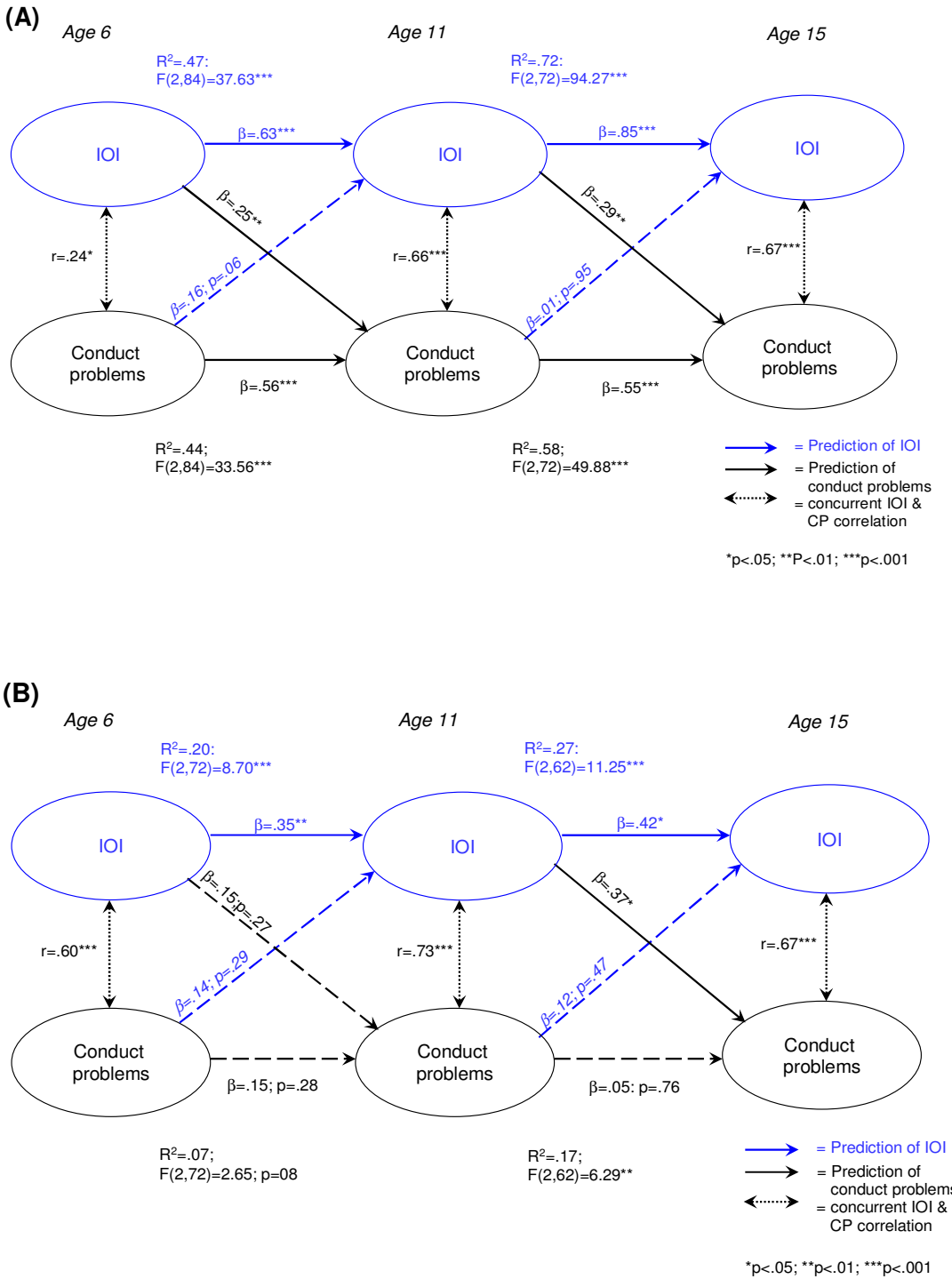


Figure 6.6

Regression & correlation model of IOI and conduct problems in Romanian high e'risk sample (A) parent report (B) teacher report

Developmental pathways from IOI and conduct problems: Parent report.
Multiple regression was used to test the independent contributions of IOI and conduct problems to each outcome domain at the subsequent assessment wave, as measured by the Rutter Scales and the SDQ (see figures 6.6). This was done in two stages: The contributions of IOI and conduct problems at age 6 were calculated in relation to each domain at age 11, and then separate regression models were used to assess the contribution of IOI and conduct problems at age 11 to variation in each domain at age 15.

Age 6 – 11 years: The age 6 to age 11 model showed that significant independent contributions were made by both IOI (β=.25, *p*=.005) and conduct problems (β=.56, *p*<.001) at age 6 to the variation in conduct problem scores at age 11. With respect to IOI at age 11, there was a highly significant independent contribution made by IOI at age 6 (β=.63, *p*<.001) but only a weak and marginal contribution from conduct problems (β=.16, *p*=.06).

Age 11 – 15 years: From age 11 to age 15 there was a similar pattern of findings. Both IOI (β=.29, *p*=.006) and conduct problems (β=.55, *p*<.001) at age 11 made independent, significant contributions to the variation in conduct problems at age 15. For IOI at age 15, again there was a large contribution to variation in the outcome made by IOI at age 11 (β=.85, *p*<.001) but no independent contribution made by conduct problems (β=.01, *p*=.95).

Developmental pathways from IOI and conduct problems: Teacher report
The same multiple regression models were applied to the teacher data on conduct problems and IOI rated on the Rutter Scales and the SDQ.

Age 6 – 11 years: In contrast the model utilizing the parent report data, the regression model using the teacher reports of IOI and conduct problems at age 6 to predict conduct problems at age 11 did not fit the available data well (R^2=.07*, p* =.08). Rather surprisingly, neither IOI (*p*=.27) or conduct problems (*p* =.28) at age 6 made significant contributions to conduct variation at age 11. The model using

the teacher report in relation to IOI as an outcome at age 11 fit the data well and the results were similar to those using the parent report data (R^2=.20, p<.001). IOI at age 6 contributed significantly to variation in IOI at age 11 (β=.35, p=.007) whereas conduct problems did not *(p*=.28).

Age 11 -15 years: With respect to the age 11 to age 15 model used to examine the prediction of variation in conduct problems, a significant independent contribution was made by IOI at age 11 (β=.37*, p*=.04) but there was no contribution from earlier conduct problems *(p*=.76). IOI at age 11 also made an independent contribution to IOI at age 15 (β=.42*, p*=.02) and again conduct problems did not *(p*=.47).

Developmental pathways – summary

Overall it seems that there is a significant developmental pathway from earlier IOI to later conduct problems in the Romanian e'risk sample, particularly with respect to reports from parents. Conduct problems do not seem to be a significant precursor to later variation in IOI. With the exception of conduct problems rated by teachers, there also seemed to be strong contributions to later variation within outcome over time.

6.5.1.2 Is deprivation-related IOI phenotypically similar to IOI/ADHD in the general, non-deprived population in terms of the association with low IQ?

In line with the findings from age 11 (Stevens et al., 2008), when taken as a whole the Romanian e'risk sample has a cognitive deficit at age 15 of around 12 IQ points compared with the population average of 100 (Rom e'risk: *M*=88.08), a deficit of close to 1 standard deviation in populations norms (1*SD*=15 IQ points). The *t* test showed there was no difference in IQ scores within the e'risk group between the subgroup with a research diagnosis of ADHD and the group below the diagnostic cut-off (*p*=.81). Furthermore, there was no bivariate correlation between IQ scores on the WISC and IOI scores on the SDQ within the e'risk sample at age 15 according to parent reports, and a moderate correlation according to teachers (age 15_{parent}: *r*= -.13*, p*=.30; age $15_{teacher}$: *r*= -.24*, p*=.05).

6.5.1.3 Is deprivation-related IOI phenotypically similar to IOI/ADHD in the general, non-deprived population in terms of the association with executive dysfunction?

At age 15 the only measure of executive function available was the backwards digit span on the WISC, which was used to test working memory performance. The difference between the two ADHD CAPA diagnostic groups within the Romanian e'risk sample was in the expected direction. That is, the ADHD group had poorer digit span scores than the group below diagnostic cut-off. However, the *t* test showed that the difference was not significant (p=.10). With respect to association between ratings of IOI on the SDQ and the digit span scores, the correlation approached but did not reach significance according to parental reports of IOI and teacher reports showed no correlation between the two measures (age 15_{parent}: r=-.22, p=.07; age $15_{teacher}$: r= -.09, p=.48).

6.5.1.4 Is deprivation-related IOI phenotypically similar to IOI/ADHD in the general, non-deprived population in terms of the gender discrepancy/prevalence amongst males?

Another salient feature of the ADHD phenotype in the general population is the substantial discrepancy in prevalence rates between males and females. Community based studies in the U.K. using the Rutter Scales or the SDQ put the ratio of boys to girls at around 3:1 (Heptinstall & Taylor, 2002; Youth in Mind, 2001). Clinic referred samples show a much larger discrepancy of around 10:1, boys to girls (Gaub & Carlson, 1997). In order to address this question regarding phenotypic similarities, the gender ratio in the Romanian high e'risk sample is compared with that seen in the normal population sample; first, in general terms by comparing the rates of deprivation-related ADHD in the e'risk sample, classified via a research diagnosis from the CAPA interview, with the rates reported in the ADHD literature. Second, a more detailed developmentally informed comparison is presented between the rates of cases above the SDQ cut-off found in the Romanian e'risk sample from childhood to mid-adolescence and the British population norms supplied on the SDQinfo website (*http://www.sdqinfo.com/bb1.html*).

Deprivation-related ADHD and gender (CAPA interview)

Table 6.6 presents the proportions of boys and girls in the Romanian high e'risk group who received a research diagnosis of ADHD. At the age 15 assessment wave 26% of males in the Romanian e'risk sample received a research diagnosis of ADHD compared with 8% of females, a ratio of 3.3:1, males to females. The discrepancy is in the same direction as that seen in relation to non deprivation-related ADHD and is roughly similar to the ratio in community sample classified using questionnaire measures (i.e. 3:1) but is not of the same magnitude as that seen in clinic referred samples (i.e. 10:1). With respect to ADHD in U.K. sample of the ERA study there were three cases (6.4% of U.K. sample) that received a research diagnosis of ADHD, all of which were males (9.7% of U.K. males). Such a low number of cases above cut-off meant that no meaningful analyses could be carried out using the U.K. subsample (see table 6.7)

Deprivation related IOI and gender (Rutter Scales/SDQ): Parent report.

Table 6.6 displays the rounded percentages of cases above the SDQ cut-off in the high e'risk group across assessment waves and split according to gender. Figure 6.7, below, shows that according to parent reports of IOI on the Rutter Scales/ SDQ, females were elevated across all three assessment waves compared with population norms. Males were elevated from age 11 onwards. In contrast to the equal proportion of boys to girls at age 6, a moderate sex difference emerged in the e'risk sample in early adolescence and continues into mid-adolescence. At age 11 years the ratio of males to females was 2:1 and at age 15 the sex difference decreased slightly to a ratio of 1.5:1.

With respect to the U.K. subsample of the ERA study, exploratory analysis revealed such low numbers above the IOI Rutter scales/SDQ cut-off, which meant that no meaningful analyses could be carried out (see table 6.7). Although, by and large, there was a clear male preponderance amongst the cases that did reach cut-off throughout the assessment waves.

Table 6.7

Cases above IOI cut-off in ERA U.K. sample as a function of gender

		Cases above cut-off	
IOI/ADHD measure		Percentage	Sample size
Parent report			
Age 6 (Rutter Scales)	male	9%	3
	female	0%	0
Age 11 (Rutter Scales)	male	6%	2
	female	6%	1
Age 15 (SDQ)	male	10%	3
	female	0%	0
Age 15 (CAPA)	male	10%	3
	female	0%	0
Teacher report			
Age 6 (Rutter Scales)	male	6%	2
	female	0%	0
Age 11 (Rutter Scales)	male	12%	4
	female	0%	0
Age 15 (SDQ)	male	7%	2
	female	6%	1

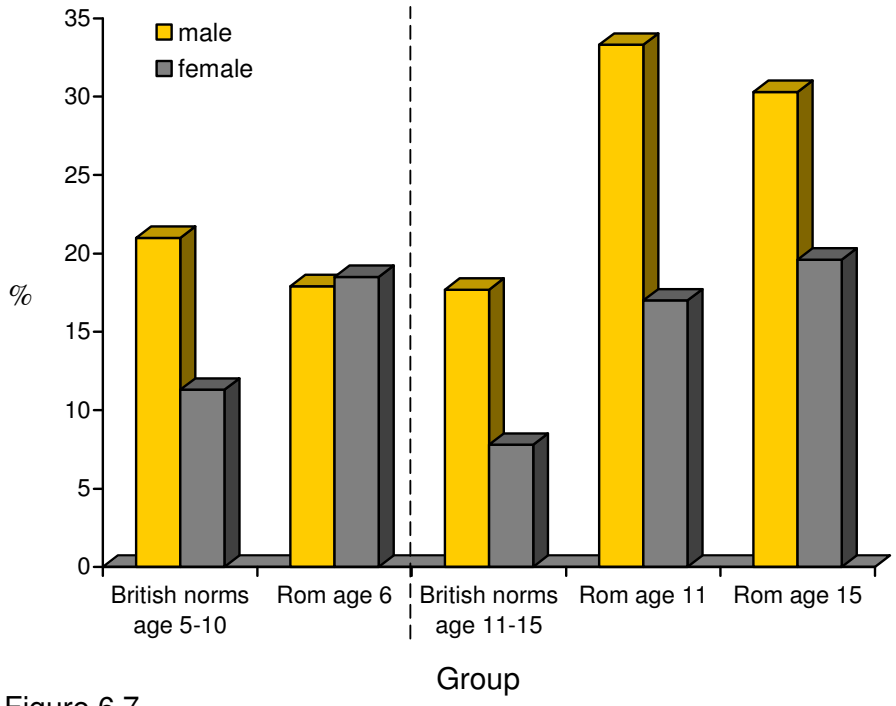


Figure 6.7

Percentages in abnormal range for IOI presented by age & gender: British norms & Romanian IR high e'risk sample aged 6-43 months at entry to U.K. (parent report)

Deprivation related IOI and gender (Rutter Scales/SDQ): Teacher report

According teacher reports of IOI behaviour in the Romanian e'risk group the proportion of both males and females was raised across all assessment waves when compared with the British population norms (see figure 6.8 below, and table 6.6 for ERA percentages). The data from teacher reports on the developmental pattern of sex differences in the high e'risk sample mirrored that reported by parents. At age 6 the sexes are roughly equal in the proportion above cut-off but by early adolescence we saw a moderate gender discrepancy emerge. The ratio of boys to girls was 1.5:1 at both ages 11 and 15 years.

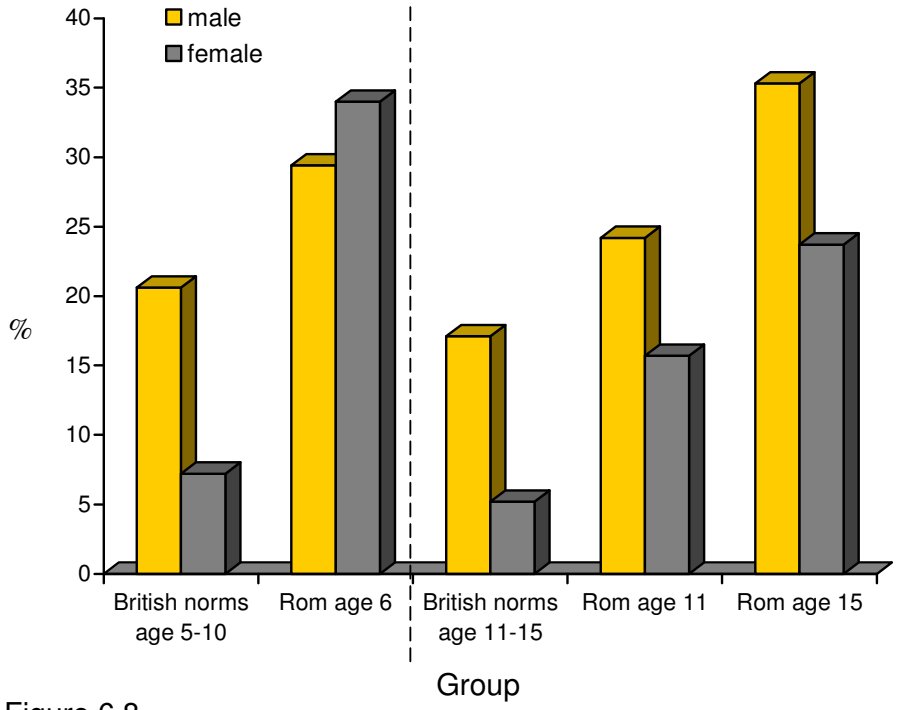


Figure 6.8

Percentages in abnormal range for IOI presented by age & gender: British norms & Romanian IR high e'risk sample aged 6-43 months at entry to U.K. (teacher report)

Gender discrepancy: Summary.

The developmental trajectory of sex differences in the Romanian high e'risk sample follows a different pathway from that seen in the nondeprived population. Unlike population and clinical samples, the proportion of boys and girls in the abnormal range for deprivation-related IOI in the e'risk sample at age 6 was roughly equal, according to both parent and teacher reports on the Rutter Scales. However, by early adolescence a discrepancy in prevalence rates had emerged. At age 11 the ratio of boys to girls was in the same direction but of a slightly smaller magnitude than that seen in population samples. This pattern of sex difference detected on the questionnaire measure in the Rom e'risk sample continued into mid-adolescence but equalized slightly compared with the rates at age 11. At age 15 the proportion of cases with a research diagnosis of ADHD from the CAPA interview showed a discrepancy between the sexes, with the ratio of boys to girls at 3:1. The difference in prevalence rates using this more comprehensive measure of ADHD mirrored that seen in population samples but

was lower than that of clinical samples, which has been reported as being closer to 10:1 boys to girls.

6.5.1.4 Phenotypic similarities: Summary

In terms of its pattern of associations the deprivation-related IOI in the Romanian e'risk sample had some similar and some discrepant features compared with the nondeprivation-related IOI phenotype. There were similarities with regards to the substantial overlap between conduct problems and IOI and the indication that early IOI may be a developmental precursor to later conduct problems in the e'risk sample. A similar pattern of sex differences was seen in adolescence, but in contrast to population and clinical samples, there was no discrepancy seen at age 6, suggesting that the developmental course of sex differences in the e'risk sample differed from that in the general population. In terms of IQ and executive function deficits the results were less clear and differences with the nondeprivation related IOI phenotype were apparent. The high e'risk sample as a whole had substantially depressed IQ scores with no detectable difference between the ADHD diagnostic groups within the e'risk group and no correlation between IQ and IOI using the questionnaire measures. The measurement of executive function at age 15 was limited to a single instrument: Backwards digit span, which taped working memory performance. Unfortunately, unlike at age 11, there was no measure of interference control done at the mid-adolescent assessment wave. The results for the digit span task showed a non-significant difference between the ADHD cut-off groups in the e'risk sample. In summary, there were phenotypic similarities in the e'risk group with nondeprivation related IOI in terms of the overlap and developmental pathways between conduct problems and IOI, the gender discrepancy in prevalence rates from early adolescence onwards, but a difference in the developmental trajectory of sex differences. The association with IQ and executive function deficit differed with no detectable differences found.

6.5.2 Is the deprivation-related phenotype characterised by particular underlying ADHD subtype symptoms?

This question addressed the issue of whether a specific ADHD subtype symptom presentation is more likely in relation to early institutional deprivation. There are two main ADHD subtypes defined in the DSM-IV-TR: Hyperactive (overactive)/

impulsive or inattentive. A 'combined type' diagnosis where all three symptom domains are present is also possible using the DSM-IV-TR criteria (American Psychiatric Association, 2000). The specific symptoms assessed by the CAPA interview fit into these three symptom domains (see appendix 4) and were used in the following analysis. A within-sample analysis was carried out to see if the association with early deprivation was being driven by particular subtype symptoms. This was done in two stages: First, an ANOVA was performed to assess the overactive/impulsive symptoms levels across the adoptee groups (U.K.; Rom non-IR; Rom IR: <6, 6 to <24, 24 to 43). The ANOVA revealed that there was an overall significant group difference in the level of overactive/impulsive symptoms in the same direction as the total ADHD symptom score presented above in table 6.3 ($F(4,194)=7.73$, $p<.001$). That is, extended institutional deprivation conferred a significant risk for elevated overactive/impulsive symptoms. Second, the same ANOVA model was run to assess the effect of deprivation adoptee group on inattentive symptom level. The pattern of results was the same and demonstrated a significant difference in inattentiveness between the groups ($F(4, 194)=6.20$, $p<.001$). This suggested that extended institutional deprivation was also associated with an increased risk for elevated levels of inattention.

6.6 Results section 3: Overlap between IOI and disinhibited attachment

6.6.1 Is there overlap between IOI and disinhibited attachment in mid-adolescence?

The children in the Romanian high e'risk sample with a research diagnosis of ADHD also had, on average, a significantly higher level of disinhibited attachment (DA) compared with the group of children below the cut-off for ADHD (see table 6.6). In line with the findings from age 11 (Stevens et al., 2008), the *t* test showed a significant difference in disinhibited attachment scores between the ADHD diagnostic groups in mid-adolescence ($p=.01$).

6.6.1.1 Developmental overlap between IOI and disinhibited attachment

The models below in figure 6.9 show: i) the concurrent overlap between IOI and disinhibited attachment tested using bivariate correlations; and ii) the developmental pathways between the domains, tested using multiple regression.

For these analyses, IOI was tested using parent and teacher reports on the Rutter Scales/SDQ. The disinhibited attachment measure combining parent interview and investigator ratings was used again in the present analysis. The same multiple regression model reported above in relation to IOI and conduct problems was used to test the independent contributions of IOI and DA to each outcome domain at the subsequent assessment wave. This was done in two stages: The contributions of IOI and DA at age 6 were calculated in relation to each domain at age 11, and then separate regression models were used to assess the contribution of IOI and DA at age 11 to variation in each domain at age 15.

Concurrent correlational analysis of overlap between IOI and DA

There were highly significant, moderately sized concurrent correlations between IOI and disinhibited attachment at all three assessment waves according to both parent and teacher (parent: $p<.001$; $p<.001$; $p<.001$; teacher: $p<.001$; $p<.001$; $p<.001$, at ages 6, 11 and 15, respectively). This corroborated the analysis above using the CAPA ADHD research diagnosis. Moreover, the relationship between IOI and disinhibited attachment in mid-adolescence remained highly significant after controlling for the shared association with duration of deprivation in a partial correlation analysis ($age15_{parent}$: $r=.47$, $p<.001$; $age15_{teacher}$: $r=.48$, $p<.001$).

IOI and DA developmental pathways: Parent report

The age 6 – 11 regression model showed significant independent contributions were made by both IOI ($\beta=.28$, $p=.006$) and DA ($\beta=.33$, $p=.001$) at age 6 to the variation in DA at age 11. However, with respect to IOI at age 11, IOI at age 6 made a significant contributed to its variation at 11 ($\beta=.66$, $p<.001$), whereas there was no contribution by DA ($\beta=.02$, $p=.80$).

A similar pattern of results is seen in the age 11 – 15 model. IOI and DA at 11 both contributed significantly to the variation in DA at 15 (IOI: β=.22, *p*=.03; DA: β=.49, *p*<.001). For IOI at age 15, again there was a large contribution to variation in the outcome made by IOI at age 11 (β=.81, *p*<.001) but no independent contribution made by DA (β=.10, *p*=.14).

IOI and DA developmental pathways: Teacher report

The analysis using teacher reports of IOI demonstrated largely similar results. The model of the age 6 to age 11 impairment domains showed that IOI and DA at 6 significantly predicted DA at 11 (IOI: β=.23*, p*=.03; DA: β=.40*, p<.001*). With respect to the variance in IOI at 11, there was a significant contribution made by IOI at age 6 (β=.33*, p*=.004). However, in contrast to the model using parent reported IOI, there was also an independent contribution made by DA (β=.26*, p*=.03). The age 11 to 15 model showed that DA at age 15 was significantly influenced by age 11 DA (β=.50*, p*<.001) and there was also a small independent contribution by IOI at 11 (β=.20*, p*=.05). IOI at age 11 also significantly predicted IOI at 15 (β=.46*, p*<.001), whereas DA at 11 did not (β=.17*, p*=.13)

IOI and DA developmental pathways: Summary

Overall, it seems that there is a significant developmental pathway from earlier IOI to later disinhibited attachment in the Romanian e'risk sample, according to both parent and teacher reported IOI behaviour. By and large, disinhibited attachment did not appear to be a significant precursor to later variation in IOI. With respect to both the outcome domains, there also seemed to be strong contributions to later variation within outcome over time.

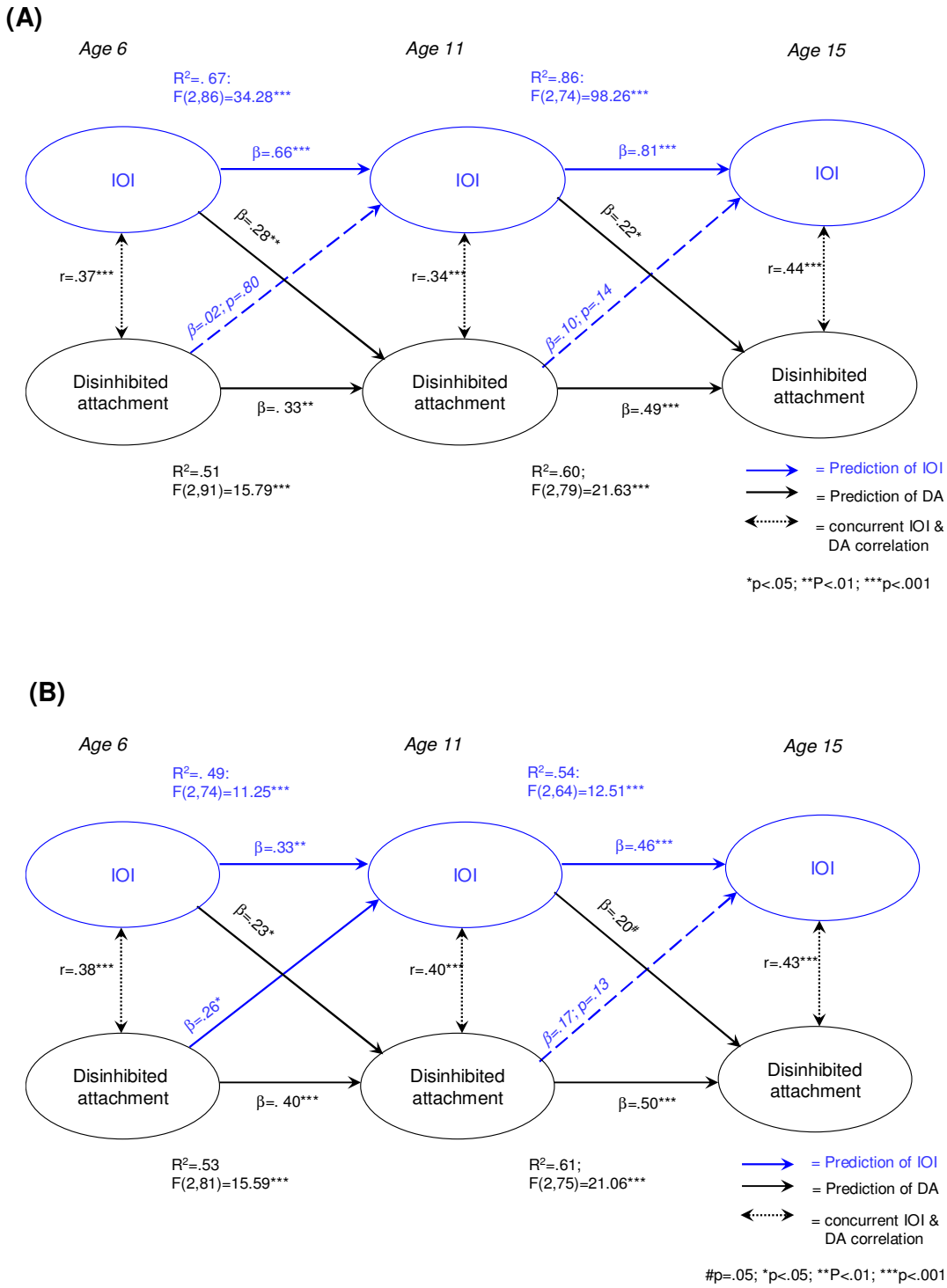


Figure 6.9

Regression and correlation model of IOI and disinhibited attachment in high e'risk sample: (A) parent report (B) teacher report

6.6.1.2 Exploratory factor analysis of IOI, disinhibited attachment and conduct problems

The next stage in the current investigation built on the exploratory analysis carried out in our recent paper on the age 11 data (Stevens et al., 2008). The analysis looked at whether IOI, conduct problems and disinhibited attachment were distinct constructs or if they were better conceptualised as part of the same underlying latent construct, given the high level of overlap and shared association with duration of deprivation. A full list of the items on the separate measures can be found in appendices 3 and 5 and method section 4.3.3.4.

At age 15 this was investigated with an exploratory principal components factor analysis using the individual IOI and conduct problems items on the SDQ and the disinhibited attachment assessment items from the parental interview and the investigator ratings. Factors with eigen values of greater than 1 were extracted using a varimax rotation to an orthogonal solution. Missing values were replaced with the mean in order to maximise the available data.

In line with that reported on the age 11 outcomes, the age 15 assessment measures seemed to be able to distinguish the three outcome domains as distinct factors. The model extracted five dimensions using the principal components method, which explained 72.4% of the total variance. The five conduct items loaded .57 or higher and accounted for 32% of the variance. The two overactivity items on the SDQ scale: *Restless, overactive* and *constantly fidgeting or squirming* also loaded .56 and .67, respectively, onto this 'conduct' factor. However, these two items also had substantial loading scores, .47 and .40, respectively, on the second factor, which accounted for a further 18% of the variance. This factor consisted of the two overactivity items alongside the remaining three IOI items from the SDQ, which tap inattentive and impulsive behaviour. The disinhibited attachment items loaded on a further three factors. The three items from the parental interview loaded .74 or higher on one factor and accounted for 7% of the variance. The investigator ratings of disinhibited attachment loaded onto two separate factors accounting for 10% and 6% of the variance, respectively, and with substantial overlap for several of the items. The first factor grouped the following items together: *Overall disinhibition, violated*

verbal boundaries and *spontaneous comments,* with loading scores of .79 or higher. The item measuring violation of social boundaries also loaded fairly highly onto this factor (.45). The final factor grouped the remaining items together: *Relationship with examiner, violated social boundaries, unsolicited physical contact* and loaded .66 or higher. The *violation of verbal boundaries* item also loaded onto this factor with a score of 0.41.

In summary, there is substantial and significant overlap between IOI and disinhibited attachment in the high e'risk sample, but the two domains of impairment do appear to be distinct outcome domains. The cases who received a research diagnosis of ADHD also had significantly higher disinhibition scores compared with those below diagnostic cut-off. The group difference was supported by significant correlations between the domains according to both parent and teacher reports on the SDQ. The overlap is in line with findings on IOI reported in previously published papers on the ERA study, suggesting that the overlap is a persistent feature of the deprivation-related IOI phenotype and is present after the shared association with duration of deprivation is accounted for. The results from the factor analysis using the age 15 data corroborate the analysis done at age 11 and suggested that IOI, disinhibited attachment are overlapping but dissociable constructs.

6.7 Chapter summary

The first section of the chapter addressed the question of the persistence of the risk associated with early institutional rearing. The findings showed that institutional deprivation, in particular of a duration lasting at least 6 months, constituted a significant and persistent risk factor for elevated levels and increased rates of IOI/ADHD impairment in the ERA sample. This was shown across settings (home and school), measurement tools (questionnaire and interview measures) and three assessment waves spanning childhood to mid-adolescence. Moreover, the high rates of impairment compared with population figures, alongside the pervasiveness and persistence of IOI/ADHD illustrates the clinical significance of this particular domain of impairment within the ERA sample.

The second section of the chapter dealt with the phenotypic similarities and differences between deprivation-related IOI/ADHD and that seen in the

general, nondeprived population there was a combination of common and unique features. The commonalities centred around the persistent association between IOI and conduct problems and the apparent trajectory from early IOI to later conduct problems. Adolescent IOI in the e'risk group showed a similar pattern of discrepancy between the genders, to nondeprived ADHD samples. The most obvious area where IOI in the Romanian e'risk sample appears to be distinctive is in terms of the overlap with disinhibition. This association was explored in the third and final section of the chapter. Although, given the scarcity of research in the area in relation to the nondeprivation-related ADHD phenotype, it is not possible to comment with any confidence on how disparate the two phenotypes actually are in this regard. Deprivation related IOI also showed a unique developmental trajectory in terms of the late emergence of sex differences. The male preponderance in rates of impairment did not become apparent until early adolescence, unlike in population and clinical samples. Low IQ and executive dysfunction were not associated with deprivation-related IOI. Although, IQ was strongly associated with extended institutional deprivation, and the high e'risk sample as a whole had substantially depressed scores, influencing the analysis. In conclusion, IOI/ADHD is a persistent, clinically significant impairment in the ERA high e'risk sample with common and unique phenotypic features when compared with the phenotype in the general population. When this is considered in conjunction with the large degree of heterogeneity in individuals' response to institutional deprivation the importance of investigating other potentially influential factors, namely genetic make-up, becomes clear.

CHAPTER 7: RESULTS

DO DOPAMINE GENES MODERATE THE EFFECTS OF INSTITUTIONAL DEPRIVATION ON THE RISK FOR IOI?

7.1 Chapter Outline

One of the key findings presented in the preceding chapter was that early institutional deprivation lasting 6 months or more constituted a significant risk factor for elevated levels and clinically significant rates of IOI/ADHD in the ERA sample. Furthermore, although there were some unique phenotypic features of the deprivation-related IOI phenotype, there was a considerable amount of commonality with IOI/ADHD found in the normal, nondeprived population. Despite the strong association between early deprivation and later IOI the relationship was not deterministic and the majority of children who experienced extended institutional care in infancy were not in the abnormal range for IOI/ADHD. This variability raises the question as to what other factors are operating to influence the development of the ERA children. A possible mechanism for the observed variability within the sample is the moderation of the adverse effects associated with institutional rearing by factors 'within' the adoptees themselves or within their environments. Such factors may operate to protect some children while leaving others more susceptible to the risks of their environment. Moderation by genetic factors represents one such possible mechanism that may help to account for some of the heterogeneity in outcome that we have observed. Moreover, small effect sizes and variability in association between susceptibility genes and nondeprivation-related IOI/ADHD reported in molecular genetics literature suggests that interactions with environmental pathogens need to be considered in the risk for IOI outcome.

The current chapter explores this possibility with a hypothesis driven investigation of the moderation of the environmental risk effects associated with institutional deprivation by genetic factors linked to the risk for ADHD in the nondeprived population. The results of the analyses presented in the previous chapter on the elevated risk associated with deprivation lasting at least 6 months allows a planned group comparison in terms of the environmental risk factor.

This investigation represents the first of two strategies used to identify candidate susceptibility genes that may operate within a moderation framework to influence the risk for IOI/ADHD in the ERA sample. That is, given the similarities between the deprivation-related IOI phenotype and that seen in the nondeprived population; do genetic factors found to be associated with ADHD in the general population influence susceptibility for later IOI impairment in the ERA sample? The second strategy for candidate gene selection relates to susceptibility genes that have functional significance in terms of the risk associated with early deprivation and will we tested in the following chapter.

Obvious candidates for testing the 'phenotype' hypothesis relate to genes within the dopamine system, in particular those shown to interact with other environmental pathogens. The putative mechanisms and evidence from the literature supporting this claim are discussed in detail in introductory chapter 3. The present chapter focuses on two VNTR polymorphisms within the dopamine transporter (DAT1) gene, a haplotype of these two VNTRs and also a polymorphism within dopamine receptor (DRD4) gene.

The analyses in the current chapter, which are used to test the phenotype hypothesis, are set out in three main sections: The first section deals with whether there is a gene-environment correlation (rGE) between the dopamine genes of interest and institutional deprivation. This analysis relates to the genetic mediation model discussed in the introduction section and addresses whether the association between institutional deprivation and later IOI/ADHD is mediated by a significant correlation between deprivation and genetic makeup. That is, do the children who experienced prolonged deprivation also possess greater genetic liability, tested by comparing genotype frequencies between the environmental risk groups. The second section forms the main thrust of the empirical chapter and sets out the analysis of the genetic moderation of environmental risk for later impairment by testing for a gene-environment (GxE) interaction. The aim of this section was to examine the role that DAT1 and DRD4 risk genotypes play in moderating the risk associated with extended institutional deprivation on IOI/ADHD symptoms in the ERA sample. In the third section, the same GxE interaction model of the interplay between specific candidate genes and institutional deprivation is applied in relation to the risk for other behavioural features, i.e. cognitive impairment, conduct problems or disinhibited attachment. This analysis was conducted to: First, investigate the specificity of GxE effects.

That is, whether there was a generalised GxE interaction effect for impaired outcome in the sample or whether GxE effects were specific to IOI outcome. Second, cognitive impairment (IQ) was included as a covariate in the GxE interaction analyses of the effects on IOI. It therefore seemed important to ascertain what the GxE effects were in relation to this, and the other outcomes themselves

7.1.1 IOI and dopamine genes

7.1.1.1 DAT1 40-bp VNTR (3'UTR)

For the following analyses using the DAT1 40-bp VNTR (3'UTR) the sample (n=127) was dichotomously split into high and low genetic risk (g'risk) groups, in line with literature in the area (Kahn et al., 2003; Brookes et al., 2006b). The high g'risk group consisted of the children who were homozygous for the 10-repeat (10R) allele of the polymorphism (n=74, 58%); the low g'risk group consisted of individuals who were heterozygous for 10R and those who possessed no 10R alleles (n=53, 42%).

7.1.1.2 DAT1 30-bp VNTR (intron 8)

The analyses using the DAT1 30-bp VNTR in intron 8 (n=127) used a similar strategy for classifying genetic risk groups and is in line with other research on the association between this genotype and ADHD (Asherson et al., 2007; Laucht et al., 2007; Brookes et al., 2006b). The high g'risk group consisted the children who were homozygous for the 6-repeat (6-R) allele of the polymorphism (n=84, 66%); the low risk group consisted of individuals who were heterozygous for 6-R and those who possessed no 6-R alleles (n=43, 34%).

7.1.1.3 DAT1 10R-6R haplotype

The DAT1 haplotype combining the 40-bp VNTR (3'UTR) and the 30-bp VNTR (intron 8) was constructed following the approach used by Brookes et al. (2006b). There were haplotype data available on 125 study participants. The high risk haplotype group comprised the individuals who were homozygous for both the 10R 40-bp VNTR and the 6R 30-bp VNTR (n=62, 49.6%). The low g'risk group comprised all other haplotype combinations (n=63, 50.4%).

7.1.1.4 DRD4 (exon III) genotype

The method applied in the current study for classifying the DRD4 genotype followed the method used in the literature (Brookes et al., 2005). DRD4 genotype data were available on 126 cases. The high g'risk group consisted of the children who possessed at least one 7 repeat allele of the 48-bp VNTR in exon III of DRD4 (n=30, 24%). The low g'risk groups consisted of those who possessed no 7-repeat alleles (n=96, 76%).

7.1.2 Data analysis

7.1.2.1 Analytical strategy

To investigate the moderation of the risk associated with institutional deprivation by specific candidate dopamine genes within sample evaluations were carried out across the environmental and genetic risk groups using data from two informants and multiple assessment waves. The main effects and interactions between institutional deprivation and dopamine genotypes/haplotype are presented in the subsequent sections.

The sample was split into the high and low environmental risk groups as defined in the methodology section. To recap: The low e'risk group consisted of the U.K., Rom non-IR and Rom IR < 6 months subsamples; the high e'risk group comprised the Rom IR children who experienced at least 6 months institutional deprivation. The sample was split in this way because the results from the previous chapter indicated that deprivation lasting at least 6 months conferred significant risk for later IOI impairment. Moreover, there were no detectable differences in IOI outcome between the adoptee groups who experienced less than 6 months or no institutional deprivation. Furthermore, given the small sample size available for the genetic analyses, by dichotomizing the sample in this way it optimised the statistical power available.

The GxE interaction analyses in relation to IOI/ADHD using dopamine genotypes and institutional deprivation are presented in two stages for each consecutive genotype/haplotype. In stage one the data are modelled without controlling for confounding factors and analysed using analysis of variance tests. In stage two an analysis of covariance (ANCOVA) model was used. The ANCOVA model includes the child characteristics: Gender and IQ. These factors were chosen because they have been shown in chapter 6 to be associated with

either duration of deprivation or IOI in the ERA study. Moreover, low IQ and a male preponderance are phenotypic features associated with ADHD in the nondeprived population and as we are focusing on genes related to this phenotype it makes sense to control for their effect within our sample.

Owing to the substantial overlap between disinhibited attachment, conduct problems and IOI in the ERA sample these features were not included as covariates. Preparatory analyses (not included in the current thesis) showed that when these features were included in the ANCOVA model it introduced significant colinearity problems, which rendered the results uninterpretable.

Within both stages of the analytical model the longitudinal results are presented first followed by cross sectional results, where appropriate. The main effects of the e'risk and g'risk factors and the interactions over time on the level of IOI were analysed using a three-way repeated measures analysis of variance test, with institutional deprivation e'risk group and genotype group as the two between-subjects factors and assessment wave (3 levels: Age 6, 11 and 15) entered as a within-subjects factor. If a significant or near significant ($p<.01$) three way GxE interaction was found between e'risk group, g'risk group and assessment age the analyses were then broken down to look at the specific, cross sectional effects, tested using a two-way analysis of variance model design.

Once again institutional deprivation e'risk group and genotype group were used as the between-subjects factors. The focus of the cross sectional analysis was on the mid-adolescent assessment wave, utilizing the age 15 data, in order to ascertain the persistence of effects. Cross sectional data from earlier assessment waves were used in support and to investigate the developmental trajectory of genotype moderation effects on IOI outcome. The size of the genotype effect (d) within the two environmental risk groups is also reported. This was done in order to compare the strength of the effect within the low e'risk versus the high e'risk group and to provide additional evidence about the developmental trajectory of effects. Following the same structure as the preceding chapter, the results from the parent and teacher reports of IOI symptoms are presented separately, in order to investigate whether there is a differential pattern of effects on IOI exhibited in the home and school setting.

The final section of the analyses in this chapter explores the specificity of the GxE interaction effects. That is, whether similar effects can be found using other behavioural measures as outcome variables, i.e. IQ, conduct problems and disinhibited attachment, or whether the effects are specific to IOI/ADHD. This is relevant also in relation to the use of IQ as a covariate in the IOI/ADHD models and the overlap between domains that was discussed in the previous chapter. The same repeated measures ANVOVA model used in the analysis of effects on IOI was applied to these other outcomes.

7.1.2.2 Multiple testing issues

It is pertinent at this point to acknowledge the increase in the probability of falsely rejecting the null hypothesis (type I error) that comes with carrying out multiple testing procedures, such as the analyses performed in the current study. The null hypothesis predicts there is no association between the independent variables (institutional deprivation and genetic risk) and the dependent variable, IOI. The null hypothesis also predicts that there is no GxE interaction between the independent variables in relation to the risk for IOI. The implications of carrying out multiple tests and the increased likelihood of type I errors are that putatively significant effects need to interpreted with caution and should be supported by similar effects across alternative measurement devices and/or by a consistent pattern of results across measurement waves, informants, or linked genotypes/haplotypes. One-off significant findings should be treated with the scepticism for the risk of capitalising on chance in order to produce said results.

7.1.3 Predictions, hypotheses and research questions

The hypothesised mechanism being (indirectly) examined in the current chapter is whether the experience of early institutional deprivation during sensitive periods of development leads to alterations in neurobiological function in carriers of specific 'risk' alleles that moderate the detrimental impact of the environmental pathogen on long term behavioural development. Directly testing the genetic mechanism, i.e. biological programming, epigenetic processes and neurobiological dysfunction, is outside the scope of the current study but this hypothesis prompts the prediction that fundamental alterations in neurobiological

development as a function of early experience expressed as GxE interaction effects will be detectable early on and persistent over time. This leads to the prediction that children who possess the dopamine risk genotypes would have been particularly susceptible to the adverse effects of early institutional deprivation and at particular risks for the development of early onset, persistent IOI/ADHD type symptoms.

The current chapter aimed to test this prediction using the following research questions:

1. Are there gene-environment correlations between the dopamine transporter (DAT1) genotypes/haplotype and institutional deprivation?
2. Is there a gene-environment correlation between dopamine receptor (DRD4) genotype and institutional deprivation?
3. Does DAT1 genotype interact with early deprivation to increase the risk for inattention/overactivity/impulsivity?
   a. DAT1 40-bp VNTR located in the 3'UTR (10 repeat = risk genotype)
   b. DAT1 30-bp VNTR located in intron 8 (6 repeat = risk genotype)
4. Does the DAT1 10R-6R haplotype to interact with early deprivation to increase the risk for inattention/overactivity/impulsivity?
5. Does DRD4 (exon III) genotype interact with early deprivation to increase the risk for inattention/overactivity/impulsivity.
6. Does DAT1 genotype/haplotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?
   a. DAT1 40-bp VNTR located in the 3'UTR (10 repeat = risk genotype)
   b. DAT1 30-bp VNTR located in intron 8 (6 repeat = risk genotype)
   c. DAT1 haplotype (10R-6R = risk haplotype)
7. Does DRD4 (exon III) genotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

7.2 Results section 1: Dopamine gene-environment correlation (rGE)

Table 7.1 below summarises the genotype frequency data presented in the methodology section in order to address questions about the correlation between exposure to the environmental pathogen, institutional deprivation and the specific dopaminergic genetic risk factors. The results of the chi-square tests of association are discussed below.

Table 7.1

Proportions of cases with low risk versus high risk dopamine genotypes/haplotypes as a function of environmental risk group

		Environmental risk groups ±				
		% with genotype		frequencies		
Genotype		Low e'risk	High e'risk	Low e'risk	High e'risk	chi-square results
Dopamine transporter						
DAT1 40-bp (3'UTR)	Low g'risk	42%	41%	32	21	$\chi^2(1, N=127)=.01$, p=.92
	High g'risk	58%	59%	44	30	
DAT1 30-bp (intron 8)	Low g'risk	31%	38%	23	20	$\chi^2(1, N=127)=.83$, p=.36
	High g'risk	69%	62%	52	32	
DAT1 haplotype	Low g'risk	47%	55%	35	28	$\chi^2(1, N=125)=.70$, p=.40
	High g'risk	53%	45%	39	23	
Dopamine receptor						
DRD4	Low g'risk	77%	75%	58	38	$\chi^2(1, N=126)=.13$, p=.72
	High g'risk	23%	25%	17	13	

± *Low e'risk: UK, Rom non-IR, Rom IR <6 months*
High e'risk: Rom IR 6 to <24 and 24 to 42 months

7.2.1 Are there gene-environment correlations between DAT1 genotypes/haplotype and institutional deprivation?

7.2.1.1 DAT1 40-bp (3'UTR) genotype and institutional deprivation

There was no appreciable difference in the frequency of cases with low and high risk genotypes between the two environmental risk groups. That is, there was no

association between DAT1 40bp (3'UTR) and institutional deprivation risk group, indicating no gene-environment correlation (rGE) was present (p=.92).

7.2.1.2 DAT1 30-bp (intron 8) genotype and institutional deprivation

Similarly, there was no association between the DAT1 30-bp (intron 8) genotype groups and the deprivation risk groups (p=.36), again demonstrating no rGE.

7.2.1.3 DAT1 haplotype and institutional deprivation

When the two genotypes were combined to form the DAT1 haplotype there was no significant difference in haplotype frequencies between the e'risk groups. That is, no association between e'risk and g'risk (p=.40) and therefore no rGE.

7.2.1.4 Summary of DAT1 rGE effects

The results showed that those children who experienced longer deprivation did not appear to have a greater genetic liability, in terms of possessing specific DAT1 risk alleles, than those in the low e'risk group. This was demonstrated by a lack of significant rGE effects.

7.2.2 Is there a gene-environment correlation between DRD4 genotype and institutional deprivation?

The dopamine receptor genotype followed the same pattern as the DAT1 genotypes. DRD4 genotype was not associated with institutional deprivation risk group (p=.72), indicating the absence of an rGE. That is, the children in the high e'risk group who resided longer in the institutions were not subject to an increased genetic liability compared with the low e'risk group.

7.3 Results section 2: Gene-environment interaction in relation to IOI

This next section examined whether individuals' genetic makeup moderated the risk associated with institutional deprivation for IOI/ADHD symptomatology. The genetic makeup aspect was tested in terms of specific dopamine transporter and dopamine receptor polymorphisms. The model was tested in two stages: First,

without covarying for other factors; second, controlling for potentially confounding effects of IQ and gender.

The overall longitudinal effects are presented for each model first (three-way repeated measures ANOVAs/ANCOVAs). In the cases where a three-way GxExAge interaction was found cross sectional analyses are then performed. Cross sectional effects were tested using two-way ANOVAs and ANCOVAs and effect size (d) analyses were used to report on the strength of the effect of genotype group across the low and high e'risk groups.

7.3.1 Does the DAT1 40-bp (3'UTR) genotype interact with early deprivation to increase the risk for IOI?

7.3.1.1 IOI and DAT1 40-bp (3'UTR) genotype effects over time (no covariates): Longitudinal analyses

A three factor repeated measures ANOVA was used to investigate the main effects and interactions over time of DAT1 40-bp genotype and early deprivation (between-subjects factors), with assessment age included as a within-subjects factor. The results are presented in table 7.2 using parent and teacher reports of IOI behaviour from the Rutter Scales at ages 6 and 11 and the SDQ at age 15.

Table 7.2

Main effects and interactions over time between DAT1 40-bp (3'UTR) genotype, institutional deprivation and assessment age on IOI *(no covariates)*

	Main effects			
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age	
Parent report ±	F(1,110)=8.60**	F(1,110)=4.52*	F(2,210)=.52, p=.59	
Teacher report	F(1,85)=24.56***	F1,85)=.24, p=.62	F(2,170)=.38, p=.69	
	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,110)=3.44, p=.07	F(2,210)=.52, p=.59	F(2,210)=.51, p=.59	F(2,210)=1.03, p=.36
Teacher report	F(1,85)=.004, p=.95	F(2,170)=1.48, p=.23	F(2,170)=1.87, p=.16	F(2,170)=1.27, p=.28

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 40-bp (3'UTR), institutional deprivation and IOI outcome over time (no covariates)

The longitudinal analysis of the parent report data on IOI behaviour at ages 6, 11 and 15 years, without controlling for confounding factors, showed there was a significant main effect of environmental risk group and genetic risk group on IOI outcome, but no effect of assessment age (p=.004; p=.03; p=.59, for the three effects, respectively). This indicated that e'risk groups differed significantly from one another in their level of IOI behaviour over the course of the study period but that average within-group levels did not change significantly over time. Moreover, the analyses showed that g'risk groups also significantly differed in their level of IOI behaviour consistently over time. With respect to the interaction effects, the gene-environment interaction (GxE) between DAT1 40-bp genotype and institutional deprivation approached significance (p=.07). This gives an initial suggestion that DAT1 genotype may moderate the effects of institutional deprivation and that the interaction is present over time. The repeated measures analysis showed no indication of a three-way interaction between g'risk group, e'risk group and assessment age (p=.36). Therefore, in-depth cross-sectional analyses were not performed. Figure 7.1 plots the e'risk and g'risk group differences over time and is discussed below.

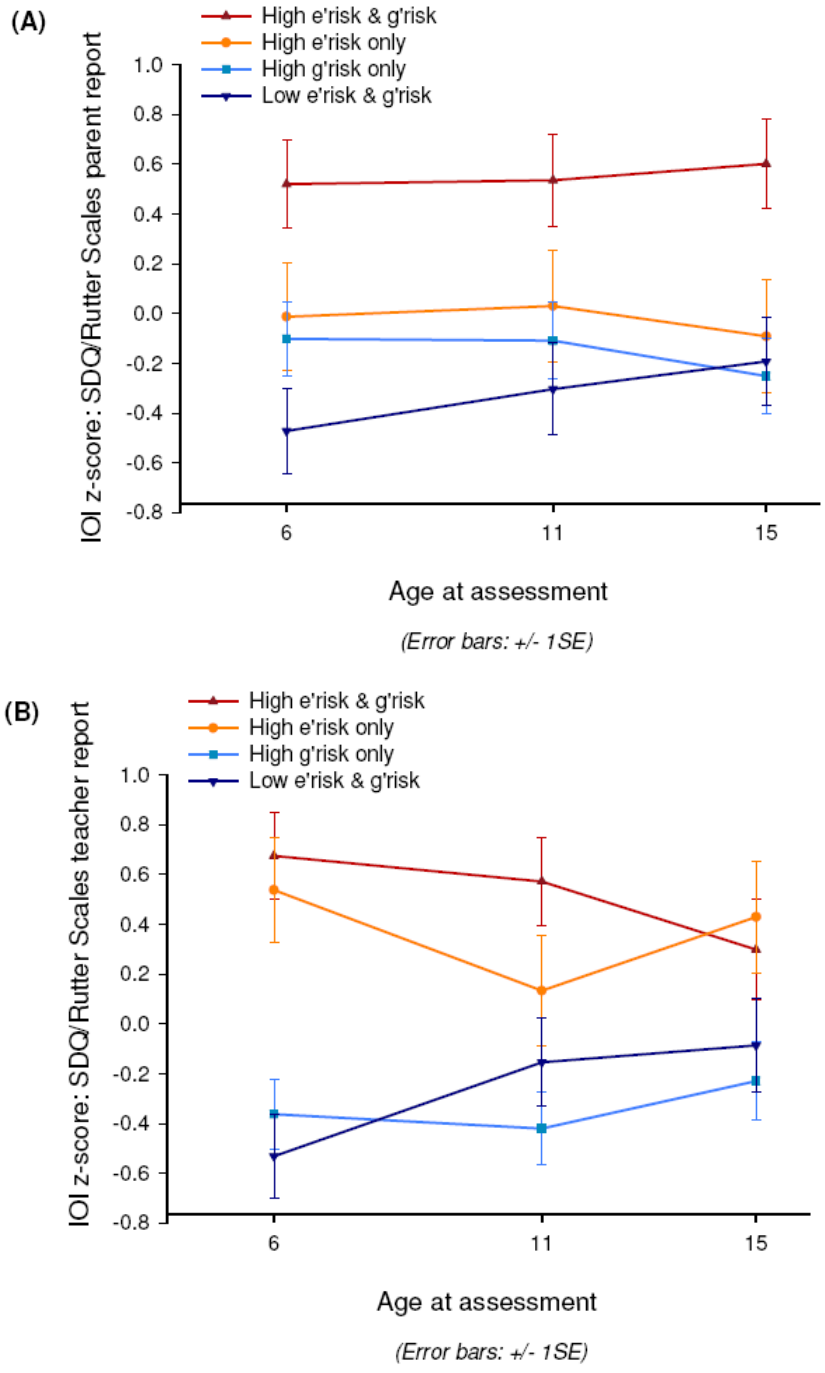


Figure 7.1

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DAT1 40-bp (3'UTR) genotype *(no covariates)*: (A) parent and (B) teacher reports

Figure 7.1 (A) indicates that the children who experienced extended deprivation and possessed the high risk 10R genotype had the highest IOI scores according to parents across all assessment waves. By the age 15 assessment this association seems to account for nearly all of the variation between the risk groups, as the both the genotype groups in the low e'risk subsample and the carriers of the low g'risk genotype in the extended deprivation group all have similar levels of IOI.

Teacher reports: Longitudinal analysis of DAT1 40-bp (3'UTR), institutional deprivation and IOI outcome over time (no covariates)

The longitudinal results using the teacher reports of IOI behaviour at ages 6 and 11 on the Rutter Scales and at age 15 on the SDQ showed a similar pattern of results from the parent reports with respect to the effects of the environmental risk factor but did not show the same genetic effects. Similar to the parent reports there was a significant main effect of the e'risk, institutional deprivation, but in contrast, no main effect of genotype was found ($p<.001$; $p=.62$ for the two effects, respectively). The main effect of deprivation is illustrated above in figure 7.1 (B), where the two high e'risk groups, who experienced extended deprivation, showed the highest IOI scores over all assessment waves. The repeated measures analysis indicated that there was no main effect of assessment age ($p=.69$). In terms of the interaction effects, no GxE interaction was found ($p=.95$) and there was no GxExAge interaction detected ($p=.28$). Therefore, no additional cross sectional analyses were undertaken.

7.3.1.2 IOI and DAT1 40-bp genotype effects over time (controlling for IQ and gender): Longitudinal analyses

As outlined in the previous section, a three factor repeated measures ANOVA was used to investigate the main effects and interactions, with IQ and gender added as covariates in order to control for their effect on IOI/ADHD. By controlling for confounding factors such it may help to clarify the nature of the interplay relationship between DAT1 40-bp genotype and institutional deprivation in the risk for IOI. The results are presented in table 7.3 and illustrated below in figure 7.2.

Table 7.3

Main effects and interactions over time between DAT1 40-bp (3'UTR) genotype, institutional deprivation and assessment age on IOI (controlling for IQ and gender)

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,106)=2.29, p=.13	F(1,106)=5.46*	F(2,207)=2.10, p=.13
Teacher report	F(1,83)=8.73**	F(1,83)=.04, p=.84	F(2,166)=1.46, p=.23

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,106)=4.28*	F(2,207)=.18, p=.83	F(2,207)=.46, p=.63	F(2,207)=1.23, p=.29
Teacher report	F(1,83)=.51, p=.48	F(2,166)=.27, p=.76	F(2,166)=1.82, p=.17	F(2,166)=1.34, p=.26

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 40-bp (3'UTR), institutional deprivation and IOI outcome over time (controlling for IQ and gender)

With the potentially confounding effects of IQ and gender controlled for in the longitudinal ANCOVA model, there was a significant GxE interaction between genotype group and exposure to institutional deprivation and a significant main effect of genotype group (p=.04; p=.02, for the two effects, respectively). However, the main effect of institutional deprivation was no longer observable (p=.13). This indicated that e'risk groups did not differ significantly from one another in their level of IOI over time once the effects of IQ and gender were controlled for, unlike for genotype groups, and also that genotype status seemed to moderate the effects of institutional deprivation persistently over the course of the study period. There was no main effect of assessment age and no interactions between age and risk factors (all n/s).

Figure 7.2 (A), below, shows a similar pattern of developmental trajectories for each risk group as the 'no covariates' model. That is, the children who were exposed to extended deprivation and carried the 10R risk allele were rated by parents as having the highest IOI scores throughout development. Despite the three-way GxE interaction with assessment age not reaching a significant level (p=.29), at age 6 the variance in scores is spread evenly between the groups but from early adolescence onwards the moderating effect of DAT1 40-bp (3'UTR)

genotype on e'risk becomes especially apparent, and by age 15 this seems to account for nearly all of the variance in the range of mean group scores.

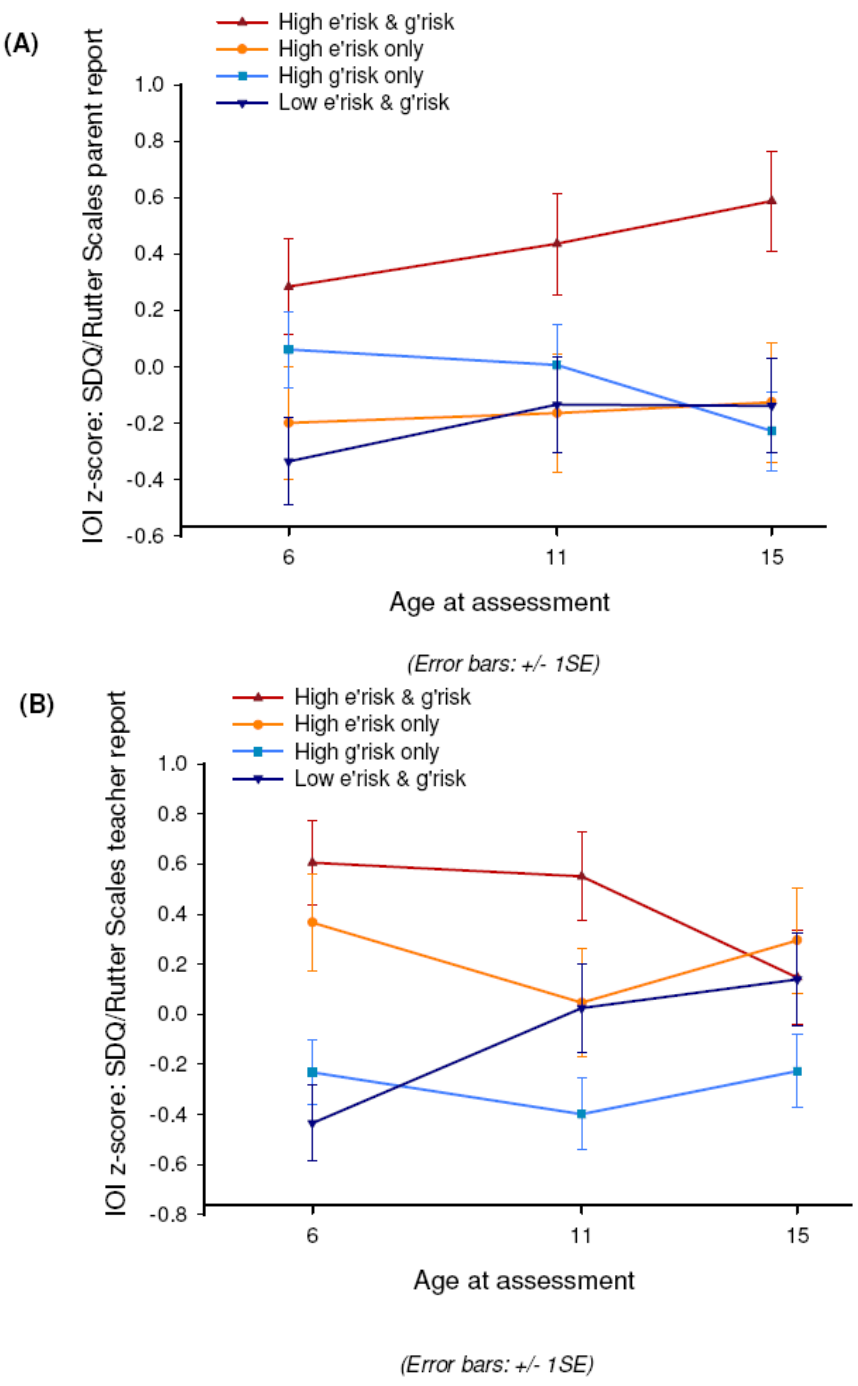


Figure 7.2

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DAT1 40-bp (3'UTR) genotype *(controlling for IQ and gender)*: (A) parent and (B) teacher reports

Teacher report: Longitudinal analysis of DAT1 40-bp (3'UTR), institutional deprivation and IOI outcome over time (controlling for IQ and gender)

Adding IQ and gender to the repeated measures longitudinal ANCOVA model did not affect the results with respect to teacher rated IOI. The only significant finding was in relation to the main effect of environmental risk group on IOI outcome over time (p=.004), indicating that there was a persistent difference between the deprivation groups. Genotype groups did not differ from one another and average e'risk and g'risk group levels did not change significantly over time (p=.62; p=.69, for the two effects, respectively). Like in the previous model, there was no GxE interaction (p=.95) and no interaction between assessment age and risk factors (see table 7.3, all effects n/s). The developmental trajectories of the risk groups were similar to the model with no covariates (see figure 7.2 (B)). The results suggest that DAT1 40-bp (3'UTR) genotype does not persistently moderate the risk effect associated with institutional deprivation on teacher rated IOI over time.

7.3.1.3 DAT1 40-bp genotype and deprivation: Summary of effects in IOI

If taken overall, the analyses demonstrate three main findings: First, there is evidence to suggest a synergistic interaction between DAT1 40-bp genotype and early institutional deprivation on the risk for IOI, as rated by parents but not teachers. This provides the first indication that DAT1 genotype appears to moderate the adverse effects of deprivation on the risk for IOI, such that the children who had experienced extended deprivation and possessed the 10R genotype had persistently elevated IOI scores compared with all other risk groupings. This interaction was evident longitudinally, with some indication from the graphical representation of the data that the effect may get stronger over time. Second, as would be predicted from the results of the previous chapter, deprivation risk groups differ significantly from one another persistently over time. Extended deprivation confers a significant risk for elevated levels of parent and teacher rated IOI within an analytical model that includes genetic risk as an independent variable. The effect is seen across assessment waves. Third, parent rated IOI and teacher rated IOI show a different pattern of main genetic effects. The predicted 10R risk genotype conferred a significant risk for elevated levels of parent rated IOI, but not teacher. This was seen across covariate models and assessment ages.

7.3.2 Does the DAT1 30-bp VNTR genotype in intron 8 interact with early deprivation to increase the risk for IOI?

The 30-bp polymorphism in the intron 8 of DAT1 was then applied to the same analytical model and used to test for a GxE interaction. The same two stage model was applied: i) no covariates; ii) controlling for IQ and gender. Once again, the overall longitudinal effects are presented for each model first (three-way repeated measures ANOVAs/ANCOVAs) followed, where appropriate, by the specific cross sectional effects, tested using two-way ANOVAs/ANCOVAs. Effect sizes (d) were used to assess the strength of the effect of genotype group across the low and high e'risk groups.

7.3.2.1 IOI and DAT1 30-bp (intron 8) genotype effects over time (no covariates): Longitudinal analyses

The results of the three way repeated measures ANOVAs are presented below in table 7.4, testing the parent and teacher reports of IOI at ages 6, 11 and 15 years, with DAT1 30-bp (intron 8) genotype group and institutional deprivation risk group as between-subject factors and assessment age as a within-subjects factor. Figures 7.3 (A&B) provide graphical representations of the developmental trajectories of the results over time.

Table 7.4
Main effects and interactions over time between DAT1 30-bp VNTR genotype, institutional deprivation and assessment age on IOI *(no covariates)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,110)=7.81**	F(1,110)=.16, p=.69	F(2,212)=.12, p=.88
Teacher report ±	F(1,84)=16.87***	F(1,84)=.34, p=.56	F(2,166)=.25, p=.78

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,110)=.77, p=.38	F(2,212)=1.07, p=.34	F(2,212)=1.63, p=.20	F(2,212)=3.74*
Teacher report ±	F(1,84)=.49, p=.49	F(2,166)=1.91, p=.15	F(2,166)=.21, p=.81	F(2,166)=.29, p=.74

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome over time (no covariates)

The longitudinal analysis using the parental reports of IOI from childhood to mid-adolescence (without covariates) showed a significant main effect of environmental risk group but no main effect of genotype group or assessment age (p=.006; p=.69; p=.88, for the three effects, respectively). There was no observable two-way interaction between: Genotype and e'risk groups (GxE interaction); age and e'risk; or age and genotype (all n/s). However, there was a significant three-way interaction between age, deprivation group and genotype group (p=.03). Taken together, the results demonstrated the e'risk groups differed significantly from each other persistently over time, average group levels did not change, with the suggestion that two risk factors interacted with each other differentially over time (see figure 7.3 (A)), but no overall GxE interaction could be detected when the data was analysed longitudinally. The significant GxE interaction with assessment age prompted additional cross sectional analysis of the data to be performed to investigate the specific GxE effects at each time point (see heading 7.3.2.2, below).

Teacher report: Longitudinal analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome over time (no covariates)

The longitudinal repeated measures analysis of variance using the teacher report data gave somewhat similar results to the parent report data above and again provided evidence for the association between extended deprivation and outcome (see figure 7.3 (B), below). There was a main effect of e'risk group on IOI over time (p<.001), no main effect of genotype group or assessment age and no significant two way GxE interaction detectable over time (p=.49). However, in contrast to the parent report data there was no three way interaction between e'risk group, g'risk group and assessment age (p=.74). Accordingly, no further cross sectional analyses were performed on the teacher reports of IOI.

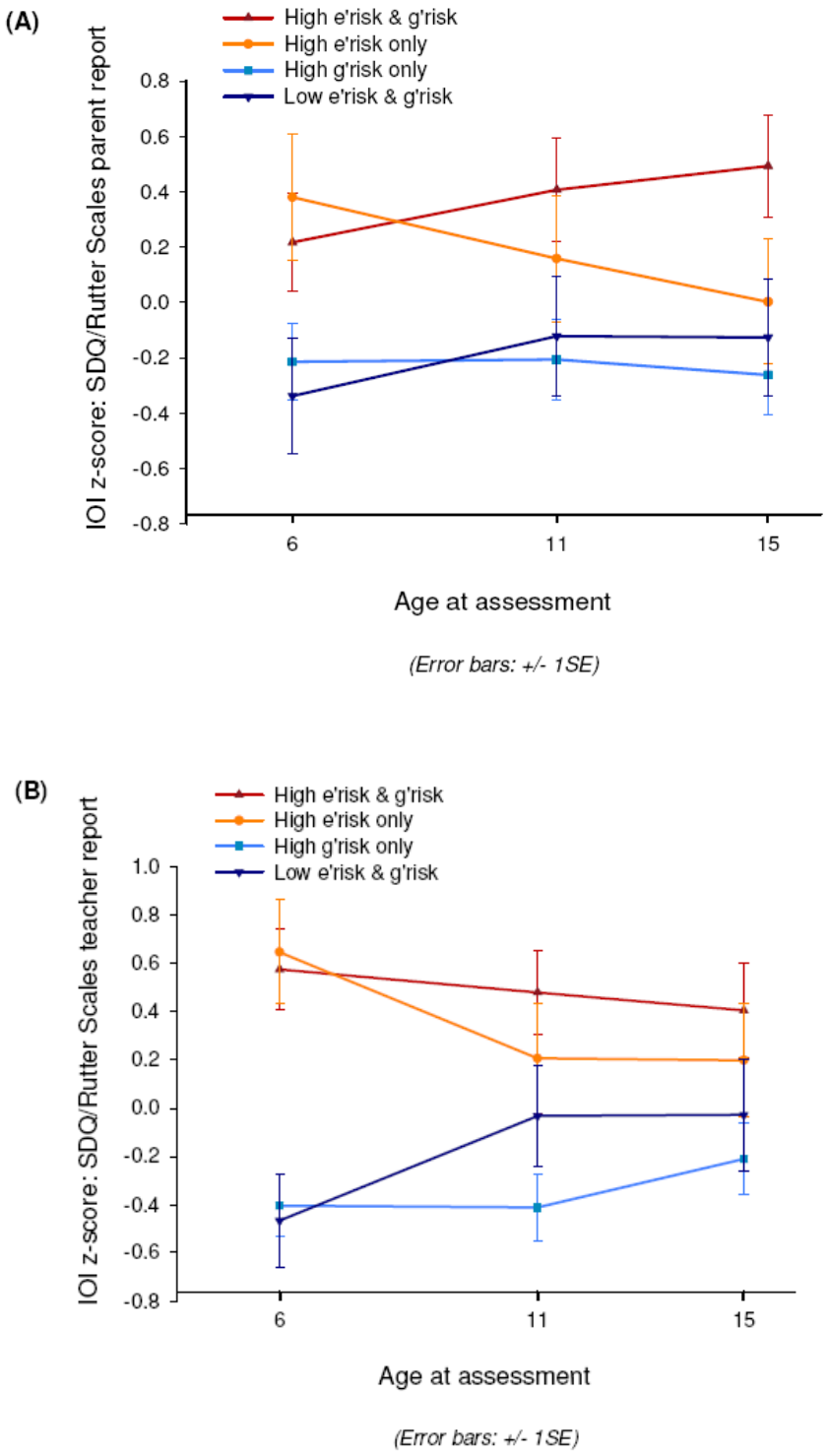


Figure 7.3

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DAT1 30-bp (intron 8) genotype *(no covariates)*: (A) parent & (B) teacher reports

7.3.2.2 IOI and DAT1 30-bp (intron 8) genotype effects (no covariates): Cross sectional analyses

In order to investigate the GxE interaction effects in more detail two-way ANOVA tests were used to investigate whether they was any detectably cross sectional moderation effects by DAT1 30-bp (intron 8) genotype of institutional deprivation at ages 6, 11 and 15. The uncorrected mean z-scores, standard deviations, sample sizes and test statistics are listed in table 7.6. Effect size estimates were used to measure the strength of the effect of genotype group across the low and high e'risk groups on IOI/ADHD. The effect sizes for both of the covariate models are presented in the following table.

Table 7.5

Effect size of DAT1 30-bp VNTR (intron 8) genotype status on IOI/ADHD scores across environmental risk groups and covariate models
(using mean z-scores and estimated marginal mean scores)

	effect sizes (d) across covariate models			
	no covariates		IQ & gender	
IOI/ADHD measure	Low e'risk	high e'risk	Low e'risk	high e'risk
Parent report				
Age 6 (Rutter Scales)	-0.14	0.14	-0.15	-0.09
Age 11 (Rutter Scales)	0.10	-0.21	0.10	-0.69
Age 15 (SDQ)	0.14	-0.50	0.08	-0.84
Age 15 (CAPA)	-0.01	-0.38	-0.05	-0.69

Table 7.6

Mean levels of IOI and ADHD symptoms (and standard deviations) across DAT1 30-bp VNTR (intron 8) genotype and institutional deprivation groups *(no covariates)*

	Genetic and environmental risk groups										
	Mean (SD)				Sample size				ANOVA results		
IOI/ADHD measure	Low G&E risk	G'risk only	E' risk only	High G&E risk	Low G&E risk	G'risk only	E'risk only	High G&E risk	e'risk main effect	g'risk main effect	GxE effect
Parent report											
Age 6 (Rutter scales)	-.34 (.80)	-.21 (.95)	.38 (1.2)	.22 (1.07)	23	51	19	32	$F(1,121)=8.95^{**}$	$F(1,121)=.01$, $p=.92$	$F(1,121)=.56$, $p=.45$
Age11 (Rutter scales)	-.12 (.74)	-.21 (.92)	.16 (1.2)	.41 (1.16)	22	48	20	30	$F(1,116)=5.18^{*}$	$F(1,116)=.18$, $p=.68$	$F(1,116)=.72$, $p=.40$
Age 15 (SDQ)	-.13 (.95)	-.26 (.99)	.002 (.95)	.49 (1.02)	22	48	19	29	$F(1,114)=5.23^{*}$	$F(1,114)=.84$, $p=.36$	$F(1,114)=2.64$, $p=.11$
Age 15 (CAPA)	-.21 (.91)	-.20 (.82)	.20 (1.10)	.66 (1.31)	23	51	20	30	$F(1,120)=10.57^{**}$	$F(1,120)=1.48$, $p=.23$	$F(1,120)=1.34$, $p=.25$

*$p<.05$; **; $p<.01$; ***$p<.001$

Parent report: Cross sectional analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome (no covariates)

The results of the cross sectional analyses supported the longitudinal results. That is, at age 15 there was a significant main effect of institutional deprivation group according to reports on the SDQ and the CAPA interview (age 15_{SDQ}: p=.02; age 15_{CAPA}: p=.001). There was no main effect of DAT1 30-bp genotype group at age 15 (age 15_{SDQ}: p=.36; age 15_{CAPA}: p=.23). There was a slight indication of moderation of e'risk by genetic factors as demonstrated by the medium size of the genotype effect in the high e'risk group (see table 7.5) and the distribution of the risk groups illustrated in figure 7.3 (A). However, the interaction fell short of significance (age 15_{SDQ}: p=.11; age 15_{CAPA}: p=.25). In line also with the longitudinal findings, the age 6 and 11 assessment waves exhibited a similar pattern of results with a main effect of deprivation group but not genotype group (see table 7.6) and some indication that the interaction between genetic and environmental factors increased over time (see figure 7.3 (A)).

7.3.2.3 IOI and DAT1 30-bp (intron 8) genotype effects over time (controlling for IQ and gender): Longitudinal analyses

Presented below are the longitudinal 3-way repeated measures ANCOVA results of the effects of DAT1 30-bp (intron 8) and deprivation on IOI over time, with assessment age as a within-subjects factor, controlling for the effects of IQ and gender within the model.

Table 7.7

Main effects and interactions over time between DAT1 30-bp VNTR genotype, institutional deprivation and assessment age on IOI *(controlling for IQ and gender)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,106)=1.59, p=.21	F(1,106)=2.16, p=.15	F(2,209)=1.82, p=.17
Teacher report ±	F(1,82)=4.90*	F(1,82)=.07, p=.79	F(2,164)=1.37, p=.26

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,106)=3.27, p=.07	F(2,209)=.31, p=.73	F(2,209)=1.82, p=.17	F(2,209)=4.42*
Teacher report ±	F(1,82)=2.80, p=.10	F(2,164)=65, p=.52	F(2,164)=.32, p=.73	F(2,164)=32, p=.72

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome over time (controlling for IQ and gender)

With the addition of IQ and gender to the longitudinal analysis of parent reports of IOI the main effect of institutional deprivation could no longer be detected (*p*=.21). There was no main effect of DAT1 30-bp genotype group or assessment age on IOI (*p*=.15; *p*=.17, for the two effects, respectively). In contrast to the model with no covariates, there was some indication that genotype moderated the effects of deprivation on outcome over time once the confounding effects of IQ and gender were controlled for, but the GxE interaction fell just short of significance (*p*=.07). Similar to the preceding analysis, a three way interaction between age, genotype and e'risk group was observed (*p*=.01). Taken together with the results presented in figure 7.4, this suggested that the GxE interaction seemed to get stronger as the children grew older. Cross sectional analysis of the data is presented below, to explore the differential GxE interaction across the assessment waves. There were no two way interactions between age and e'risk group or age and genotype (*p*=.73; *p*=.17 for the two effects, respectively).

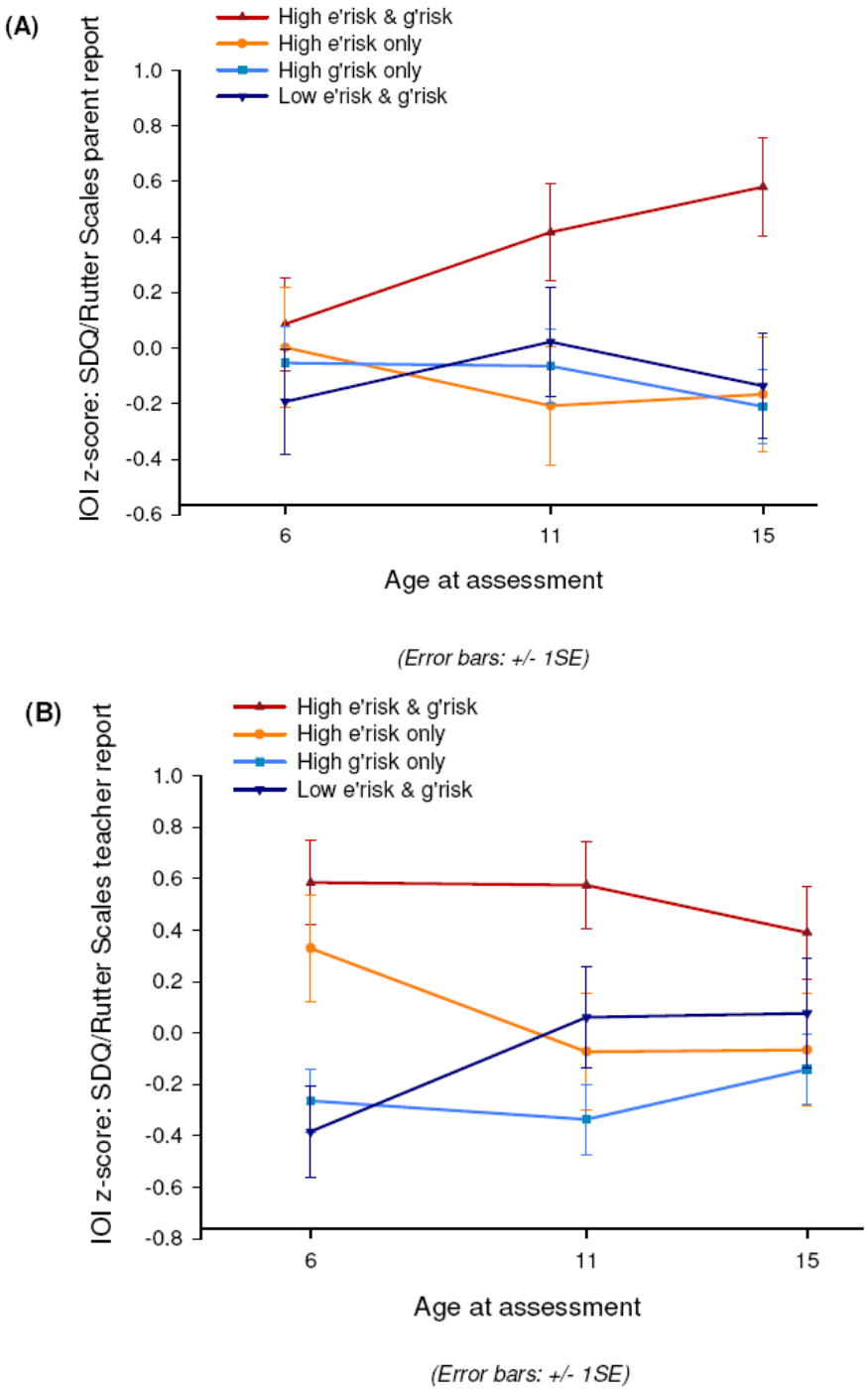


Figure 7.4

IOI at ages 6, 11 & 15 years as a function of early deprivation experience & DAT1 30-bp (intron 8) *(controlling for IQ & gender)*:(A) parent & (B) teacher reports

Teacher report: Longitudinal analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome over time (controlling for IQ and gender)

By and large the addition of IQ and gender to the longitudinal model using teacher reports of IOI did not alter the effects substantially. There was a significant main effect of institutional deprivation but no main effect of genotype or assessment age (p=.03; p=.79; p=26, for the three effects, respectively). Moreover, although still significant, the overall effect of e'risk group was somewhat diminished compared with the model that did not control for IQ and gender effects, and there was some suggestion of an interaction observable between genotype and deprivation over time on IOI (GxE: p=.10, see figure 7.4 (B) above). There were no interactions between age and risk factors (all n/s, see table 7.7).

7.3.2.4 IOI and DAT1 30-bp (intron 8) genotype effects (controlling for IQ and gender): Cross sectional analyses

A two way ANCOVA test was used to investigate the cross sectional effects of DAT1 30-bp group and institutional deprivation on IOI at ages 6, 11 and 15 years, with IQ and gender added as covariates. The estimated marginal means, standard errors, sample sizes and test results are given in table 7.8. The genotype effect sizes within e'risk groups are listed above in table 7.5.

Table 7.8

Estimated marginal mean levels of IOI and ADHD symptoms (and standard errors) across DAT1 30-bp VNTR (intron 8) genotype and institutional deprivation groups (c*ontrolling for IQ and gender)*

	Genetic and environmental risk groups										
	Mean (SE)				Sample size				ANOVA results		
IOI/ADHD measure	Low G&E risk	G'risk only	E'risk only	High G&E risk	Low G&E risk	G'risk only	E'risk only	High G&E risk	e'risk main effect	g'risk main effect	GxE effect
Parent report											
Age 6 (Rutter scales)	-.19 (.19)	-.05 (.13)	.002(.22)	.09 (.17)	23	51	19	30	$F(1,117)=.78$, $p=.38$	$F(1,117)=.40$, $p=.53$	$F(1,117)=.02$, $p=.88$
Age11 (Rutter scales)	.02 (.20)	-.07 (.13)	-.21 (.22)	.42 (.18)	22	48	20	28	$F(1,112)=.41$, $p=.52$	$F(1,112)=2.28$, $p=.13$	$F(1,112)=3.97$*
Age 15 (SDQ)	-.14 (.19)	-.21 (.13)	-.17 (.21)	58 (.18)	22	47	19	26	$F(1,108)=4.33$*	$F(1,108)=3.55$, $p=.06$	$F(1,108)=5.41$*
Age 15 (CAPA)	-.21 (.20)	-.16 (.14)	-.02 (.21)	.67 (.18)	23	50	20	29	$F(1,116)=8.08$**	$F(1,116)=3.65$, $p=.06$	$F(1,116)=2.72$, $p=.10$

*$p<.05$; **; $p<.01$; ***$p<.001$

Parent report: Cross sectional analysis of DAT1 30-bp (intron 8), institutional deprivation and IOI outcome (controlling for IQ and gender)

The findings from the longitudinal analysis of the combined effects of deprivation and DAT1 30-bp on IOI over time are borne out in the results of the cross sectional analyses. As reported in table 7.8, at age 15, using the SDQ parent report of IOI, there was a main effect of deprivation group, a significant GxE interaction between genotype and deprivation group and the main effect of genotype approached significance (p=.04; p=.02; p=.06, for the three effects, respectively). This suggested that the environmental risk groups differed in their level of IOI in mid-adolescence but that genotype status moderated the risk associated with the deprivation, demonstrated graphically in figure 7.5 (A) and by the large effect of genotype group in the high e'risk sample (d=-.84, see table 7.5 on page 198). There was also some evidence for an overall group difference as a function of DAT1 30-bp genotype. The data from the CAPA interview at age 15 supported these findings with a similar pattern of results. There was a main effect of e'risk group and some suggestion that genotype moderated these effects, although the GxE interaction did not reach significance (p=.005; p=.10, for the two effects, respectively). This is illustrated below by figure 7.5 (B) and evident in the large effect of genotype group on CAPA rated ADHD symptoms in the high e'risk subsample (d=-.69). Like the SDQ findings, the main effect of genotype group approached but just fell short of significance (p=.06). The developmental trajectories of the effects on IOI from childhood to mid-adolescence can be seen above in figure 7.4(A). In brief, after controlling for the effects of IQ and gender, the moderation of the risk associated with institutional deprivation by genotype group on IOI impairment appears to strengthen over time and was apparent from early adolescence onwards (GxE: Age 6: p=.88; age 11: p=.049). The children who experienced extended deprivation and possessed the risk genotype were rated by parents as having the highest level of IOI across time. There was no main effect of deprivation group or of genotype group at age 6 or age 11 (all n/s, see table 7.8).

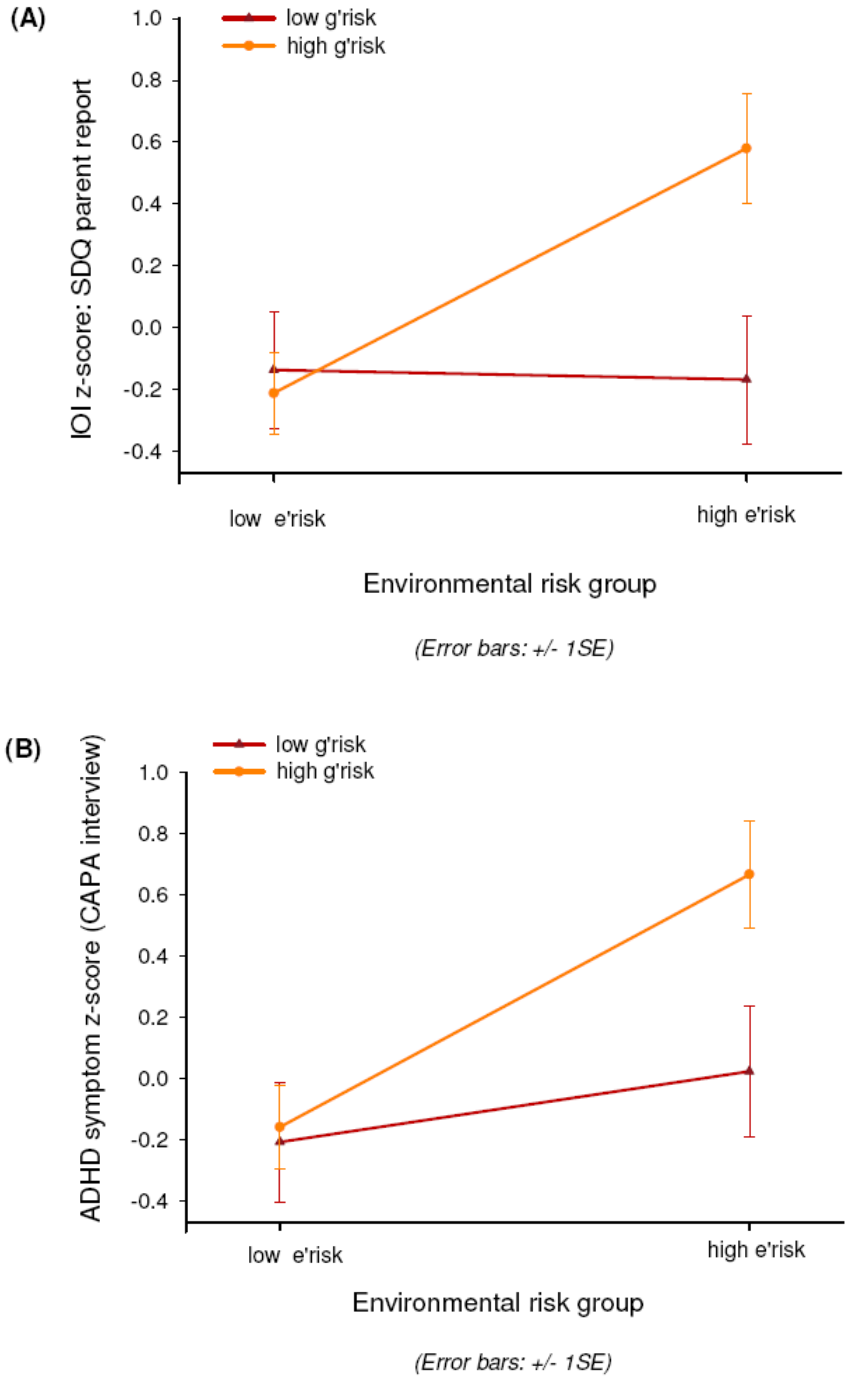


Figure 7.5

IOI & ADHD symptoms at age 15 years as a function of early deprivation experience & DAT1 30-bp (intron 8) genotype *(controlling for IQ and gender)*: Parent report (A) SDQ (B) CAPA interview

7.3.2.5 DAT1 30-bp genotype and deprivation: Summary of effects on IOI

By and large the findings followed a similar overall pattern as those reported above for DAT40-bp (3'UTR) genotype. The three main findings were: First, the analyses provided further support for the moderation by dopamine transporter genotype of the adverse effects of extended deprivation on the risk for parent rated IOI in the same direction as in interaction reported for the DAT1 40-bp (3'UTR) polymorphism. That is, the children who possessed the 6R risk genotype and experience 6 months or more institutional deprivation had the highest IOI scores. According to parent reports of IOI, the cross sectional analyses suggested that the effect got stronger over time and was apparent mainly from age 11 onwards. This was reflected in the longitudinal analyses by the GxE interaction with assessment age. The cross sectional and longitudinal GxE interaction ANOVA test results were significant when IQ and gender effects were controlled for but GxE effect is demonstrated throughout the models when effect sizes are considered. Moreover, there is some suggestion of the GxE interaction from the graphical representation of teacher reports as well from early adolescence onwards, particularly in the model controlling for IQ and gender, although the effect did not reach the required significance level. Second, the main effect of institutional deprivation on the risk for IOI impairment can be seen throughout. However, with the addition of covariates to the models of parent reported IOI the longitudinal effects fell short of significance. Third, there was some indication of a main genetic effect on parent rated IOI in adolescence, in line with that reported above for DAT1 40-bp (3'UTR), but the results fell short of statistical significance.

7.3.3 Does the DAT1 10R-6R haplotype to interact with early deprivation to increase the risk for IOI?

The two dopamine transporter genotypes were merged to form the DAT1 haplotypes in order to explore the linked functional impact of the two polymorphisms on the moderation of risk associated with institutional deprivation for elevated levels of IOI in the ERA sample. The same analytical strategy employed above was applied to the investigation of haplotype effects.

7.3.3.1 IOI and DAT1 10R-6R haplotype effects over time (no covariates): Longitudinal analyses

The results of the three way repeated measures ANOVA analysis of longitudinal effects on IOI (no covariates) are presented below in table 7.9, with DAT1 haplotype and institutional deprivation risk group entered as between-subjects factors and assessment age as a within-subjects factor. The scores over time are presented graphically in figure 7.6 (A&B).

Table 7.9
Main effects and interactions over time between DAT1 10R-6R haplotype, institutional deprivation and assessment age on IOI *(no covariates)*

	Main effects			
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age	
Parent report ±	F(1,108)=11.41**	F(1,108)=2.34, p=.13	F(2,208)=.44, p=.63	
Teacher report ±	F(1,83)=22.03***	F(1,83)=.02, p=.88	F(2,164)=.31, p=.73	
	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,108)=1.70, p=.20	F(2,208)=.28, p=.75	F(2,208)=.11, p=.89	F(2,208)=3.41*
Teacher report ±	F(1,83)=.59, p=.44	F(2,164)=1.62, p=.20	F(2,164).50, p=.60	F(2,164)=.64, p=.53

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and IOI outcome over time (no covariates)

The repeated measures analysis of uncorrected parental reports of IOI behaviour demonstrated a significant main effect of environmental risk group over time, but not for haplotype group or assessment age (p=.001; p=.13; p=.63, for the three effects, respectively). This indicated that the deprivation risk groups differed significantly from one another persistently over time. There was a significant three-way interaction between g'risk group, e'risk group and assessment age, suggesting the GxE interaction between haplotype and deprivation effects changed over time (p=.04). The subsequent cross sectional analyses investigate

the GxE effect further. However, there was no clear two way interactions between haplotype and e'risk (p=.20) over time, nor between age and the separate risk factors (age x e'risk: p=.75; age x g'risk: p=.89).

Teacher report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and IOI outcome over time (no covariates)

The overall analysis of teacher reports of IOI over time showed a similar pattern of results to the parents' accounts presented above. That is, there was a main effect of e'risk group over time, but the genotype groups did not significantly from each other and there was no overall effect of assessment age (p<.001; p=.88; p=.73, for the three effects, respectively). The effect of extended deprivation on poor IOI outcome can be seen graphically in figure 7.6 (B), below. There was no GxE interaction effect over time (p=.44). This suggested that haplotype status did not moderate deprivation risk for IOI according to teacher reports. However, unlike for parent reports of IOI, there were no interactions between age and risk factors (all n/s, see table 7.9) demonstrating that overall group differences and the interaction between e'risk and g'risk did not differ over time.

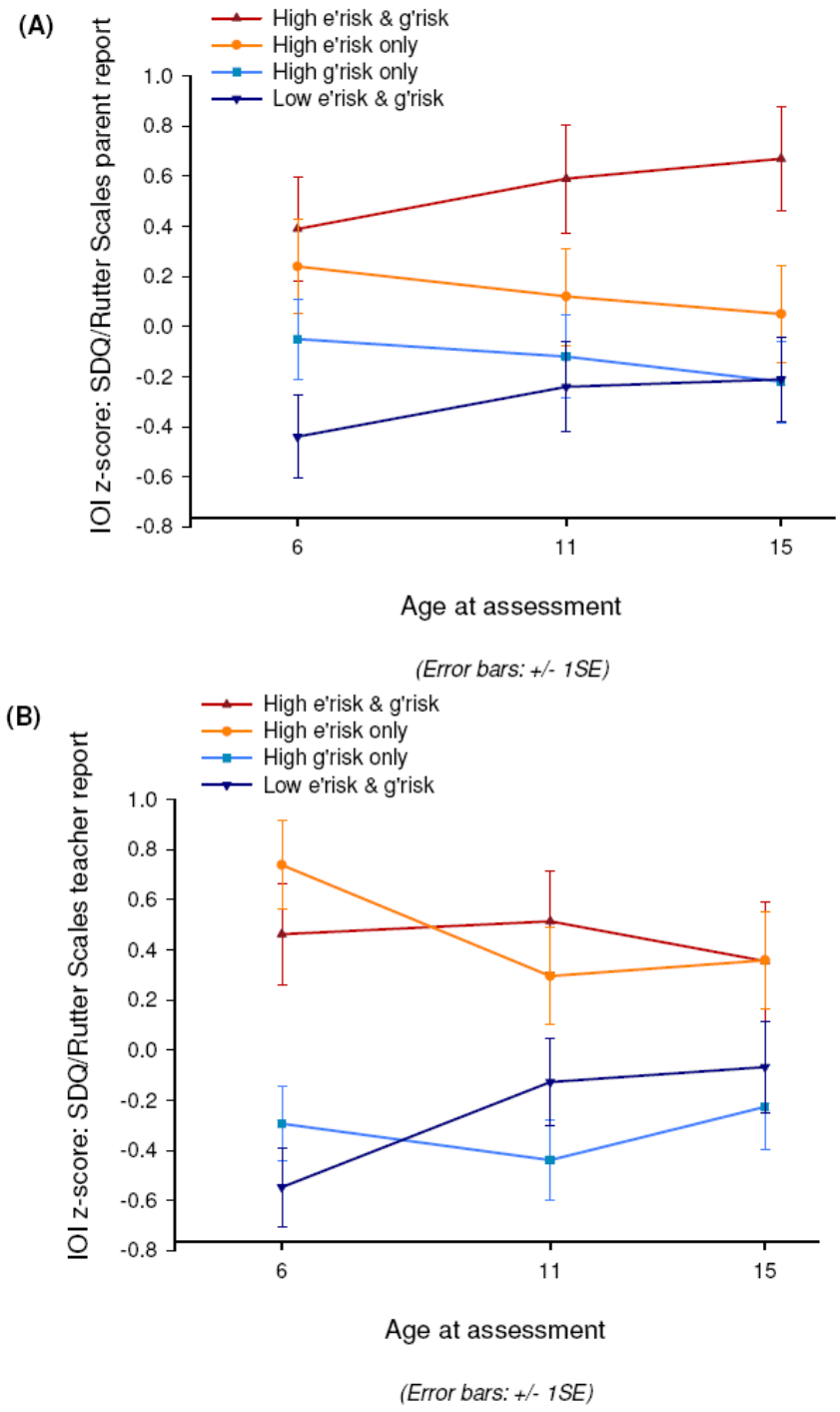


Figure 7.6

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DAT1 haplotype *(no covariates)*: (A) parent and (B) teacher reports

7.3.3.2 IOI and DAT1 10R-6R haplotype effects (no covariates): Cross sectional analyses

The specific cross sectional effects of DAT1 haplotype and institutional deprivation were then explored using a two way ANOVA model and applied to parent reports of IOI/ADHD at ages 6, 11 and 15 years. Table 7.11 gives the mean IOI z-scores, standard deviations, sample sizes and test statistic, uncorrected for the effects of covariates. Effect size estimates were used to measure the strength of the effect of genotype group across the low and high e'risk groups on IOI/ADHD. The effect sizes for both models are presented in the following table.

Table 7.10

Effect size of DAT1 haplotype status on IOI/ADHD scores across environmental risk groups and covariate models

(using mean z-scores and estimated marginal mean scores)

	Effect sizes (d) across covariate models			
	no covariates		IQ & gender	
IOI/ADHD measure	Low e'risk	high e'risk	Low e'risk	high e'risk
Parent report				
Age 6 (Rutter Scales)	-0.44	-0.13	-0.45	-0.39
Age 11 (Rutter Scales)	-0.14	-0.40	-0.08	-0.89
Age 15 (SDQ)	0.01	-0.65	0.07	-0.92
Age 15 (CAPA)	-0.06	-0.42	-0.002	-0.64

Table 7.11

Mean levels of IOI and ADHD symptoms (and standard deviations) across DAT 1 haplotype and institutional deprivation groups *(no covariates)*

	Genetic and environmental risk groups										
	Mean (SD)				Sample size				ANOVA results		
IOI/ADHD measure	Low G&E risk	G'risk only	E' risk only	High G&E risk	Low G&E risk	G'risk only	E'risk only	High G&E risk	e'risk main effect	g'risk main effect	GxE effect
Parent report											
Age 6 (Rutter scales)	-.44 (.76)	-.05 (.98)	.24 (1.15)	.39 (1.10)	35	38	27	23	$F(122)=9.53^{**}$	$F(1,122)=2.20$; $p=.14$	$F(1,122)=.45$; $p=.50$
Age11 (Rutter scales)	-.24 (.85)	-.12 (.89)	.12 (.14)	.59 (1.22)	32	37	27	22	$F(1,117)=7.93^{**}$	$F(1,117)=2.49$; $p=.12$	$F(1,117)=.87$; $p=.35$
Age 15 (SDQ)	-.21 (1.02)	-.22 (.96)	.05 (.87)	.67 (1.02)	33	36	25	22	$F(1,115)=9.68^{**}$	$F(1,115)=2.75$; $p=.10$	$F(1,115)=2.95$; $p=.09$
Age 15 (CAPA)	-.22 (.88)	-.17 (.82)	.25 (1.11)	.78 (1.39)	35	38	28	21	$F(1,121)=14.02^{***}$	$F(1,121)=2.28$; $p=.13$	$F(1,121)=1.58$; $p=.21$

*$p<.05$; **; $p<.01$; ***$p<.001$

Parent report: Cross sectional analysis of DAT1 haplotype, institutional deprivation and IOI outcome (no covariates)

The two way ANOVA analysis presented in table 7.11 showed that there was a main effect of the environmental risk factor, institutional deprivation, on IOI. The children who had experienced extended institutional deprivation had elevated levels of IOI/ADHD at age 15 (age 15_{SDQ}: p=.002; age 15_{CAPA}: p<.001) The main effect of extended institutional deprivation was evident in childhood and early adolescence, with significant associations at ages 6 and 11 years according to parents (age 6: p=.003; age 11: p=.006). Moreover, in line with the longitudinal findings and the separate genotype results, the cross sectional main effect of the genetic risk factor fell short of significance, that is, haplotype group did not independently significantly influence levels of IOI/ADHD at age 15, or at either of the earlier assessment ages (age 6: p=.14; age 11: p=.12; age 15_{SDQ}: p=.10; age 15_{CAPA}: p=.13).

With respect to the GxE interaction between institutional deprivation and DAT1 haplotype, the children who were exposed to extended deprivation and also carried the DAT1 risk haplotype had higher levels of IOI and ADHD at age 15 (figure 7.6 (A)). This was reflected in the medium effect size of haplotype status for the high e'risk group (see table 7.10: Age $15_{SDQ:}$ d= -.65; age 15_{CAPA}*:* d= -.42). However, the interaction only approached statistical significant according to parent reports on the SDQ and fell short of significance on the CAPA (age 15_{SDQ}: p=.09; age 15_{CAPA}: p=.21). Although the results were in the same direction, no significant GxE interaction could be detected in the ANOVA model at the age 6 or 11 assessment waves according to parent reports of IOI behaviour (age 6: p=.50; age 11: p=.35). The GxE interaction can be seen emerging in the increasing effect size of haplotype status in the high e'risk group according to parental accounts of IOI behaviour, which may account for the significant three way GxE interaction with assessment age reported in the longitudinal analysis section above. Between ages 6 and 11 years there is an increase from a very small (d= -.13) to approaching a medium effect size at age 11 (d= -.40).

7.3.3.3 IOI and DAT1 10R-6R haplotype effects over time (controlling for IQ and gender): Longitudinal analyses

Gender and IQ were then added to the three-way repeated measures ANCOVA model with DAT1 haplotype and institutional deprivation as the predictors of IOI at 6, 11 and 15 (assessment age as within-subjects factor). The results are presented below in table 7.12.

Table 7.12
Main effects and interactions over time between DAT1 10R-6R haplotype, institutional deprivation and assessment age on IOI *(controlling for IQ and gender)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,104)=4.22*	F(1,104)=5.69*	F(2,204)=2.10, p=.13
Teacher report ±	F(1,81)=9.01**	F(1,81)=.10, p=.75	F(2,162)=1.15, p=.32

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,104)=5.24*	F(2, 204)=.33, p=.72	F(2,204)=.35, p=.70	F(2,204)=4.26*
Teacher report ±	F(1,81)=.27, p=.60	F(2,162)=.54, p=.59	F(2,162)=.71, p=.49	F(2,162)=.68, p=.51

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and IOI outcome over time (controlling for IQ and gender)

The analysis showed that after controlling for effects of IQ and gender in the model there was an overall significant GxE interaction between DAT1 haplotype and institutional deprivation on parent reports of IOI over time (p=.02). Moreover, the significant three way GxE interaction with assessment age suggested that the effect of the interaction became stronger as the children grew older (p=.02; see figure 7.7(A)). In accordance with the previously described analytical strategy, subsequent cross sectional analyses explore this interaction further. With respect to the longitudinal main effects, significant effects were observed for both institutional deprivation group and for haplotype status (e'risk: p=.04; g'risk:

p=.02). This suggested that the extended deprivation group differed significantly from the low e'risk in their level of IOI persistently over time, as did the group carrying the high risk 10R-6R haplotype compared with the low risk carriers.

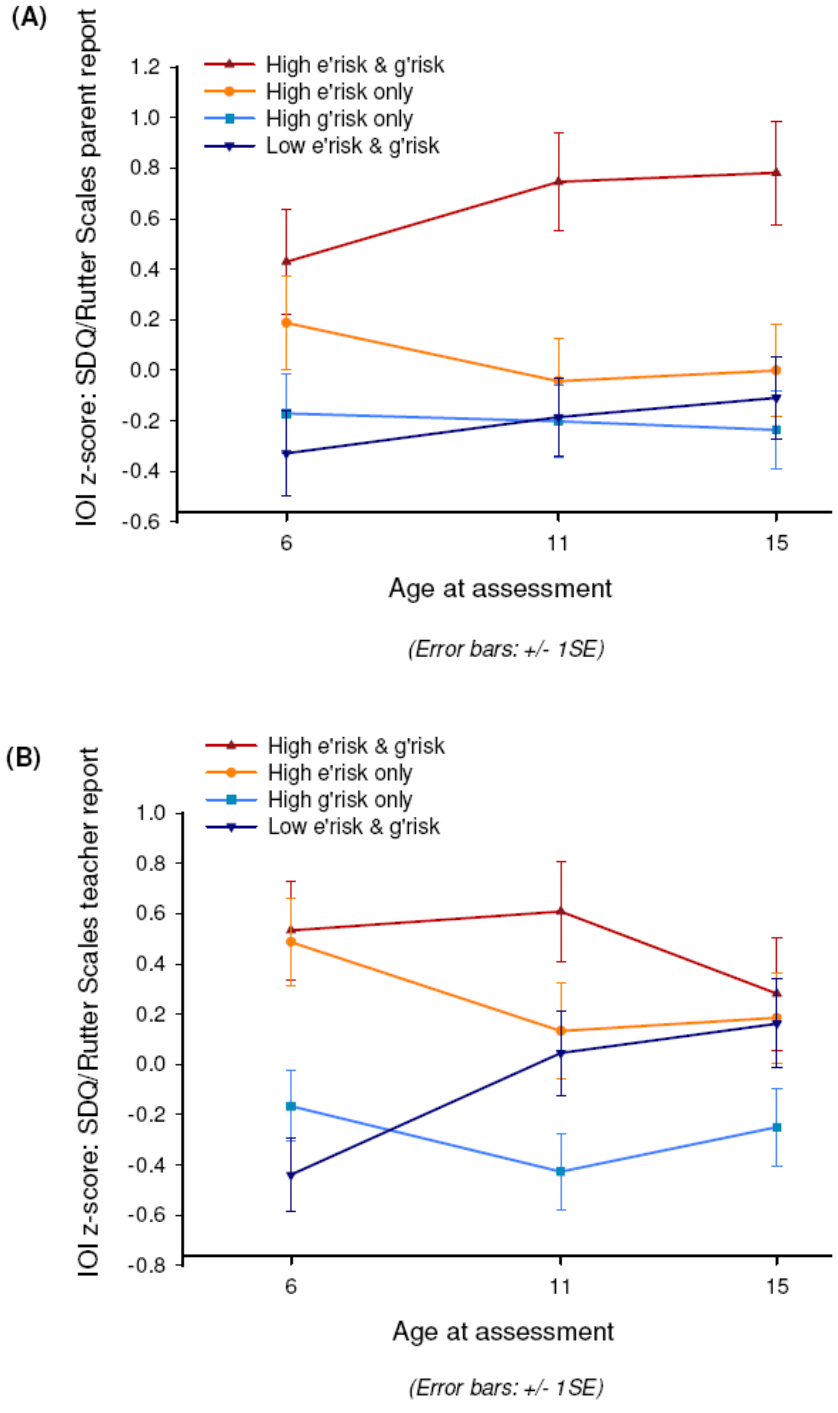


Figure 7.7

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DAT1 haplotype (controlling for IQ & gender): (A) parent & (B) teacher reports

Teacher report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and IOI outcome over time (Controlling for IQ and gender)

Teacher reports of IOI over time, after controlling for the effects of IQ and gender, showed a similar pattern to that seen when no covariates were included. That is, there was an overall main effect of environmental risk group, suggesting the institutional deprivation groups differed in their level of IOI over time (p=.004). There were no main effects of haplotype group or assessment age (p=.75; p=.32, for the two effects, respectively). However, unlike the analysis of parent reports, there was no GxE interaction detectable over time using the DAT1 haplotype as the genetic predictor (p=.60) and no significant interactions between assessment age and risk factors (all n/s: p's =.49 - .59). The combined effect of the two risk factors over time are displayed in figure 7.7 (B), on the previous page.

7.3.3.4 IOI and DAT1 10R-6R haplotype effects (controlling for IQ and gender): Cross sectional analyses

Following the previously outlined analytical framework, gender and concurrent IQ scores were then added to the cross sectional two-way ANCOVA analysis of specific effects of DAT1 haplotype and deprivation group on parent reported IOI at each assessment wave. The estimated marginal means, standard errors, sample sizes and ANCOVA test results are presented below in table 7.13 and illustrated by figures 7.7 and 7.8. Table 7.10, on page 211 lists the size of the effect of haplotype on IOI within each e'risk group, across the assessment ages, controlling the effects of IQ and gender.

Table 7.13

Estimated marginal mean levels of IOI and ADHD symptoms (and standard errors) across DAT 1 haplotype and institutional deprivation groups (c*ontrolling for IQ and gender)*

	Genetic and environmental risk groups										
	Mean (SE)				Sample size				ANOVA results		
IOI/ADHD measure	Low G&E risk	G'risk only	E'risk only	High G&E risk	Low G&E risk	G'risk only	E'risk only	High G&E risk	e'risk main effect	g'risk main effect	GxE effect
Parent report											
Age 6 (Rutter scales)	-.29 (.15)	.10 (.15)	-.07 (.18)	.27 (.19)	35	38	27	21	F(1,120)=1.19; p=.28	F(1,120)=4.87*	F(1,120)=.02; p=.89
Age11 (Rutter scales)	-.07 (.16)	.003 (.15)	-.16 (.18)	.63 (.20)	32	37	27	20	F(1,115)=2.03; p=.16	F(1,115)=6.34*	F(1,115)=4.49*
Age 15 (SDQ)	-.15 (.16)	-.21 (.15)	-.04 (.18)	.76 (.20)	32	36	25	19	F(1,111)=9.22**	F(1,111)=4.72*	F(1,111)=6.38*
Age 15 (CAPA)	-.16 (.16)	-.16 (.16)	.14 (.18)	.75 (.21)	34	38	28	20	F(1,119)=10.57**	F(1,119)=2.86; p=.09	F(1,119)=2.84; p=.095

*p<.05; **; p<.01; ***p<.001

Parent report: Cross sectional analysis of DAT1 haplotype, institutional deprivation and IOI outcome (controlling for IQ and gender)

The main effect of institutional deprivation on levels of IOI/ADHD, although still fairly strong, was somewhat diminished once the effect of IQ and gender are controlled. There was an effect of institutional deprivation group at age 15 according to parents, with higher IOI scores given to the children in the extended deprivation group (age 15_{SDQ}: p=.003; age 15_{CAPA}: p=.002). However, like the models discussed above that analysed the DAT1 genotypes separately, the main effect of e'risk group was not significant at ages 6 and 11 (age 6: p=.28; age 11: p=.16). Interestingly, with covariates in the model the main effect of haplotype status on levels of IOI, which was indicated in the separate genotype analyses, was significant at age15 according to parent reports on the SDQ, and a marginal association was found using the results of the CAPA interview (age 15_{SDQ}: p=.03; age 15_{CAPA}: p=.09). The effect of DAT1 haplotype status was supported longitudinally by parental reports of IOI at age 6 and 11 (age 6: p=.03; age 11: p=.01).

With respect to the interaction between DAT1 haplotype and institutional deprivation on risk for IOI/ADHD, there was a clear synergistic gene-environment interaction at age 15 according to parent reports. The children who carry the risk haplotype, 10R-6R, and were exposed to over 6 months institutional deprivation were rated as having by far the highest IOI scores (SDQ_{parent} : 4 SD increase). The GxE interaction effect at age 15 was highly significant according to parental questionnaire reports (age 15_{SDQ}: p=.013). With respect to the results from the CAPA interview reports of ADHD symptomatology, the GxE interaction approached significance (age 15_{CAPA}: p=.095). This was reflected in the large effect size of haplotype status on IOI scores at age 15 within the high e'risk group but the small effect size in the low e'risk group (high e'risk: SDQ_{parent}*: d*=-.92; CAPA*: d*=-.64; low e'risk: SDQ_{parent}*: d*=.07; CAPA*: d*=-.002). The cross sectional interaction is displayed graphically in figure 7.8 (A&B). The results from parent reports of IOI at age 6 and 11 support the repeated measures analysis presented in the preceding section by showing that that the GxE interaction gets stronger over time and was significant from early adolescence onwards (age 6: p=.89; age 11: p=.04). The change in effect size within the high e'risk group reflects this (age 6*: d*= -.39; age 11*: d*= -.89). At each assessment wave the children who

possessed the risk haplotype and had experienced extended deprivation were rated as having the highest IOI scores.

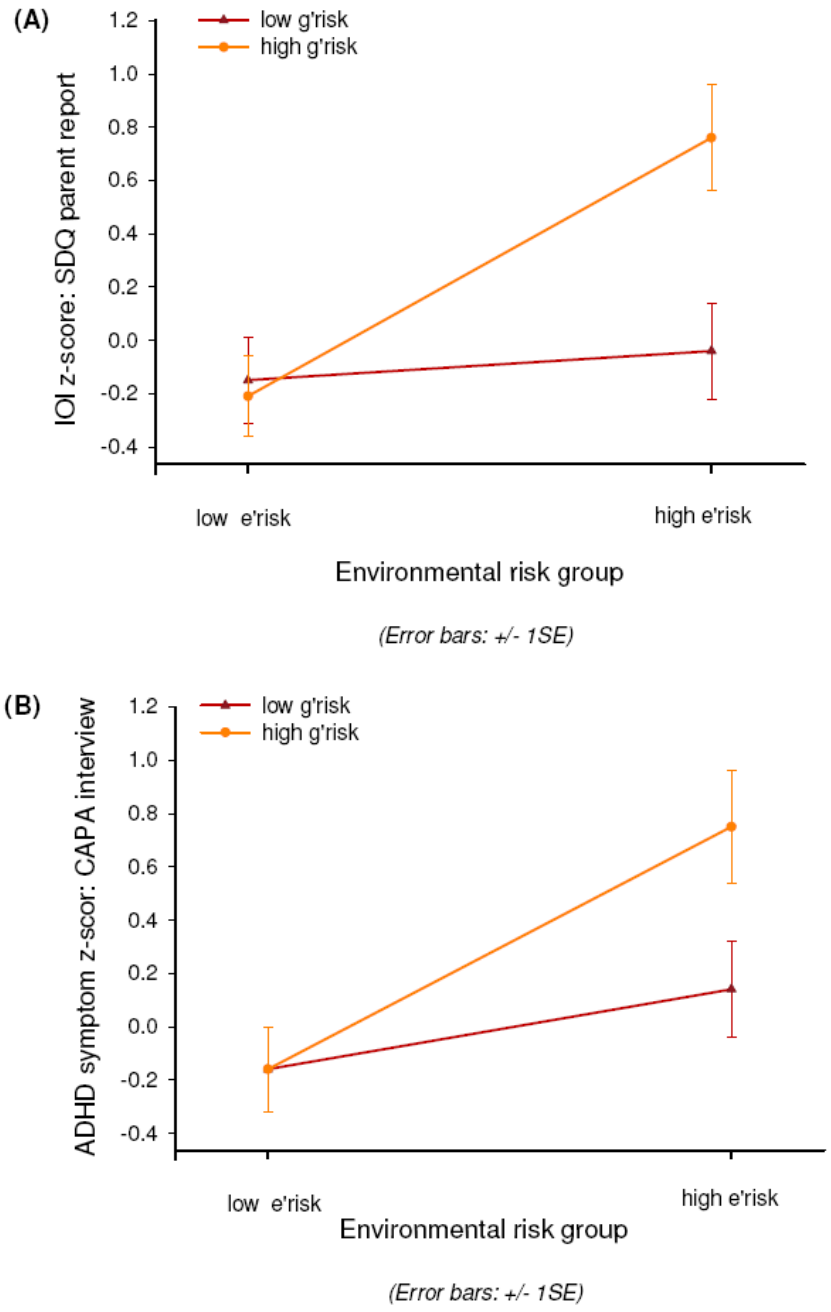


Figure 7.8

IOI & ADHD symptoms at age 15 years as a function of early deprivation experience & DAT1 haplotype *(controlling for IQ and gender)*: Parent report (A) SDQ (B) CAPA interview

7.3.3.5 DAT1 10R-6R haplotype and deprivation: Summary of effects on IOI

By combining the two genotypes into a single haplotype model it helped to clarify somewhat the independent and the interaction effects of DAT1 genetic risk and institutional deprivation on IOI outcome. There were five main findings: First, the analyses presented above provide further support for the suggested synergistic gene-environment interaction between DAT1 and institutional deprivation in relation to IOI/ADHD symptoms. Second, the GxE interaction appears to get stronger over time. The effect is weak in childhood and becomes significant only in early adolescence. By controlling for the effects of IQ and gender on IOI/ADHD the distinctions between the groups that were beginning to emerge in the 'uncontrolled' model became more refined. Third, the main effect of institutional deprivation on IOI/ADHD was evident throughout the analyses but was reduced once confounding factors were controlled for in the model. Fourth, there was a main effect of haplotype status on levels of IOI, which was only evident once the covariates were controlled for in the model but only according to parent and not teacher reports. Fifth, because of the problem with small cell sizes and its effect on significance values the effect size values provide an important test of the strength of the GxE effect.

7.3.4 Does the DRD4 genotype interact with early deprivation to increase the risk for IOI?

To investigate moderation of the risk associated with institutional deprivation by dopamine receptor (DRD4) genotype for IOI/ADHD in the ERA sample, the ANOVA models and analytical framework used above in relation to dopamine transporter genotypes were then applied to the DRD4 (exon III) VNTR polymorphism. This analysis also allows us to explore whether the moderation effects reported above are specific only to the DAT1 gene or whether similar effects can be observed with a second alternative dopamine gene, which has been shown in other studies to have a slightly higher direct gene to disorder link than the transporter gene.

7.3.4.1 IOI and DRD4 genotype effects over time (no covariates): Longitudinal analysis

A repeated measures ANOVA was used to test the effects of the DRD4 genotype and institutional deprivation exposure on IOI over time, with assessment age

entered as a within-subjects factor. Parent and teacher reports at ages 6, 11 and 15 on the Rutter Scales and SDQ were used in the longitudinal model, uncorrected for confounding factors in the first instance, and the results are presented below in table 7.14

Table 7.14

Main effects and interactions over time between DRD4 genotype, institutional deprivation and assessment age on IOI *(no covariates)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,109)=4.01*	F(1,109)=.02, p=.90	F(2,210)=.08, p=.92
Teacher report ±	F(1,84)=17.76***	F(1,84)=.15, p=.70	F(2,164)=1.52, p=.22

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,109)=2.01, p=.16	F(2,210)=1.17, p=.31	F(2,210)=.64, p=.52	F(2,210)=1.39, p=.25
Teacher report ±	F(1,84)=.04, p=.85	F(2,164)=1.95, p=.15	F(2,164)=1.97, p=.15	F(2,164)=.29, p=.75

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of DRD4 genotype, institutional deprivation and IOI outcome over time (no covariates)

The results of the three-way repeated measures ANOVA showed that there was a significant effect of environmental risk group on parent rated IOI over time (p=.048). There were no main effects of g'risk group or of assessment age (p=.90; p=.92, for the two effects, respectively). This indicated that the children who experienced over 6 months deprivation differed significantly and persistently in their level of IOI from the low e'risk group, but that there was no such group difference between DRD4 genotypes. The interaction between genetic and environmental risk factors was not significant, suggesting that the dopamine receptor genotype did not moderate the effects of deprivation on the risk for IOI over time (p=.16). This is illustrated by figure 7.9 (A), which shows the developmental trajectory of the combined effects of DRD4 genotype and institutional deprivation on parent rated IOI outcome. Likewise, there was no

three way GxE interactions between assessment age and risk factors, suggesting that the pattern of associations in relation to IOI did not change significantly from childhood to mid-adolescence, according to parent reports on the Rutter Scales and SDQ (p=.25).

Teacher report: Longitudinal analysis of DRD4 genotype, institutional deprivation and IOI outcome over time (no covariates)

The analysis of reports of IOI behaviour from teachers, without controlling for the effects of relevant covariates, showed a similar pattern of longitudinal results to the parent reports (see table 7.14). That is, there was a highly significant group difference between institutional deprivation risk groups persistently over time but no other main effects were detectable (e'risk: $p<.001$; g'risk: $p=.70$; age: $p=.22$). Figure 7.9 (B) displays these results graphically. Likewise, no GxE interaction between DRD4 genotype and deprivation on IOI outcome was observed ($p=.85$) and there were no interactions between assessment age and risk factors (all n/s; p's=.15 - .75).

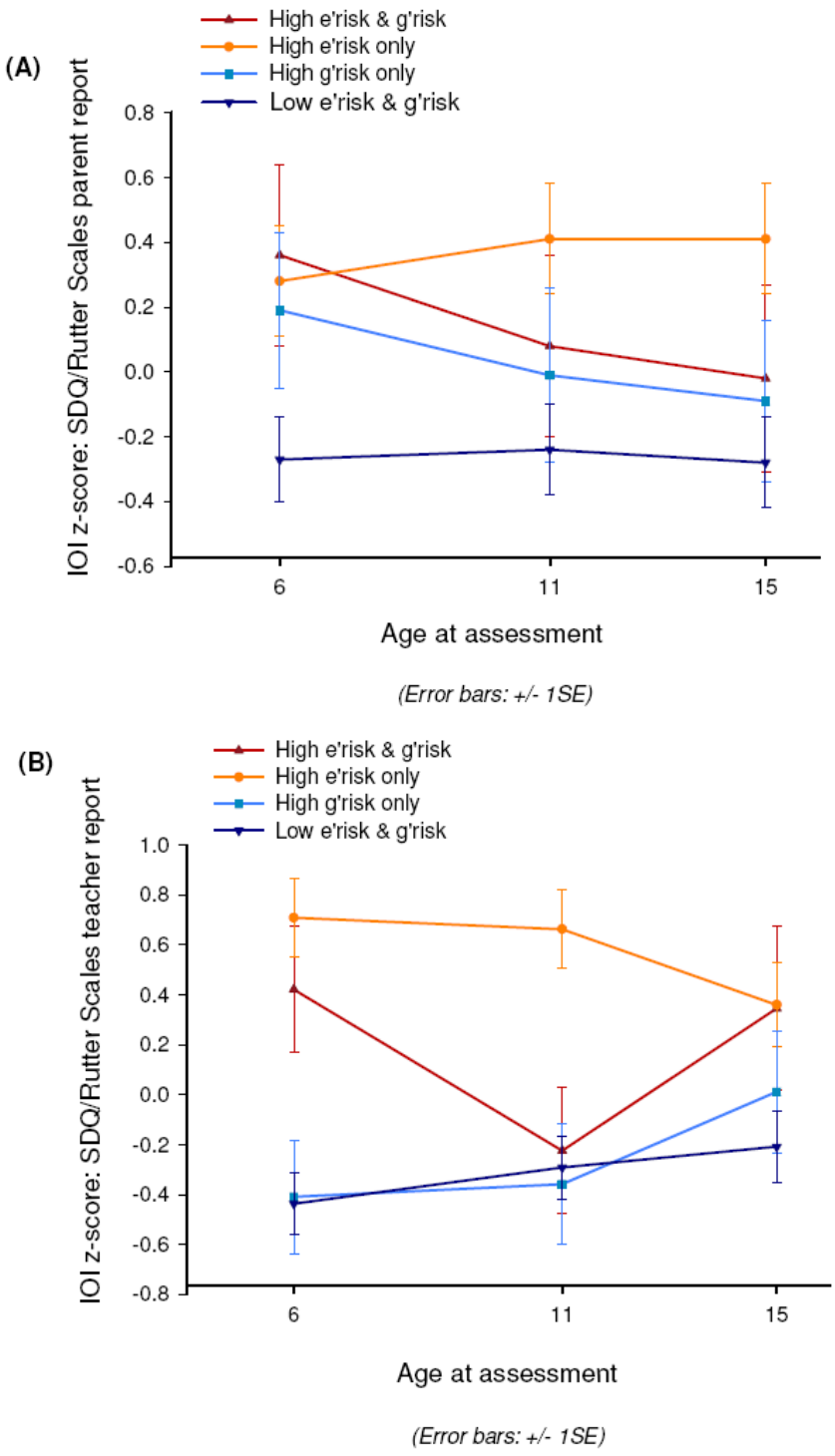


Figure 7.9

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DRD4 genotype *(no covariates)*: (A) parent and (B) teacher reports

7.3.4.2 IOI and DRD4 genotype effects over time (controlling for IQ and gender): Longitudinal analysis

The next stage of the longitudinal analysis of DRD4 and deprivation effects on IOI was to include IQ and gender as covariates in the repeated measures (ANCOVA) model. The main effects and interactions between risk factors over time are presented below in table 7.15.

Table 7.15

Main effects and interactions over time between DRD4 genotype, institutional deprivation and assessment age on IOI *(controlling for IQ and gender)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report	F(2,210)=1.89, p=.17	F(2,210)=.02, p=90	F(2,210)=2.42, p=.09
Teacher report ±	F(1,82)=7.49**	F(1,82)=.54, p=.46	F(2,164)=1.53, p=.22

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report	F(2,210)=.01, p=.92	F(2,210)=.20, p=.82	F(2,210)=.13, p=.88	F(2,210)=1.38, p=.26
Teacher report ±	F(1,82)=.02, p=.90	F(2,164)=.80, p=.45	F(2,164)=1.62, p=.20	F(2,164)=.25, p=.78

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent reports: Longitudinal analysis of DRD4 genotype, institutional deprivation and IOI outcome (controlling for IQ and gender)

The results following the inclusion of IQ and gender as covariates to the three-way repeated measures ANCOVA analysis were comparable to the 'no covariates' model, reported above. However, the overall main effect of institutional deprivation group, although perhaps suggestive of a similar effect was no longer significant (*p*=.17). Again, there was no main effect of genotype over time (*p*=.90). The main effect of assessment age approached, but did not reach significance (*p*=.09). There was no indication of a persistent GxE interaction effect between DRD4 genotype and institutional deprivation groups (*p*=.92). Likewise, there were no interactions between risk factors and

assessment age (all n/s, *p*'s = .26 - .88). The developmental trajectory of the combined effects of DRD4 genotype and institutional deprivation on IOI outcome, controlling for IQ and gender effect, can be seen graphically in figure 7.10 (A). These suggest that the pattern of differences between the specific risk groups show a slightly different arrangement compared with the uncorrected trajectories. However, this did not result in significant changes to the findings.

Teacher reports: Longitudinal analysis of DRD4 genotype, institutional deprivation and IOI outcome (controlling for IQ and gender)

The addition of IQ and gender to the model using teacher report data did not change the pattern of effects. There was a significant overall main effect of e'risk group, indicating that deprivation groups differed significantly from one another persistently over time (p=.008). There was no main effect of DRD4 genotype and no GxE interaction between risk factors (p=.46; p=.90, for the two effects, respectively). No interactions between assessment age and e'risk and/or g'risk were found (all n/s, see table 7.15; *p*'s =.20 - .78).

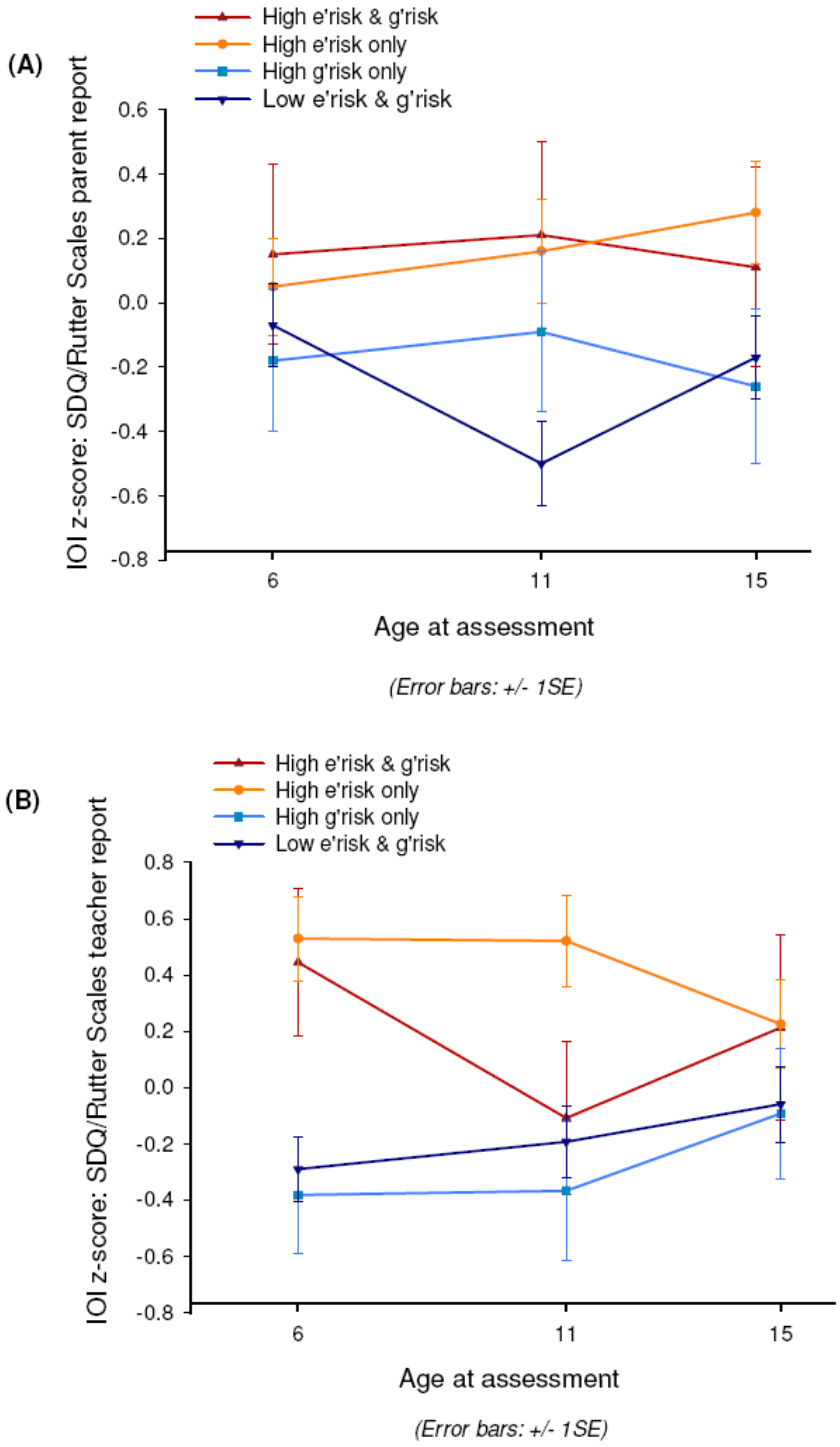


Figure 7.10

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & DRD4 genotype *(controlling for IQ and gender)*: (A) parent and (B) teacher reports

7.3.4.3 DRD4 and deprivation – summary of effects on IOI

The analysis of the moderation of deprivation effects on IOI by DRD4 genotype produced a different pattern of findings from the analysis above on DAT1. The two main findings were: First, DRD4 genotype did not influence the risk for IOI. There were no longitudinal main effects of genotype and no GxE interactions in relation to DRD4 genotype and experience of institutional deprivation on levels of IOI. This suggested GxE interaction effects reported in the preceding sections were specific to the DAT1 genotypes. Second, there was a main effect of extended institutional deprivation on elevated IOI/ADHD scores. This was demonstrated across assessment ages and informants. However, the introduction of additional covariates to the models reduced the variation in IOI scores, which resulted in nonsignificant parental report findings.

7.4 Results section 3: Gene-environment interaction in relation the risk for other associated features

7.4.1 Does DAT1 40-bp genotype (3'UTR) interact with early deprivation to increase the risk for cognitive impairment (IQ), disinhibited attachment or conduct problems?

The aim of the final part of the current chapter was to explore the association between DAT1 and DRD4 genotypes/haplotype, institutional deprivation and alternative domains of impairment. These domains were chosen on the basis of their association with either deprivation or IOI, and/or its use as a covariate in the ANOVA models presented above. In the following analysis the behavioural measures: Cognitive impairment, disinhibited attachment and conduct problems, were used as dependent outcome measures. The rationale for this analysis was to explore whether the genetic effects described above were specific to IOI or represented part of a more generalised risk mechanism for poor outcome in the ERA sample. The same repeated measures ANOVA model used in relation to IOI was applied here to test for the main effects of institutional deprivation risk group, genotype/haplotype group and GxE interaction with respect to the risk for cognitive impairment (lowered IQ), disinhibited attachment and conduct problems over time. Institutional deprivation was classified in the same way, i.e. the sample was dichotomously split into high risk (≥ 6 months institutional rearing in Romania) and low risk (U.K., Rom non-IR and Rom IR < 6months) groups. The same high and low risk genotype groups used in the analyses above were also

applied here. Outcome scores from age 6, 11 and 15 were entered into the three-way repeated measures ANOVA model, with assessment age as a between subject factor. Full details of how cognitive impairment (IQ), disinhibited attachment and conduct problems were measured is given in the method section under heading 4.3.4. In brief: IQ (cognitive impairment) was measured at age 6 using the McCarthy Scales of Children's Abilities (McCarthy, 1972) and at age 11 and 15 using a short form of the Wechsler Intelligence Scale for Children (WISC III$^{U.K.}$; Wechsler, 1992; disinhibited attachment was assessed using a composite measure that comprised items from the parental interview and investigator ratings; conduct problems were measured using parent and teacher reports on the Rutter Scales at ages 6 and 11, and the SDQ at age 15. No covariates were used.

The first section of the current analysis focuses on the DAT1 40-bp genotype (3'UTR). This is followed by parallel analyses using the DAT1 30-bp (intron 8) genotype, the DAT1 10R-6R haplotype and the DRD4 genotype. Separate repeated measures ANOVA analyses were performed for each behavioural outcome. The main effects and GxE interaction between DAT1 40-bp genotype, institutional deprivation and assessment age on the risk for cognitive impairment (lowered IQ), disinhibited attachment or conduct problems over time are presented below in table 7.16.

Table 7.16

Main effects & interactions over time between DAT1 40-bp genotype (3'UTR), institutional deprivation & assessment age on ERA outcomes *(no covariates)*

	Main effects		
Outcome	Environmental risk	Genetic risk	Assessment age
IQ	F(1,115)=26.60***	F(1,115)=.01, p=.95	F(2,191)=.85, p=.41
Disinhibited attachment	F(1,120)=33.06***	F(1,120)=.43, p=.51	F(2,219)=1.34, p=.26
Conduct problems *(parent report)*	F(1,110)=2.24, p=.14	F(1,110)=2.09, p=.15	F(2,188)=.04, p=.94
Conduct problems *(teacher report)*	F(1,85)=5.05*	F(1,85)=.58, p=.45	F(2,170)=.26, p=.78

	Interactions			
Outcome	G x E	E x age	G x age	G x E x age
IQ	F(1,115)=.21, p=.65	F(2,191)=4.50*	F(2,191)=.11, p=.86	F(2,191)=1.34, p=.26
Disinhibited attachment	F(1,120)=.38, p=.54	F(2,219)=1.54, p=.22	F(2,219)=.00, p=.999	F(2,219)=1.24, p=.29
Conduct problems *(parent report)*	F(1,110)=.19, p=.67	F(2,188)=.28, p=.72	F(2,188)=.14, p=.84	F(2,188)=3.46*
Conduct problems *(teacher report)*	F(1,85)=.06, p=.81	F(2,170)=.40, p=.67	F(2,170)=4.33*	F(2,170)=.16, p=.85

*p<.05; **p<.01; ***p<.001

± Sphericity assumption not met, Huynh-Feldt correction applied

7.4.1.1 IQ (cognitive impairment) and the effects of DAT1 40-bp genotype (3'UTR) and institutional deprivation over time

The three-way repeated measures ANOVA analysis of IQ at ages 6, 11 and 15 years showed there was a significant main effect of environmental risk group on cognitive outcome, but no effect of genetic risk group or assessment age (p<.001; p=.95; p=.41, for the three effects, respectively). This indicated that institutional deprivation risk groups differed significantly from one another in their level of IQ over the course of the study period but that average within-group levels did not change significantly over time. Moreover, DAT1 40-bp g'risk groups did not differ significantly from one another in their level of IQ. With respect to the interaction effects, the GxE interaction between DAT1 40-bp genotype and institutional deprivation was not significant in relation to level of IQ over time (p=.65). This indicated that DAT1 genotype did not moderate the effects of institutional deprivation on the risk for cognitive impairment. There was a significant interaction between deprivation risk group and assessment age in relation to IQ (p=.02). This suggested that there was a different pattern of change in the levels of IQ between the e'risk groups over time. This issue is discussed in more details

in the ERA study paper by Beckett et al (2006). There were no other significant interactions with assessment age (G'risk x age: *p*=.86; GxExAge: *p*=.26).

7.4.1.2 Disinhibited attachment and the effects of DAT1 40-bp genotype (3'UTR) and institutional deprivation over time

The repeated measures ANOVA model was then performed using disinhibited attachment as the outcome measure. A similar pattern of results was found. There was an overall significant main effect of institutional deprivation risk group over time, but no effect of DAT1 40-bp genotype group or assessment age (*p*<.001; *p*=.51; *p*=.26, for the three effects, respectively). This suggested that deprivation e'risk groups differed from one another in their level of disinhibited attachment persistently over time. Genotype status did not influence level of disinhibition, with no difference between the groups over time.

The test of GxE interaction with respect to disinhibited attachment was not significant, indicating that there was no moderation by DAT1 40-bp genotype of the risk associated with extended deprivation for disinhibited attachment. None of the interactions between risk factors and assessment age were significant, indicating there was no differential effect of assessment age between the e'risk or g'risk groups on disinhibited attachment outcome (see table 7.16).

7.4.1.3 Conduct problems and the effects of DAT1 40-bp genotype (3'UTR) and institutional deprivation over time

The same three way repeated measure ANOVA model was then used to test for the main effects and interactions between institutional deprivation and DAT140-bp genotype in relation to the risk for conduct problems.

Parent report: Longitudinal analysis of DAT1 40-bp genotype (3'UTR), institutional deprivation and conduct problems over time

According the analysis of parent reports of conduct problems at age 6, 11 and 15, there were no main effects of deprivation e'risk group, DAT1 40-bp genotype group or assessment age on outcome (*p*=.14; *p*=.15; *p*=.94, for the three effects, respectively). Moreover, there was no GxE interaction between e'risk and g'risk factors in relation to conduct problems over time (p=.67). The overall results

suggested that neither institutional deprivation risk groups nor genotype groups differed from one another in their level of conduct problems. Overall group levels did not change over time and there were no two-way interactions between assessment age and risk factors (E x age: p=.72; G x age: p=.84). However, a significant three-way interaction between genotype, deprivation group and assessment age was observed, suggesting that the two risk factors interacted with each other differentially over time but no overall GxE interaction was observed.

Teacher report: Longitudinal analysis of DAT1 40-bp genotype (3'UTR), institutional deprivation and conduct problems over time

When teacher reports of conduct problems were used as the dependent measure in the repeated measures ANOVA a main effect of deprivation group was detected (p=.03). There was still no main effect of DAT1 40-bp genotype risk group or of assessment age (p=.45; p=.78, for the two effects, respectively). Moreover there was no indication of moderation of environmental effects by genotype group on conduct problems scores over time (p=.81). The only other significant finding related the interaction between g'risk group and assessment age (p=.02). This suggested that there was a different pattern of change in the levels of conduct problems between the genotype groups over time.

7.4.1.4 Summary of the effects of DAT1 40-bp genotype (3'UTR) and institutional deprivation on IQ, disinhibited attachment and conduct problems over time

The series of ANOVA tests presented above revealed no indication that DAT1 40-bp genotype interacted with institutional deprivation to influence the risk for cognitive impairment, disinhibited attachment or conduct problems. This suggested that the genetic moderation effects observed in relation to the risk for IOI from extended institutional deprivation were specific to that outcome. Moreover, by controlling for the effects of IQ, disinhibition and conduct problems in the analysis of GxE interaction and IOI this did not introduce bias to the model by masking shared moderation effects in relation to the covariates.

The association between extended institutional deprivation and cognitive impairment and also disinhibited attachment has been well documented in previous papers so will not be discussed in more detail in the current thesis (Beckett et al., 2006; Rutter et al., 2007a).

7.4.2 Does DAT1 30-bp genotype (intron 8) interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

The investigation of the interaction between genotype and deprivation in relation to cognitive impairment, disinhibited attachment and conduct problems was then performed with DAT1 30-bp genotype (intron 8) as the between-subjects genetic risk factor. Again, institutional deprivation was included as the other between-subjects risk factor and assessment age as the within-subjects factor in the three-way repeated measures ANOVA model. The separate test results for each behavioural outcome are presented below in table 7.17

Table 7.17
Main effects and interactions over time between DAT1 30-bp genotype (intron 8), institutional deprivation and assessment age on ERA outcomes *(no covariates)*

	Main effects		
Outcome	Environmental risk	Genetic risk	Assessment age
IQ	F(1,115)=24.35***	F(1,115)=1.29, p=.26	F(2,190)=.60, p=.52
Disinhibited attachment	F(1,120)=29.41***	F(1,120)=.06, p=.81	F(2,220)=1.81, p=.17
Conduct problems *(parent report)*	F(1,110)=1.81, p=.18	F(1,110)=1.46, p=.23	F(2,191)=.31, p=.71
Conduct problems *(teacher report)*	F(1,84)=3.02, p=.08	F(1,84)=.12, p=.72	F(2,168)=.57, p=.57

	Interactions			
Outcome	G x E	E x age	G x age	G x E x age
IQ	F(1,115)=.46, p=.50	F(2,190)=7.20**	F(2,190)=.25, p=.74	F(2,190)=1.57, p=.21
Disinhibited attachment	F(1,120)=.20, p=.65	F(2,220)=1.07, p=.34	F(2,220)=.16, p=.83	F(2,220)=.67, p=.50
Conduct problems *(parent report)*	F(1,110)=1.07, p=.30	F(2,191)=.75, p=.46	F(2,191)=1.41, p=.25	F(2,191)=3.47*
Conduct problems *(teacher report)*	F(1,84)=.34, p=.56	F(2,168)=1.22, p=.30	F(2,168)=1.67, p=.19	F(2,168)=2.5, p=.09

#p=.05; *p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

7.4.2.1 IQ (cognitive impairment) and the effects of DAT1 30-bp genotype (intron 8) and institutional deprivation over time

The DAT1 30-bp results mirror those in relation to DAT1 40-bp genotype (3'UTR). That is, the repeated measures analysis of variance with IQ from childhood to mid-adolescence as the outcome measure and DAT1 30-bp genotype group and institutional deprivation group as the independent measures demonstrated an overall a main effect of deprivation group on level of IQ over time ($p<.001$). There was no main effect of genotype group or of assessment age ($p=.26$; $p=.52$, for the two effects, respectively). Similarly, there was no GxE interaction between DAT1 30-bp genotype and e'risk group, suggesting that genotype did not moderate the risk associated with deprivation group for cognitive impairment ($p=.50$).

There was a significant interaction between deprivation group and assessment age ($p=.002$). This was not surprising given there was no influence of genetic factors on outcome and the same interaction was observed in the previous DAT1 40-bp analysis. There were no other interaction between assessment age and risk factors (G x age: $p=.74$; GxExAge: $p=.21$).

7.4.2.2 Disinhibited attachment and the effects of DAT1 30-bp genotype (intron 8) and institutional deprivation over time

The repeated measures ANOVA performed to test for the main effects and interaction of DAT1 30-bp genotype and deprivation on overall risk for disinhibited attachment demonstrated exactly the same pattern of results as the model that used DAT1 40-bp genotype. There was a main effect of deprivation group on outcome but no effect of DAT1 30-bp genotype or assessment age ($p<.001$; $p=81$; $p=.17$, for the three effects, respectively. There was no GxE interaction between deprivation and genotype ($p=.65$) and no interactions between assessment age and risk factors (all n/s; p's = .34 - .83).

7.4.2.3 Conduct problems and the effects of DAT1 30-bp genotype (intron 8) and institutional deprivation over time

The repeated measures ANOVA model was applied to the conduct problems phenotype to assess the main effects and interaction of DAT1 30-bp genotype

and institutional deprivation group in relation to this outcome. Parent and teacher reports are presented separately.

Parent report: Longitudinal analysis of DAT1 30-bp genotype (intron 8), institutional deprivation and conduct problems over time

According to parental reports of conduct problems at age 6, 11 and 15, there was no main effect of deprivation group, or DAT1 30-bp genotype or assessment age (p=.18; p=.23; p=.71, for the three effects, respectively). The test for GxE interaction between risk factors was nonsignificant (p=.30) and there was no interaction between assessment age and deprivation group or DAT1 30-bp genotype (E x age: p=.46; G x age: p=.25). A significant three-way interaction between genotype, deprivation group and assessment age was observed (p=.04), suggesting that the two risk factors interacted with each other differentially over time but no overall GxE interaction was observed. The results are in line with those reported above in relation to the DAT1 40-bp genotype.

Teacher report: Longitudinal analysis of DAT1 30-bp genotype (intron 8), institutional deprivation and conduct problems over time

By and large, the results of the repeated measures ANOVA model of the effects of DAT1 30-bp genotype and deprivation group on teacher rated conduct problems mirrored those of the DAT1 40-bp genotype, reported above. However, the overall main effect of deprivation group on conduct problems over time fell short of significance in the current analysis (p=.08). The main effect of DAT1 30-bp genotype group or assessment age was not significant (p=.72; p=.57, for the two effects, respectively). There was no GxE interaction between genotype and deprivation group (p=.56) and no interaction between assessment age and risk factors (all n/s; p's = .09 - .30).

7.4.2.4 Summary of the effects of DAT1 30-bp genotype (intron 8) & institutional deprivation on IQ, disinhibited attachment and conduct problems over time

The results of the current analysis demonstrated that DAT1 30-bp genotype (intron 8) did not interact with institutional deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems. The pattern of associations was in line with those from the analysis of DAT1 40-bp genotype.

There were main effects of institutional deprivation on the risk for cognitive impairment and disinhibited attachment, but not in relation to conduct problems. There were no main effects of DAT1 30-bp genotype (intron 8) on the risk for any of the outcomes assessed. This indicated that exposure to institutional deprivation influenced the risk for cognitive impairment and disinhibited attachment persistently over time, but genotype group did not significantly affect any of the outcomes directly or through the moderation of environmental risk.

7.4.3 Does DAT1 (10R-6R) haplotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

The three-way repeated measures ANOVA model described above was then applied to the DAT1 (10R-6R) haplotype. The results of this analysis are set out below in Table 7.18. The aim was to investigate whether this haplotype, which combines the two DAT1 genotypes, interacts with extended institutional deprivation to influence the risk for cognitive impairment, disinhibited attachment and conduct problems over time. Again, the purpose of this analysis was to explore whether the interaction effects observed in relation to IOI were specific to that outcome and to check the validity of using IQ, disinhibited attachment and conduct problems as covariates in the GxE interaction analyses presented in the previous sections of this chapter.

Table 7.18

Main effects & interactions over time between DAT1 (10R-6R) haplotype, institutional deprivation & assessment age on ERA outcomes *(no covariates)*

	Main effects		
Outcome	Environmental risk	Genetic risk	Assessment age
IQ	F(1,113)=23.66***	F(1,113)=.52, p=.47	F(2,190)=.63, p=.51
Disinhibited attachment	F(1,118)=29.73***	F(1,118)=.78, p=.38	F(2,217)=1.71, p=.19
Conduct problems *(parent report)*	F(1,108)=2.55, p=.11	F(1,108)=1.87, p=.17	F(2,186)=.20, p=.79
Conduct problems *(teacher report)*	F(1,83)=4.92*	F(1,83)=1.37, p=.25	F(2,166)=.08, p=.92

	Interactions			
Outcome	G x E	E x age	G x age	G x E x age
IQ	F(1,113)=.77, p=.38	F(2,190)=4.07*	F(2,190)=1.18, p=.31	F(2,190)=.39, p=.64
Disinhibited attachment	F(1,118)=1.92, p=.17	F(2,217)=.96, p=.38	F(2,217)=.16, p=.83	F(2,217)=1.79, p=.17
Conduct problems *(parent report)*	F(1,108)=.83, p=.37	F(2,186)=.08, p=.90	F(2,186)=3.14#	F(2,186)=5.22**
Conduct problems *(teacher report)*	F(1,83)=.01, p=.91	F(2,166)=.53, p=.59	F(2,166)=3.34*	F(2,166)=.58, p=.37

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

7.4.3.1 IQ (cognitive impairment) and the effects of DAT1 (10R-6R) haplotype and institutional deprivation over time

The results mirrored those from the analyses of the separate genotypes. That is, there was a main effect of institutional deprivation group on IQ level, but no effect of genotype status or assessment age (p<.001; p=.47; p=.51, for the three effects, respectively). Again, there was no genetic moderation by DAT1 haplotype of the risk associated with extended deprivation for cognitive impairment, demonstrated by the nonsignificant GxE interaction test results (p=.38). The significant interaction between environmental risk group and assessment age, described in the previous analyses using the separate DAT1 genotypes, was also observed in the current analysis (p=.03). There were no other interactions with assessment age (G x age: p=.31; GxExage: p=.64)

7.4.3.2 Disinhibited attachment and the effects of DAT1 (10R-6R) haplotype and institutional deprivation over time

Using DAT1 haplotype in the repeated measures ANOVA model did not change the pattern of results in relation to the risk for disinhibited attachment described in the preceding sections on the separate DAT1 genotypes. The main effect of institutional deprivation group on disinhibited attachment over time was highly significant ($p<.001$), indicating that disinhibited attachment score varied as a function of e'risk group. There was no main effect of haplotype group or assessment age ($p=.38$; $p=.19$, for the two effects, respectively). There was no GxE interaction between DAT1 haplotype and deprivation group on the risk for disinhibited attachment ($p=.17$), suggesting no genetic moderation of the environmental risk. There were no interactions between assessment age and risk factors (all n/s: p's = .17 - .83).

7.4.3.3 Conduct problems and the effects of DAT1 (10R-6R) haplotype and institutional deprivation over time

The three-way repeated measure ANOVA model was then applied to conduct problems at ages 6, 11 and 15 with DAT1 haplotype and institutional deprivation remaining as the predictor variables. The reports of conduct behaviour from parents and teachers were analysed separately and listed above in table 7.18.

Parent report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and conduct problems over time

The analysis using the DAT1 haplotype as the genetic risk factor in the GxE ANOVA model showed the same pattern of results as that from the DAT1 genotypes analyses (40-bp and 30-bp polymorphisms). The overall main effect of deprivation group did not reach a significant level, according to parent reports of conduct problems ($p=.11$). There was no main effect of genotype group or assessment age ($p=.17$; $p=.79$, for the two effects, respectively). Moreover, there was no GxE interaction between DAT1 haplotype and institutional deprivation exposure on the risk for conduct problems over time ($p=.37$). Again, there was a significant three-way interaction between age, deprivation group and haplotype

(*p*=009). This suggested that e'risk and g'risk factors interacted with each other differentially over time, but no overall GxE interaction was detected. The interaction between DAT1 haplotype and assessment age just reached a significant level (*p*=.05). This provided some suggestion that the pattern of differences between haplotype groups may have changed over time but, as noted above, the overall difference between the groups was not significant.

Teacher report: Longitudinal analysis of DAT1 haplotype, institutional deprivation and conduct problems over time

The analysis of the effects of DAT1 haplotype and deprivation on the risk for teacher reported conduct problems demonstrated a significant main effect of deprivation group, but no overall effect of haplotype group or assessment age (*p*=.03; *p*=.25; *p*=.92, for the three effects, respectively). However, despite there being no main effect of haplotype there was a significant interaction between haplotype group and assessment wave (*p*=.04). As noted above, this indicated that relative difference between haplotype groups changed during the course of the study period. There was no GxE interaction between deprivation group and DAT1 haplotype (*p*=.91) and no other interactions between assessment age and risk factors (E x age: *p*=.59; GxExage: *p*=.37).

7.4.3.4 Summary of the effects of DAT1 10R-6R haplotype and institutional deprivation on IQ, disinhibited attachment and conduct problems over time

Unsurprisingly, the testing of the GxE model using a repeated measures ANOVA with combined DAT1 haplotype and institutional deprivation risk groups produced results that were in line with those from the analyses of the separate DAT1 40-bp (3'UTR) and 30-bp (intron 8) genotypes. That is, there was no moderation of the risk association with the environmental pathogen by the dopamine transporter 10R-6R haplotype in relation to any of the behavioural outcomes I tested. There was a main effect of e'risk group in relation to IQ, disinhibited attachment and conduct problems (teacher rated); i.e. the institutional deprivation groups were rated persistently over time as having significantly different scores from one another. Moreover, the scores did not differ as a function of DAT1 haplotype, as indicated by nonsignificant main effects of g'risk group across the outcomes. The

results provide further support for the specificity of the DAT1 moderation of environmental susceptibility effects reported in the previous section in relation to IOI outcome.

7.4.4 Does DRD4 genotype (exon III) interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

The same three-way repeated measures ANOVA model was then run using DRD4 genotype and institutional deprivation risk groups as between-subjects factors and assessment age as a within-subject factor. Separate tests again for were performed for the following outcomes: Cognitive impairment (lowered IQ), disinhibited attachment and conduct problems. The results are reported below in table 7.19. Although no genetic moderation by DRD4 genotype was found with regards to the risk for IOI from extended institutional deprivation, the analyses were performed to validate the use of the behavioural measures as covariates in said analyses.

Table 7.19

Main effects & interactions over time between DRD4 genotype (exon III), institutional deprivation & assessment age on ERA outcomes *(no covariates)*

	Main effects		
Outcome	Environmental risk	Genetic risk	Assessment age
IQ ±	F(1,114)=13.38***	F(1,114)=2.58, p=.11	F(2,187)=.14, p=.83
Disinhibited attachment ±	F(1,119)=16.36***	F(1,119)=3.17, p=.08	F(2,218)=2.16, p=.12
Conduct problems *(parent report)* ±	F(1,109)=.47, p=.49	F(1,109)=.19, p=.66	F(2,185)=.10, p=.88
Conduct problems *(teacher report)*	F(1,84)=3.64, p=.06	F(1,84)=.84, p=.36	F(2,168)=.68, p=.51

	Interactions			
Outcome	G x E	E x age	G x age	G x E x age
IQ ±	F(1,114)=.53, p=.47	F(2,187)=4.78*	F(2,187)=.56, p=.54	F(2,187)=.27, p=.72
Disinhibited attachment ±	F(1,119)=2.28, p=.13	F(2,218)=.72, p=.48	F(2,218)=.68, p=.49	F(2,218)=.16, p=.83
Conduct problems *(parent report)* ±	F(1,109)=2.25, p=.14	F(2,185)=.01, p=.98	F(2,185)=.08, p=.89	F(2,185)=.13, p=.84
Conduct problems *(teacher report)*	F(1,84)=.001, p=.97	F(2,168)=.28, p=.76	F(2,168)=1.21, p=.30	F(2,168)=.66, p=.52

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

7.4.4.1 IQ (cognitive impairment) and the effects of DRD4 genotype (exon II) and institutional deprivation over time

Like in the analyses above using DAT1 genotypes/haplotype, the ANOVA using DRD4 genotype showed there was no moderation of the risk associated with institutional deprivation for lowered IQ using this genotype either. The main effect of deprivation group still came through as significant, (p<.001). Similarly, the interaction between deprivation group and assessment age was still significant, indicating that the e'risk groups showed different developmental trajectories (p=.03). There was no main effect of genotype or assessment age and no GxE interaction between DRD4 genotype and deprivation group (p=.11; p=.83; p=.47, for the three effects, respectively). No interaction between assessment age and genotype was detected, or between e'risk, g'risk and age (p=.54; p=.72, for the two effects, respectively).

7.4.4.2 Disinhibited attachment and the effects of DRD4 genotype (exon II) and institutional deprivation over time

The ANOVA test was then performed in relation to the outcome of disinhibited attachment. Like in the previous analyses, the main effect of deprivation group on the risk for disinhibited attachment was highly significant (p<.001). The main effect of DRD4 genotype on outcome approached, but did not reach, significance (p=.08). This represented a change from the tests using DAT1 genotypes and gave some weak indication that disinhibited attachment scores may vary as a function of DRD4 genotype. There was also a faint suggestion that DRD4 genotype might moderate the risk associated with deprivation for disinhibited attachment. The GxE interaction did reach the required level of significance (p=.13) but the G-E interplay may warrant further investigation as there is evidence in the literature on the link between DRD4 genotype and disorganised attachment in infants (Lakatos et al., 2000). However, it is beyond the scope of the current study to explore the association further at the current time. There was no indication of any interactions between assessment age and risk factors (all n/s; p's =.48 - .83).

7.4.4.3 Conduct problems and the effects of DRD4 genotype (exon II) and institutional deprivation over time

The final stage of this analysis was to explore the effect of DRD4 and institutional deprivation on conduct problems over time. The same three-way repeated measures ANOVA model was used and the results of the analysis on parent and teacher reports of conduct behaviour are presented above in table 7.19.

Parent report: Longitudinal analysis of DRD4 genotype, institutional deprivation and conduct problems over time

The results showed that according to parent reports of conduct problems over time, there was no main effect of institutional deprivation group, or DRD4 genotype risk group or assessment age (p=.49; p=.66; p=.88, for the three effects, respectively). The GxE interaction between e'risk and g'risk groups was not significant (p=.14) and there were no interactions between assessment wave and risk factors (all n/s; p's =. 84 - .98). The results are in line with those reported in relation to the dopamine transporter genotypes/haplotype.

Teacher report: Longitudinal analysis of DRD4 genotype, institutional deprivation and conduct problems over time

The results of the analysis using the DRD4 genotype and institutional deprivation as risk factors for teacher reported conduct problems also demonstrated a similar pattern of findings to those relating to the DAT1 genotypes/haplotype. The overall main effect of deprivation group on the risk for conduct problems approached, but fell just short, of significance (p=.06) and there were no other significant effects. There was no effect of DRD4 genotype or assessment age on outcome (p=.36; p=.51, for the two effects, respectively). No GxE interaction between e'risk and g'risk factors was found (p=.97) and there were no interactions between assessment age and DRD4 genotype and/or deprivation group (all n/s; p's =.30 - .76).

7.4.4.4 Summary of the effects of DRD4 genotype (exon III) and institutional deprivation on IQ, disinhibited attachment and conduct problems over time

By and large, the result of the analysis of the effects of DRD4 genotype and institutional deprivation on cognitive impairment, disinhibited attachment and conduct problems over time was in line with the parallel analyses using DAT1 genotypes/haplotype. The main finding was that there was no significant genetic moderation of environment risk on the risk for the behavioural outcomes tested still held. The main effect of exposure to institutional deprivation on the risk for cognitive impairment, disinhibited attachment and, to some degree, teacher rated conduct problems was apparent also in this analysis. There was no main effect of DRD4 genotype on cognitive impairment or conduct problems. However, the one distinguishing feature of the current analysis using DRD4 was the indication that genotype may influence disinhibited attachment outcome. The results were not significant but did suggest that a future investigation of the association may produce some interesting findings.

7.5 Results chapter summary

The analyses produced an interesting set of results, an integrative discussion of which is provided in the final chapter, number 9. In summary, there were nine main findings: First, the analyses demonstrated compelling evidence of a specific synergistic gene-environment interaction in relation to the risk for IOI following early institutional deprivation. The results show the power of specific genetic polymorphisms to moderate the risk associated with institutional deprivation for elevated levels of IOI. Levels of IOI were highest in those children who were exposed to extended deprivation and possessed the risk genotype and the combination of these two risk factors appeared to account for nearly all the variance in parent rated IOI scores within the GenERA sample by mid-adolescence.

Second, this interaction was specific to the dopamine transporter genotypes studied and was not observed in relation to the analysis of the dopamine receptor polymorphism. Similar effects were found across both the DAT1 40-bp (3'UTR) and the DAT1 30-bp (intron 8) genotypes, which have been functionally linked to one another in the literature (Brookes et al., 2006b).

Moreover, combining the two polymorphisms into the DAT1 10R-6R haplotype helped to clarify and strengthen the GxE interaction effect. A table summarising the GxE interaction results in relation to IOI outcome is given below.

Table 7.20

Summary of longitudinal GxE interaction ANOVA findings for dopamine genotypes and institutional deprivation on the risk for IOI

	repeated measures ANOVA models			
	no covariates		IQ & gender	
Genotype	GxE	GxExAge	GxE	GxExAge
Dopamine transporter (parent report IOI)				
DAT1 40-bp (3'UTR)	p=.07	p=.36	p=.34	p=.29
DAT1 30-bp (intron 8)*	p=.38	p=.03	p=.07	p=.01
DAT1 haplotype*	p=.20	p=.04	p=.02	p=.02
Dopamine transporter (teacher report IOI)				
all GxE effects non significant				
Dopamine receptor DRD4 (parent report IOI)				
all GxE effects non significant				
Dopamine receptor DRD4 (teacher report IOI)				
all GxE effects non significant				

* results supported by cross sectional analyses

Third, the DAT1 GxE interaction effect was observed only in relation to parent reported symptoms of IOI. The statistically significant results from the ANOVA tests of group differences were found for the parent reports on the questionnaire measure of IOI (Rutter Scales and SDQ). However, the results from the parental CAPA interview data on ADHD symptomatology indicated the same pattern of results and it is fair to conclude that they show support for the GxE interaction effect. There was some indication from the graphical representations of the data that teacher reported IOI symptoms showed similar elevations for the children who experienced extended deprivation and were in possession of the risk genotype. This pattern was not reflected in significant statistical results, was

mainly in relation to the results at age 11, but could not be seen to any significant degree at the other assessment waves.

Fourth, the DAT1 GxE interaction was most apparent once the effects of IQ and gender were controlled for in the analytical model. That is, by controlling for the variance in IOI attributable to these factors the risk for elevated outcome scores associated with the interaction between extended institutional deprivation and dopamine genotype could be seen more clearly. However, a similar pattern of results was seen in the initial 'uncorrected' analyses, providing support for the GxE interaction model of effects and reassurance that the results were not just chance effects. Because of the small sample size available for significance testing of the GxE interaction effects it was especially important to partial out the specific effects of the risk factors of interest. Moreover, because of the effect of adding IQ into the model it was essential to ascertain whether there was an interaction between genetic and environmental risk factors in relation to IQ as the dependent variable. Reassuringly, the results of the analyses in section 7.4 showed that there was no GxE interaction effect with respect to IQ outcome.

Fifth, the longitudinal analyses provided important developmental support for the GxE interaction finding. There was support for an overall GxE interaction over the whole study period and the cross sectional analyses indicated that the GxE effect seemed to get stronger over time and was significant from early adolescence onwards.

Sixth, the analysis of GxE interaction effects in relation to other cognitive and behavioural outcomes provided support for the specificity of the effect in relation to IOI and the inclusion of these outcomes as covariates in the analytical models. That is, DAT1 genotypes/haplotype did not moderate the risk for cognitive impairment, conduct problems or disinhibited attachment following early institutional deprivation. The same lack of GxE interaction effect was found with respect to DRD4 and the risk for conduct problems and cognitive impairment. However, there was some indication that this preliminary analysis of the moderation of the risk for disinhibited attachment from early deprivation by DRD4 genotype may warrant further investigation in the future.

Seventh, the main effect of institutional deprivation for IOI impairment was apparent throughout the majority of the analyses. That is, those children who

resided in the institutions for at least 6 months were persistently rated by parents and teacher as having elevated levels of IOI over the whole study period.

Eighth, there was a main effect of DAT1 40-bp genotype and DAT1 haplotype on the risk for IOI. The high risk genotype/haplotype groups were rated by parents as having elevated levels of IOI compared with the carriers of the low risk alleles. However, this effect was not seen in relation to the DAT1 30-bp (intron 8) polymorphism.

Ninth, there was no indication of a gene-environment correlation between any of the genotypes and institutional deprivation. That is, the children in the high e'risk group who resided longer in the institutions were not subject to an increased genetic liability for IOI compared with the low e'risk group.

The above results show support for the phenotype hypothesis that specific genetic polymorphism associated with ADHD in the nondeprived population moderate the risk for IOI following extreme early deprivation.

CHAPTER 8: RESULTS

DOES THE GLUCOCORTICOID RECEPTOR GENE MODERATE THE EFFECTS OF INSTITUTIONAL DEPRIVATION ON THE RISK FOR IOI?

8.1 Chapter outline

The current chapter used a process-based model of gene-environment interplay to select the candidate gene to examine the moderation of the risk for associated with institutional deprivation. Like in the previous chapter, this investigation of gene-environment interplay represents one possible mechanism that could help to account for the variability in outcome observed in the ERA sample. Specific polymorphisms of the glucocorticoid receptor (GR) gene were used to test this hypothesis driven investigation. The hypothesis is that genes that influence biological factors implicated in a child's response to their adverse environment represent a possible candidate for GxE interaction even where these genes have not been associated with the outcome of interest in previous candidate gene or GxE interaction studies.

The GR genes were chosen because they have been shown to moderate an individual's physiological and psychological response to stress. The early experience of global deprivation in the Romanian institutions is likely to be stressful. It was hypothesised that adoptees' responses to this psychosocial stress associated with early institutional deprivation will be moderated by functional polymorphisms in the GR genes leading to early and significant alterations in brain development. This may in turn influence later behavioural outcomes across a range of outcomes associated with stress response systems. As outlined in chapter 3 of the introduction (heading 3.5.2.2), the hypothesised pathway that could influence levels of IOI could operate through GR modulated dysregulations of HPA axis activity, which are associated with chronic elevations of cortisol activity, that may have long lasting and profound down-stream effects on brain development and functioning in circuits and neurotransmitter systems implicated in the patho-physiology of ADHD.

The format of the current chapter and the rationale for each analysis follows that used in the previous chapter on dopamine genotypes but with the addition of a section on the direct association between IOI and specific GR haplotypes. The chapter is set out in four sections: First, an analysis of the gene-environment correlations is presented in order to investigate the mediation model set out in the introduction (heading 3.4.2) and discussed in the previous chapter. Second, the results are given of a preliminary analysis examining the levels of IOI across the GR four-way haplotype groups (details given below). Third, the main investigation of the moderation of the risk associated with institutional deprivation for elevated levels of IOI by a specific GR polymorphism is presented. Fourth, like in the final section of the previous chapter, the same GxE interaction model used in relation to IOI is then applied to the other relevant behavioural outcomes.

8.1.1 Glucocorticoid receptor genes and IOI

8.1.1.1 GR BclI genotypes

For the following analyses using the GR *Bcl*I SNP the sample (n=120) were split dichotomously into two groups based on preliminary analyses of the risk for IOI associated with the specific alleles and published work on the associations glucocorticoid sensitivity and hormonal stress responses (Wust et al., 2004; van Rossum et al., 2003; van Rossum & Lamberts, 2004) One group comprised the C homozygotes (n=59, 49%) and the other was made up those who possessed at least one G allele (n=61, 51%).

8.1.1.2 GR 9beta genotypes

Following the same approach as above, this genotype was also categorised dichotomously based on preliminary analysis on the link with IOI and published reports of functionality of the gene (Kumsta et al., 2007; Derijk et al., 2001). The sample (n=127) was divided into one group made up of individuals who were homozygous for the A allele (n=80, 63%) and one group made up of those who possessed at least one G allele (n=47, 37%).

8.1.1.3 GR BclI-9beta haplotype

In addition to SNP based analyses, haplotype based analyses were performed (n=120). Previous studies have indicated that the G allele at the *Bcl*I locus and the G allele at the 9beta locus occur independently of one another (van den

Akker et al., 2008; Kumsta et al., 2007). The sample was therefore split into four genotype groups based on 3 haplotypes and following the approach used in Kumsta et al (2007). The four genotype groups were: Homozygotes for the most common haplotype (MCH; C-A); *Bcl*I G carriers (G-A heterozygotes or homozygotes); 9beta G carriers (C-G heterozygotes or homozygotes) or the mixed group (G-A paired with C-G). See appendix 6 and method section 5.5.2 for details.

8.1.2 Data analysis

The same analytical strategy used in the previous chapter to test for genetic mediation or moderation of the risk for IOI associated with institutional deprivation was applied here to the GR genotypes (see section 7.1.2). The correlations between environmental risk groups and the GR genotypes/haplotypes are presented first and analysed using chi-square tests of association. In section two, the results of the preliminarily analysis of the association between haplotype groups and IOI outcome are given. This was conducted as there have been no previous reports on the risk for IOI associated with the specific GR SNPs or haplotypes in the literature. This was tested using an analysis of variance model and post hoc Tukey's tests. The analysis of GxE interaction was conducted in the same manner as in the previous chapter and presented in the same way under section three of the current chapter. The final section, four, presents the analysis of the specificity of the GR effects in relation to IOI by applying the same GxE model to the outcomes of IQ, disinhibited attachment and conduct problems. This is done is the same way as in the previous chapter.

8.1.3 Predictions, hypotheses and research questions

In contrast to the phenotype-based strategy used in chapter 7, the current chapter employs a process-based model of candidate gene selection whereby variations of specific GR genetic polymorphism were selected based on their hypothesised impact on the effects of the environmental pathogen, rather than on their association with ADHD in the general population.

It was hypothesised that adoptees' responses to the psychosocial stress associated with early institutional deprivation will be moderated by functional polymorphisms in the GR gene leading to early and significant alterations in brain development that in turn influences IOI outcome. This leads to the prediction that children who possessed specific GR alleles would be particularly susceptible to the adverse effects of institutional deprivation and at particular risk for the development of early onset, persistent IOI.

The current chapter aimed to test this prediction using the following research questions:

1. Are there gene-environment correlations between the glucocorticoid receptor (GR) genotypes/haplotype and institutional deprivation?
2. Is there a specific GR 9beta/*Bcl*I haplotype associated with IOI in the GenERA sample as a whole?

The aim of the preliminary haplotype analysis is to isolate a specific SNP for the GxE interaction analysis may confer increased risk for or protections from IOI in the GenERA sample.

3. Does GR *Bcl*I or 9beta genotype interact with early deprivation to increase the risk for IOI?
4. Does GR *Bcl*I or 9beta genotype interact with early deprivation to increase the risk for cognitive impairment, disinhibited attachment or conduct problems?

8.2 Results section 1: GR Gene-environment correlation

Table 8.1 presents the GR genotype and haplotype frequencies across the environmental risk groups in order to address questions about the correlation between genetic risk and exposure to institutional deprivation. The results of the chi-square tests of association are discussed below.

Table 8.1

Percentage of cases across GR SNPs/haplotype groups within duration of deprivation environmental risk groups

		Environmental risk groups ±				
		% with genotype		frequencies		
Genotype		Low e'risk	High e'risk	Low e'risk	High e'risk	chi-square results
GR genotypes						
GR *Bcl*I	C:C	39%	65%	29	30	$\chi^2(1, N=120)=7.69, p<.01$
	G:G, G:C	61%	35%	45	16	
GR 9beta	A:A	69%	54%	53	27	$\chi^2(1, N=127)=2.86, p=.09$
	G:G, G:A	31%	46%	24	23	
GR haplotype						
*Bcl*I-9beta haplotype	MCH	24%	30%	18	14	$\chi^2(3,N=120)=10.86, p<.05$
	*Bcl*I G	43%	24%	32	11	
	9beta G	15%	37%	11	17	
	Mixed	18%	9%	13	4	

± *Low e'risk: UK, Rom non-IR, Rom IR <6 months*
High e'risk: Rom IR 6 to <24 and 24 to 42 months

8.2.1 Are there gene-environment correlations between glucocorticoid receptor (GR) genotypes/haplotype and institutional deprivation?

8.2.1.1 GR BclI SNP and institutional deprivation

The analysis of genotype frequencies across the environmental risk groups revealed an interesting pattern of results. There was a significant association between e'risk group and GR *Bcl*I genotype ($p<.01$), which suggested that there may be a gene-environment correlation between exposure to prolonged institutional deprivation and GR *Bcl*I genotype. Further investigation of the specific allelic frequencies (i.e. C vs. G allele) revealed that the distribution in the low e'risk group roughly corresponded to that seen in the general population (e.g. van den Akker et al., 2008, >6000 subjects; Kumsta et al., 2007, 600 subjects). However, the high e'risk there appears to be an underrepresentation of the G allele (19%) compared with the low e'risk group (38%).

8.2.1.2 GR 9beta SNP and institutional deprivation

The difference in the frequency of cases with A:A genotype and those with at least one G allele between the two environmental risk groups approached but did not reach significance (p =.09). That is, there was no significant association between deprivation risk groups and genotype groups, indicating no rGE. However, further investigation of the allelic frequencies indicated that the 9beta G allele may be slightly overrepresented in the high e'risk group compared with the low e'risk group (28% vs. 16%). Similar to above, the frequencies in the low e'risk roughly corresponded to those observed in the population (e.g. Kumsta et al., 2007; van den Akker et al., 2008).

8.2.1.3 GR BclI-9beta haplotype and institutional deprivation

The anomalies reported above on the two separate SNPs corresponded to a significant difference in the frequency of cases with each haplotype between the environmental risk groups (p<.05). The analysis suggested there may be correlation between haplotype group and exposure to institutional deprivation. To test whether combining two ethnic groups (U.K. and Romanian) within the low e'risk group confounded the results, the U.K. children were excluded from the subsample and the allelic frequencies were recalculated. The frequencies of the low e'risk Romanian children still matched those reported from the general population indicating that ethnicity was not confounding the results.

8.3 Results section 2: GR and the risk for IOI

8.3.1 Is there a specific GR 9beta/BclI haplotype associated with IOI in the GenERA sample as a whole?

There have been no previous reports on the risk for IOI associated with the specific GR SNPs or haplotypes in the literature. Therefore, a preliminary analysis was carried out to investigate the levels of IOI across the GR four-way haplotype groups to see if there was any indication of a particular genotype in the haplotype conferring risk for, or protection from, IOI/ADHD symptoms in the GenERA sample. Analysis of variance tests were used to assess this. The haplotype was selected for this analysis following the procedure used in previous

studies of GR on the association with physiological stress response to psychosocial stressors (Kumsta et al., 2007).

Table 8.2

Mean levels of IOI/ADHD symptoms (and standard deviations) across glucocorticoid receptor *Bcl*I-9beta haplotypes

	GR haplotype*				
	Mean z-scores (SD)				
IOI/ADHD measure	MCH	*Bcl*I G	9Beta G	Mixed	ANOVA
Parent report					
Age 6 (Rutter scales)	.23 (1.19)	-.23 (.94)	-.07 (1.13)	-.11 (.80)	*F*(3,118)=1.18; *p*=32
Age11 (Rutter scales)	.37 (1.27)	-.23 (.73)	.05 (1.17)	-.12 (94)	*F*(3,115)=2.10, *p*=.10
Age 15 (SDQ)	.28 (1.07)	-.30 (.87)	.13 (1.10)	.05 (1.09)	*F*(3,113)=2.11, *p* =.10
Age 15 (CAPA)	.40 (1.23)	-.32 (.63)	.46 (1.28)	-.14 (1.03)	*F*(3,117)=4.57, *p* =.005
Teacher report					
Age 6 (Rutter scales)	.16 (1.05)	-.10 (1.04)	.16 (1.11)	-.40 (.88)	*F*(3,111)=1.34, *p* =.27
Age 11 (Rutter scales)	.19 (1.22)	-.09 (.86)	-.17 (.94)	.17 (1.15)	*F*(3,105)=.77, *p* =.51
Age 15 (SDQ)	.18 (1.07)	-.06 (.96)	.03 (.91)	-.37 (1.04)	*F*(3,101)=.99, *p* =.40

* *MCH: Most common haplotype: two C:A alleles*
BclI G: One or two G:A alleles
9Beta G: one or two C:G alleles
Mixed: One C:G, one G:A allele

Table 8.2 shows that in mid-adolescence the haplotype groups differed in their ADHD symptom scores, as rated on the CAPA interview (*p*=.005). There was also a suggestive difference between the groups on the parental questionnaire of IOI symptoms (*p*=.10). The children who were in the *Bcl*I G group (one or two G:A alleles) had the lowest IOI/ADHD scores followed by the 'mixed' group (who possessed one G:A and one C:G allele), suggesting that the G:A genotype may confer some protective value against IOI/ADHD symptomatology. This pattern was mirrored longitudinally in the parent rated symptoms at ages 6 and 11, but the group differences did not reach significance (age 6*: p*=.32; age 11*: p*=.10).

The pattern of results from the teacher ratings of IOI behaviour gave general support to the findings above, but was a little more mixed. By and large

the *Bcl*I G and mixed haplotype groups were rated as having the lowest IOI scores, but no significant group differences were found (age 6*: p*=27; age 11*: p*=51; age 15*: p*=.40.

Post hoc Tukey's tests of the age 15 data corroborated the suggested distinction of the *Bcl*I G group. This group had significantly lower ADHD symptom scores on the CAPA than both the MCH (*p*=.02) and the 9beta G groups (*p*=.01). Unsurprisingly, there was no difference between the *Bcl*I G and mixed groups (*p*=.98) as they share an allele in common, G:A. The difference in IOI scores between the *Bcl*I G and MCH groups was also marginally significant, as rated on the parental questionnaire measure ($SDQ_{parent}15$*: p*=.09; age 11_{parent}*: p*=.08). This suggested that the G allele in the *Bcl*I genotype may confer some protective influence over levels of IOI/ADHD in the sample and that it would make sense to investigate the *Bcl*I SNP genotype separately. However, given the findings of the rGE analysis, much caution must be used when interpreting the following analysis of the influence by this SNP of the risk association with institutional deprivation for IOI/ADHD.

8.4 Results section 3: GR Gene-environment interaction in relation to IOI

This next section examined whether individuals' genetic makeup moderated the risk associated with institutional deprivation for IOI/ADHD symptomatology. This was analysed in relation to the specific GR *Bcl*I SNP. Following the protective effect of the G allele suggested by the previous haplotype analysis the C:C genotype was classified as the 'risk' genotype (i.e. carriers were allocated to high g'risk group) and individuals with at least one G allele were classified as being in the low g'risk group. The ANOVA models that were used in the previous chapter were applied here to investigate the main effects and interaction between the environmental risk factor, early institutional deprivation, and the genetic risk factor, GR *Bcl*I genotype, on levels of IOI/ADHD in longitudinally and specifically in mid-adolescence. Following the same format as the previous chapter, the results are presented in two stages. In stage one the data are modelled without controlling for confounding factors and analysed using analysis of variance tests. In stage two an analysis of covariance model was used. The ANCOVA model controlled for the effects of gender and IQ. Within each stage the longitudinal analyses are presented first, followed by cross sectional analyses where appropriate (i.e. when a three way interaction between assessment age,

genotype and e'risk group is found). The data were analysed using the same three-way repeated measures analysis of variance models as in the previous chapter.

8.4.1 Does the GR BclI genotype interact with early deprivation to increase the risk for IOI?

8.4.1.1 IOI and GR BclI genotype effects over time (no covariates): Longitudinal analyses

The first stage of the analysis investigated the main effects and GxE interaction using an ANOVA model without controlling for potentially confounding factors. A three factor repeated measures ANOVA was used to investigate the main effects and interactions over time of GR *Bcl*I genotype and early deprivation (between-subjects factors), with assessment age included as a within-subjects factor. The results are presented in table 8.3 using parent and teacher reports of IOI behaviour from the Rutter Scales at ages 6 and 11 and the SDQ at age 15.

Table 8.3
Main effects and interactions over time between GR *Bcl*I genotype, institutional deprivation and assessment age on IOI *(no covariates)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report	F(1,106)=7.93**	F(1,106)=1.29, p=.26	F(2,212)=.34, p=.71
Teacher report	F(1,78)=19.92***	F(1,78)=.08, p=.78	F(2,156)=2.28, p=.12

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report	F(1,106)=.11, p=.74	F(2,212)=1.46, p=.24	F(2,212)=1.83, p=.16	F(2,212)=.66, p=.52
Teacher report	F(1,78)=.75, p=.39	F(2,156)=6.06**	F(2,156)=3.83*	F(2,156)=1.40, p=.25

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

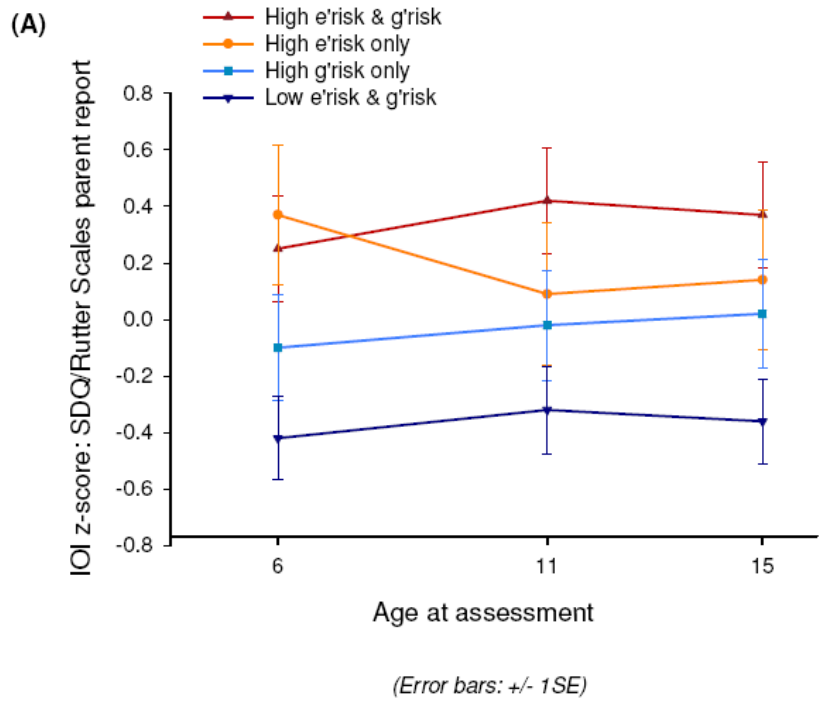
Parent report: Longitudinal analysis of GR BclI, institutional deprivation and IOI outcome over time (no covariates)

The longitudinal analysis of the parent report data on IOI behaviour at ages 6, 11 and 15 years, without controlling for confounding factors, showed there was a significant main effect of environmental risk group on IOI outcome, but no effect of genetic risk group or assessment age (p=.006*; p*=.26*; p*=.71, for the three effects, respectively). This indicated that e'risk groups differed significantly from one another in their level of IOI behaviour over the course of the study period but that average within-group levels did not change significantly over time. G'risk groups did not significantly differ in their level of IOI behaviour over time. With respect to the interaction effects, there was no gene-environment interaction (GxE) between GR *Bcl*I genotype and institutional deprivation (p=.74). That is, there was no indication that GR *Bcl*I genotype moderated the effects of institutional deprivation over time. Figure 8.1 (A), below, displays these effects graphically. The analysis showed there was no three-way interaction between age, genotype and e'risk group (p=.52). Accordingly, no additional cross sectional analyses were conducted. None of the interactions between risk factors and assessment age were significant, indicating there was no differential effect of assessment age between the e'risk or g'risk groups on IOI outcome (see table 8.3).

Teacher reports: Longitudinal analysis of GR BclI, institutional deprivation and IOI outcome over time (no covariates)

The longitudinal analysis using teacher reports of IOI behaviour produced a somewhat similar set of findings to that of the parent reports. There was an overall main effect of e'risk group on IOI behaviour, but not of genetic risk group or assessment age (p<.001*; p*=.78*; p*=.12, for the three effects, respectively). Again there was no GxE interaction between GR *Bcl*I genotype and early deprivation (p=.39). In contrast to parent reported behaviour, the evidence suggested a differential pattern of change in levels of IOI between the e'risk groups over time (p=.003) and also in the levels of IOI between the g'risk groups over time (p=.03). This was indicated by the significant interactions between age and deprivation risk group and between age and genotype group (see figure 8.1

(B), below). However, similar to the parent report data, there was no three-way interaction between age and e'risk and g'risk factors (*p*=.25).



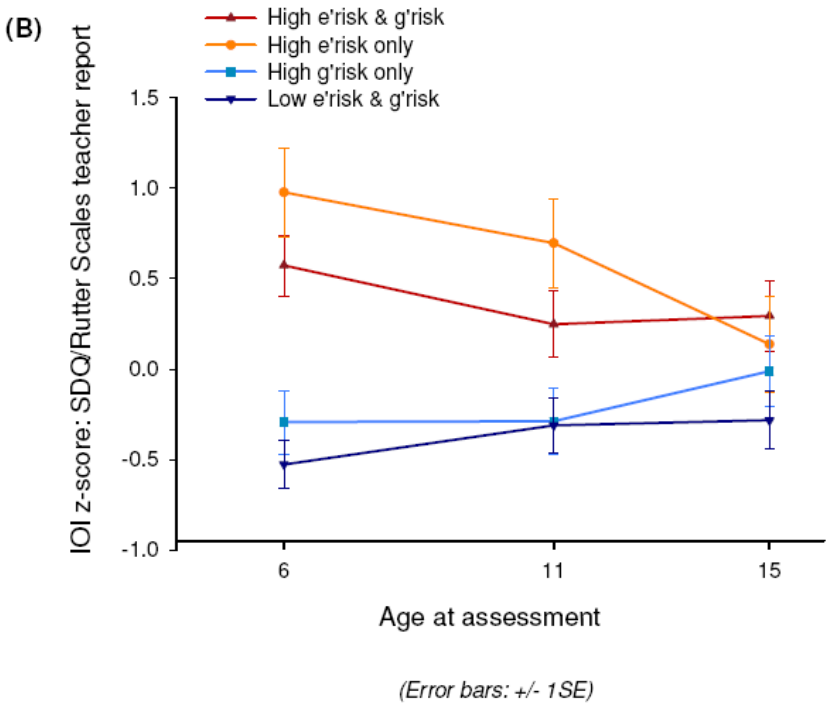


Figure 8.1

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & GR *Bcl*I genotype *(no covariates)*: (A) parent and (B) teacher reports

8.4.1.2 IOI and GR BclI genotype effects over time (controlling of IQ and gender): Longitudinal analyses

As outlined above, the same three-way repeated measures ANOVA model used to examine the effects of institutional deprivation and GR *Bcl*I on IOI over time, with assessment wave as a within-subjects factor. At this stage of the analysis IQ and gender were added as covariates to the model. The results are presented below in table 8.6

Table 8.4

Main effects and interactions over time between GR *Bcl*I genotype, institutional deprivation and assessment age on IOI *(Controlling of IQ and gender)*

	Main effects		
IOI age 6-11-15	Environmental risk	Genetic risk	Assessment age
Parent report ±	F(1,102)=2.06, p=.15	F(1,102)=4.01*	F(2,201)=2.00, p=.14
Teacher report	F(1,76)=8.36**	F(1,76)=.04, p=.84	F(2,156)=1.98, p=.14

	Interactions			
IOI age 6-11-15	G x E	E x age	G x age	G x E x age
Parent report ±	F(1,02)=.35, p=.56	F(2,201)=.28, p=.75	F(2,201)=2.50, p=.09	F(2,201)=.98, p=.38
Teacher report	F(1,76)=2.21, p=.14	F(2,156)=3.99*	F(2,156)=3.97*	F(2,156)=1.52, p=.22

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

Parent report: Longitudinal analysis of GR BclI, institutional deprivation and IOI outcome over time (controlling of IQ and gender)

Unlike in the uncorrected model above, once the effects of IQ and gender were controlled the overall main effect of environmental risk group on parent rated IOI fell short of significance (*p*=.15). However, the main effect of genotype group reached significance, indicating the GR *Bcl*I groups differed from one another in their level of IOI behaviour persistently over time (*p*=.048). The main effects of both the risk factors can be seen below in figure 8.2 (A), which displays the developmental trajectory of effects. Like in the uncorrected model there was no

GxE interaction (p=.56), no main effect of assessment age (p=.14) and no significant three-way interaction between age and risk factors (p=.38).

Teacher report: Longitudinal analysis of GR BclI, institutional deprivation and IOI outcome over time (controlling of IQ and gender)

The addition of IQ and gender as covariates to the repeated measures longitudinal analysis of teacher reported IOI did not change the overall results of the main effects and interactions. There was an overall main effect of environmental risk group (p=.005), indicating that there was a persistent difference between the deprivation groups. Genotype groups did not differ from one another and average e'risk and g'risk group levels did not change significantly over time (p=.84*;* p=.14, for the two effects, respectively). Like in the uncorrected model, there were significant interactions between age and genotype group (p=.02) and between deprivation risk group and age (p=.02), but no three way interaction between factors over time (p=.22). The developmental trajectories of the separate risk groups can be seen below in figure 8.2 (B).

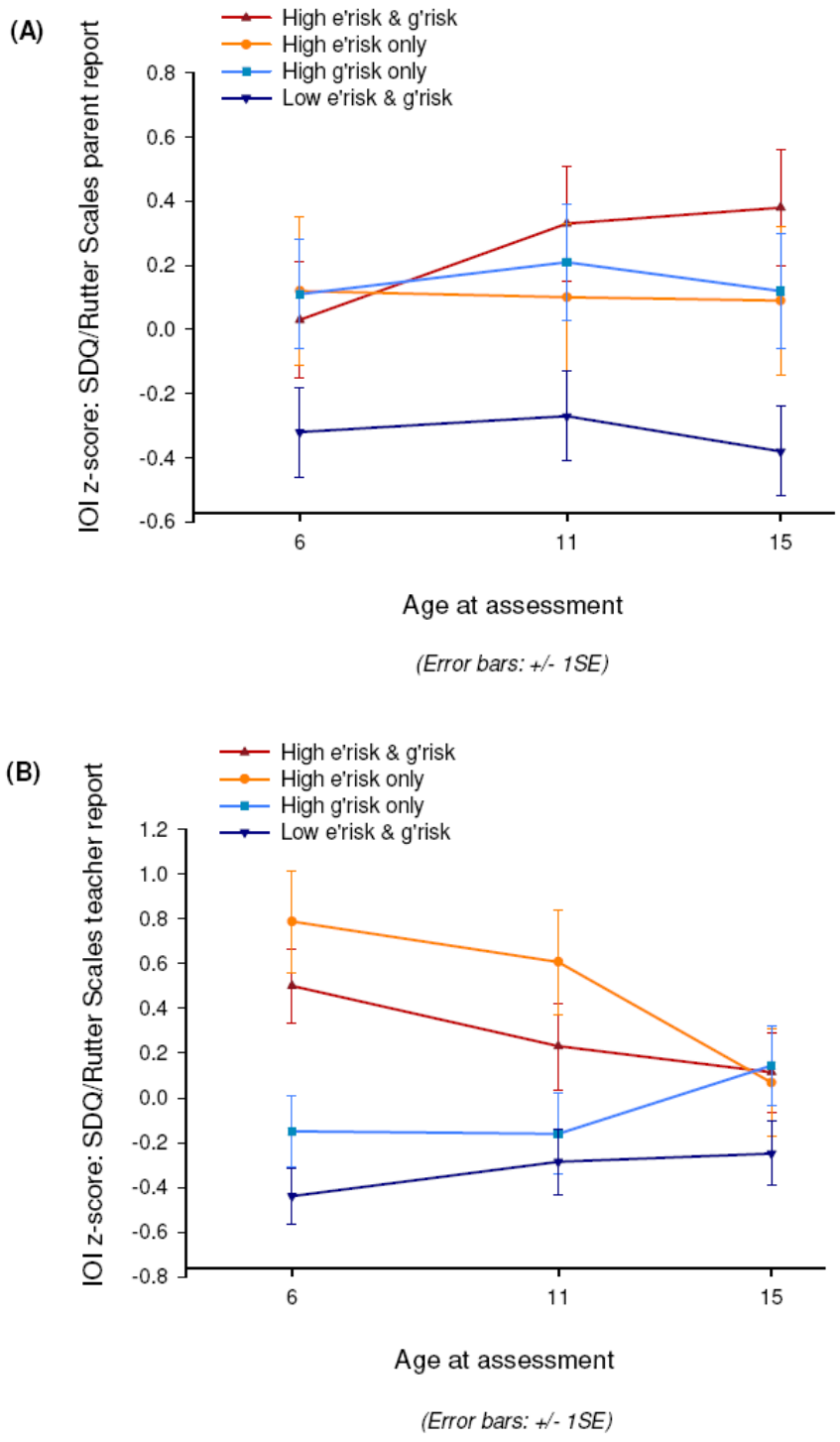


Figure 8.2

IOI at ages 6, 11 and 15 years as a function of early deprivation experience & GR *Bcl*I genotype *(Controlling of IQ and gender)*: (A) parent and (B) teacher reports

8.5. Results section 4: Gene-environment interaction in relation the risk for other associated features

8.5.1 Does GR BclI genotype interact with early deprivation to increase the risk for cognitive impairment (IQ), disinhibited attachment or conduct problems?

The aim of the final section of the current empirical chapter was to explore the association between GR *Bcl*I genotype, institutional deprivation and the various covariates used in the ANOVA models presented above to investigate the risk for IOI. This analysis follows the same format as the one presented in the final part of the previous chapter on the influence of dopamine genes (refer to section 7.4.1 for the rationale and analytical technique). In brief, the same repeated measures ANOVA model used above in relation to IOI was applied here to test for the main effects of institutional deprivation risk group, GR *Bcl*I genotype group and GxE interaction with respect to the risk for cognitive impairment (lowered IQ), disinhibited attachment and conduct problems over time. Institutional deprivation was classified in the same way, i.e. the sample was dichotomously split into high risk (≥ 6 months institutional rearing in Romania) and low risk (U.K., Rom non-IR and Rom IR < 6months) groups. The same high and low risk genotype groups used in the analyses above were also applied here. Outcome scores from age 6, 11 and 15 were entered into the three-way repeated measures ANOVA model, with assessment age as a between subject factor.

Table 8.5

Main effects & interactions over time between GR *Bcl*I genotype, institutional deprivation & assessment age on ERA outcomes *(no covariates)*

	Main effects		
Outcome	Environmental risk	Genetic risk	Assessment age
IQ ±	F(1,109)=20.10***	F(1,109)<.01, p=.98	F(2,181)=.88, p=.40
Disinhibited attachment ±	F(1,113)=22.38***	F(1,113)=.01, p=.92	F(2,207)=1.44, p=.24
Conduct problems *(parent report)* ±	F(1,106)=1.59, p=.21	F(1,106)=2.87, p=.09	F(2,181)=.14, p=84
Conduct problems *(teacher report)*	F(1,78)=3.76, p=.06	F(1,78)=.31, p=.58	F(2,156)=1.10, p=.34

	Interactions			
Outcome	G x E	E x age	G x age	G x E x age
IQ ±	F(1,109)=.29, p=.59	F(2,181)=6.73**	F(2,181)=.28, p=71	F(2,181)=.01, p=.98
Disinhibited attachment ±	F(1,113)=.20, p=.66	F(2,207)=.1.53, p=.22	F(2,207)=.54, p=57	F(2,207)=.52, p=.58
Conduct problems *(parent report)* ±	F(1,106)=.06, p=.81	F(2,181)=.13, p=.85	F(2,181)=1.36, p=.26	F(2,181)=1.11, p=.33
Conduct problems *(teacher report)*	F(1,78)=93, p=.34	F(2,156)=2.52, p=.08	F(2,156)=4.32*	F(2,156)=.57, p=.57

*p<.05; **p<.01; ***p<.001
± Sphericity assumption not met, Huynh-Feldt correction applied

8.5.1.1 IQ (cognitive impairment) and the effects of GR BclI genotype and institutional deprivation over time

The three-way repeated measures ANOVA analysis of IQ at ages 6, 11 and 15 years demonstrated a significant main effect of environmental risk group on cognitive outcome, but no effect of genetic risk group or assessment age (p<.001*;* p=.98*;* p=.40, for the three effects, respectively). This indicated that institutional deprivation risk groups differed significantly from one another in their level of IQ over the course of the study period, but average within-group levels did not change significantly over time. Importantly for the specificity of the findings reported above in relation to IOI, GR *Bcl*I g'risk groups did not differ significantly from one another in their level of IQ. With respect to the interaction effects, the GxE interaction between GR *Bcl*I genotype and institutional deprivation was not significant in relation to level of IQ over time (p=.59). This indicated that GR *Bcl*I genotype did not moderate the effects of institutional deprivation on the risk for cognitive impairment. There was a significant interaction between deprivation risk group and assessment age in relation to IQ (p=.003). This suggested that there

was a different pattern of change in the levels of IQ between the e'risk groups over time. There were no other significant interactions with assessment age (G'risk x age*: p*=.71; GxExAge*: p*=.98).

8.5.1.2 Disinhibited attachment and the effects of GR BclI genotype and institutional deprivation over time

The repeated measures ANOVA model was performed using disinhibited attachment as the outcome measure and produced a similar pattern of results to above. There was an overall significant main effect of institutional deprivation risk group over time, but no effect of GR *Bcl*I genotype group or assessment age (*p*<.001*; p*=.92*; p*=.24, for the three effects, respectively). This suggested that deprivation e'risk groups differed from one another in their level of disinhibited attachment persistently over time. Genotype status did not influence level of disinhibition, with no difference between the groups over time.

The test of GxE interaction with respect to disinhibited attachment was not significant, suggesting that there was no moderation by GR *Bcl*I genotype of the risk associated with extended deprivation for disinhibited attachment. None of the interactions between risk factors and assessment age were significant, indicating there was no differential effect of assessment age between the e'risk or g'risk groups on disinhibited attachment outcome (all n/s: see table 8.10).

8.5.1.3 Conduct problems and the effects of GR BclI genotype and institutional deprivation over time

The same three way repeated measure ANOVA model was then used to test for the main effects and interactions between institutional deprivation and GR *Bcl*I genotype in relation to the risk for conduct problems.

Parent report: Longitudinal analysis of GR BclI genotype, institutional deprivation and conduct problems over time

The repeated measures analysis with parent reported conduct problems over time as the dependent variable yielded no significant results. There was no main effect of deprivation group, GR *Bcl*I genotype group or assessment age on the overall level of conduct problems (*p*=21*; p*=.09*; p*=.84, for the three effects,

respectively). There was no detectable GxE interaction (*p*=81) and no interactions between assessment age and the risk factors (all n/s).

Teacher report: Longitudinal analysis of GR BclI genotype, institutional deprivation and conduct problems over time

The pattern of effects using teacher reported conduct behaviour showed some subtle differences in effects. The main effect of e'risk group approached significance (*p*=.06) suggesting that the deprivation groups may have differed in their levels of teacher rated conduct problems over time. There was still no main effect of genotype group or assessment age (*p*=58*; p*=.34, for the two effects, respectively). Again, there was no GxE interaction between GR *Bcl*I genotype and early deprivation (*p*=.34). The analysis of the interactions between risk factors and age revealed that there was a differential pattern of change between the g'risk groups over time (*p*=.02). The other interactions did not reach significance (E'risk x age*: p*=.08; GxExAge*: p*=.57).

8.6 Results chapter summary

The analysis of the GR gene produced a different pattern of results from those reported in the previous chapter in relation to the dopamine genotypes. In summary, there were five main findings:

First, the analyses suggested there were significant gene-environment correlations between the GR genotypes/haplotypes and institutional deprivation. That is, the genotype frequencies in the group of children who resided longer in the institutions were significantly different from that observed in the low e'risk group. Moreover, the high e'risk group demonstrated a different distribution of alleles from that reported in the general population. There appeared to be an under representation of the GR *Bcl*I G allele and a slight over representation of the 9beta G allele in the high e'risk group. This was not confounded by the different ethnic makeup of the low versus high e'risk groups.

Second, the analysis of the risk for IOI from the separate GR haplotypes suggested that GR *Bcl*I G allele may be associated with a decreased risk for IOI. Accordingly, the *Bcl*I SNP was isolated for further investigation of the

hypothesised interaction with institutional deprivation in relation to the development of IOI symptoms.

Third, the analysis of the interplay between GR *Bcl*I and early deprivation indicated that there was no interaction between the risk factors. Therefore GR *Bcl*I genotype did not moderate the risk for IOI following early institutional deprivation.

Fourth, the main effect of institutional deprivation on IOI impairment was apparent throughout the majority of the analyses. That is, those children who resided in the institutions for at least 6 months were persistently rated by parents and teachers as having elevated levels of IOI over the whole study period. With respect to the analyses of parent reported IOI, in the model that included covariates the variation between the risk groups was reduced to such a degree that the main effect of deprivation fell short of significance.

Fifth, the analysis of effects in relation to other cognitive and behavioural outcomes showed that GR *Bcl*I genotype did not influence the risk for cognitive impairment, conduct problems or disinhibited attachment following early institutional deprivation either as a direct main effect or via an interaction with the environmental pathogen.

CHAPTER 9: DISCUSSION

9.1 Chapter outline

Longitudinal studies of severe early institutional deprivation provide an important naturalistic opportunity to investigate the effects of early adverse experience on later development. The present study investigated the persistence and presentation from childhood into mid-adolescence of a specific sequela associated with early deprivation - inattention/overactivity/impulsivity. Once the pattern of risk associated with deprivation was established, the study then set out to examine the potential role that genetic factors play in moderating the risk for IOI following early deprivation. This was tested using two strategies for selecting candidate genes derived from two models of the mechanistic pathway from risk to IOI disorder. The first 'phenotype' model looked at the role of dopamine genes, previously found to be associated with ADHD in the general population, as moderators of early deprivation risk. The second 'process' model examined whether the glucocorticoid receptor gene, which has a regulatory role within the stress response system, interacted with the stressful early deprivation experience to increase the risk for the development of IOI.

This chapter first reviews the study's findings in relation to the research questions set out at the start of the thesis. Second, it provides an interpretation of the results in the context of the candidate gene selection strategies, the hypothesised mechanisms and wider research issues in order to provide an understanding of the moderation of the effects of early deprivation by individual genetic makeup. Third, the strengths and limitations of the current study are discussed and finally future research possibilities are explored.

9.2 Empirical findings 1: IOI as an outcome of early deprivation

The first set of empirical analyses (chapter 6) set out to: i) investigate the developmental and aetiological pathways between the environmental pathogen, early institutional deprivation, and IOI in the ERA sample; and ii) to examine the associated features of the deprivation-related IOI phenotype. The results of these analyses helped to identify a number of important characteristics of IOI as an

outcome of early institutional deprivation. These are set out against the research questions used to investigation this association.

9.2.1 Does the risk for IOI associated with severe early institutional deprivation persist to age 15 years?

The key finding was that the experience of institutional deprivation had a significant and persistent influence on levels of IOI from childhood to mid-adolescence. The subsample of children who experienced institutional rearing in Romania had, on average, consistently elevated IOI scores over time and across home and school settings compared with U.K. and non institution-reared Romanian children. Specifically, the age 15 results showed that the effect of deprivation held across different measures of IOI/ADHD, informants and analysis methods. That is, the institution-reared children had significantly higher scores when assessed using the questionnaire measure of IOI (SDQ) completed by parents and teachers and also the parental interview measure of ADHD symptoms (CAPA). In contrast, the IOI scores of the U.K. children could not be differentiated from those of the non institution-reared Romanians.

9.2.2 What effect does duration of deprivation have on IOI?

The analysis of the effect of duration of deprivation demonstrated that there was a marked increase in the risk for elevated levels of IOI at around the 6 months of deprivation point. This step-increase in risk showed a pervasive, persistent and relatively stable pattern of effects from childhood to mid-adolescence. The children who experienced at least 6 months deprivation were rated as having a marked and significant elevation of IOI scores, but there was no further linear association beyond the 6 month threshold between duration of deprivation and levels of IOI. Accordingly, all institution-reared children adopted at 6 months or older were grouped together to form a high e'risk subsample. In contrast, there were no detectable differences in the level of IOI between the groups of children who experienced less than 6 months or no institutional rearing in Romania or were adopted within the U.K. On those grounds, they were grouped together as a low e'risk subsample. These between-group differences were obtained longitudinally, cross sectionally, across informants and across different measurement devices. Moreover, these results corroborate the ERA findings reported in the Stevens et al. (2008) paper and provide further support for a

threshold model of early risk, in which the risk for lasting deficits is substantially increased if early adverse events occur within a critical developmental window (Bruer, 2001). The findings also fit within the framework of developmental programming theory, which proposes that a mechanism for intra-organismic change may be through some form of developmental programming during critical periods of early development (Rutter & O'Connor, 2004), discussed in more detail in sections 9.3.1 and 9.7.1, below.

9.2.3 Are the rates of deprivation-related IOI/ADHD found in the adolescent Romanian high e'risk sample clinically significant?

The elevated levels of IOI in the high e'risk sample, reported above, translated into clinically significant rates of IOI/ADHD impairment, which were persistent across settings, assessment ages, gender and different assessment tools. The current study utilised the comprehensive data gathered from the CAPA interview and applied criteria for a research diagnosis of ADHD. This enabled an important validation of the longitudinal analyses using the questionnaire measures by showing that rates of IOI impairment were similarly elevated across a different measurement tool that required more stringent cut-off criteria. The rates of deprivation-related IOI/ADHD were around three to four times the rate observed in the within-sample low e'risk group and around two times the rates reported in a large representative population sample (*http://www.sdqinfo.com/bb1.html*; Meltzer et al., 2000).

9.2.4 Is there individual continuity in IOI behaviour over time?

In general, the findings supported individual developmental continuity in deprivation-related IOI within the ERA sample. The level of individual persistence in deprivation-related IOI was illustrated by high correlations in behaviour from age 6 to age 11 and again from age 11 to age 15, according to both parents and teachers. The findings from the categorical analysis using a cut-off to distinguish normality from abnormality/impairment were less clear cut. High levels of IOI only moderately predicted later ratings of abnormality and teachers in particular reported an inconsistent pattern of impairment. However, there are several caveats to this analysis that warrant discussion. First, there was a substantial

amount of missing data in the categorical analysis, as only cases that had data from all three assessment waves could be included. This was particularly apparent with the model of teacher reports where over 40% of the cases were not included in the analysis because one or more of their data-points were missing. This suggests that the categorical analysis of teacher reports should be interpreted with caution.

Second, unlike with parent reports, different teachers completed the questionnaires on children's IOI behaviour at each time point. It may be that some teachers knew the participants better than others and so were able to provide more accurate reports. At the age 11 assessment the questionnaires were completed by each child's primary school main class teacher, i.e. before they moved to secondary education. Whereas, at age 15 students are taught by several teachers and we had less control over how well and for how long the informant teacher knew the ERA study participant. Third, cut-off analyses are not good at capturing the level of impairment of the cases that hover around the designated threshold. For example, some cases may be above the cut-off at a particular assessment wave but fall just below at others. These cases would still show persistently elevated levels compared with the mean but would not be picked up consistently as such in a dichotomous categorical analysis, like the one used in the current investigation. For a discussion of the predictive validity of categorically measured ADHD see Fergusson and Horwood (1995).

9.2.5 Is deprivation-related IOI similar to IOI/ADHD as seen in the general population?

This question was addressed by taking several features typically associated with the ADHD phenotype in the general population and looking at their association with deprivation-related IOI in the ERA high e'risk sample (i.e. the Romanian children who experienced at least 6 months institutional rearing). The aim of this question and the following two questions on ADHD subtypes and disinhibited attachment was to characterize the deprivation-related phenotype by examining distinctiveness and commonality in relation to the phenotype seen in the general nondeprived population. Four key features were examined: the developmental link and comorbidity with conduct problems; the association with lowered IQ; the association with executive dysfunction; and the pattern of gender discrepancy.

9.2.5.1 IOI and the developmental link and overlap with conduct problems

The findings suggested that there were high contemporaneous correlations between conduct problems and IOI (assessed using the questionnaire measures) that held over time, across settings and were particularly apparent from early adolescence onwards. Moreover, there was a strong association in mid-adolescence between a research diagnosis of ADHD (derived from the CAPA interview) and elevated conduct problems scores according to both parents and teachers (assessed through the SDQ). This demonstrated that the overlap between domains could be observed across IOI/ADHD assessment tools. This high level of overlap is consistent with the common pattern on comorbidity reported in the literature on ADHD and conduct problems in the general population (Willcutt et al., 1999). Also in line with the literature on nondeprivation-related IOI/ADHD was the evidence for a developmental pathway from IOI to later conduct problems within the ERA sample (Taylor et al., 1996; Burke et al., 2005). The analysis demonstrated that there was a contribution to variation in conduct problems from earlier IOI behaviour in the high e'risk subsample, but that conduct problems did not predict IOI.

9.2.5.2 IOI and the association with lowered IQ

There was no evidence of an association between deprivation-related IOI and IQ in mid-adolescence. This was in line with the age 11 findings reported in Stevens et al. (2008) but was in contrast to the lowered IQ scores reported in the literature on the relationship between IQ and nondeprivation-related IOI (Frazier et al., 2004). However, the nonsignificant association between the IOI and IQ reported in the current thesis may not accurately represent the nature of the effects. One possible explanation is that the effect of duration of deprivation on IQ may be overshadowing any relationship between IOI and IQ. That is, the high e'risk sample (≥6 months deprivation) used in the analysis had depressed IQ scores overall and owing to this strong association between institutional deprivation and IQ (Beckett et al., 2006) it is difficult to disentangle the specific relationship with IOI. Future investigations of the overlap may consider looking at the association between IQ and IOI in a restricted sample of children with IQs in the normal range, e.g. those with an IQ score of 70 or greater on the WISC.

9.2.5.3 IOI and the association with executive dysfunction

The association between IOI and executive dysfunction in mid-adolescence approached, but fell just short of, statistical significance. At age 15 executive functioning was assessed using just one test that tapped working memory performance, the backwards digit span task on the WISC III$^{U.K.}$ (Wechsler, 1992). Poorer digit span scores were weakly correlated with elevated parent rating of IOI behaviour and were displayed by children who received a research diagnosis of ADHD. However, the statistical tests did not reach significance and the findings were not reflected in teacher rated IOI. The age 15 findings were broadly in line with those from the age 11 assessment wave (Stevens et al., 2008). At age 11 the assessment of executive functioning was based on the backwards digit span task and an additional test of interference control (Stroop Color-Word Interference Test; Stroop, 1935). In early adolescence both tests, and in particular the Stroop test, demonstrated a stronger association with IOI. This gave the first suggestion that deprivation-related IOI may bear the same hallmark of deficits in this domain that characterises ADHD in the wider nondeprived population. However, as noted in the 2008 paper, one must be cautious about over interpreting the finding of deficient executive functioning based only on two tests at age 11 and particularly as the age 15 test of the association with working memory performance fell short of statistical significance. Nonetheless, the results indicate that further research in this area with a more comprehensive battery of neuropsychological tests is necessary to contrast and compare deprivation-related and nondeprivation-related IOI.

9.2.5.4 IOI and gender discrepancy/prevalence amongst males

A discrepancy in prevalence rates between the sexes emerged in relation to the rates of IOI in early adolescence and persisted to age 15. By the age 11 assessment wave, IOI impairment was more common in boys than it was in girls, as is seen generally in nondeprivation-related IOI/ADHD samples (Biederman et al., 2002; Heptinstall & Taylor, 2002). The discrepancy in adolescent prevalence rates in the high e'risk sample, for IOI impairment rated on the Rutter Scales and the SDQ, approached the ratio observed in epidemiological studies (Youth in Mind, 2001). Moreover, at age 15 the availability of prevalence rates of ADHD

from the CAPA interview was particularly informative as it incorporated a more comprehensive assessment of impairment and corroborated the longitudinal findings from the questionnaire measures. The proportion of boys to girls with a research diagnosis of ADHD mirrored the rates seen in population samples (Heptinstall & Taylor, 2002). This was a different picture from that seen at age 6 where there were roughly equal numbers of boys to girls classified with deprivation-related IOI impairment. This shift in the ratio between the sexes may reflect a developmental process by which the general risk factors for IOI, other than those specifically related to early deprivation, come into play as one moves further away from the institutional experience.

9.2.6 Is the deprivation-related phenotype characterized by particular underlying ADHD subtype symptoms?

The DSM-IV-TR diagnostic criteria specify a subtype based on inattentive symptoms and a separate overactivity/impulsivity subtype. According to observed levels of ADHD symptomatology in the ERA sample, assessed using the CAPA interview, the association with extended institutional deprivation did not seem to be driven by the symptoms from one particular diagnostic subtype. A significant association between duration of deprivation and outcome was found for both inattentive symptoms and for overactive/impulsive symptoms.

9.2.7 Is there overlap between IOI and disinhibited attachment in mid-adolescence?

The primary finding in the analysis of deprivation-related IOI and disinhibited attachment in mid-adolescence was the substantial level of overlap between the two domains of impairment, as has been identified in other studies of institution-reared children (Roy et al., 2004). This was demonstrated by the significantly higher level of disinhibited attachment observed for cases with a research diagnosis of ADHD and corroborated by the significant correlation with elevated IOI scores as rated by both parents and teachers on the SDQ. Moreover, the two domains were found to be dissociable constructs and the association was not accounted for by as shared association with duration of deprivation. The pattern of concurrent overlap was observed longitudinally with significant correlations

between the domains at both the age 6 and age 11 assessment waves. Interestingly, the exploratory analysis of developmental pathways between the domains indicated that earlier IOI contributed to later disinhibited attachment behaviour in the ERA high e'risk sample. Whereas, earlier disinhibited attachment did not seem to predict later variation in IOI.

9.3 Interpretation of IOI phenotype findings

There were two main aims with respect to the current thesis' empirical study of the characteristics of the deprivation-related IOI phenotype in the ERA study: First, to examine the persistence of IOI impairment into adolescence; second, to investigate the presentation of the phenotype in terms of its associated features. The results help to identify several key features of IOI as an outcome of early severe institutional deprivation. [4]

9.3.1 Persistence and characterisation of the risk associated with early deprivation for IOI

There are two key findings in this regard: First, IOI following prolonged early deprivation persisted into mid-adolescence and second, the persistence of IOI can be characterised by a step-increase in the risk associated with institutional deprivation at around the 6 months deprivation point.

The first finding of persistence to age 15 of IOI as specific sequela of early deprivation was evident on a group level, demonstrated by the substantial differences between the adoptee groups, and on an individual level, indicated by the high correlation between assessment waves over time. However, IOI impairment at a previous assessment wave only moderately predicted later ratings of abnormality in this domain. In general, the findings supported developmental continuity in impairment and reinforce the published report of the age 11 results and the mechanisms suggested therein (Stevens et al., 2008). That is, this level of persistence, despite the radical change in social environment following adoption, makes it highly unlikely that the effects are the result of an initial behavioural reaction to the poor conditions of the early institutional rearing

[4] A discussion of the age 11 findings has been reported in Stevens et al. (2008). The current discussion extends this account into mid-adolescence and links it with the GxE investigation.

environment; the influence of which one would expect to decrease with duration of time spent in 'good' environments. Rather, this is perhaps suggestive of some form of intra-organismic or fundamental neurobiological alteration. Rutter & O'Connor (2004) hypothesised that persistent problems, such as IOI, following exposure to early severe adverse events, were the result of experience-adaptive biological programming; whereby the brain adapts to certain experiences during a critical period to optimise the specific conditions of that environment. This lends itself to the proposition that an alternative neurodevelopmental pathway is initiated during an early critical period that is adapted to the stressful rearing environment (Teicher et al., 2003). This model may hold some relevance for the persistent adverse effects presented above, when exposure to extreme deprivation is viewed as a stressful experience.

Animal models support the existence of long lasting effects of early stress on brain development and on later psychological and behavioural functioning. This includes altered structure and function (e.g., HPA axis and associated brain structures) and effects on neurochemical and developmental processes such as neurogenesis, synaptic overproduction and pruning and myelination (McEwan, 1999; Teicher et al., 2003). One such model suggests that antenatal exposure to glucocorticoids (due to maternal stress or administration of a synthetic analogue during pregnancy) has long term effects on the HPA axis development and functioning of offspring and impacts on later locomotor activity in animals and ADHD-type behaviours in humans (Kapoor et al., 2008).

Recent MRI work on a subsample of ERA participants is consistent with this model (Mehta et al., 2008). Future research is needed to focus on the role of stress reactivity in humans following early deprivation in developmental outcomes such as IOI. Moreover, these propositions fit into the mechanistic framework being indirectly tested in the sections of the current thesis on GxE interactions in relation to the risk for IOI in the ERA sample. That is, whether the hypothesised neurobiological alterations are influenced by individual genetic makeup and the possession of specific risk alleles that may be associated with increased susceptibility to the adverse effects of early institutional deprivation.

The second main finding was that the persistence of the dose-response relationship between early deprivation and IOI can be characterized by a marked step-increase in the risk for elevated levels of IOI at around the 6 months of

deprivation point. This pattern was particularly apparent from early adolescence onwards and is again consistent with accounts in which early adverse events need to occur within a critical developmental window for negative outcomes to follow (Bruer, 2001). Due to the inevitable confound between age and duration of deprivation in the ERA study, these models cannot be tested definitively using the current data. That is, it is not possible to isolate a group of children who experienced less than 6 months deprivation that wasn't in the first 6 months of life (i.e. no deprivation from age 6 – 12 months, but exposed to deprivation before and after).

However, the finding of a step-increase at around the 6 month point does help to disentangle whether institutional rearing may in fact be a marker for some underlying genetic predisposition or prenatal risk for problem behaviours, such as IOI, because, if such processes were operating within the ERA sample, one would expect the adverse effects to be seen across institution-reared groups. Parental ADHD and prenatal risk factors, such as low birth weight, maternal smoking or alcohol use during pregnancy or premature birth, are reported to be associated with ADHD in the general population (Taylor & Sonuga-Barke, 2008). It is possible that such factors may have had some impact on the elevated levels and rates of IOI found in our sample. However, if these factors were driving the association between institutional deprivation and IOI then the increased risk for IOI should be spread across the adoptee age groups, and not just for those who experienced over 6 months deprivation.

A further possibility is that dose of deprivation was acting as a marker for genetic or prenatal risk. If this were the case then one would predict that those children who experienced an extended period of deprivation would have greater genetic liability or prenatal adversity than earlier adopted children. Although prenatal risk processes cannot be definitively tested in the current study, the investigation of the influence of genetic factors was tested using specific genetic polymorphisms. There was no evidence to suggest greater genetic liability from the analysis of the gene-environment correlation between dopamine transporter or receptor genotypes and exposure to deprivation. There was a surprising correlation between glucocorticoid receptor genotypes and deprivation risk group. However, the meaning of the correlation is unclear in relation to genetic liability from this specific gene and the finding should, therefore, be treated with caution. The finding is discussed in more detail below in sections 9.5.1 and 9.7.3.

One reason why it is unlikely that those children who were adopted at an older age would be differentially affected by genetic and/or prenatal risk factors is that the ERA children could not be adopted out of the institutions until the fall of the Ceauşescu regime. Therefore, it is unlikely that the children who resided longer in the institutions were those who had not been chosen for adoption sooner, possibly due to developmental or behavioural problems (which may have been influenced by genetic predisposition or prenatal adversity).

There are also several other potentially confounding factors that warrant mention here but were unfortunately outside the scope of the current thesis to deal with empirically. These have been addressed in other papers by the ERA study. Factors such as differences in quality of care between individual children and between institutions (Castle et al., 1999), physical health status (Beckett et al., 2003) and the adoptive family rearing environment may all potentially have had some impact on persistence and prevalence of IOI impairment. However, it is worth noting that the quality of care in the institutions ranged from poor to abysmal and that the postadoption rearing environments have not been found to mediate the impact of institutional deprivation on other areas of impairment, although this may be due to a lack of variation within the sample of adoptive parents (Colvert et al., 2008; Kreppner et al., 2007). The adoptive families were generally middle-class, were slightly better educated than the general U.K. population and there was little variation between them on the measures we had available.

9.3.2 Presentation of deprivation-related IOI

The presentation of concurrently and developmentally associated features of deprivation-related IOI in adolescence displayed key commonalities with the nondeprivation related phenotype, but also some important distinguishing characteristics. These are summarised in detail above in relation to the separate research questions. Overall, there were several key characteristics identified.

First, deprivation-related IOI showed a similar presentation to the nondeprivation-related phenotype in terms of its concurrent and developmental association with conduct problems and the male preponderance in the rates of impairment in adolescence. Albeit weak, there was some indication that there

was a shared underlying pathophysiology from the evidence suggesting executive function deficits may play a role. Moreover, the evidence in relation to IQ was inconclusive owing to the possibly overriding association between the duration of deprivation and cognitive functioning. Symptoms from both the ADHD subtypes were found to be associated with duration of deprivation, which provides the first evidence to suggest that effects were not being driven by a particular symptom phenotype. Taken overall, these findings suggested that there were sufficient similarities between deprivation and nondeprivation-related IOI to justify looking at the putative genetic mechanisms implicated in the aetiology of ADHD in the general population for candidate genes to apply to the current study of GxE interaction in the ERA study.

Second, the findings also demonstrated several distinctive features of the deprivation-related IOI. A differential developmental trajectory of sex differences was found. There were roughly equal numbers of boys to girls with IOI in childhood but a gender discrepancy, which resembled that seen in nondeprived samples, emerged in early adolescence with substantially more boys than girls rated as having abnormal levels of IOI/ADHD. This sex difference persisted to age 15 and was observed across measures, suggesting that the effects reported on the age 11 findings were not transient and instead indicated a stable developmental trend. Examination of the gender discrepancy at a further follow-up would be desirable in order to corroborate this claim.

The association with disinhibited attachment represents another key feature where deprivation-related IOI may differ from the presentation in the wider nondeprived population. This overlap has been reported in relation to other institution-reared samples but, unfortunately, there is insufficient evidence from nondeprived samples to assess whether disinhibited attachment of the sort displayed by the deprived children in the current sample might also be present as an important clinical feature in at least a subsample of ADHD cases. Alternatively, it is possible that disinhibited attachment may form part of a deprivation-specific syndrome alongside other distinctive domains of impairment that have been observed, such as quasi autistic features. If so, then the overlap presented in the current thesis may indicate that IOI behaviours represent a feature of this syndrome. The findings from the exploratory analysis on whether the domains represented dissociable constructs, conducted in the current thesis and the paper on the age 11 findings (Stevens et al., 2008), do not seem to support this idea.

Further examination of IOI/ADHD subtypes and the overlap with specific symptoms may help to elucidate this issue.

9.4 Empirical findings 2: Do dopamine genes moderate the effects of institutional deprivation on the risk for IOI?

The results of the analyses in chapter 7 are set out below against the research questions and discussed in relation to the original path marker and moderator models proposed in chapter 3 of the introduction. It is important to acknowledge at this point in the discussion the risks associated with multiple testing strategies, such as those carried out in the current study, in terms of capitalising on chance results. By conducting analytical tests under several covariate conditions, genotypes, informants and outcomes there was an increased risk for the detection of false positive results. However, one-off positive results were treated with the utmost caution with more confidence being placed in significant results that were reflected across linked genotypes and covariate models.

9.4.1 Are there gene-environment correlations (rGE) between DAT1 or DRD4 genotypes and institutional deprivation?

This question aimed to test the path marker rGE model proposed in the introductory chapter (heading 3.4.2). This model related to the hypothesis that individual differences in levels of IOI, especially those related to the duration of deprivation, come about because those children who experienced longer periods of deprivation have greater genetic liability than earlier adopted children, i.e. is dose of deprivation associated with genetic risk? Do later adopted children also carry more 'risk genes'? There was no reason to predict that this was the operative mechanism, but it was important to test for it in order to rule such processes out of the causal pathways to disorder. The analyses showed there was no difference in the frequency of cases with low and high DAT1 genotypes/haplotype between the two environmental risk groups and therefore did not support the path marker rGE model of effects. That is, there was an absence of rGE across the DAT1 40-bp (3'UTR) and DAT1 30-bp (intron 8) genotypes and the combined DAT1 10R-6R haplotype in relation to exposure to extended institutional deprivation. Similarly, the analysis of the DRD4 (exon III)

genotype showed no evidence in support of the path marker rGE model of effects. There was no detectable difference between the institutional deprivation risk groups in the frequency of cases with the low risk vs. high risk genotype. In summary, the children who resided longer in the institutions did not have a greater liability, in terms of the specific DRD4 or DAT1 genotypes, than the low environmental risk group, i.e. extended deprivation exposure was not a 'marker' for underlying genetic liability.

9.4.2 Does DAT1 genotype/haplotype interact with early deprivation to increase the risk for IOI?

The key overall finding from the chapter as a whole was the compelling evidence indicating the presence of a synergistic interaction in relation to the DAT1 gene and institutional deprivation, which provided support for the phenotype-based hypothesis of GxE effects. The research question above sought to test the moderation model put forward in chapter 3 of the introduction using the phenotype-based hypothesis of effects (heading 3.4.3). That is, the two contrasting queries were: i) whether there was a simple additive co-action of genetic factors (associated with the ADHD phenotype) and environmental risk factors on the risk for deprivation-related IOI; or ii) whether there was a synergistic interaction between factors, whereby one factors alters the impact of another on outcome.

The analyses demonstrated seven main findings in relation to the interplay between the DAT1 gene and early institutional deprivation: First, the adverse effects of institutional deprivation on the risk for IOI were significantly moderated by the presence or absence of specific DAT1 genotypes/haplotypes.

Second, two findings suggested the interplay between genetic and environmental factors operated by way of a synergistic GxE interaction rather than an additive G+E effect: i) the presence of a statistical interaction effect in the analysis of variance tests; ii) the substantially larger effect size estimates in the high e'risk group compared with the low e'risk group.

Third, the genetic risk effect was in the expected direction from the predicted risk alleles: 10R allele of the 40-bp (3'UTR) polymorphism; 6R allele of the 30-bp (intron 8) polymorphism; and the combined 10R-6R haplotype.

Fourth, there was a parallel pattern of GxE interaction effects across the DAT1 genotypes (40-bp 3'UTR and 30-bp intron 8) and the combined DAT1 10R-6R haplotype. The effects seemed to be stronger in relation to the DAT1 40-bp (3'UTR) genotype than the 30-bp (intron 8) genotype, but by combining the two in the DAT1 haplotype the manifest GxE interaction became more clear cut.

Fifth, the GxE interaction was found in relation to parental reports of IOI/ADHD symptoms but not from teacher reports. Although speculative, one explanation for why the effects are seen for parents' reports but not teachers' is that: i) there were different teachers ratings the children's behaviour at each assessment wave; and ii) the teachers were less likely to be involved on a one-to-one basis with the young people as they reached mid- adolescence (when the interaction effect can be seen most clearly using the data from parent reports of IOI).

Sixth, the longitudinal and cross sectional findings provided evidence that the GxE interaction appeared to get stronger over time but can also be detected as an overall effect over the whole study period.

Seventh, in order to control for the effects of IQ and gender, GxE interaction analyses were also conducted with these two factors as covariates. Two factors that showed considerable overlap with IOI, disinhibited attachment and conduct problems, were not added as covariates owing to substantial overlap with IOI and associated colinearity problems. The findings indicated that by controlling for the variance in IOI attributable to IQ and gender the risk for elevated outcome scores associated with the interaction between extended institutional deprivation and dopamine genotype could be seen more clearly. Reassuringly, the initial 'uncorrected' analyses showed a similar pattern of results.

9.4.3 Does DRD4 (exon III) genotype interact with early deprivation to increase the risk for IOI?

The key message from this analysis was a negative finding in terms of the presence of a GxE interaction. The analysis demonstrated that DRD4 genotype did not moderate the risk associated with early deprivation for the development of IOI. This was found longitudinally, cross sectionally and across informants and

measurement tools. There was no evidence to suggest a main effect of the DRD4 7-repeat allele on the risk for IOI, thus no support was found for the additive model of G-E effects, either. Moreover, there was no support for the finding in the literature on the direct association between this allele and ADHD in the general population.

9.4.4 Does DAT1 or DRD4 genotype/haplotype interact with early deprivation to increase the risk for other cognitive and behavioural outcomes?

It was important to test whether the effects of DAT1 on IOI were specific to that outcome or whether there was a general effect on a range of outcome associated with deprivation or with IOI. Moreover, the question addressed the validity of including these outcomes as covariates in the models testing for the presence of DAT1 and DRD4 GxE interactions with early deprivation. The key finding from this analysis was that there was no moderation by the DAT1 genotypes/haplotype of the risk for cognitive impairment, disinhibited attachment or conduct problems following early institutional deprivation. The results of the analysis provided support for the specificity of DAT1 effects in relation to the risk for IOI and thus the internal validity of the effect with the specific phenotype being considered.

The DRD4 polymorphism did not demonstrate any significant effects with IOI, but a parallel analysis of whether this genotype was associated with other outcomes was still conducted. The results produced a similar set of findings to those above. DRD4 did not significantly moderate the environmental risk associated with early deprivation for any of the three alternative cognitive or behavioural outcomes, although there was a nonsignificant preliminary indication that this genotype may hold some relevance for the risk for disinhibited attachment.

9.5 Does the glucocorticoid receptor gene moderate the effects of institutional deprivation on the risk for IOI?

The findings reported in chapter 8 of the current thesis are set out below against the relevant research questions. The analyses aimed to test the same path marker and moderator models examined in the chapter on the effects of

dopamine genes but were applied in this instance to the glucocorticoid receptor gene.

9.5.1 Are there gene-environment correlations (rGE) between glucocorticoid receptor (GR) genotypes/haplotype and institutional deprivation?

The findings on the GR genotypes/haplotype provided evidence in that seemed to support the path marker rGE model of effects. This was in contrast to the results reported above in relation to the dopamine genotypes and in contrast to the predicted absence of significant rGE in the ERA study. It appeared that GR genotype was associated with deprivation risk group. There was evidence of an association across the separate GR SNPs (9beta *p*-value suggestive, not significant) and the combined haplotype. This indicated the presence of a gene-environment correlation and suggested that exposure to extended institutional deprivation could possibly be a marker for an underlying genetic liability with respect to these genotype and developmental outcome. The high e'risk group, who had experienced extended deprivation, had a significantly different distribution of GR alleles from the low risk group and from the distribution observed in the wider population. There was an underrepresentation of the GR *Bcl*I G allele and a slight overrepresentation of the GR 9beta G allele in the high e'risk sample. These two SNPs have been associated with alterations in glucocorticoid sensitivity in different ways. The *Bcl*I G allele has been associated with hypersensitivity to glucocorticoids (van Rossum et al., 2003), whereas the 9beta G allele confers relative resistance to glucocorticoids (Kumsta et al., 2007).

9.5.2 Is Glucocorticoid receptor haplotype associated with IOI in the GenERA sample as a whole? If so, which genotype(s) confer risk?

The analysis used to address this question isolated the GR *Bcl*I SNP for the subsequent investigation of the GxE interaction model used to test the hypothesis that a specific GR genotype moderated the risk for IOI from early institutional deprivation. This was based on the significantly lower IOI scores associated with G allele of this SNP, which suggested that this allele may confer some protective influence over the risk for IOI in the sample and it would therefore make sense to investigate the *Bcl*I SNP genotype separately. However, given the significant rGE

findings reported above any GxE interaction or G+E additive effects that are detected need to be interpreted with caution.

9.5.3 Does the GR BclI genotype interact with early deprivation to increase the risk for IOI?

The main finding from the analyses in this section relates to a non-significant GxE interaction finding. That is, using GR *Bcl*I genotypes to test for moderation of the risk for IOI from early deprivation provided no support for the synergistic GxE interaction model of effects, put forward in section 3.4.3.2 of the introduction. This is in contrast to the positive results reported above with respect to the DAT1 gene.

9.6 Interpretation of findings on the role of genetic factors on the risk for IOI

The current PhD investigation has provided the first evidence of the power of genes to alter the expression of the risk effects associated with severe early institutional deprivation on outcome. The DAT1 gene moderated the impact of extended deprivation on the risk for IOI by heightening its effect in the presence of specific risk alleles. This finding has implications for our understanding of the probabilistic nature of risk factors and thus helps to account for the variability in IOI outcome that was observed in the ERA sample. It provides insight into why some children exposed to extreme early adversity develop long term psychological impairment, whereas others do not. Quite strikingly, by mid-adolescence the synergistic GxE interaction appeared to account for nearly all the variation in parent reported IOI scores, once confounding factors were controlled.

The findings of the current study confirm those reported in the literature on the interaction between environmental risk and specific DAT1 genotypes/haplotypes on the risk for IOI behaviours (Brookes et al., 2006b; Laucht et al., 2007; Kahn et al., 2003). Moreover, results were strongest in the current investigation when the DAT1 10R-6R haplotype was used. This finding provided support for the suggestion put forward by Brookes et al. (2006b) that the inconsistencies found in the literature on the association between the DAT1 40-bp (3'UTR) VNTR and ADHD may be due in part to it being a marker for other

functional sites on the gene or it interacting with a second functional polymorphic site. The current study's GxE interaction finding also provides support for the idea that the inconsistencies in direct DAT1 – ADHD association studies could also be due to the need to account for the interaction between the specific polymorphisms and early adversity. Taken together, the GxE interaction findings with respect to the DAT1 gene seem to indicate that particular polymorphisms within the gene exert their influence via a moderating effect on a range of environmental risks (e.g. maternal prenatal smoking: Kahn et al., 2003; maternal prenatal alcohol use: Brookes et al., 2006b; psychosocial risk: Laucht et al., 2007; and early institutional deprivation: current study).

It is remarkable that the influence of individual genetic makeup was detectable even with an environmental pathogen as severe as the one experienced by the children in the ERA project. One could have assumed that the adverse effect of such extreme early deprivation would have been powerful enough to override any susceptibilities from other risk factors. The current study makes an important contribution to the literature in this regard, as previous studies on ADHD and other mental health outcomes have investigated GxE interplay in relation to variations within the 'normal' range of experiences. For example, the study on the moderating effect of the serotonin transporter gene on the risk associated with stressful life events (e.g. employment, financial, housing stressors) on the development of depression (Caspi et al., 2003).

Interestingly, the developmental trajectory of GxE effects observed in the current study seemed to suggest a relative increase in the influence of genetic makeup over time. It is possible that variation in DAT1 polymorphisms account for a larger proportion of the heterogeneity in IOI outcome as the participants move away from the deprivation experience (in time). One explanation is that the relative influence of deprivation was lessening and/or other risk factors, including genetic makeup, which influence the development of ADHD more generally, may be playing an increasing role. The capacity of the study to provide some insight into GxE interaction effects longitudinally represents another important addition to the current literature.

There are several aspects of the present study's identified GxE interaction that add support to the internal validity of the claim. First, in terms of specificity, the effects were seen only in relation to DAT1 genotypes but not in relation to

another gene (DRD4) that has been linked to ADHD in the general population or with one that operates within a different neurotransmitter system, the GR gene. However, we cannot rule out that the different scaling properties (i.e. genotype frequencies) of these other genes compared with the DAT1 polymorphism did not have an effect. Second, the GxE effect was seen across both DAT1 40-bp (3'UTR) and 30-bp (intron) VNTRs and the DAT1 10R-6R haplotypes. This is reassuring given that the two risk alleles (10R and 6R) have been linked to each other and with ADHD (Asherson et al., 2007; Brookes et al., 2006b). Third, evidence of the GxE interaction could be detected across different measures of IOI. The significant results were mainly from parent reports of IOI symptoms on the Rutter Scales and the SDQ but the results from the CAPA interview measure supported the findings. Fourth, variation in the DAT1 gene was not found to affect other deprivation related outcomes, such as cognitive impairment, or outcomes with substantial overlap with IOI, such as conduct disorder. Moreover, disinhibited attachment showed a large degree of overlap with IOI and was also related to deprivation experience but behaviour levels were not influenced by an interaction between genotype and deprivation risk factors.

The absence of the GxE effect in relation to disinhibited attachment provided further support for the conceptualisation of these two outcomes as dissociable constructs (discussed above in section 9.3.2). If the outcomes represented parts of a common underlying latent construct, being measured in different ways, then one would expect there to be an indication from the analyses that DAT1 moderated the risk for disinhibited attachment as well as IOI. It is possible that genetic factors do not influence the risk for the non-IOI outcomes in the same way, or if they do then different genes may be involved. The results of the analysis of DRD4 and disinhibited attachment indicate the latter may be the case and is therefore worth investigation in the future. This is particularly so in the light of findings from other research studies that suggest an association between the DRD4 7-repeat exon III polymorphism and disorganised attachment in infants (Lakatos et al., 2000; Lakatos et al., 2002; Gervai et al., 2005). Moreover, not only do these results help to characterise the deprivation-related IOI phenotype in relation to other domains of impairment within the ERA study, they also further our understanding of the phenotype in relation to ADHD in the wider population and complement the discussion above on the presentation of the phenotype (section 9.3). The GxE interaction was observed with a genotype that operates

within the dopaminergic system, which has been implicated in the pathophysiology of nondeprivation related IOI.

The neurobiological pathway that the observed GxE interaction effects operated through can only be speculated about at the current time. A possible mechanism was put forward in the introduction (section 2.3) and the observed GxE interaction in relation to DAT1 gene variation and institutional deprivation on the risk for IOI fits into the hypothesised framework. One can extend the hypothesised mechanism by including the observed GxE interaction in relation to a specific gene: DAT1. One possibility is that DAT1 10R-6R haplotype alters dopamine function which then influences the individual's susceptibility to the adverse effects of early institutional deprivation. Even after the current study's claim to have identified a GxE interaction, a key question remains as to how it is that an environmental pathogen, which is external to the person, can "get inside the nervous system and alter its elements to generate the symptoms of a disordered mind" (Caspi & Moffitt, 2006). Caspi and Moffitt (2006) advise that collaborations between psychiatry, epidemiology and neuroscience will help to further our knowledge in this field. However, there are several possible models of the operative processes by which early experience influences later development that could be of relevance here, namely, developmental programming and epigenetics (Rutter & O'Connor, 2004; Rutter, 2006). Both are addressed below.

9.6.1 Developmental programming

In brief, a possible mechanism for intra-organismic change may be through some form of developmental programming during critical periods of early development (Rutter & O'Connor, 2004). This may be through experience-expectant programming, i.e. certain experiences are required for the development of normal brain functioning (e.g. sufficient visual input during sensitive periods in infancy for later normal visual functioning), or through experience-adaptive programming, i.e. the brain adapts to certain experiences during a critical period to optimise the specific conditions of that environment (e.g. language learning through early phonological discrimination) (Rutter & O'Connor, 2004). Experience-adaptive programming theory lends itself to a different possible hypothesis that early adverse experiences elicit an alternative neural developmental pathway adapted to the current stressful rearing environment, rather than early stress leading to impaired structural and functional neural development. That is, the brain may

develop along what has been termed a 'stress-responsive pathway' due to significant stress being experienced during sensitive periods in early life which has prompted a chain of modified neurobiological effects (Teicher et al., 2003). The current study has provided the first, albeit indirect, evidence that genetic variation may alter or interact with these processes following early institutional deprivation. The extent to which programming effects influence development and the biological and genetic basis for the processes are only beginning to be studied and are not yet well understood.

9.6.2 Epigenetics

It is possible that programming effects may be operating through epigenetic mechanisms. One could speculate that in the case of deprivation-related IOI, genetic variation may be interacting with these epigenetic processes to influence susceptibility from environmental risk factors for long term adverse outcome. However, as noted in chapter 3, a limitation of nonexperimental studies of GxE interplay, is that it is not possible to determine whether any GxE interaction that is detected may actually be reflecting mediation of environmental effects via epigenetic processes rather than moderation by genetic variation.

9.6.3 Glucocorticoid receptor findings

The significant rGE findings in relation to the GR genotypes/haplotype were a somewhat surprising result as there was no reason to suspect that those children who resided longer in the institutions had greater genetic liability for adverse outcome than those adopted out early or from within the U.K. Accordingly, caution should be employed when interpreting these results. One can only speculate as to the reason for this finding and also what functional impact it may have on IOI, and developmental outcome more generally, in the ERA sample. Indeed, it is speculative to suggest that the rGE translates to a so called increase in genetic liability in the high e'risk sample given that the influence of these SNPs is being investigated for the first time in the current thesis in relation to early institutional deprivation and, more specifically, on the risk for IOI. One could speculate that the anomalous GR *Bcl*I and 9beta genotype frequencies may have come about through adoption selection processes or genotyping sample selection

biases. That is, the children who were adopted out of the institutions after an extended period of deprivation were less likely to be carriers of the *Bcl*I G allele than those who remained in the institutions. It is not possible however to accurately test for the reason why this could be the case. One could also speculate that carriers of the *Bcl*I G allele were subject to a higher rate of mortality due to alterations in HPA axis stress response to their early adverse experience. However, to draw such a conclusion on the basis of one allele, in one gene, in one small sample would be a leap of logic that again cannot be empirically tested. Moreover, the GenERA sample constitutes only a part of the wider ERA sample. There could be biases introduced by the participation rate of high e'risk sample in the GenERA study (due to high refusal rate).

9.7 Strengths and limitations

There were a number of strengths and limitations to the current study which warrant discussion. The advantages of the study included: First, a large sample of children, randomly selected within age bands, who suffered severe early deprivation with an adopted comparison group. Second, the unique opportunity to study effects of early deprivation largely unconfounded by selection biases. Third, the study utilises a nonclinical sample to study the risk for IOI behaviours. Fourth, there are data available from a wide range of measures, from multiple informants and assessment waves.

The limitations included: First, the limited sample size available for the genetic interaction analyses. Second, the sampling constraints of using a natural experiment, e.g. there were very limited data available on biological background, pre and perinatal risk, and mortality rates within the institutions. Third, the inability to distinguish between different aspects of the deprivation experience, e.g. psychological, social, nutritional. Fourth, the uniqueness of sample makes generalising the results difficult. Fifth, measurement of IOI in childhood and early adolescence was based on a single questionnaire measure.

9.7.1 Strengths

There are several strengths to the study design of the main ERA project: First, the sample is large and was stratified and randomly selected (except for the

Romanian group who were over 2 years at arrival to U.K., where all available children were included). The use of the U.K. comparison group controlled for the effects of adoption but differed with respect to the post natal deprivation experience. Moreover, like the Romanian adoptees, the U.K. children also came from backgrounds where it is possible they were exposed to a higher level of prenatal risk than the average population. For instance, many of the U.K. birth mothers were only teenagers, they may have endured stress associated with an unwanted pregnancy, possible financial disadvantage, plus around 20% of the birth mothers concealed their pregnancies, making it unlikely they received proper care (Castle et al 2000).

Second, the unique circumstances in Romania at the time meant that children, including the ones in the ERA study, were only adopted within a limited time frame following the end of Ceauşescu's rule. Therefore, it was unlikely that those children who were older at the time of their adoption had not been chosen for adoption at a younger age (and thus had been left for longer in the institutions) possibly owing to some existing impairment. The children were placed in the institutions in soon after birth, largely for reasons of extreme poverty, making it unlikely that the children were given up because of their disabilities, which would have made separating out the effects of post natal deprivation difficult. Although adoptive parents did have some choice over which child they could adopt, they chose children across the age and ability range.

Third, the current study employs a nonclinical study to investigate the risk and developmental pathways to IOI and its presentation. This represents a design advantage as many studies of ADHD, particularly genetic studies, use clinical samples which inherently include a clinic referral bias in their sampling strategy.

Fourth, the study had the advantage of data being available on IOI from multiple informants (parents and teachers) across multiple assessment waves (age 6, 11 and 15) and across different measurement tools (questionnaire and interview techniques). Moreover, there were also extensive and systematic data available on the other behavioural and cognitive measures, e.g. disinhibited attachment, conduct problems and cognitive functioning, plus in-depth background information on the families.

9.7.2 Limitations

9.7.2.1 Limited sample size for genetic analysis

Perhaps the most obvious limitation of the current study was the restricted sample size used in the genetic analyses. The total GenERA sample was made up of 129 cases but this was reduced further with the need to include IOI and covariate outcome data. In the longitudinal repeated measures ANOVA analyses data were required from multiple assessment waves, and if one data point was missing then the case was excluded from the analysis (due to the statistical model, not by the author's choice). Owing to the high refusal rate and the other reasons discussed below, the GenERA sample only included part of the wider ERA sample and was not randomly selected from within that sample. Therefore biases could have been introduced by the decision of individual families about whether or not to participate in the GenERA study. However, there were several reasons for the limited participation rate: First, although the study has had a very good overall participation rate from families over the course of the study period, contact had been lost with several families. There was also a drop in rate of young people agreeing to take part in the study by the age 15 assessment wave. Accordingly, many families could not be approached to participate in the current study. Second, there are sensitivities around the collection and storing of DNA in the general population due to fears about what the material may be used for in the future, which may have caused families to decide not to take part (although explicit explanations were provided by the author as to what their data can and cannot be used for). There are also particular sensitivities that may affect vulnerable adopted groups, such as the ERA sample, e.g. the young people may not want to be reminded that they are biologically different from their adoptive family. Third, there were several participants where it was not possible to collect DNA due to the level of their behavioural, cognitive and/or physical impairment. The author made every effort to collect as many samples as possible, including providing extensive information on the process as a matter of course, providing extra information when requested, sending out multiple reminders and replacement DNA collection packs, making follow-up phone calls to families and collecting samples in person. However, one must be sensitive no to pressure families to participate in something they are not comfortable with, particularly in an at-risk sample and one that has been so committed to the research of the ERA

project over the last decade. A systematic analysis was conducted to examine whether there were any differences between those who participated and those who didn't (see section 4.1.2.2 in chapter 4). No differences were found on a range of background and outcome measures. This lends support to the assumption that the available sample is representative of the larger ERA sample.

The main repercussion of the limited sample size was that the current study lacked the power to conduct categorical GxE analyses, as the cell sizes became too small for the results to be meaningful. However, the power of the dimensional GxE analyses was increased by manipulating the measurement groups of the environmental risk factor, institutional deprivation. The adoptee groups were combined, following the analysis of risk associated with deprivation for IOI in chapter 6, to form combined low e'risk and high e'risk subsamples. Moreover, because of the large group differences that were observed in mean levels of IOI it was possible to detect a significant GxE interaction effect in relation to DAT1 gene and early deprivation.

9.7.2.2 Limited knowledge of biological background and mortality rates

The second main limitation was that the use of a 'naturally' occurring experiment meant that aspects of the background of the participants were not known (or only limited information was available). There was very limited information on the biological background of the participants that may possibly have mediated the effects of deprivation on outcome, including whether there was a family history of psychopathology. Hardly anything was known about the pre and perinatal risk factors, such as maternal alcohol or drug use during pregnancy and malnutrition in utero. This may hold particular relevance for the current investigation as prenatal adversity has been implicated in the aetiology of nondeprivation-related IOI (Taylor & Rogers, 2005). Additionally, little is know about the mortality rate within the institutions, although anecdotal reports suggest that it was high, and more so in some institutions compared with others. Mortality rates may have had an impact particularly on the late placed adoptee group as those who were available for adoption were obviously the ones that had survived the severe deprivation experience. Survival may have been influenced by genetic makeup and/or favourable treatment from staff in the institutions, both of which may have played a role on later outcome. However, the strong effect of duration of

deprivation on IOI and other outcomes, across measures and over time suggests that extended exposure to adverse rearing conditions was key in shaping the children's future development. Needless to say that adoption out of the institutions and the subsequent experience of being reared in nurturing families in the U.K. was a significant positive intervention for the children in our study.

9.7.2.3 Multiple risk factors within the deprivation experience

The third limitation related to there being multiple aspects to the risk associated with the deprivation experience, e.g. psychological, social, nutritional. It was not possible to distinguish with confidence between specific aspects of deprivation and therefore to be able to ascertain whether the effects were being driven by one or more of the factors. Furthermore, only a proxy measure of malnutrition was available: weight at entry to U.K. This may not have accurately reflected the scope of the children's nutritional deprivation as it could not capture the level of nutrition at different periods of the children's early development in the institutions. Malnutrition during different sensitive periods may have different effects on outcome.

9.7.2.4 The unique sample inhibited ability to generalize

There were also a number of draw-backs that need to be considered in terms of the ability to generalise to other samples. For example, whether the findings following such extreme early institutional deprivation can be placed within the wider literature on early adversity is open to debate. The findings of the ERA study make an important contribution to the field but direct comparisons with other groups of children, e.g. from neglected or abused backgrounds, need to be done with caution. In terms of the current study's investigation of the interaction between deprivation and genetic risk, the findings advance the literature on GxE by showing that even when examining the process in relation to such a severe environmental pathogen one can still observe genetic moderation of the risk for psychiatric impairment.

9.7.2.5 Measurement of IOI

At ages 6 and 11 the measurement of IOI was based on a single questionnaire measure, the Rutter Scales. An important check of the validity of findings is that they generalise across different measurement devices. Therefore, the findings on IOI from these assessment waves lack that level of validity. However, the association between IOI and deprivation does seem to be robust as similar findings were observed across informants (parents and teachers) over 4 assessment waves (ages 4, 6, 11 and 15; although age 4 results are not reported in the current thesis) and were found when IOI was defined both dimensionally and categorically. Moreover, the results using the data on ADHD symptomatology from the CAPA interview at the mid-adolescent assessment wave corroborated the questionnaire findings. The evidence of the GxE interaction in relation to the risk for IOI was found longitudinally and across the questionnaire and interview measurement tools.

9.8 Future directions

Many of the ideas for future research have been touched upon in previous sections of the thesis but are brought together here. First, neuroimaging research could help to elucidate the biological mechanisms involved in the GxE interaction process by investigating structural and functional alterations to specific brain regions. The recent pilot study on a small subsample of the ERA study began to explore these issues, with promising results (Mehta et al., 2008). A pivotal question for future research is on the neuroanatomy of deprivation-related IOI. Are there functional or structural alterations in the dorsal striatum or prefrontal cortex circuits, implicated in the pathophysiology of ADHD, or are alterations in brain regions targeted by the stress response system (amygdala, hippocampus) more likely to be mediating the observed impairment?

Second, the investigation on the presentation of the deprivation-related IOI phenotype could be expanded to include a more thorough examination of subtype symptomatology. Although the preliminary analysis conducted in the current thesis did not suggest that a particular subtype (inattentive vs. hyperactive/impulsive) was driving the association with deprivation, this could be explored in more detail. Future research could include a discriminate function

analysis of symptoms, categorical analyses, longitudinal analyses, and also examine subtypes in relation to the interaction with genetic factors.

Third, the current study does not examine the overlap between IOI and autistic features in the investigation of the IOI phenotype. Given that these two domains are reported to overlap in the literature on ADHD, and that quasi-autistic features constitute a specific sequela to early institutional deprivation (Rutter et al., 1999; 2007) this represents a possible area for future research.

Fourth, there was a weak suggestion of an interaction between early deprivation and DRD4 genotype on the risk for disinhibited attachment. There are reports in the literature on an association between this genotype and disordered attachment (Lakatos et al., 2000; Lakatos et al., 2002), which suggests that further research in the area may prove fruitful.

Fifth, further work is also planned to extend the GxE interaction analyses to include two DAT1 SNPs: rs40184 and rs2550946, which have been investigated in relation to ADHD previously in the literature (Brookes et al., 2006b). A cumulative model of genetic risk may be explored, combining these SNPs with the two DAT1 polymorphisms examined in the current thesis.

Finally, replication of the observed GxE interaction findings reported in the thesis using other deprived samples is needed in order to confirm their validity.

9.9 Conclusions

This study examined inattention/overactivity/impulsivity in a group of children who had experienced severe early institutional deprivation. Their development was assessed at ages 6, 11 and 15 years, with particular attention given to their outcome in mid-adolescence. The role that specific genetic factors played in moderating the link between early deprivation and IOI provided the central focus to the thesis. The pivotal finding was that, although not deterministic in its effects, institutional deprivation continued to exert adverse influence on IOI behaviour into mid-adolescence and, crucially, the influence of this environmental pathogen was moderated by individual genetic makeup.

In summary, the evidence suggests that IOI is a fairly stable impairment for this group of children and that the risk for IOI continued to be associated with

early deprivation into mid-adolescence. This highlights the persistent effects of severe early adversity on development and perhaps intimates that there was some form of fundamental neurobiological alteration. The risk effect appears to show a stepwise increase in relation to adverse IOI outcome at around the six months of deprivation mark. Furthermore, the analyses suggest that deprivation-related IOI shares a number of the features of IOI/ADHD from nondeprived samples and highlights whether early deprivation should be seen as one uncommon route to a common disorder (ADHD) or whether it should defined as a distinct phenotype with a distinct aetiology. Indeed, the hypothetical biological mechanism, put forward in the current thesis, linking deprivation and persistent IOI impairment included neurobiological features that have been related to the pathophysiology of ADHD. In brief, the putative biological mechanism may involve long term negative down-stream effects on neuro-transmitter branches (e.g. dopamine and norepinephrine systems; Pani et al., 2000) and brain circuits (e.g. dorsal striatum, prefrontal cortex) implicated in the patho-physiology of ADHD (Sanchez et al., 2001) of early stress-related dysregulations of the hypothalamic-pituitary–adrenal axis (Kaufman & Charney, 2001). Moreover, the selection of candidate genes for the study's investigation of GxE interaction from within a neurotransmitter system that has been implicated in the aetiology and treatment of ADHD developed this idea one step further and helped to indirectly test this mechanism.

The second key finding from the thesis was the presence of a GxE interaction between polymorphisms within the dopamine transporter gene and institutional deprivation, demonstrating that variation within this gene moderated the risk for IOI from early deprivation. Taken together with the persistence in IOI impairment in the sample and the observed commonalities in presentation of deprivation-related IOI and that seen in the population, the current study goes some way to supporting the hypothesised mechanism and broadening our understanding of the risk processes associated with institutional deprivation for the development of IOI behaviours. Moreover, the study provides evidence that deprivation-related IOI can be characterised in much the same way as ADHD in the wider population, but with a distinct aetiology. However, much more research is still needed into the underlying neurobiology, to test the mechanistic pathways directly, and also the overlap between IOI and other deprivation-related features in order to validate these claims.

APPENDICES

Appendix 1:

Scatterplots of IOI z-scores as a function of participants' age at entry to U.K.

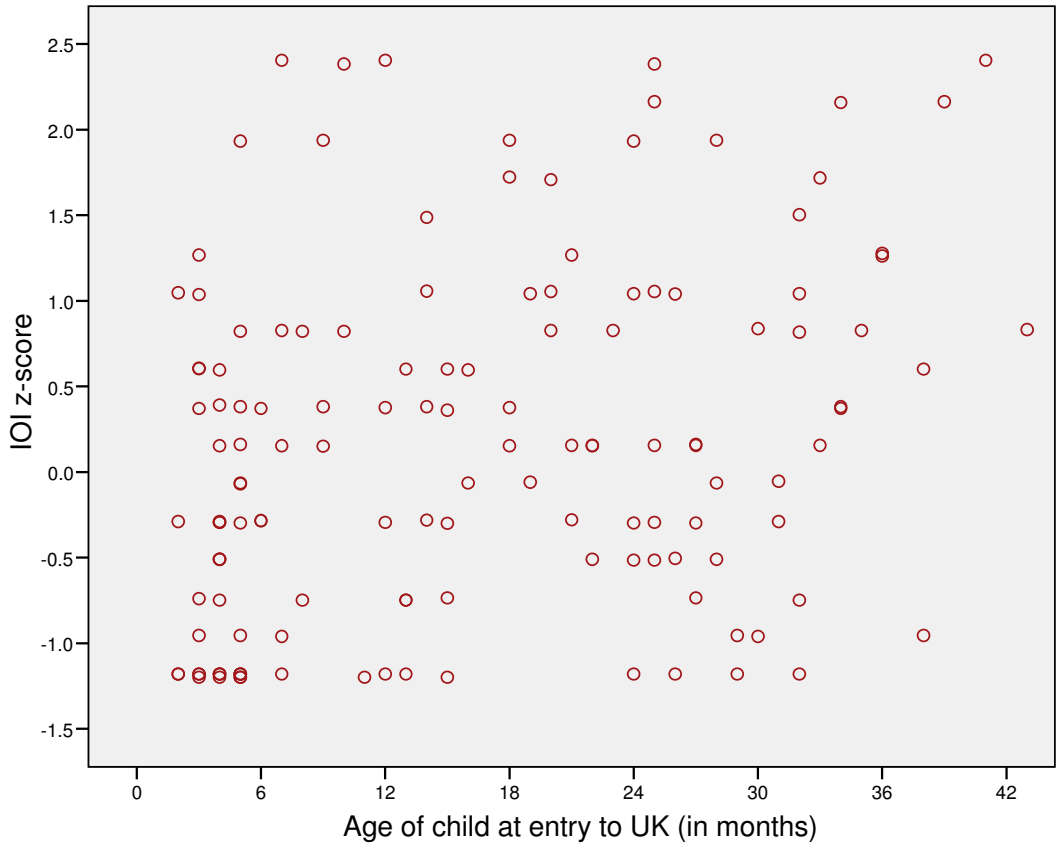


Figure A1a:

IOI at 6 years: Rutter scales, parent report *(Romanian institution-reared sample)*

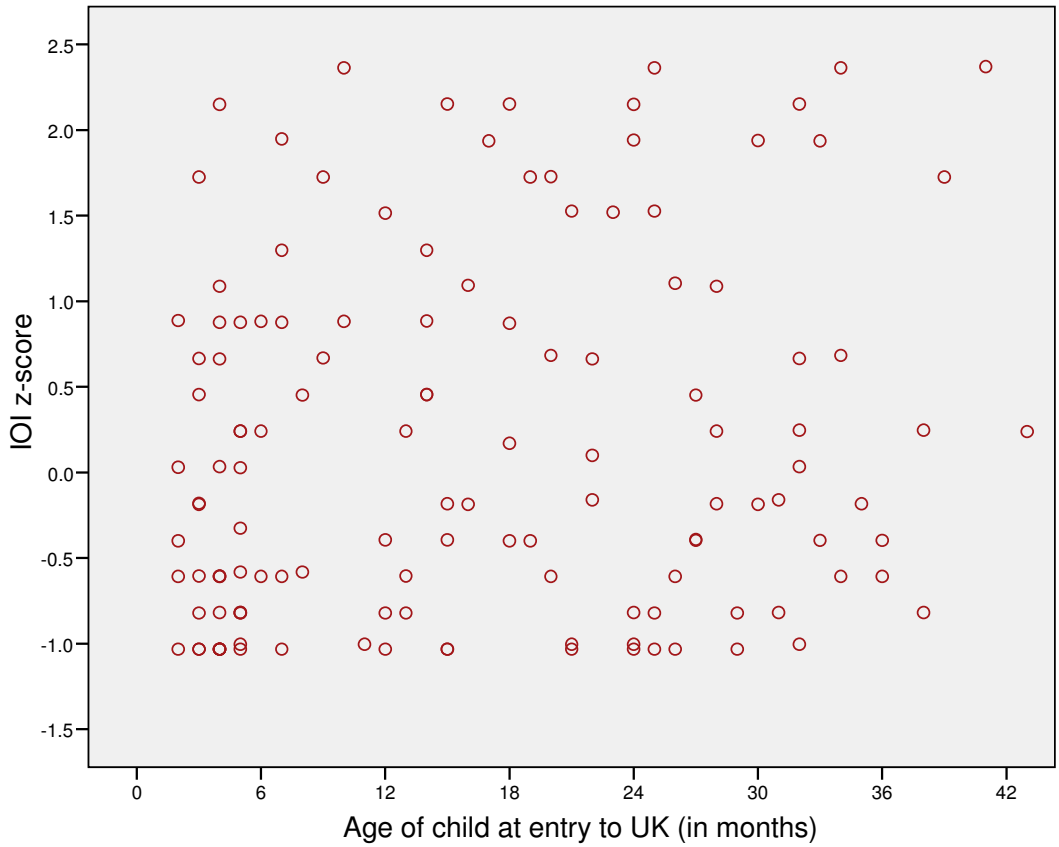


Figure A1b:

IOI at 11 years: Rutter scales, parent report *(Romanian institution-reared sample)*

Appendix 2

Information and consent forms relating to the collection of DNA samples

Copies of the DNA data collection information and consent forms for parents and participants are provided on the following pages

2a DNA collection information and consent form given to parents not yet seen as part of the main ERA assessment when DNA collection commenced

2b seen DNA collection information and consent form posted to parents already as part of the main ERA assessment when DNA collection commenced

2c Developmental assessment information sent to participants in advance of visit

2d Participant information and consent form

2e Frequently asked questions information sheet for parents

2f Frequently asked questions information sheet for participants

2g Instruction sheet for collection of mouth cells using buccal swabs

Appendix 2a

ID_____

KING'S College LONDON
University of London

KCL Family Research Project
SGDP Centre
Box Number PO80
King's College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Fax: 020-7848-0866
Email: s.stevens@iop.kcl.ac.uk

KCL Family Research Project

GenERA Study

We would like thank you and your family for the all the help you have given to the project in the past and to ask your permission for your son or daughter to take part in an extension of the main Family Research Project looking at young people's development from infancy to adolescence. This strand of the study aims to look at possible genetic factors that may influence activity and attention levels in young people. Research has shown that both environment and genes are important influences on many types of behaviour, including activity levels and the ability to focus attention. By studying your child's DNA we will find out more about the role genetics play in determining why different people respond differently to life events.

If you agree for your son or daughter to take part in this strand of the project then we will ask him or her to provide us with a DNA sample taken from the cells on the inside of his/her mouth using a cotton bud swab. This is easy to do, does not hurt at all and can be done by you or your child can do it for him/herself.

The samples will be used for research only and do not constitute a genetic test of any sort. Therefore we will not be releasing the individual results of genetic data to anyone. All genetic data will be stored anonymously and identified by code number only. The DNA will be stored until the research is completed and destroyed after that time.

Your son or daughter's participation in this study is completely voluntary and he or she may withdraw at any time. The decision on whether to participate or not will not effect your family's involvement in the main study. The sample will be treated as strictly private and confidential and will be used for our research only. It will not under any circumstances be passed on to your child's school, the police, your doctor or anyone else.

An information sheet for you and your son or daughter is attached with the answers to some frequently asked questions. Please complete the parental consent form at the bottom of this letter. If you have any questions, now or in the future, please feel free to contact us on 020 7848 0477.

ID_____

Very many thanks to you and your family for your time and participation. Your continuing help and support is greatly appreciated by the Family Research Project team.

Parental consent for son/daughter to provide DNA sample

☐ I have read the information myself and had the chance to ask any questions about the study. I agree for my son or daughter to give a sample of his/her DNA using a simple mouth swab.

☐ I would like more information before giving consent for my son or daughter to participate in this phase of the study. Please call me on _____

☐ I do not wish my son or daughter to participate in this phase of the study

Signed ……..

Date……..

Professor Michael Rutter
Professor Edmund Sonuga-Barke
SGDP Research Centre
King's College London
SE5 8AF
Ph: 020 7848 0477

Appendix 2b

KING'S College LONDON
University of London

KCL Family Research Project
SGDP Centre
Box Number PO80
King's College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Fax: 020-7848-0866
Email: s.stevens@iop.kcl.ac.uk

KCL Family Research Project

GenERA Study

Dear Mr and Mrs <<NAME>>,

We would like thank you and your family for the all the help you have given to the project in the past and to ask your permission for <<NAME>> to take part in an extension of the main Family Research Project looking at young people's development from infancy to adolescence. This strand aims to look at possible genetic factors that may influence activity and attention levels in young people. Research has shown that both genes and environment are important influences on many types of behaviour, including activity levels and the ability to focus attention. By studying your child's DNA we hope to find out more about the role genetics play in determining why different people respond differently to life events.

If you agree for <<NAME>> to take part in this strand of the project then we would like to ask <<him/her>> to provide us with a DNA sample taken from the cells on the inside of <<his/her>> mouth using a cotton bud swab. This does not hurt at all and <<NAME>> can easily do it for <<him/herself>>.

The samples will be used for research only and do not constitute a genetic test of any sort. Therefore, we will not be releasing the individual results of genetic data to anyone. All genetic data will be stored anonymously and identified by code number only. The DNA will be stored until the research is completed and destroyed after that time.

Your <<son's/daughter's>> participation in this study is completely voluntary and <<he/she>> may withdraw at any time. The decision on whether to participate or not will not effect your family's involvement in the main study. The sample will be treated as strictly private and confidential and will be used for our research only. It will not under any circumstances be passed on to your <<son's/daughter's>> school, the police, your doctor or anyone else.

To provide you and your child with more information and the answers to some frequently asked questions the following documents are enclosed:

- Information sheet with frequently asked questions
- Participant's information/consent form to provide DNA sample
- Instruction sheet for collecting mouth swabs

- Collection pack – 1 tube with 10 cotton buds
- Stamped addressed return envelope

Please complete the parental consent form at the bottom of this letter and participant consent form and return them with the mouth swabs in the envelope provided. Please hold on to the information sheet with frequently asked questions for future reference. If you have any questions, now or in the future, please feel free to contact us on 020 7848 0477

Very many thanks to you and your family for your time and participation. Your continuing help and support is greatly appreciated by the Family Research Project team.

Parental consent for son/daughter to provide DNA sample

☐ I have read the information myself and had the chance to ask any questions about the study. I agree for my son or daughter to give a sample of his/her DNA using a simple mouth swab.

☐ I would like more information before giving consent for my son or daughter to participate in this phase of the study. Please call me on _____

☐ I do not wish my son or daughter to participate in this phase of the study

Signed ……...

Date……..

Professor Michael Rutter
Professor Edmund Sonuga-Barke
SGDP Research Centre
King's College London
SE5 8AF
Ph: 020 7848 0477

Appendix 2c

KING'S College LONDON
University of London

KCL Family Research Project
SGDP Centre
Box Number PO80
King's College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Fax: 020-7848-0866
Email: *******@iop.kcl.ac.uk

«Title» «FirstName» «LastName»
«Address1»
«Address2»
«City»
«County»
«PostalCode»

«Date»

Dear «FirstName» «LastName»,

CHILDREN'S DEVELOPMENT FROM INFANCY TO ADOLESCENCE

Thank you very much for your interest in this study. The study aims to help us gain an understanding of young people's development over the years, and is a continuation of the work that we did with you when you were younger. Now we'd like to come and see you again to find out what has happened to you since then and to find out how you've been getting along. This usually takes two sessions, but if it is better for you, we can arrange to do it all in one visit.

If you agree to take part, you will be interviewed about how you have been getting along at school, how you've been getting along with friends and family and whether you've got any particular difficulties. We would also like to ask you a few questions about being adopted. As well as the interviews, there are also some short assessment activities such as puzzles. There are also some questionnaires, which we will leave for you to fill in, in your own time. As at age 11, we would like to get an up to date measure of your head size, so we'll be asking to measure this too. Also just like at age 11, we would like to video and audio tape some parts of the work that we do together, if that is ok with you.

In addition to the interviews and puzzles we would like to ask you to provide us with a DNA sample taken from the saliva cells on the inside of your mouth using a cotton bud swab. This does not hurt at all; you can do it yourself or your parents can do it for you. An information sheet with some frequently asked questions about DNA sampling is attached explaining why we would like to look at some of your genes.

The DNA samples will be used for research only and do not constitute a genetic test of any sort. Therefore, your sample will be totally private and confidential and we will not be releasing the results of your individual genetic data to anyone. All genetic data will be stored anonymously and identified by code number only. The DNA will be stored until the research is completed and destroyed after that time.

If you agree to take part in the study, you can still decide not to answer some questions or complete some of the tasks and you can withdraw from the study at any time without telling why you want to do so. All the information that you give us will be treated as strictly private a confidential within the limits of the law. The information that you give us won't be discuss with anyone outside the research team. No personal information you give us will be passed on your school, your family or anyone else.

If you have any questions now, or in the future, please feel free to contact us (tel no. 020 78 0477). We really look forward to seeing you soon and once again thank you for your interes

Yours sincerely,

Professor Michael Rutter
Professor Edmund Sonuga-Barke
Social, Genetic and Developmental Psychiatry Research Centre
London SE5 8AF

Appendix 2d

KING'S College LONDON
University of London

KCL Family Research Project
SGDP Centre
Box Number PO80
Kings College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Fax: 020-7848-0866
Email: s.stevens@iop.kcl.ac.uk

Participant consent to provide a DNA sample **ID** _____

KCL FAMILY RESEARCH PROJECT

GenERA Study

We would like you to participate in an extension of the main Family Research Project looking at young people's development from infancy to adolescence. This study aims to look at possible genetic factors that may influence activity and attention levels in young people. Research has shown that both environment and genes are important influences on many types of behaviour and by studying your DNA we will find out more about the role genetics play in determining why different people respond differently to life events.

If you agree to take part in this strand of the project then we will ask you to provide us with a DNA sample taken from the cells on the inside of your mouth using a cotton bud swab. This does not hurt at all; you can do it yourself or your parents can do it for you. The samples will be used for research only and do not constitute a genetic test of any sort. Therefore, we will not be giving the results of your individual genetic data to anyone. All genetic data will be stored anonymously and identified by code number only. The DNA will be stored until the research is completed and destroyed after that time.

Your participation is completely voluntary and you will be free to withdraw at any time. The decision on whether to participate or not will not effect your involvement in the main study. The sample will be treated as strictly private and confidential and will be used for our research only. It will not under any circumstances be passed on to your school, your family, the police, your doctor or anyone else. If you have any questions now, or in the future, please feel free to contact us.

☐ I have read the information myself and had the chance to ask any questions about the study. I agree to provide a sample of my DNA using a simple mouth swab.

☐ I would like more information before giving consent to participate in this phase of the study. Please call me on _____

☐ I do not wish to participate in this phase of the study

Signed ……..

Date……..

Professor Michael Rutter
Professor Edmund Sonuga-Barke
SGDP Centre, King's College London

Appendix 2e

KCL Family Research Project
SGDP Centre
Box Number PO80
King's College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Email: s.stevens@iop.kcl.ac.uk

What about this DNA sample? Here are some frequently asked questions and answers…

Q: Why do you want to perform studies using my child's DNA?
Research has shown that both genes and environment are important influences on many types of behaviour including activity levels and the ability to focus attention. Studies looking at the effects of life experiences have found that even with severely depriving and stressful experiences there is huge individual variation in response, with some people being more sensitive to the effects than others. Our study has found this too in relation to deprivation in Romanian institutions and the range of individual responses to that. By studying your child's DNA we will be able to look at whether normal genetic variations might play a role in shaping different people's susceptibility, or resistance, to stressful experiences and the influence this may have on levels of hyperactivity and inattention.

Q: Who can take part in this study?
We are asking everyone who is already part of the Family Research project. Your son or daughter does not have to take part in this project if she or he doesn't want to. But we hope your family will be able to help in this research.

Q: What does this part of the study involve?
Your child will be asked to provide a mouth swab using a set of cotton buds to wipe the inside of his/her mouth. This is easy to do and does not hurt at all. The sample will be sent to our own laboratory in the Social, Genetic and Developmental Research Centre where the DNA will be extracted and stored. The DNA will only be used by scientists to further our understanding about how genes influence behaviour. Any future, new use of the samples for research will only occur after appropriate ethical approval has been given.

Q: Will my child's DNA data be confidential?
The DNA laboratory will not receive any names so that the stored DNA samples will be totally anonymous. All the information we receive will be strictly confidential and only used for research. The information your family provides will not be shared with anyone else. You and your child's name, address and other personal information will be kept separately and will only be known by the Family Research Project team.

PTO

Q: Can the DNA samples ever be used to link a member of our family to crime?
No. The Family Research Project DNA bank will always be completely confidential, without exception. In addition, if a criminal court ever wishes to conduct a DNA test, they can easily take their own DNA sample from saliva, so there would be no need for them to contact the Family Research Project. Our DNA bank will be used for research on activity and attention levels only.

Q: Can the DNA be used to test whether any family member will develop a disease later in life (such as breast cancer or Alzheimer's Disease)?
No. We will not use the DNA to test for genetic risk markers that are already known. The goal of our research is to search for possible new markers, but these would have to be confirmed by other studies before it is known if they are medically useful. If there is a risk marker that is already used medically to test for an individual's risk of disease, study members can ask their GPs about testing for it. In addition, the Family Research Project DNA will not be analysed for any individual participant. The DNA will be used by the Research Group's scientists to compare groups of study members.

Q: If I am ever asked by anyone if my child has had genetic screening, what should I say?
You should say that your son or daughter has not had genetic screening, as we are not conducting screening tests on the DNA.

Q: Can our doctor contact you to find out the results of the DNA tests?
No. The Family Research Project DNA bank will be used for research purposes only. In addition, if your doctor ever wishes to conduct a DNA test for your child, the doctor can easily take a DNA sample from your son or daughter's saliva or blood, so there will be no need for your doctor to contact the study.

Any questions?
If you have any questions about the Family Research Project DNA sampling please feel free to contact Suzanne Stevens on 020 7848 0477.

Contact:
Suzanne Stevens
Family Research Project
SGDP Research Centre
Box no. P080
King's College London
De Crespigny Park
London SE5 8AF

Tel: 020 7848 0477
E-mail: s.stevens@iop.kcl.ac.uk

Appendix 2f

KCL Family Research Project
SGDP Centre
Box Number PO80
King's College London
De Crespigny Park
LONDON SE5 8AF

Tel: 020-7848-0477
Email: s.stevens@iop.kcl.ac.uk

What about this DNA sample? Here are some frequently asked questions and answers…

Q: Why do you want to perform studies using my DNA?
Research has shown that both genes and environment are important influences on many types of behaviour including activity levels and the ability to focus attention. Studies looking at the effects of life experiences have found that even with severely depriving and stressful experiences there is huge individual variation in response, with some people being more sensitive to the effects than others. Our study has found this too in relation to deprivation in Romanian institutions and the range of individual responses to that. By studying your DNA we will be able to look at whether normal genetic variations might play a role in shaping different people's susceptibility, or resistance, to stressful experiences and the influence this may have on levels of hyperactivity and inattention.

Q: Who can take part in this study?
We are asking everyone who is already part of the Family Research project. You do not have to take part in this project if you do not want to, but we hope you will be able to help in this research.

Q: What does this part of the study involve?
You will be asked to provide a mouth swab using a set of cotton buds to wipe the inside of your mouth. This is easy to do and does not hurt at all. The sample will be sent to our own laboratory in the Social, Genetic and Developmental Research Centre where your DNA will be extracted and stored. Your DNA will only be used by scientists to further our understanding about how genes influence behaviour. Any future, new use of the samples for research will only occur after appropriate ethical approval has been given.

Q: Will my DNA data be confidential?
The DNA laboratory will not receive any names so that the stored DNA samples will be totally anonymous. All the information we receive will be strictly confidential and only used for research. The information you provide will not be shared with anyone else. Your name, address and other personal information will be kept separately and will only be known by the Family Research Project team.

PTO

Q: Can the DNA samples ever be used to link a member of our family to crime?
No. The Family Research Project DNA bank will always be completely confidential, without exception. In addition, if a criminal court ever wishes to conduct a DNA test, they can easily take their own DNA sample from saliva, so there would be no need for them to contact the Family Research Project. Our DNA bank will be used for research on activity and attention levels only.

Q: Can the DNA be used to test whether any family member will develop a disease later in life (such as breast cancer or Alzheimer's Disease)?
No. We will not use the DNA to test for genetic risk markers that are already known. The goal of our research is to search for possible new markers, but these would have to be confirmed by other studies before it is known if they are medically useful. If there is a risk marker that is already used medically to test for an individual's risk of disease, study members can ask their GPs about testing for it. In addition, the Family Research Project DNA will not be analysed for any individual participant. The DNA will be used by the Research Group's scientists to compare groups of study members.

Q: If I am ever asked by anyone if I have had genetic screening, what should I say?
You should say that you have not had genetic screening, as we are not conducting screening tests on the DNA.

Q: Can my doctor contact you to find out the results of the DNA tests?
No. The Family Research Project DNA bank will be used for research purposes only. In addition, if your doctor ever wishes to conduct a DNA test for you, the doctor can easily take a DNA sample from your saliva or blood, so there will be no need for your doctor to contact the study.

Any questions?
If you have any questions about the Family Research Project DNA sampling please feel free to contact Suzanne Stevens on 020 7848 0477.

Contact:
Suzanne Stevens
Family Research Project
SGDP Research Centre
Box no. P080
Kings College London
De Crespigny Park
London SE5 8AF

Tel: 020 7848 0477
E-mail: s.stevens@iop.kcl.ac.uk

Appendix 2g

Instructions for Collecting Mouth Cells

Your pack contains: 1 Tube with storage liquid and 10 cotton wool buds.

**AS WITH ALL SMALL OBJECTS AND UNDRINKABLE LIQUIDS,
PLEASE KEEP OUT OF REACH OF CHILDREN**

Now we will tell you how to collect the mouth cells. There 1 tube and 1 pack of cotton buds. Please put all 10 buds into the 1 tube.

Timing
Please try to use the cotton wool buds all in one go. It would be best if you could do them first thing in the morning before you eat anything! It is important to make sure that THERE ARE NO BITS OF FOOD left in the mouth when swab is being taken

If you do not want to collect mouth cells using all of the buds in one day, don't worry. Try to find time in the next day to finish collecting the cells.

How to Use the Cotton Wool Buds
1) Stand the tube upright in a cup or mug and unscrew the lid.

2) Try to use half of the buds to rub inside the upper part of the mouth and half to rub inside the lower part of the mouth.

3) The best way to collect the mouth cells is by rubbing the cotton wool bud firmly, backwards and forwards, along the inside of the mouth, (including the cheek, gums and inside the lip) with a little pressure against the mouth as you do so. Try to do this for about 20 seconds with each bud (*time it, as it takes longer than you think*). If this proves to be too long, please just do it for as long as you can. It does not hurt at all.

4) Each time a bud has been used, please place it in the tube containing the storage liquid - cotton wool end downwards, into the liquid. Be sure to do the lids up tightly. Please DO NOT store the samples in the fridge, room temperature conditions are fine.

5) Repeat this procedure, for 20 seconds, with each of the buds.

6) When all the buds have been used and put in their tubes, please put the tubes, with the consent forms in the envelopes provided and post to us as soon as possible.

If you drop a bud, just throw it away - but please try not to waste many!

THANK YOU FOR YOUR HELP AND CONTRIBUTION TO THE STUDY

Please contact Suzanne Stevens on 020 7848 0477 should you require further information

Appendix 3

IOI: questionnaire items (Rutter Scales & SDQ)

Table A1

Questionnaire items measuring IOI: Revised Rutter Parent & Teacher Scales for school-age children*; the Strengths and Difficulties Questionnaire**

Questionnaire measure	
Rutter Scales	SDQ
Mother and father items	
• Very restless, has difficulty staying seated for long	• Restless, overactive, cannot stay still for long
• Squirmy, fidgety child	• Constantly fidgeting or squirming
• Cannot settle to anything for more than a few moments	• Easily distracted, concentration wanders
• Inattentive, easily distracted	• Thinks things out before acting *(coding reversed)*
	• Sees tasks through to the end, good attention span *(coding reversed)*
Teacher items	
• Very restless, has difficulty staying seated for long	• Restless, overactive, cannot stay still for long
• Squirmy, fidgety child	• Constantly fidgeting or squirming
• Inattentive, easily distracted	• Easily distracted, concentration wanders
• Excessive demands for teacher's attention	• Thinks things out before acting *(coding reversed)*
• Cannot settle to anything for more than a few moments	• Sees tasks through to the end, good attention span *(coding reversed)*
• Fails to finish things started – short attention span	

* Hogg, Rutter & Richman, 1997; ** SDQ; Goodman, 1997

Appendix 4: ADHD symptom items (CAPA interview & DSM-IV-TR)

Table A2

CAPA interview items measuring IOI/ADHD symptoms*

Outcome domain	Measurement items			
Inattention	Difficulty concentrating on tasks requiring sustained attention	Difficulty following instructions (not due to oppositional behaviour or failure to understand)	Often shifts from one uncompleted activity to another	Easily distracted by extraneous stimuli
	• Since she was 11 has s/he been able to concentrate on things s/he had to, such as reading or homework? • Has s/he had more problems concentrating than other young people his/her age?	*• Since s/he was 11 how good has s/he been at following through instructions from others? • Did s/he tend to complete things s/he'd been asked to do?*	*• Since s/he was 11 has s/he frequently jumped from one thing to another without finishing what s/he was doing?*	*• Since s/he was 11 has s/he found it difficult to pay attention when s/he could look out of the window or when s/he could hear people talking in the next room?*
Overactivity	Fidgetiness	Restlessness	Rushing about	
	• Since s/he was 11 how much has s/he tended to squirm or wiggle in his/her seat? Was this more than other children? • How much has s/he fidgeted with his/her hands or feet?	*• Since s/he was 11 has s/he usually been able to remain in his/her seat when s/he's supposed to? • Did s/he get up much more than other children (young people)?*	*• Did s/he tend to rush about more than other children?*	
Impulsivity	Often acts before thinking	Difficulty waiting for turn in games or group situations	Often blurts out answers to questions before they have been completed	Often interrupts or interrupts or intrudes on others
	• Since s/he was 11 has s/he usually tended to think about things before s/he did them? • Or did s/he tend to jump straight in impulsively without thinking about what might happen?	*• Since s/he was 11 has s/he been able to wait his/her turn for things? • As well as most children?*	*• Since s/he was 11 has s/he often blurted out the answers to questions before the person had finished the question?*	*• Since s/he was 11 has s/he tended to interrupt other people when they were talking to someone else? • What about butting into games or other activities without having been invited to join?*

* Rutter et al., 2004.

Table A3

DSM-IV-TR diagnostic criteria: Symptom items*

Outcome domain	Measurement items
Inattention	• Often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities
	• Often has difficulty sustaining attention in tasks or play activities
	• Often does not seem to listen when spoken to directly
	• Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behavior or failure to understand instructions)
	• Often has difficulty organizing tasks and activities
	• Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework
	• Often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books, or tools)
	• Is often easily distracted be extraneous stimuli
	• Is often forgetful in daily activities
Overactivity	• Often fidgets with hands of feet and squirms in seat
	• Often leaves seat in classroom or in other situations in which remaining seated is expected
	• Often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
	• Often has difficulty playing or engaging in leisure activities quietly
	• Is often "on the go" or often acts as if "driven by a motor"
	• Often talks excessively
Impulsivity	• Often blurts out answers before questions have been completed
	• Often has difficulty awaiting turn
	• Often interrupts or intrudes on others (e.g., butts into conversations or games)

* American Psychiatric Association, 2000

Appendix 5

Conduct problems: questionnaire items (Rutter Scales & SDQ)

Table A4

Questionnaire items measuring conduct problems: Revised Rutter Parent & Teacher Scales for school-age children*, the Strengths and Difficulties Questionnaire**

Questionnaire measure	
Rutter Scales	SDQ
Mother and father items	
• Frequently fights or is extremely quarrelsome with other children	• Often has temper tantrums or hot tempers
• Blames others for things	• Generally obedient, usually does adults request *(coding reversed)*
• Has stolen things on more than one occasion in the past 12 months	• Often fights with other children or bullies them
• Is often disobedient	• Often lies or cheats
• Kicks or bites other children	• Steals from home, school or elsewhere
• Often tells lies	
• Bullies other children	
• Inconsiderate of others	
Teacher items	
• Often destroys or damages own or others' property	• Often has temper tantrums or hot tempers
• Frequently fights or is extremely quarrelsome with other children	• Generally obedient, usually does adults request *(coding reversed)*
• Is often disobedient	• Often fights with other children or bullies them
• Often tells lies	• Often lies or cheats
• Has stolen things on one or more occasions in the past 12 months	• Steals from home, school or elsewhere
• Disturbs other children	
• Bullies other children	
• Blames others for things	
• Inconsiderate of others	
• Kicks, bites other children	

* Hogg, Rutter & Richman, 1997; ** SDQ; Goodman, 1997

Appendix 6

Genomic organisation of glucocorticoid receptor *BclI*– 9beta haplotypes

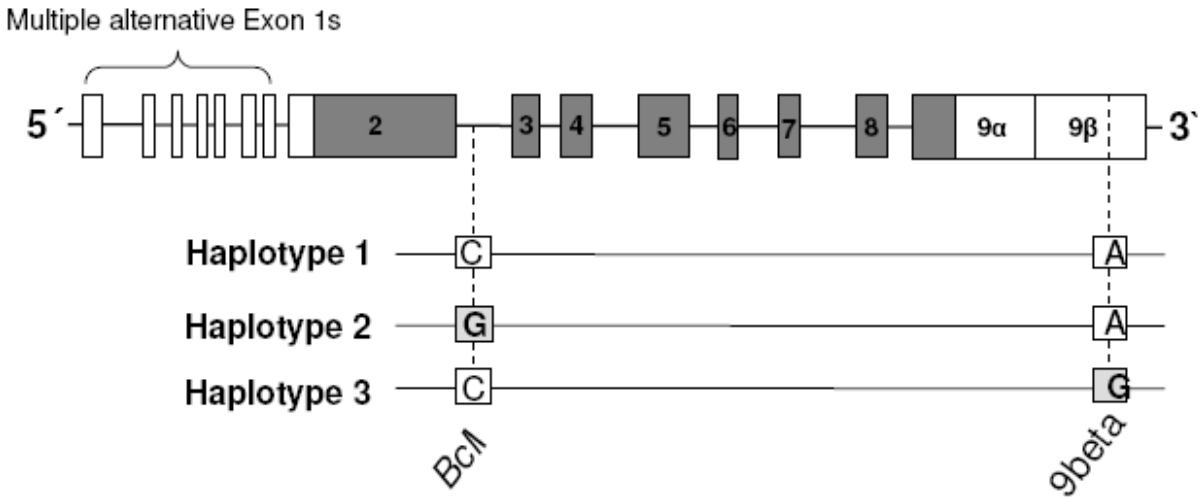


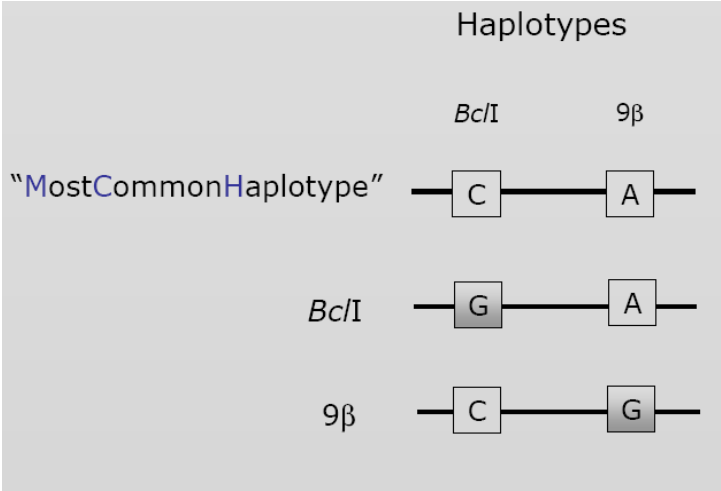
Figure A2

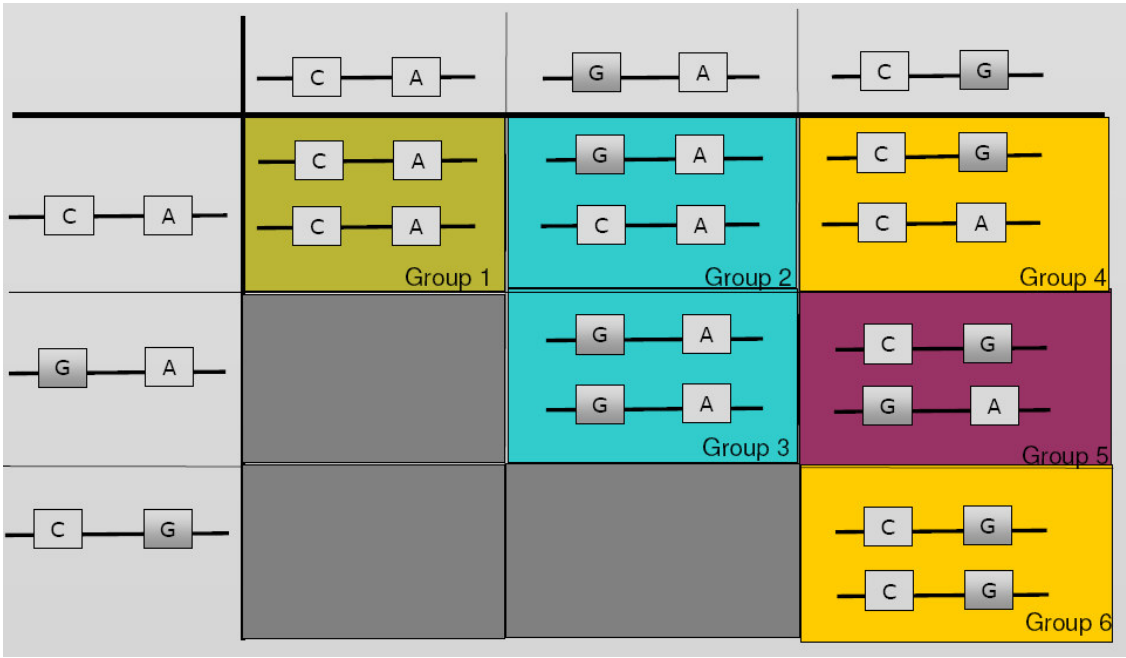
Genomic organisation of glucocorticoid receptor *BclI*-9beta haplotypes

---

Upper portion shows genomic organization of the human glucocorticoid receptor gene (*NR3C1).* Exons are indicated by boxes and the translated part of the gene is shown in darker shade. Lower portion of the diagram indicated the haplotype structure. Base pair substitutions are denoted by bold letters

R. Kumsta. (personal communication, November 7th 2007); adapted from Kumsta et al. (2007)





H-Group 1: two C-A alleles (Group 1 above) "MCH group"

H-Group 2: one or two G-A alleles (Groups 2+3 above) "BclI G group)

H-Group 3: one or two C-G alleles (Groups 4+6 above) "9beta G group)

H-Group 4: one C-G, one G-A allele (Group 5 above) "Mixed Group")

Figure A3

Glucocorticoid receptor *Bcl*I-9beta haplotype construction

Upper portion shows the possible GR *Bcl*1 – 9beta haplotypes.

The lower portion indicates how the haplotype groups were determined

R. Kumsta. (personal communication, November 7th 2007); adapted from Kumsta et al. (2007)

Appendix 7

Distribution of CAPA ADHD symptom scores

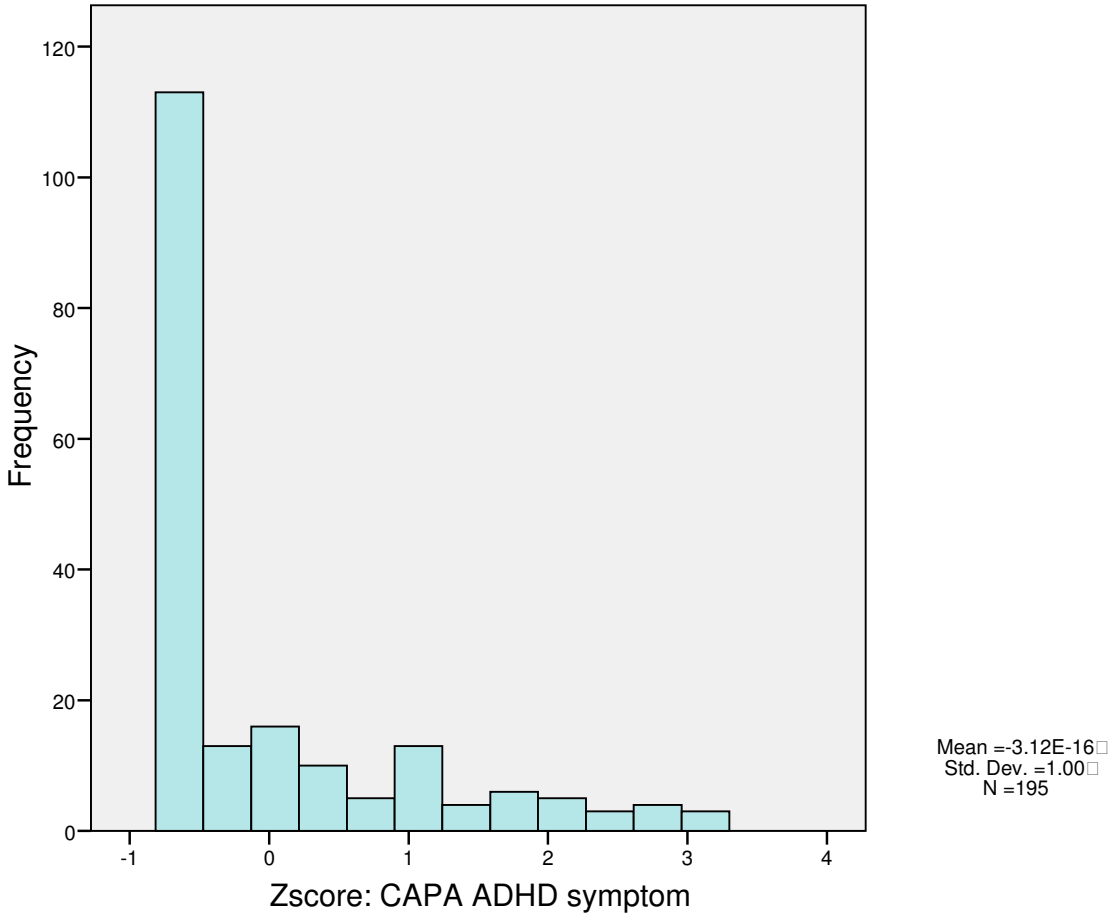


Figure A4

Distribution of CAPA ADHD z-scores

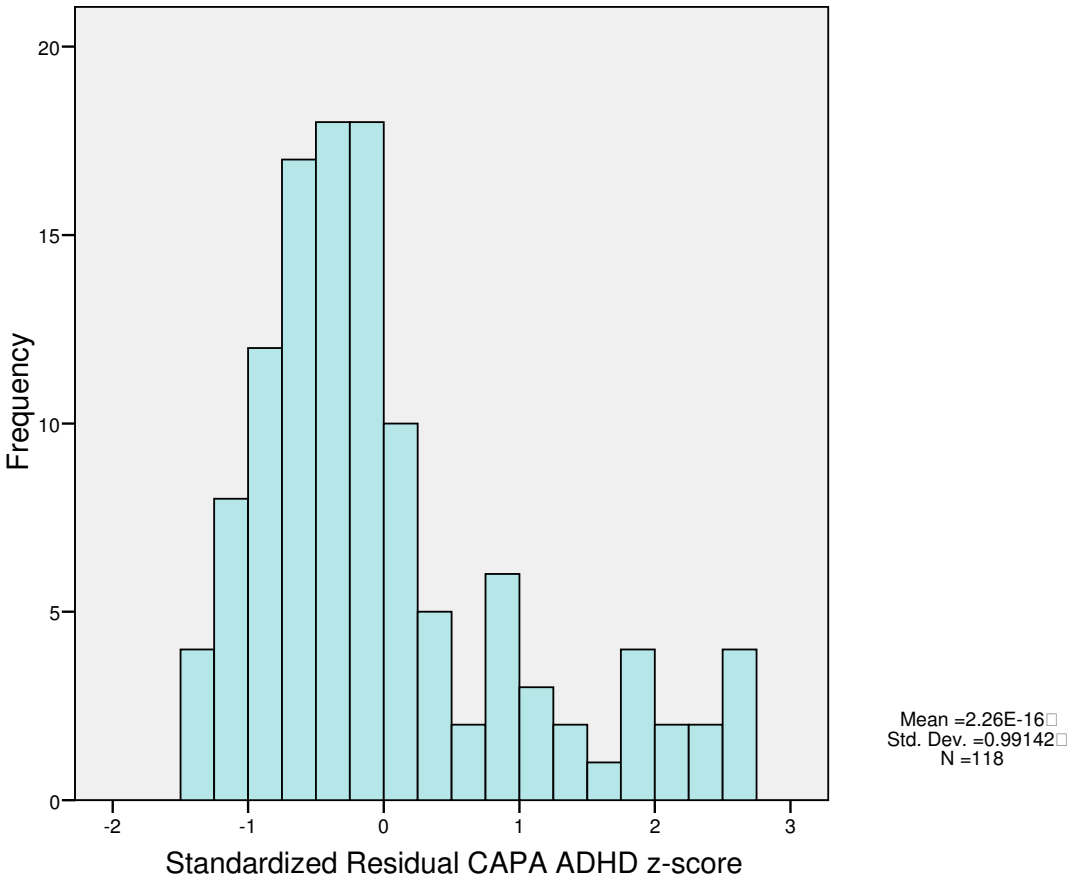


Figure A5

Distribution of standardised residual CAPA ADHD scores, from a regression analysis covarying for the effects of IQ and gender

Appendix 8

Ethical approval

Appendix 8a

Ethical approval of main ERA study: Mid-adolescent follow-up

Institute of Psychiatry

at The Maudsley

Ethical Committee
(Research)
Research Ethics Co-ordinator
Margaret M Chambers Msc

P006, Room W109
De Crespigny Park
Denmark Hill
London SE5 8AF
Tel 020 7848 0797
Fax 020 7848 0147
Email m.chambers@iop.kcl.ac.uk

KING'S College LONDON
University of London

ETHICAL COMMITTEE (RESEARCH)

1 December 2003

Prof M Rutter
SGDP Research Centre
Institute of Psychiatry

Dear Prof Rutter

Re: Developmental deficit and catch-up following profound early privation (59/92)

At its meeting on 21 November 2003, the Ethical Committee (Research) considered and confirmed Chair's action to approve the amendment to Study No. 59/92, as requested in your letter of 14 October 2003, from an ethical point of view.

Yours sincerely

Margaret M Chambers.

Margaret M Chambers
Research Ethics Co-ordinator

Appendix 8b

Ethical approval of GenERA study: Approval for initial year

Institute of Psychiatry

at The Maudsley

Ethical Committee
(Research)
Research Ethics Co-ordinator
Margaret M Chambers Msc

P006, Room W109
De Crespigny Park
Denmark Hill
London SE5 8AF
Tel 020 7848 0797
Fax 020 7848 0147
Email m.chambers@iop.kcl.ac.uk

KING'S College LONDON
University of London

ETHICAL COMMITTEE (RESEARCH)

28 July 2005

Prof E Sonuga-Barke
SGDP
PO80
Institute of Psychiatry

Dear Prof Sonuga-Barke

Re: Early deprivation, genetic risk and behavioural outcomes (107/05 or 05/Q0706/174)

The Ethical Committee (Research) considered and approved the above study at its meeting on 22 July 2005. **This approval is subject to the addition of a sentence in the DNA sampling information sheet, to the effect that any future, new use of the samples for research will only occur after ethical approval has been given. (In the paragraph – 'What does this part of the study involve?)**

Initial approval is given for one year. This will be extended automatically only on completion of annual progress reports on the study when requested by the EC(R). Please note that as Principal Investigator you are responsible for ensuring these reports are sent to us.

Please note that projects which have not commenced within two years of original approval must be re-submitted to the EC(R).

Any serious adverse events which occur in connection with this study should be reported to the Committee using the attached form.

Please quote Study No. 107/05 in all future correspondence with the IOP/SLAM Research Ethics Office.

When corresponding with other LRECs, please quote Study No. **05/Q0706/174**, which is the study number as registered on the national Research Ethics Database.

Yours sincerely,

Margaret M. Chambers

Margaret M Chambers
Research Ethics Coordinator

Appendix 8c

Ethical approval of GenERA study: Approval for duration of project

NHS
London

The Joint South London and Maudsley and the Institute of Psychiatry Research Ethics Committee

Camberwell Building
94 Denmark Hill
London SE5 9RS

Telephone: 020 3299 5033

15 January 2007

Prof Edmund Sonuga-Barke
PO 80 SGDP Centre
Institute of Psychiatry
De Crespigny Park
London SE5 8AF

Dear Prof Sonuga-Barke

Full title of study: **The role of genetic risk in the heterogeneity of outcome following early institutional deprivation**

REC reference number: **05/Q0706/174**

Thank you for sending the progress report for the above study dated 15 December 2006. The report has now been reviewed by the Chair of the Research Ethics Committee and I can confirm that the favourable ethical opinion for the study continues to apply for the duration of the research.

05/Q0706/174	**Please quote this number on all correspondence**

Yours sincerely

Jenny Liebscher
Committee Co-ordinator

Email: ethics.office@iop.kcl.ac.uk

An advisory committee to the London Strategic Health Authority

REFERENCES

Aardoom, H. A., Hirasing, R. A., Rona, R. J., Sanavro, F. L., vandenHeuvel, E. W., & Leeuwenburg, J. (1997). Food intolerance (food hypersensitivity) and chronic complaints in children: The parents' perception. *European Journal of Pediatrics, 156,* 110-112.

Allhusen, V., Belsky, J., Kersey, H. B., Booth-Laforce, C., Bradley, R., Brownell, C. A. et al. (2005). Predicting individual differences in attention, memory, and planning in first graders from experiences at home, child care, and school. *Developmental Psychology, 41,* 99-114.

American Psychiatric Association. (2000). Diagnostic and Statistical Manual of Mental Disorders, version IV, text revision. Washington, DC: American Psychiatric Association.

Angold, A. & Costello, E. J. (2000). The Child and Adolescent Psychiatric Assessment (CAPA). *Journal of the American Academy of Child & Adolescent Psychiatry.39 (1):* 39-48.

Angold, A., Prendergast, M., Cox, A., Harrington, R., Simonoff, E., & Rutter, M. (1995). The Child and Adolescent Psychiatric-Assessment (Capa). *Psychological Medicine, 25,* 739-753.

Antrop, I., Roeyers, H., Van Oost, P., & Buysse, A. (2000). Stimulation seeking and hyperactivity in children with ADHD. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 41,* 225-231.

Antrop, I., Stock, P., Verte, S., Wiersema, J. R., Baeyens, D., & Roeyers, H. (2006). ADHD and delay aversion: the influence of non-temporal stimulation on choice for delayed rewards. *Journal of Child Psychology and Psychiatry, 47,* 1152-1158.

Arnold, L. E. (1996). Sex differences in ADHD: Conference summary. *Journal of Abnormal Child Psychology, 24,* 555-569.

Applied Biosystems. TaqMan SNP genotyping information. Retrieved August 28th 2008, from *http://www.appliedbiosystems.com*

Asherson, P., Brookes, K., Franke, B., Chen, W., Gill, M., Ebstein, R. P. et al. (2007). Confirmation that a specific haplotype of the dopamine transporter gene is associated with combined-type ADHD. *American Journal of Psychiatry, 164,* 674-677.

Asherson, P. & IMAGE Consortium (2004). Attention-Deficit Hyperactivity Disorder in the post-genomic era. *European Child & Adolescent Psychiatry.13 Suppl 1:* I50-70.

Asherson, P., Kuntsi, J., & Taylor, E. (2005). Unravelling the complexity of attention-deficit hyperactivity disorder: a behavioural genomic approach. *British Journal of Psychiatry, 187,* 103-105.

Auerbach, J. G., Landau, R., Berger, A., Arbelle, S., Faroy, M., & Karplus, M. (2005). Neonatal behavior of infants at familial risk for ADHD. *Infant Behavior & Development, 28,* 220-224.

Banaschewski, T., Hollis, C., Oosterlaan, J., Roeyers, H., Rubia, K., Willcutt, E. et al. (2005). Towards an understanding of unique and shared pathways in the psychopathophysiology of ADHD. *Developmental Science, 8,* -140.

Banerjee, T. D., Middleton, F., & Faraone, S. V. (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatr, 96*.

Barkley, R. A. (1997). Behavioral Inhibition, Sustained Attention, and Executive Functions: Constructing a Unifying Theory of ADHD. *Psychological Bulletin, 121,* 65-94.

Baron, R. M. & Kenny, D. A. (1986). The Moderator-Mediator Variable Distinction in Social Psychological Research: Conceptual, Strategic, and Statistical Considerations. *Journal of Personality & Social Psychology, 51,* 1173-1182.

Becker, L. A. (1998/1999). Effect size calculator. *http://web.uccs.edu/lbecker/Psy590/escalc3.htm*. Retrieved April 5th 2008.

Beckett, C., Castle, J., Groothues, C., O'Connor, T. G., Rutter, M. and the English and Romanian Adoptees (E.R.A) study team (2003). Health problems in children adopted from Romania: Association with duration of deprivation and behavioural problems. *Adoption and Fostering, 27,* 19-29.

Beckett, C., Maughan, B., Rutter, M., Castle, J., Colvert, E., Groothues, C. et al. (2006). Do the Effects of Early Severe Deprivation on Cognition Persist Into Early Adolescence? Findings From the English and Romanian Adoptees Study. *Child Development, 77,* -711.

Behar, L. & Stringfield, S. (1974). Behavior Rating Scale for Preschool Child. *Developmental Psychology, 10,* 601-610.

Belsky, J., Fearon, R. M. P., & Bell, B. (2007). Parenting, attention and externalizing problems: testing mediation longitudinally, repeatedly and reciprocally. *Journal of Child Psychology and Psychiatry, 48,* 1233-1242.

Bentler, P. M. & Bonett, D. G. (1980). Significance Tests and Goodness of Fit in the Analysis of Covariance-Structures. *Psychological Bulletin, 88,* 588-606.

Bhutta, A. T., Cleves, M. A., Casey, P. H., Cradock, M. M., & Anand, K. J. S. (2002). Cognitive and behavioral outcomes of school-aged children who were born preterm - A meta-analysis. *Jama-Journal of the American Medical Association, 288,* 728-737.

Biederman, J. & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet, 366,* 237-248.

Biederman, J., Faraone, S. V., Spencer, T., Wilens, T., Norman, D., Lapey, K. A. et al. (1993). Patterns of Psychiatric Comorbidity, Cognition, and Psychosocial Functioning in Adults with Attention-Deficit Hyperactivity Disorder. *American Journal of Psychiatry, 150,* 1792-1798.

Biederman, J., Mick, E., Faraone, S. V., Braaten, E., Doyle, A., Spencer, T. et al. (2002). Influence of gender on attention deficit hyperactivity disorder in children referred to a psychiatric clinic. *American Journal of Psychiatry, 159,* 36-42.

Bitsakou, P., Antrop, I., Wiersema, J. R., & Sonuga-Barke, E. J. S. (2006). Probing the limits of delay intolerance: Preliminary young adult data from the Delay Frustration Task (DeFT). *Journal of Neuroscience Methods, 151,* 38-44.

Brieber, S., Neufang, S., Bruning, N., Kamp-Becker, I., Remschmidt, H., Herpertz-Dahlmann, B. et al. (2007). Structural brain abnormalities in adolescents with autism spectrum disorder and patients with attention deficit/hyperactivity disorder. *Journal of Child Psychology and Psychiatry, 48,* 1251-1258.

Briscoe-Smith, A. M. & Hinshaw, S. P. (2006). Linkages between child abuse and attention-deficit/hyperactivity disorder in girls: Behavioral and social correlates. *Child Abuse & Neglect, 30,* 1239-1255.

Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N. et al. (2006a). The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. *Molecular Psychiatry, 11,* 934-953.

Brookes, K. J., Xu, X. H., Chen, C. K., Huang, Y. S., Wu, Y. Y., & Asherson, P. (2005). No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. *Bmc Medical Genetics, 6*.

Brookes, K.-J., Mill, J., Guindalini, C., Curran, S., Xu, X., Knight, J. et al. (2006b). A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Archives of General Psychiatry.Vol.63(1)()(pp 74-81), 2006.,* 74-81.

Browne, M. W., & Cudeck, R. (1993). Alternative ways of assessing model fit. In K. A. Bollen & J. S. Long (Eds.), *Testing structural equation models* (pp. 136-162). Newburry Park, CA.: Sage.

Bruer, J. T. (2001). A critical and sensitive period primer. In D. B. Bailey, J. T. Bruer, F. J. Symons & J. W. Lichtman (Eds.), *Critical thinking about critical periods* (pp.3-26). Baltimore: Brookes.

Burgess, K. B., Marshall, P. J., Rubin, K. H., & Fox, N. A. (2003). Infant attachment and temperament as predictors of subsequent externalizing problems and cardiac physiology. *Journal of Child Psychology and Psychiatry, 44,* 819-831.

Burke, J. D., Loeber, R., Lahey, B. B., & Rathouz, P. J. (2005). Developmental transitions among affective and behavioral disorders in adolescent boys. *Journal of Child Psychology and Psychiatry, 46,* 1200-1210.

Carlson, E. A., Sampson, M. C., & Sroufe, L. A. (2003). Implications of Attachment Theory and Research for Developmental-Behavioral Pediatrics. *Journal of Developmental & Behavioral Pediatrics, 24,* -379.

Carlson, M. & Earls, F. (1997). Psychological and neuroendocrinological sequelae of early social deprivation in institutionalized children in Romania. In C. Carter, I. Lederhendler, B. Kirkpatrick, (Eds.). *The integrative neurobiology of affiliation*. (pp. 419-428). New York: New York Academy of Sciences

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W. et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science.297:* 851-4.

Caspi, A. & Moffitt, T. E. (2006). Opinion - Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nature Reviews Neuroscience, 7,* 583-590.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H. et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.301:* 386-9.

Castellanos, F. X., Lee, P. P., Sharp, W., Jeffries, N. O., Greenstein, D. K., Clasen, L. S. et al. (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *Jama-Journal of the American Medical Association, 288,* 1740-1748.

Castellanos, F. X., Sonuga-Barke, E. J. S., Milham, M. P., & Tannock, R. (2006). Characterizing cognition in ADHD: Beyond executive dysfunction. *Trends in Cognitive Sciences, 10,* 117-123.

Castle, J., Beckett, C., Groothues, C., & the English and Romanian Adoptees Study Team (2000). Infant adoption in England: a longitudinal account of social and cognitive progress. *Adoption and Fostering,* 24, 26 - 35.

Castle, J., Groothues, C., Bredenkamp, D., Beckett, C., O'Connor, T., Rutter, M. et al. (1999). Effects of qualities of early institutional care on cognitive attainment. *American Journal of Orthopsychiatry, 69,* 424-437.

Children's Health Care Collaborative Study Group (1992). Romanian Health and Social Care System for Children and Families - Future-Directions in Health-Care Reform. *British Medical Journal, 304,* 556-559.

Chisholm, K. (1998). A three year follow-up of attachment and indiscriminate friendliness in children adopted from Romanian orphanages. *Child Development, 69,* 1092-1106.

Christiansen, H., Chen, W., Oades, R. D., Asherson, P., Taylor, E. A., Lasky-Su, J. et al. (2008). Co-transmission of conduct problems with attention-deficit/hyperactivity disorder: familial evidence for a distinct disorder. *Journal of Neural Transmission, 115,* 163-175.

Chronis, A. M., Lahey, B. B., Pelham, W. E., Williams, S. H., Baumann, B. L., Kipp, H. et al. (2007). Maternal depression and early positive parenting predict future conduct problems in young children with attention-deficit/hyperactivity disorder. *Developmental Psychology, 43,* 70-82.

Clarke, L., Ungerer, J., Chahoud, K., Johnson, S., & Stiefel, I. (2002). Attention deficit hyperactivity disorder is associated with attachment insecurity. *Clinical Child Psychology and Psychiatry, 7,* 179-198.

Coghill, D., Nigg, J., Rothenberger, A., Sonuga-Barke, E., & Tannock, R. (2005). Whither causal models in the neuroscience of ADHD? *Developmental Science, 8,* 105-114.

Colvert, E., Rutter, M., Beckett, C., Castle, J., Groothues, C., Hawkins, A. et al. (2008). Emotional difficulties in early adolescence following severe early deprivation: Findings from the English and Romanian adoptees study. *Development and Psychopathology, 20,* 547-567.

Comings, D., Gade-Andavolu, R., Gonzalez, N., Wu, S., Muhleman, D., Blake, H. et al. (2000). Comparison of the role of dopamine, serotonin, and noradrenaline genes in ADHD, ODD and conduct disorder: Multivariate regression analysis of 20 genes. *Clinical Genetics, 5.,* 178-196.

Cook, E. H., Stein, M. A., Krasowski, M. D., Cox, N. J., Olkon, D. M., Kieffer, J. E. et al. (1995). Association of Attention-Deficit Disorder and the Dopamine Transporter Gene. *American Journal of Human Genetics, 56,* 993-998.

Croft, C., Beckett, C., Rutter, M., Castle, J., Colvert, E., Groothues, C. et al. (2007). Early adolescent outcomes of institutionally-deprived and non-deprived adoptees. II: Language as a protective factor and a vulnerable outcome. *Journal of Child Psychology and Psychiatry, 48,* 31-44.

Crosbie, J. & Schachar, R. (2001). Deficient inhibition as a marker for familial ADHD. *American Journal of Psychiatry, 158,* 1884-1890.

de Kloet, E. R., Sibug, R. M., Helmerhorst, F. M., & Schmidt, M. (2005). Stress, genes and the mechanism of programming the brain for later life. *Neuroscience and Biobehavioral Reviews, 29,* 271-281.

Derijk, R. H., Schaaf, M. J. M., Turner, G., Datson, N. A., Vreugdenhil, E., Cidlowski, J. et al. (2001). A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *Journal of Rheumatology, 28,* 2383-2388.

Dickstein, S. G., Bannon, K., Castellanos, F. X., & Milham, M. P. (2006). The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. *Journal of Child Psychology and Psychiatry, 47,* 1051-1062.

DiMaio, S., Grizenko, N., & Joober, R. (2003). Dopamine genes and attention-deficit hyperactivity disorder: a review. *Journal of Psychiatry & Neuroscience, 28,* 27-38.

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., & Fischman, A. J. (1999). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet, 354,* 2132-2133.

Doyle, A. E. (2006). Executive functions in attention-deficit/hyperactivity disorder. *Journal of Clinical Psychiatry, 67,* 21-26.

Elander, J. & Rutter, M. (1996). Use and development of the Rutter parents' and teachers' scales. *International Journal of Methods in Psychiatric Research.Vol 6(2),* 63-78.

Eley, T. C., Sugden, K., Corsico, A., Gregory, A. M., Sham, P., McGuffin, P. et al. (2004). Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Molecular Psychiatry, 9.,* 908-915.

Ellison-Wright, I., Ellison-Wright, Z., & Bullmore, E. (2008). Structural brain change in Attention Deficit Hyperactivity Disorder identified by meta-analysis. *Bmc Psychiatry, 8*.

Faraone, S. V., Perlis, R. H., Doyle, A. E., Smoller, J. W., Goralnick, J. J., Holmgren, M. A. et al. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry.57(11):1313-23*.

Faraone, S V., Sergeant, J., Gillberg, C. & Biederman, J. (2003). The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry*, 2, 104-113.

Fearon, R. M. P. & Belsky, J. (2004). Attachment and attention: Protection in relation to gender and cumulative social-contextual adversity. *Child Development, 75,* 1677-1693.

Fergusson, D. M. & Horwood, L. J. (1995). Predictive validity of categorically and dimensionally scored measures of disruptive childhood behaviors. *Journal of the American Academy of Child & Adolescent Psychiatry, 34,* -485.

Finzi-Dottan, R., Manor, I., & Tyano, S. (2006). ADHD, temperament, and parental style as predictors of the child's attachment patterns. *Child Psychiatry & Human Development, 37,* 103-114.

Fisher, L., Ames, E. W., Chisholm, K., & Savoie, L. (1997). Problems reported by parents of Romanian orphans adopted to British Columbia. *International Journal of Behavioral Development, 20,* 67-82.

Fleury, I., Beau-Lieu, P., Primeau, M., Labuda, D., Sinnett, D., & Krajinovic, M. (2003). Characterization of the BclI polymorphism in the glucocorticoid receptor gene. *Clinical Chemistry, 49,* 1528-1531.

Frankenburg, W. K., van Doornick, W. J., Liddell, T. N., & Dick, N. P. (1986). *Revised Denver Prescreening Developmental Questionnaire (R-PDQ)*. High Wycombe, UK: DDM Incorporated/The Test Agency.

Frazier, T. W., Demaree, H. A., & Youngstrom, E. A. (2004). Meta-analysis of intellectual and neuropsychological test performance in attention-deficit/hyperactivity disorder. *Neuropsychology, 18,* 543-555.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., & Craig, I. W. (2003). DNA from buccal swabs recruited by mail: Evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. *Behavior Genetics, 33,* 67-72.

French, N. P., Hagan, R., Evans, S. F., Mullan, A., & Newnham, J. P. (2004). Repeated antenatal corticosteroids: Effects on cerebral palsy and childhood behavior. *American Journal of Obstetrics and Gynecology, 190,* 588-595.

Gainetdinov, R. R. & Caron, M. C. (2001). Genetics of childhood disorders: XXIV. ADHD, part 8: Hyperdopaminergic mice as an animal model of ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry, 40,* 380-382.

Galler, J. R. & Ramsey, F. (1989). A follow-up study of the influence of early malnutrition on development: Behavior at home and at school. *Journal of the American Academy of Child & Adolescent Psychiatry 28*, 254-261.

Gaub, M. B. & Carlson, C. L. P. (1997). Gender Differences in ADHD: A Meta-Analysis and Critical Review. *Journal of the American Academy of Child & Adolescent Psychiatry, 36,* 1036-1045.

Gervai, J., Nemoda, Z., Lakatos, K., Ronai, Z., Toth, I., Ney, K. et al. (2005). Transmission disequilibrium tests confirm the link between DRD4 gene polymorphism and infant attachment. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics,1,* 126-30.

Geurts, H. M., Vertie, S., Oosterlaan, J., Roeyers, H., & Sergeant, J. A. (2004). How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism? *Journal of Child Psychology and Psychiatry, 45,* 836-854.

Giros, B., Elmestikawy, S., Godinot, N., Zheng, K. Q., Han, H., Yangfeng, T. et al. (1992). Cloning, Pharmacological Characterization, and Chromosome Assignment of the Human Dopamine Transporter. *Molecular Pharmacology, 42,* 383-390.

Glod, C. A. & Teicher, M. H. (1996). Relationship between early abuse, posttraumatic stress disorder, and activity levels in prepubertal children. *Journal of the American Academy of Child and Adolescent Psychiatry, 35,* 1384-1393.

Goldfarb, W. (1945). Effects of psychological deprivation in infancy and subsequent stimulation. *American Journal of Psychiatry, 102,* 18-33.

Goodman, R. (1997). The strengths and difficulties questionnaire: A research note. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 38,* 581-586.

Goodman, R. (1999). The extended version of the Strengths and Difficulties Questionnaire as a guide to child psychiatric caseness and consequent burden. *Journal of Child Psychology & Psychiatry & Allied Disciplines.40(5):791-9*.

Goodman, R. (2001). Psychometric Properties of the Strengths and Difficulties Questionnaire. *Journal of the American Academy of Child & Adolescent Psychiatry, 40,* 1337-1345.

Goodman, R., Simonoff, E., & Stevenson, J. (1995). The impact of child IQ, parent IQ and sibling IQ on child behavioural deviance scores. *Journal of Child Psychology and Psychiatry, 36,* 409-425.

Groothues, C., Beckett, C., O'Connor, T. G. (1998/1999). The outcomes of adoptions from Romania: predictors of parental satisfaction. *Adoption and Fostering*, 22, 30-40.

Gunnar, M. & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58,* 145-173.

Gunnar, M. R., Morison, S. J., Chisholm, K., & Schuder, M. (2001). Salivary cortisol levels in children adopted from romanian orphanages. *Development & Psychopathology.13(3):611-28*.

Gunnar, M. R., Larson, M. C., Hertsgaard, L., & Harris, M. L. (1992). The stressfulness of separation among nine-month-old infants: Effects of social context variables and infant temperament. *Child Development, 63,* 290-303.

Gunnar, M. R. & van Dulmen, M. H. M. (2007). Behavior problems in postinstitutionalized internationally adopted children. *Development and Psychopathology, 19,* 129-148.

Hadders-Algra, M. & Groothuis, A. M. C. (1999). Quality of general movements in infancy is related to neurological dysfunction, ADHD, and aggressive behaviour. *Developmental Medicine and Child Neurology, 41,* 381-391.

Harfstrand, A., Fuxe, K., Cintra, A., Agnati, L. F., Zini, I., Wikstrom, A. C. et al. (1986). Glucocorticoid Receptor Immunoreactivity in Monoaminergic Neurons of Rat-Brain. *Proceedings of the National Academy of Sciences of the United States of America, 83,* 9779-9783.

Hartung, C. M., Willcutt, E. G., Lahey, B. B., Pelham, W. E., Loney, J., Stein, M. A. et al. (2002). Sex differences in young children who meet criteria for attention deficit hyperactivity disorder. *Journal of Clinical Child and Adolescent Psychology, 31,* 453-464.

Henikoff, S. & Matzke, M. A. (1997). Exploring and explaining epigenetic effects. *Trends in Genetics, 13,* 293-295.

Heptinstall, E. & Taylor, E. (2002). Sex differences and their significance. In S. Sandberg (Ed.), *Hyperactivity and attention disorders of childhood,* (2nd ed., pp. 99-125). Cambridge, UK: Cambridge University Press.

Hill, S. Y., Lowers, L., Locke-Wellman, J., & Shen, S. A. (2000). Maternal smoking and drinking during pregnancy and the risk for child and adolescent psychiatric disorders. *Journal of Studies on Alcohol, 61,* 661-668.

Hinshaw, S. P. (1987). On the Distinction Between Attentional Deficits Hyperactivity and Conduct Problems Aggression in Child Psychopathology. *Psychological Bulletin, 101,* 443-463.

Hirshfeld-Becker, D. R., Biederman, J., Faraone, S. V., Violette, H., Wrightsman, J., & Rosenbaum, J. F. (2002). Temperamental correlates of disruptive behavior disorders in young children: Preliminary findings. *Biological Psychiatry, 51,* 563-574.

Horvath, J. & Markman, B. (2008). Attachment disorganization and ADHD - Are they overlapping conditions? An evaluation of executive functioning. *Clinical Neuropsychologist, 22,* 445-446.

Ising, M., Depping, A. M., Siebertz, A., Lucae, S., Unschuld, P. G., Kloiber, S. et al. (2008). Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *European Journal of Neuroscience, 28,* 389-398.

Jablonka, E. & Lamb, M. J. (2002). The changing concept of epigenetics. *Annals of the New York Academy of Sciences 981* (1), 82–96

Jaffee, S. R. & Price, T. S. (2007). Gene-environment correlations: a review of the evidence and implications for prevention of mental illness. *Molecular Psychiatry, 12,* 432-442.

Jensen, P. S. M., Martin, D. B., & Cantwell, D. P. M. (1997). Comorbidity in ADHD: Implications for Research, Practice, and DSM-V. *Journal of the American Academy of Child & Adolescent Psychiatry, 36,* 1065-1079.

Johnson, D. E. (2001). Medical and developmental sequelae of early childhood institutionalization in international adoptees from Romania and the Russian Federation. In C. Nelson (Ed.), *Effects of early adversity on neurobehavioral development* . Rahwah, NJ: Lawrence Erlbaum.

Juffer, F. & van IJzendoorn, M. H. (2005). Behavior problems and mental health referrals of international adoptees - A meta-analysis. *Jama-Journal of the American Medical Association, 293,* 2501-2515.

Kahn, R. S., Khoury, J., Nichols, W. C., & Lanphear, B. P. (2003). Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. *Journal of Pediatrics.143(1):104-10*.

Kaler, S. R. & Freeman, B. J. (1994). Analysis of Environmental Deprivation - Cognitive and Social-Development in Romanian Orphans. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 35,* 769-781.

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. *Journal of Physiology-London, 572,* 31-44.

Kapoor, A., Petropoulos, S., & Matthews, S. G. (2008). Fetal programming of hypothalamic-pituitary-adrenal (HPA) axis function and behavior by synthetic glucocorticoids. *Brain Research Reviews, 57,* 586-595.

Kaufman, J. & Charney, D. (2001). Effects of early stress on brain structure and function: Implications for understanding the relationship between child maltreatment and depression. *Development and Psychopathology, 13,* 451-471.

Kaufman, J., Plotsky, P. M., Nemeroff, C. B., & Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: Clinical implications. *Biological Psychiatry, 48,* 778-790.

Kbioscience. KASPar genotyping information. Retrieved April 25th 2007, from *http://www.kbioscience.co.uk*

Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression - A replication. *Archives of General Psychiatry, 62,* 529-535.

Keown, L. J. & Woodward, L. J. (2002). Early parent-child relations and family functioning of preschool boys with pervasive hyperactivity. *Journal of Abnormal Child Psychology, 30,* 541-553.

Kessler, R. C., Adler, L., Barkley, R., Biederman, J., Conners, C. K., Demler, O. et al. (2006). The prevalence and correlates of adult ADHD in the United States: Results from the National Comorbidity Survey Replication. *American Journal of Psychiatry, 163,* 716-723.

Kleeberger, S. & Cho, H.Y. (2008). Gene-environment interactions in environmental lung diseases. In M. Rutter (Ed.) *Genetic effects on environmental vulnerability to disease*. Chicester, UK: Wiley.

Kooij, J. J. S., Buitelaar, J. K., Van den Oord, E. J., Furer, J. W., Rijnders, C. A. T., & Hodiamont, P. P. G. (2005). Internal and external validity of Attention-Deficit Hyperactivity Disorder in a population-based sample of adults. *Psychological Medicine, 35,* 817-827.

Kreppner, J. M., Rutter, M., Beckett, C., Castle, J., Colvert, E., Groothues, C. et al. (2007). Normality and impairment following profound early institutional deprivation: A longitudinal follow-up into early adolescence. *Developmental Psychology, 43,* 931-946.

Kreppner, J. M., O'Connor, T. G., Rutter, M., Beckett, C., Castle, J., Croft, C. et al. (2001). Can inattention/overactivity be an institutional deprivation syndrome? *Journal of Abnormal Child Psychology, 29,* 513-528.

Kumsta, R., Entringer, S., Koper, J. W., van Rossum, E. F. C., Hellhammer, D. H., & Wust, S. (2007). Sex specific associations between common glucocorticoid receptor gene variants and hypothalamus-pituitary-adrenal axis responses to psychosocial stress. *Biological Psychiatry, 62,* 863-869.

Kumsta, R., Entringer, S., Koper, J.W., van Rossum, E.F.C., Hellhammer, D.H. & Wust, S. (2008). Glucocorticoid receptor gene polymorphisms and glucocorticoid sensitivity of subdermal blood vessels and leukocytes. *Biological Psychology. 79.* 179-184.

Kuntsi, J., Eley, T. C., Taylor, A., Hughes, C., Asherson, P., Caspi, A. et al. (2004). Co-occurrence of ADHD and low IQ has genetic origins. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics, 124B,* 41-47.

Lahey, B. B., Pelham, W. E., Chronis, A., Massetti, G., Kipp, H., Ehrhardt, A. et al. (2006). Predictive validity of ICD-10 hyperkinetic disorder relative to DSM-IV attention-deficit/hyperactivity disorder among younger children. *Journal of Child Psychology and Psychiatry, 47,* 472-479.

Lakatos, K., Nemoda, Z., Toth, I., Ronai, Z., Ney, K., Sasvari-Szekely, M. et al. (2002). Further evidence for the role of the dopamine D4 receptor (DRD4) gene in attachment disorganization: Interaction of the exon III 48-bp repeat and the -521 C/T promoter polymorphisms. *Molecular Psychiatry,1,* 27-31.

Lakatos, K., Toth, I., Nemoda, Z., Ney, K., Sasvari-Szekely, M., & Gervai, J. (2000). Dopamine D4 receptor (DRD4) gene polymorphism is associated with attachment disorganization in infants. *Molecular Psychiatry,5, 633-7*.

Lander, E. S. & Schork, N. J. (1994). Genetic Dissection of Complex Traits. *Science, 265,* 2037-2048.

Laucht, M., Skowronek, M. H., Becker, K., Banaschewski, T., Schmidt, M. H., Esser, G. et al. (2007). Psychosocial adversity moderates the impact of the dopamine transporter gene on inattention and hyperactivity-impulsivity among 15-year-olds. *Journal of Neural Transmission, 114,* LXXXIII.

Levitt, M. (1999). Toxic metals, preconception and early childhood development. *Social Science Information Sur les Sciences Sociales, 38,* 179-201.

Li, D. W., Sham, P. C., Owen, M. J., & He, L. (2006). Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). *Human Molecular Genetics, 15,* 2276-2284.

Linnet, K. M., Dalsgaard, S., Obel, C., Wisborg, K., Henriksen, T. B., Rodriguez, A. et al. (2003). Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: Review of the current evidence. *American Journal of Psychiatry, 160,* 1028-1040.

Little, T. D., Henrich, C. C., Jones, S. M., & Hawley, P. H. (2003). Disentangling the "whys" from the "whats" of aggressive behaviour. *International Journal of Behavioral Development, 27,* 122-133.

Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology, 12,* 5-12.

MacLean, K. (2003). The impact of institutionalization on child development. *Development & Psychopathology.15(4):853-84*.

Maniadaki, K., Sonuga-Barke, E., & Kakouros, E. (2005). Parents' causal attributions about attention deficit/hyperactivity disorder: the effect of child and parent sex. *Child Care Health and Development, 31,* 331-340.

Marcovitch, S., Goldberg, S., Gold, A., & Washington, J. (1997). Determinants of behavioural problems in Romanian children adopted in Ontario. *International Journal of Behavioral Development, 20,* -31.

McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K. et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet, 370,* 1560-1567.

McCarthy, D. (1972). The McCarthy scales of children's abilities. New York: The Psychological Corporation/Harcourt Brace Jovanovich, Inc.

McEwan, B. S. (1999). The effects of stress on structural and functional plasticity in the hippocampus. In D. S. Charney, E. J. Nestler & B. S. Bunney (Eds.), *Neurobiology of mental illness* (pp. 475-493), New York: Oxford University Press.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience, 24,* 1161-1192.

Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology, 27,* 127-138.

Meehl, P. E. (1992). Factors and Taxa, Traits and Types, Differences of Degree and Differences in Kind. *Journal of Personality, 60,* 117-174.

Meehl, P. E. (2004). What's in a taxon? *Journal of Abnormal Psychology, 113,* 39-43.

M.A., Golembo N.I., Nosarti C., Colvert E., Mota A., Williams S.C.R., Rutter M., Sonuga-Barke E.J.S. (2008). *Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: The English and Romanian Adoptees study*. Manuscript submitted for publication.

Meltzer, H., Gatward, R., Goodman, R., and Ford, F. (2000). *Mental health of children and adolescents in Great Britain*. London: The Stationery Office.

Mick, E. & Faraone, S. V. (2008). Genetics of attention deficit hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America, 17,* 261-284.

Mick, E., Biederman, J., Faraone, S. V., Sayer, J., & Kleinman, S. (2002). Case-control study of attention-deficit hyperactivity disorder and maternal smoking, alcohol use and drug use during pregnancy. *Journal of the American Academy of Child and Adolescent Psychiatry, 41,* 378-385.

Mill, J. & Petronis, A. (2008). Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): the potential role of epigenetic processes in mediating susceptibility. *Journal of Child Psychology and Psychiatry*. *49*, 120-130.

Moffitt, T. E., Caspi, A., & Rutter, M. (2005). Strategy for Investigating Interactions Between Measured Genes and Measured Environments. *Archives of General Psychiatry, 62,* 473-481

Muthén, L. K., & Muthén, B. O. (1998-2006). *Mplus. Statistical analyses with latent variables. User's guide* (4.1 ed.). Los Angeles: Muthén & Muthén.

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

Needleman, H. L. (1982). Lead and Impaired Abilities. *Developmental Medicine and Child Neurology, 24,* 196-197.

Newcorn, J. H., Halperin, J. M., Jensen, P. S., Abikoff, H. B., Arnold, E., Cantwell, D. P. et al. (2001). Symptom profiles in children with ADHD: Effects of comorbidity and gender. *Journal of the American Academy of Child & Adolescent Psychiatry, 40,* 137-146.

Nigg, J. T. (2005). Neuropsychologic Theory and Findings in Attention-Deficit/Hyperactivity Disorder: The State of the Field and Salient Challenges for the Coming Decade. *Biological Psychiatry, 57,* 1424-1435.

Nutt, D. J., Fone, K., Asherson, P., Bramble, D., Hill, P., Matthews, K. et al. (2007). Evidence-based guidelines for management of attention-deficit/hyperactivity disorder in adolescents in transition to adult services and in adults: recommendations from the British Association for Psychopharmacology. *Journal of Psychopharmacology, 21,* 10-41.

O'Connor, T. G., Caspi, A., Defries, J. C., & Plomin, R. (2003a). Genotype-environment interaction in children's adjustment to parental separation. *Journal of Child Psychology & Psychiatry,.44, 849-56*.

O'Connor, T. G., Heron, J., Golding, J., & Glover, V. (2003b). Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 44,* 1025-1036.

O'Connor, T. G., Rutter, M., Beckett, C., Keaveney, L., Kreppner, J. M., & English & Romanian Adoptees Study Team (2000). The effects of global severe privation on cognitive competence: Extension and longitudinal follow-up. *Child Development, 71,* 376-390.

Pani, L., Porcella, A., & Gessa, G. L. (2000). The role of stress in the pathophysiology of the dopaminergic system. *Molecular Psychiatry, 5,* 14-21.

Piazza, P. V. & LeMoal, M. (1996). Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annual Review of Pharmacology and Toxicology, 36,* 359-378.

Pineda, D. A., Puerta, I. C., Merchan, V., Arango, C. P., Galvis, A. Y., Velasquez, B. et al. (2003). Perinatal factors associated with attention deficit/hyperactivity diagnosis in Colombian 'paisa' children. *Revista de Neurologia, 36,* 609-613.

Pliszka, S. R. (1998). Comorbidity of attention-deficit/hyperactivity disorder with psychiatric disorder: An overview. *Journal of Clinical Psychiatry, 59,* 50-58.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57,* 1385-1390.

Plotsky, P. M. & Meaney, M. J. (1993). Early, Postnatal Experience Alters Hypothalamic Corticotropin-Releasing Factor (Crf) Messenger-Rna, Median-Eminence Crf Content and Stress-Induced Release in Adult-Rats. *Molecular Brain Research, 18,* 195-200.

Polanczyk, G., De Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: A systematic review and metaregression analysis. *American Journal of Psychiatry.164,* 942-948.

Ramsay, M. C. & Reynolds, C. R. (2000). Does smoking by pregnant women influence IQ, birth weight, and developmental disabilities in their infants? A methodological review and multivariate analysis. *Neuropsychology Review, 10,* 1-40.

Rohde, L. A., Szobot, C., Polanczyk, G., Schmitz, M., Martins, S., & Tramontina, S. (2005). Attention-Deficit/Hyperactivity Disorder in a Diverse Culture: Do Research and Clinical Findings Support the Notion of a Cultural Construct for the Disorder? *Biological Psychiatry, 57,* 1436-1441.

Roy, P., Rutter, M., & Pickles, A. (2000). Institutional care: Risk from family background or pattern of rearing? *Journal of Child Psychology & Psychiatry & Allied Disciplines, 41,* 139-149.

Roy, P., Rutter, M., & Pickles, A. (2004). Institutional care: Associations between overactivity and lack of selectivity in social relationships. [References]. *Journal of Child Psychology & Psychiatry & Allied Disciplines, 45,* 866-873.

Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G. et al. (1997). Mice Lacking Dopamine D4 Receptors Are Supersensitive to Ethanol, Cocaine, and Methamphetamine. *Cell, 90,* 991-1001.

Rucklidge, J. J. & Tannock, R. (2001). Psychiatric, psychosocial, and cognitive functioning of female adolescents with ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry, 40,* 530-540.

Rutter, M. (1983). Statistical and personal interactions: Facets and Perspectives. In. D. Magnusson & V. Allen (Eds.), *Human development: an interactional perspective* (pp. 295-319). New York: Academic Press .

Rutter, M. L. (1999). Psychosocial adversity and child psychopathology. *British Journal of Psychiatry, 174,* 480-493.

Rutter, M. (2006). *Genes and behavior: Nature-nurture interplay explained*. Oxford: Blackwell Publishing.

Rutter, M. (2008). Introduction: Whither gene-environment interactions? In M. Rutter (Ed.), *Genetic effects on environmental vulnerability to disease* (pp.1-12). Chicester, UK: Wiley.

Rutter, M., Andersen-Wood, L., Beckett, C., Bredenkamp, D., Castle, J., Groothues, C. et al. (1999). Quasi-autistic patterns following severe early global privation. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 40,* 537-549.

Rutter, M., Beckett, C., Castle, J., Colvert, E., Kreppner, J., Mehta, M., Stevens, S. & Sonuga-Barke, E. (2007). Effects of profound early institutional deprivation: An overview of findings from a UK longitudinal study of Romanian adoptees. *European Journal of Developmental Psychology, 4,* 332-350.

Rutter, M., Colvert, E., Kreppner, J., Beckett, C., Castle, J., Groothues, C. et al. (2007a). Early adolescent outcomes for institutionally-deprived and non-deprived adoptees. I: Disinhibited attachment. *Journal of Child Psychology and Psychiatry, 48,* 17-30.

Rutter, M. & English & Romanian Adoptees Study Team (1998). Developmental catch-up, and deficit, following adoption after severe global early privation. *Journal of Child Psychology & Psychiatry & Allied Disciplines, 39,* 465-476.

Rutter, M. L., Kreppner, J. M., O'Connor, T. G., & English and Romanian Adoptees (ERA) study team (2001). Specificity and heterogeneity in children's responses to profound institutional privation.[erratum appears in Br J Psychiatry 2001 Oct;179:371]. *British Journal of Psychiatry.179:97-103*.

Rutter, M., Kreppner, J., Croft, C., Murin, M., Colvert, E., Beckett, C. et al. (2007b). Early adolescent outcomes of institutionally deprived and non-deprived adoptees. III. Quasi-autism. *Journal of Child Psychology and Psychiatry, 48,* 1200-1207.

Rutter, M., Moffitt, T. E., & Caspi, A. (2006). Gene-environment interplay and psychopathology: multiple varieties but real effects. *Journal of Child Psychology and Psychiatry, 47,* 226-261.

Rutter, M., O'Connor, T., Beckett, C., Castle, J., Croft, C., Dunn, J. et al. (2000). Recovery and deficit following profound early deprivation. In P.Selman (Ed.), *Edited Collection on Inter-country Adoption* (pp. 107-125). London: BAAF.

Rutter, M. & O'Connor, T. G. (2004). Are there biological programming effects for psychological development? Findings from a study of Romanian adoptees. *Developmental Psychology, 40,* 81-94.

Rutter, M. & Pickles, A. (1991). Person-environment interactions: Concepts, mechanisms, and implications for data analysis. In T. Wachs & R. Plomin (Eds.) Conceptualisation and measurement of organism-environment interaction, pp. 105-141. Washington, DC: American Psychological Association.

Rutter, M., Roy, P. & Kreppner, J. (2002). Institutional care as a risk factor for inattention/overactivity. In S. Sandberg (Ed.), *Hyperactivity and attention disorders of childhood,* (2nd ed., pp. 417-434). Cambridge, UK. Cambridge University Press.

Rutter M, Silberg J, Colvert E, Kreppner J. (2004). *CAPA-C Lifetime Version, Parent Interview (Version 1.0)*. Lifetime Version developed for use in the English and Romanian Adoptees Study. SGDP Centre, Institute of Psychiatry, London

Sanchez, M. M., Ladd, C. O., & Plotsky, P. M. (2001). Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Development and Psychopathology, 13,* 419-449.

Sattler, J. (2002) *Assessment of children's intelligence and special abilities.* Boston: Allyn & Bacon.

Scime, M. & Norvilitis, J. M. (2006). Task performance and response to frustration in children with attention deficit hyperactivity disorder. *Psychology in the Schools, 43,* 377-386.

Seidman, L. J., Valera, E. M., & Makris, N. (2005). Structural brain imaging of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57,* 1263-1272.

Seipp, C. M. & Johnston, C. (2005). Mother-son interactions in families of boys with attention-deficit/hyperactivity disorder with and without oppositional behavior. *Journal of Abnormal Child Psychology, 33,* 87-98.

Smyke, A. T., Dumitrescu, A., & Zeanah, C. H. (2002). Attachment disturbances in young children. I: The continuum of caretaking casualty. *Journal of the American Academy of Child and Adolescent Psychiatry, 41*.

Sonuga-Barke, E. J. S. (1994). Annotation - on Dysfunction and Function in Psychological Theories of Childhood Disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 35,* 801-815.

Sonuga-Barke, E. (2008). Attention-deficit/hyperactivity disorder: is a developmental perspective needed? In P. Zelazo (Ed.). *Oxford handbook of developmental psychology.* Manuscript in preparation.

Sonuga-Barke, E. J. S., Dalen, L., Daley, D., & Remington, B. (2002). Are planning, working memory, and inhibition associated with individual differences in preschool ADHD symptoms? *Developmental Neuropsychology, 21,* 255-272.

Sonuga-Barke, E. J. S., Sergeant, J. A., Nigg, J., & Willcutt, E. (2008). Executive dysfunction and delay aversion in attention deficit hyperactivity disorder: Nosologic and diagnostic implications. *Child and Adolescent Psychiatric Clinics of North America, 17,* 367-384.

Sonuga-Barke, E. J. S., Taylor, E., Sembi, S., & Smith, J. (1992). Hyperactivity and Delay Aversion .1. the Effect of Delay on Choice. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 33,* 387-398.

Sonuga-Barke, E. J. S., Thompson, M., Stevenson, J., & Viney, D. (1997). Patterns of behaviour problems among pre-school children. *Psychological Medicine, 27,* 909-918.

Sonuga-Barke, E. J. S. (2005). Causal Models of Attention-Deficit/Hyperactivity Disorder: From Common Simple Deficits to Multiple Developmental Pathways. *Biological Psychiatry, 57,* 1231-1238.

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

Spencer, T., Biederman, J., Harding, M., O'Donnell, D., Wilens, T., Faraone, S. et al. (1998). Disentangling the overlap between Tourette's disorder and ADHD. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 39,* 1037-1044.

Spencer, T., Biederman, J., & Wilens, T. (2000b). Pharmacotherapy of attention deficit hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America, 9,* 77-+.

Spencer, T., Biederman, J., & Wilens, T. (2000a). Pharmacotherapy of attention deficit hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America, 9,* 77-+.

SPSS for Windows, version 14.0 & 15.0. (2005). Chicago: SPSS Inc.

Stanfield, A. C., McIntosh, A. M., Spencer, M. D., Philip, R., Gaur, S., & Lawrie, S. M. (2008). Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry, 23*.

Stevens, S., Sonuga-Barke, E., Asherson, P., Kreppner, J. & Rutter, M. (2006). A consideration of the potential role of genetic factors in individual differences in response to early institutional deprivation: The case of inattention/overactivity in the English and Romanian Adoptees study. *Occasional Paper: Association of Child and Adolescent Mental Health, 25*, 63-76.

Stevens, S. E., Sonuga-Barke, E. J. S., Kreppner, J. M., Beckett, C., Castle, J., Colvert, E. et al. (2008). Inattention/overactivity following early severe institutional deprivation: Presentation and associations in early adolescence. *Journal of Abnormal Child Psychology, 36,* 385-398.

Stiefel, I. (1997). Can disturbance in attachment contribute to attention deficit hyperactivity disorder? A case discussion. *Clinical Child Psychology and Psychiatry, 2,* 45-64.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18,* 643-662.

Swanson, J. M., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G. A., Volkow, N. et al. (2007). Etiologic subtypes of attention-deficit/hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychology Review, 17,* 39-59.

Swanson, J., Arnold, L. E., Kraemer, H., Hechtman, L., Molina, B., Hinshaw, S. et al. (2008a). Evidence, interpretation, and qualification from multiple reports of long-term outcomes in the Multimodal Treatment study of Children With ADHD (MTA): part I: executive summary. *J Atten Disord, 12*.

Swanson, J., Arnold, L. E., Kraemer, H., Hechtman, L., Molina, B., Hinshaw, S. et al. (2008b). Evidence, interpretation, and qualification from multiple reports of long-term outcomes in the Multimodal Treatment Study of children with ADHD (MTA): Part II: supporting details. *J Atten Disord, 12,* 15-43.

Tabery, J. (2007). Biometric and developmental gene-environment interactions: Looking back, moving forward. *Development and Psychopathology, 19,* 961-976.

Taylor, E. (1998). Clinical foundations of hyperactivity research. *Behavioural Brain Research, 94,* 11-24.

Taylor, E. & Rogers, J. W. (2005). Practitioner review: Early adversity and developmental disorders. *Journal of Child Psychology and Psychiatry, 46,* 451-467.

Taylor, E. M., Chadwick, O. P., Heptinstall, E. P., & Danckaerts, M. P. (1996). Hyperactivity and Conduct Problems as Risk Factors for Adolescent Development. *Journal of the American Academy of Child & Adolescent Psychiatry, 35,* 1213-1226.

Taylor, E. & Sonuga-Barke, E. (2008). Disorders of attention and activity. In M. Rutter, D. Bishop, D. Pine, S. Scott, J. Stevenson, E. Taylor, A. Thapar (eds.) *Rutter's Child and Adolescent Psychiatry,* (5th ed., pp. 521-542). Massachusetts, USA: Blackwell.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience & Biobehavioral Reviews.27(1-2):33-44,* -Mar.

Thapar, A., Fowler, T., Rice, F., Scourfield, J., van den Bree, M., Thomas, H. et al. (2003). Maternal smoking during pregnancy and attention deficit hyperactivity disorder symptoms in offspring. *American Journal of Psychiatry, 160,* 1985-1989.

Thapar, A., O'Donovan, M., & Owen, M. J. (2005). The genetics of attention deficit hyperactivity disorder. *Human Molecular Genetics, 14,* R275-R282.

Thapar, A., Rice, F., Hay, D., Boivin, J., Langley, K., van den Bree, M., Rutter, M. & Harold, G. (2008). Prenatal smoking does not cause ADHD: Evidence from a novel design. Manuscript submitted for publication.

Thapar, A., van den Bree, M., Fowler, T., Langley, K., & Whittinger, N. (2006). Predictors of antisocial behaviour in children with attention deficit hyperactivity disorder. *European Child & Adolescent Psychiatry, 15,* 118-125.

Thapar, A., Harrington, R., & McGuffin, P. (2001). Examining the comorbidity of ADHD-related behaviours and conduct problems using a twin study design. [References]. *British Journal of Psychiatry, 179,* 224-229.

Thunstrom, M. (2002). Severe sleep problems in infancy associated with subsequent development of attention-deficit/hyperactivity disorder at 5.5 years of age. *Acta Paediatrica, 91,* 584-592.

Tiret, L. (2002). Gene-environment interaction: a central concept in multifactorial diseases. *Proceedings of the Nutrition Society, 61,* 457-463.

Tizard, B. & Hodges, J. (1978). The effect of early institutional rearing on the development of eight year old children. *Journal of Child Psychology & Psychiatry & Allied Disciplines, 19,* 99-118.

van den Akker, E. L. T., Koper, J. W., van Rossum, E. F. C., Dekker, M. J. H., Russcher, H., de Jong, F. H. et al. (2008). Glucocorticoid receptor gene and risk of cardiovascular disease. *Archives of Internal Medicine, 168,* 33-39.

van Rossum, E. F., Koper, J. W., van den Beld, A. W., Uitterlinden, A. G., Arp, P., Ester, W. et al. (2003). Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. *Clinical Endocrinology, 59,* 585-592.

van Rossum, E. F. C., Binder, E. B., Majer, M., Koper, J. W., Ising, M., Modell, S. et al. (2006). Polymorphisms of the glucocorticoid receptor gene and major depression. *Biological Psychiatry, 59,* 681-688.

van Rossum, E. F. C. & Lamberts, S. W. J. (2004). *Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition*. (vols. 59) BETHESDA: ENDOCRINE SOC.

Vandenbergh, D. J., Thompson, M. D., Cook, E. H., Bendahhou, E., Nguyen, T., Krasowski, M. D. et al. (2000). Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. *Molecular Psychiatry, 5,* 283-292.

von Stauffenberg, C. & Campbell, S. B. (2007). Predicting the early developmental course of symptoms of attention deficit hyperactivity disorder. *Journal of Applied Developmental Psychology, 28,* 536-552.

Vorria, P., Papaligoura, Z., Sarafidou, J., Kopakaki, M., Dunn, J., van IJzendoorn, M. H. et al. (2006). The development of adopted children after institutional care: a follow-up study. *Journal of Child Psychology and Psychiatry, 47,* 1246-1253.

Wechsler, D. (1992). *Manual for the Wechsler Intelligence Scale for Children* (3rd ed.). London: Psychological Corporation.

Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Progress in Neurobiology, 65,* 427-451.

Weiss, G., Hechtman, L., Milroy, T., & Perlman, T. (1985). Psychiatric Status of Hyperactives As Adults - A Controlled Prospective 15-Year Follow-Up of 63 Hyperactive-Children. *Journal of the American Academy of Child and Adolescent Psychiatry, 24,* 211-220.

Welsh, M. C. & Pennington, B. F. (1988). Assessing Frontal-Lobe Functioning in Children - Views from Developmental-Psychology. *Developmental Neuropsychology, 4,* 199-230.

Willcutt, E. G., Doyle, A. E., Nigg, J. T., Faraone, S. V., & Pennington, B. F. (2005). Validity of the Executive Function Theory of Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. *Biological Psychiatry, 57,* 1336-1346.

Willcutt, E. G., Pennington, B. F., Chhabildas, N. A., Friedman, M. C., & Alexander, J. (1999). Psychiatry comorbidity associated with DSM-IV ADHD in a nonreferred sample of twins. *Journal of the American Academy of Child & Adolescent Psychiatry, 38,* -1362.

World Health Organization (WHO). (1992). *The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organization.

Wust, S., van Rossum, E. F., Federenko, I. S., Koper, J. W., Kumsta, R., & Hellhammer, D. H. (2004). Common polymorphisms in the glucocorticoid receptor gene are associated with adrenocortical responses to psychosocial stress. *Journal of Clinical Endocrinology & Metabolism.89(2):565-73*.

Yang, B. R., Chan, R. C. K., Jing, J., Li, T., Sham, P., & Chen, R. Y. L. (2007). A meta-analysis of association studies between the 10-repeat allele of a VNTR polymorphism in the 3 '-UTR of dopamine transporter gene and attention deficit hyperactivity disorder. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics, 144B,* 541-550.

Youth in Mind; SDQ: Normative SDQ data from Britain. (2001, February 21). Retrieved June 13 2008, from *http://www.sdqinfo.com*

Yu, C. Y., & Muthén, B. (2002). *Evaluation of model fit indices for latent variable models with categorical and continuous outcomes* (Technical Report). Los Angeles: University of California at Los Angeles, Graduate School of Education & Information Studies.

Zeanah, C. H., Nelson, C. A., Fox, N. A., Smyke, A. T., Marshall, P., Parker, S. W. et al. (2003). Designing research to study the effects of institutionalization on brain and behavioral development: The Bucharest Early Intervention Project. *Development and Psychopathology, 15,* 885-907.

Zeanah, C. H., Smyke, A. T., Koga, S. F., & Carlson, E. (2005). Attachment in Institutionalized and Community Children in Romania. *Child Development, 75,* 1015-1028.