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EXPLORING THE CONTRIBUTION OF PRENATAL STRESS TO THE PATHOGENESIS OF AUTISM AS A NEUROBIOLOGICAL DEVELOPMENTAL DISORDER: A DIZYGOTIC TWIN STUDY

by

MARLEEN CLAASSEN

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SUPERVISOR:

PROF. H. NAUDé (Department of Educational Psychology)

CO-SUPERVISORS:

PROF. E. PRETORIUS (School of Medicine, Faculty of Health Sciences)

PROF. M.C. BOSMAN (School of Medicine, Faculty of Health Sciences)

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This dissertation is dedicated to all parents who have children diagnosed with autism. May our Lord bless these families in many different ways.

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Psalm 119:130

'Wanneer U woord vir mense oopgaan; bring dit lig;
Dit gee insig aan die wat nog onervare is.'

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DECLARATION

I declare that **EXPLORING THE CONTRIBUTION OF PRENATAL STRESS TO THE PATHOGENESIS OF AUTISM AS A NEUROBIOLOGICAL DEVELOPMENTAL DISORDER: A DIZYGOTIC TWIN STUDY** is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

…….. ……

MARLEEN CLAASSEN DATE

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BY: MARLEEN CLAASSEN
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SUPERVISOR: PROFESSOR H. NAUDé
CO-SUPERVISORS: PROFESSORS E. PRETORIUS and M.C. BOSMAN

SUMMARY

Exploring the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder: a dizygotic twin study

This research project explores the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder. The neurobiological impact of stress prior to the 28th week of gestation might produce structural neural changes, specifically regarding the cerebellum, the brain stem and limbic pathways, including the hippocampal area, which concept relates closely to the pathogenesis of autism. In this research project a significant focus is placed on prenatal hipothalamic-pituary-adrenal (HPA) activity due to the HPA axis' interactivity with cortisol, digoxin and serotonin, as these biochemicals are significantly implicated in programmed foetal development, postnatal cortical behaviour, postnatal learning, as well as in functional impairment of socialization, communication and imagery associated with autism. Based upon the rationale of this research project and the conceptualisation of the topic of interest, the **research problem** was formulated as follows: *In what unique ways does prenatal stress contribute to the pathogenesis of autism as a neurobiological developmental disorder?* **Sub questions** included: Did the mother of the dizygotic twins experience significant stress during the period of gestation? What structural brain differences can be observed among the dizygotic twins at hand of MR-imaging? To which periods of prenatal development can these structural differences be related? How do these differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins? What plasma differences can be observed among the dizygotic twins at hand of blood sampling? How does elevation of pre- and postnatal

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glucocorticoids relate to plasma difference among the dizygotic twins? How do these plasma differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins? This research project represents **quantitative research**. The mode of inquiry is non-experimental at hand of a single dizygotic twin study. The following **data generating strategies** were employed: clinical intake interviews, administration of a diagnostic stress inventory and the 16-PF Questionnaire, MR-imaging, and the collection of blood plasma pathology results.

Key words: autistic disorder, prenatal stress, neurobiological developmental disorder, glucocorticoids, serotonin, digoxin, HPA-axis, intra-uterine deprivation, sub-optimal placental nutrient supply.

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In order to simplify the reading task the masculine gender is used within the text. This type of referencing should not be seen as a form of gender discrimination, since all references implicitly include the female gender, except if indicated otherwise.

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CHAPTER 1

INTRODUCTION, AWARENESS OF THE PROBLEM, RATIONALE FOR THE STUDY AND ANALYSIS OF THE RESEARCH PROBLEM, LITERATURE REVIEW, DEFINITION OF KEY CONCEPTS, PROBLEM STATEMENT, PURPOSE OF THE STUDY, THEORETICAL FRAMEWORK AND PARADIGMATIC PERSPECTIVE, RESEARCH DESIGN AND METHODOLOGY, ETHICAL CONSIDERATIONS AND CHAPTER OUTLINE

> "Although some investigators still choose to believe that human emotions are unique and acquired through social learning, the data-based premise is that psychology is neurodynamics and that the ultimate sources of human cognition and feelings are biological, and that these foundations are essential for all of the many acquired complexities that characterize the detailed expressions of human emotions in the real world. The traditional distinction between bodily and psychological processes becomes blurred as we come to increasingly appreciate that mental abilities are bodily functions of the brain. Psychological and brain analyses must remain two-way streets. To understand the nature of the brain, we must understand emotions, but to understand the diversity of real-life emotions, we must understand the intrinsic operating systems of the brain" (Panksepp 1998:20).

1.1 INTRODUCTION

By means of this research project the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder will be explored. Autism is characterised by serious functional impairment pertaining to socialisation, communication and imagery (Panksepp 1998:276; Trevarthen 2000: 4; Kates et al 2004:539). In 1943 Kanner established the concept *autism* (also known as *Kanner's autism*) and remarked that children with autism "... have come into the world with an innate inability to form the usual, biologically provided affective contact with people" (Kanner 1943:50 cited in Panksepp 1998:276). After a period during which claims were made that autism is caused by faulty parenting, researchers are currently united in their view that autism is primarily a neurobiological developmental disorder (Bauman & Kemper 1994; Herman 1996;

Panksepp 1998; Trevarthen 2000; Clark 2002; Courchesne 2002; Keller & Persico 2003; Schmidt & Rotenberg 2005). Research findings suggest that this neurobiological developmental disorder might be ascribed to disrupted neural development during the second trimester of gestation when the foetal brain stem, cerebellum and limbic pathways must be generated (Bauman & Kemper 1995:1-26). In keeping with these findings, Beversdorf in 2004 pointed out a significant relation between prenatal stress and the development of autism (Beversdorf 2004). The neurobiological impact of stress prior to the 28th week of gestation might produce structural neural changes, specifically regarding the cerebellum, the brain stem and limbic pathways, including the hippocampal area (Sapolsky 2000:925-935). Sapolsky (2000) found that programmed apoptosis is affected due to the neurobiological impact of stress on foetal development, which concept relates closely to the pathogenesis of autism. Sapolsky (2000:925-935) established that increased levels of cortisol in response to chronic stress (maternal or foetal) might kill nerve cells in the hippocampus. If hippocampal activity is thus compromised, excessive cortisol is secreted and, over time, the ability to turn off the stress response decreases, which leads to further atrophy of the hippocampus. These findings indicate that chronic stress leading to chronic secretion of cortisol may have long-lasting effects on physical functioning, including brain damage. Programmed apoptosis may be grossly interfered with, especially within the areas of the hippocampus and the cerebellum. MR-imaging confirmed structural differences of the cerebellum, the brain stem and limbic system associated with autism (Beversdorf 2004), and these structural differences were further associated with elevated levels of glucocorticoids and endogenous opiates during gestation (Bertram & Hanson 2002:459–467). Elevated glucocorticoids inhibit foetal growth and are associated with altered programmed foetal cortical development (Bertram & Hanson 2002:460). Thus, there appears to be a link between the pathogenesis of autism and prenatal endogenous and exogenous glucocorticoids as well as endogenous opiates (Panksepp 1998). In this research project a significant focus is placed on prenatal hipothalamic-pituary-adrenal (HPA) activity due to the HPA axis' interactivity with ß-endorphins, cortisol, oxytocin, insulin, endogenous opiates, digoxin and serotonin (Kurup & Kurup 2003:1537-1559), as these hormones are significantly implicated in programmed foetal development, postnatal cortical behaviour, postnatal learning, as well as in functional impairment of socialization, communication and imagery associated with autism (APA 2000:75).

Recently various studies concerning monozygotic twins have been undertaken in order to investigate the neurobiological and cortical similarities and differences between children with and without autism (Trevarthen 2000; Clark 2002; Courchesne 2002; Bertram & Hanson 2002; Kurup & Kurup 2003; Keller & Persico 2003; Beversdorf 2004; Schmidt & Rotenberg 2005). This research project differs from previous studies by tracking the neurobiological and cortical differences and similarities in a pair of dizygotic twins, where only one of the pair meets the criteria for autism (APA 2000:75). Dizygotic twins develop from two different ovaries that were fertilized at the same time. Consequently, these dizygotic siblings share no more genetic similarities than normal siblings (Plug, Meyer, Louw & Gouws 1987:67). The reason why a dizogotic twin study was decided upon is to explore whether HPA activity manifests differently among a pair of dizogotic siblings, since it is expected that both dizygotic foetuses were exposed to elevated glucocorticoids during gestation, yet only one of the siblings is affected and meets the diagnostic criteria for autism (refer to Annexture B). In addition, a dizygotic twin study offers the ideal research control, since all significant variables are uniform and constant. If prenatal stress does play a role in the pathogenesis of autism, why was only one sibling affected? This phenomenon might only be explained by means of a neurobiological enquiry that forms the basis for a neuropsychological explanation of this complex phenomenon.

1.2 AWARENESS OF THE PROBLEM

During my training as student in psychology I was stationed at the Unica School for learners with Autism in Pretoria. Following my observations of these children's behaviour, i.e., socialisation, communication, and learning, and after various discussions on the aetiology of autism with the psychologist employed at this school, I concluded that there must be a neural substrate to autism. I then started to investigate the foundations of human emotions, which led me to the field of affective neuroscience. With current advances in neurobiology, neuroscience, and neuropsychology, the bulk of literature pointed to the role of neurobiology in the understanding of human cognitive behaviour and emotions. The literature on these research findings provided me with recent information about the brain-operating systems involved in foetal brain development, how these brain-operating systems organize the fundamental emotional tendencies of all human beings, as well the neuropsychological implications of these findings.

Following this personal interest, the parents of a pair of dizygotic twins consulted me during my internship training this year. As this couple suffered significant emotional discomfort due to their personal guilt feelings and their misunderstanding of autism as a developmental disorder, I proceeded with in-depth reading in order to support the parents with accurate information about autism. The couple's misconceptions were demystified through information about the structural brain differences inherent to autism and how these differences relate to different affective, social, and cognitive behaviour in their two siblings. The parents found consolation through the power of knowledge, and were intrigued by the neurobiological basis underpinning autism as a developmental disorder. They concluded that they might contribute significantly to the demystification of autism and initiated further exploration by offering the participation of their dizygotic twins in a research project. Keeping in mind that they might later reconsider involvement, I discussed the advantages and disadvantages of such an endeavour with the parents; they anyway wanted to proceed and gave informed consent, upon which I discussed the proposed study with my supervisor.

1.3 RATIONALE FOR THE STUDY AND ANALYSIS OF THE RESEARCH PROBLEM

> "To understand the basic operating systems of the brain, we have to begin relating incomplete sets of neurological facts to poorly understood psychological phenomena that emerge from many interacting brain activities. Why has it taken us so long to recognize the general organizational principles for mind and behaviour that are found within the primitive genetically dictated areas of the brain? It is partly because until recently we simply did not know enough about the brain. It is also because for a long time, 20th century psychology insisted that we should seek to explain everything in human behaviour via environmental events that assail human beings in their real-life interactions with the world rather than via the evolutionary skills that are constructed in their brains as genetic birthrights" (Panksepp 1998:4-5).

During various discussions with my supervisor and co-supervisors we envisioned that a research project of this nature might be met with resistance; however, the rationale for this study refutes potential resistance and arguments that a research project of this nature cannot be pursued within the field of Educational Psychology, although the rationale for this study cannot be defined using a single disciplinary approach. The premise of the rationale is that all human beings come into the world with a variety of abilities, i.e.,

intrinsic psycho-behavioural control systems, that do not require previous learning, but which provide immediate opportunities for learning to occur. Specific hormones activate these intrinsic psycho-behavioural control systems during gestation, as well as postnatally (Pretorius 2005). In each individual the influence of these intrinsic psycho-behavioural control systems varies as a function of the individual's life span. As these systems mature and interact with higher brain areas, where they undergo both re-representation and refinement, the individual learns to make effective behavioural choices. Emotional tendencies such as those related to fear, anger and separation distress**,** emerge at early developmental stages. Following gradual maturation these systems, through their effects on other parts of the brain, allow the individual to experience more subtle social feelings and to anticipate important events and deal with them in increasingly complex ways, i.e., one develops a certain 'theory of mind' or 'social cognition' (Kolb & Whishaw 2003:375). When these intrinsic psycho-behavioural control systems are overtaxed or operate outside the normal range, we call the end results *psychiatric* or *mental disorders*, in keeping with the diagnostic criteria for autism ([APA] American Psychiatric Association 2000:75). Under activity of certain systems may cause depression and variants of personality disorders. Over activity may contribute to mania, paranoid schizophrenia, anxiety, obsessive-compulsive, and posttraumatic stress disorders. Other disorders such as autism and schizophrenia appear to emerge from lesioned brain areas and associated "wiring" problems in brain circuits (Kolb & Whishaw 2003:602). Because of the social importance of these human problems, a substantive understanding of the neurobiological systems and the manner in which they can become compromised or overtaxed pre- and postnatally is of great scientific and societal concern, and therefore this research project becomes firmly based within the field of Educational Psychology.

Based upon the preceding premise, it is essential to synthesize biological, psychological, and neurological perspectives on the pathogenesis of autism as a developmental disorder, and this synthesis is reflected by the choice of supervisors/co-supervisors for this research project. Various disciplines have contributed towards this needed synthesis, but there is presently no umbrella discipline to bridge these neurobiological, neurological and psychological approaches, or have managed to synthesize the nature of neural systems within the human brain under one umbrella. The various cognitive sciences are beginning to address the complexities of the human mind (Eysenck 2001:5), but until recently these approaches have ignored the evolutionary neural systems upon which our vast cortical

potentials are built and to which those potentials may still be subservient. The *experimental cognitive psychological approach* involves carrying out experiments on normal individuals, typically under laboratory conditions, whereas the *cognitive neuropsychological approach* involves studying patterns of cognitive impairment shown by brain-damaged patients in order to understand normal human cognition (Eysenck 2001:5). The *cognitive neuroscientific approach* involves using several techniques for studying brain functioning (e.g., brain scans) in order to identify the processes and structures used in cognition (Eysenck 2001:5). *Clinical psychology* and *psychiatry* attempt to deal at a practical level with the underlying disturbances in brain mechanisms, but neither has an adequate neuroconceptual foundation of the sources of cognition and emotionality upon which systematic understanding can be constructed (Panksepp 1998:5). *Neuropsychology* aims to diagnose the presence of cortical damage or dysfunction and to localize it where possible. In doing so, there is an attempt to provide an accurate and unbiased estimate of a person's cognitive capacity (Kolb & Whishaw 2003:756). The contribution of some of these approaches will be discussed under the heading that deals with the embedded paradigm; yet these approaches are mentioned here in order to illustrate the blurred distinctions and the complexities inherent to a suitable rationale. Nonetheless, this research project seeks to combine several elements of more than one approach; yet the missing piece that could bring all these disciplines together as part of a suitable rationale is a neurobiological understanding of the basic pre- and postnatal cognitive and emotional operating systems of the human brain, without compromising the psychological inputs. This synthesis could be found in the *affective neuroscience* (Panksepp 1998:5). Affective neuroscience is deeply rooted within *physiological psychology, behavioural biology,* and *behavioural neuroscience,* and provides the modernized umbrella label for various physiological, affective, and cognitive psychological approaches to disorders. The affective neuroscience deals with pre- and postnatal neurobiological development and provides a suitable premise for the rationale of this study, as it will become clear from the following paragraphs.

Affective neuroscience offers a unique perspective on autism, which approach supplements the Educational Psychological perspective, since autism is defined as a developmental disorder first evident in early childhood (APA 2000:75). Why do we define autism as a developmental disorder *per se*? Almost all disorders included in the DSM-IV-TR (APA 2000) could be defined as developmental disorders, since with time the characteristic

symptoms undergo certain changes, and since the condition in itself often only develops over time – most disorders originate in childhood, although the full presentation of the condition only manifests itself much later during adulthood. In layperson's terminology this group of disorders is called 'adult psychopathology', mostly because the full clinical image is only met during adulthood, namely after the age of 18 years. In contradistinction, there is a group of developmental disorders that manifest early in life, the full clinical image is met during the early developmental years, and the disorder often persists as the individual grows older. The concept 'early developmental disorder' or 'childhood disorder' therefore seems to be a misnomer, because these conditions are relatively permanent in nature and the duration thereof persists through adulthood (Barlow & Durand 2002:455). Thus, the majority of these 'childhood' developmental disorders are by nature not unique to early childhood, e.g., autism (Barlow & Durand 2002:455). However, Educational Psychologists take special interest in these developmental disorders, since significant focus is placed on the social, affective and cognitive development of children diagnosed with these disorders. These disorders are of clinical significance within the field of Educational Psychology, since the child's normal development is affected by the condition, implicating compromised mastery of developmental milestones. What are the differences between these two categories of disorders then? When the full range of diagnostic criteria only manifests as a disorder during adulthood, the mastery of basic developmental skills is not compromised; yet, adult psychopathology might contribute to sensory, motor or cognitive impairment or deterioration. In addition to non-mastery of certain developmental milestones, developmental disorders usually first diagnosed in infancy or childhood also affect family life, educational needs, education planning and provision (Naudé 2005).

"Autism is characterized by markedly abnormal or impaired development in social interaction and communication and a markedly restricted repertoire of activity and interests. The impairment in reciprocal social interaction is gross and sustained. The impairment in communication is also marked and sustained and affects both verbal and nonverbal skills" (APA 2000:70). This brief description of impairment relates to a compromised neural substrate, in keeping with affective neuroscience, which approach deals with pre- and post neurobiological development. In addition, if speech does develop in children with autism, the pitch, intonation, rate, rhythm, or stress may be abnormal (APA 2000:70), which points to compromised neural involvement, specifically cerebellum

involvement (Naudé, Marx, Pretorius & Hislop-Esterhuyzen 2006). "In most cases, there is no period of unequivocally normal development, although in perhaps 20% of cases parents may report relatively normal development for one or two years. In such cases, parents may report that the child adquired a few words and lost these or seemed to stagnate developmentally. By definition, if there is a period of normal development, it cannot extend past age three years" (APA 2000:71). It is thus concluded that autism is characterised by developmental delays, in keeping with compromised or overtaxed neurobiological systems, which seems to occur prenatal, as suggested by various researchers (Trevarthen 2000; Clark 2002; Courchesne 2002; Bertram & Hanson 2002; Kurup & Kurup 2003; Keller & Persico 2003; Beversdorf 2004; Schmid & Rotenberg 2005).

The following principles thus underlie the rationale for this research project namely, developmental delay or a total lack of mastery of certain developmental skills characterise autism (APA 2000:70–75; Kolb & Whishaw 2003:375); it is suggested that this developmental delay or lack of mastery of developmental skills might be ascribed to compromised neural development during the second trimester of gestation when the foetal brain stem, cerebellum and limbic pathways must be generated (Bauman & Kemper 1995:1-26); research findings point towards a significant relation between prenatal stress and the development of autism (Beversdorf 2004); autism deals primarily with development in the areas of socialisation, communication, and imagery, and exploration of this phenomenon is therefore firmly based in both the Affective Neuroscience and the Educational Psychology. If prenatal risk factors associated with the pathogenesis of autism could be identified, such findings might inform Educational Psychologists to better understand and provide for children with autistic disorder. In addition, such data might significantly implicate prevention and treatment regimes (Burd, 1999: 441). The rationale of this study is in keeping with Barlow and Durand's' views, "… we cannot study behavioural, cognitive, or emotional processes without appreciating the contribution of biological and social factors to psychological and psychopathological expression. Thus, we have abandoned the traditional compartmentalized approach to psychopathology. Instead, we use a more accessible approach that accurately reflects the current state of our clinical science" (Barlow & Durand 2002:xvii).

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1.4 LITERATURE REVIEW

Recent research projects focused on the role of glucocorticoids in programmed foetal development (Benediktsson, Lindsay, Lindsay & Seckl 1993:339-341; Coleman 1994:104; Levitt, Lindsay, Holmes & Seckl 1996:1200-1204; Nyirienda, Lindsay, Kenyon, Burchell & Seckl 1998:2174-2181; Levitt, Lambert, Woods, Hales, Andrew & Seckl 2000:4611-4618; Courchesne 2002:21-23; Bertram & Hanson 2002:459; Clark 2002:7-10). An increase in foetal exposure to maternal glucocorticoids can cause increased glucocorticoid receptor density and disruption of programmed neural development (Lindsay et al 1996:1200-1204; Bertram & Hanson 2001:103-121), yet despite these adverse risks it has become common practice to administer glucocorticoids to pregnant mothers to promote foetal maturation of organs if they are in danger of pre-term delivery (Matthews 2001:309-317).

Various studies point to the involvement of the hypothalamic–pituitary–adrenal (HPA) axis in neural programming. This is not surprising as glucocorticoids produced in the adrenal cortex in response to signals from the HPA axis have wide-ranging effects on a number of systems both in foetal and adult life, playing key roles in regulation of salt and water homeostasis, blood pressure, immunological responses and metabolism. The HPA axis is controlled by a classic negative feedback system, in which glucocorticoids released into the circulation by the adrenal gland interact with glucocorticoid receptors (GRs) located in the pituitary, hypothalamus and hippocampus. Thus over-activity at any stage along this pathway should result in negative feedback to the corticosteroid releasing hormone (CRH) corticotrophins in the hypothalamus and consequent reduced CRH release.

Pre-clinical research conducted over the past decade has shown that excess levels of glucocorticoids can result in functional and morphological hippocampal changes (Sapolsky 1994:294; Gould 1994:73; Miller et al 1993:391; Squire & Zola-Morgan 1991:1380; Zola-Morgan & Squire 1993; Zola-Morgan et al 1994; Alvarez et al 1995:3976). In addition, McEwen in 1997 noted that hippocampal shrinkage is usually accompanied by deficits in declarative, episodic, spatial and contextual memory performance, in keeping with the associated features of autistic disorder. These hippocampal changes provide a neural substrate for changes in cognitive functioning among children with autistic disorder (APA

2000:75). MR imaging revealed decreased benzodiazepine receptor binding in the medial prefrontal cortex, as well as reduced hippocampal volumes due to over-exposure to glucocorticoids (Stein et al 1997:951; Villarreal & King 2001:131). In addition to this, PET-scans demonstrated decreased N-acetyl aspartame (NAA) ratios and absolute concentrations in the medial temporal lobe and hippocampus, in keeping with the structural brain differences observed in autistic disorder (Panksepp 1998:358). Furthermore, fMRI studies demonstrated different patterns of limbic and paralimbic structure activation due to an excess of glucocorticoids. Of theoretical importance are findings of failure to activate the anterior cingulate, as well as amygdala activation during symptom provocation studies, in keeping with similar observations that were made in autistic disorder (Panksepp 1998). Villarreal and King (2001) suggested that anterior cingulate dysfunction produces failure to inhibit amygdala activation and/or an intrinsic lower threshold of amygdala response to fearful stimuli. These observations are in keeping with research findings implicating disrupted neural development, i.e., brain stem, cerebellum, hippocampal and limbic pathway abnormalities among individuals with autistic disorder (Bauman & Kemper 1995:1-26; Sapolsky 2000:925-935). In addition, Sapolsky (2000:925-935) found that programmed apoptosis is affected due to the neurobiology of stress, which concept relates closely to the pathogenesis of autism (Kalat 2001:346).

Glucocorticoids in the foetus can be derived from three sources: (i) through increasing basal secretion as the foetal adrenal system matures, or in response to foetal stress; (ii) from the mother by transplacental transfer; or (iii) by maternal glucocorticoids crossing into the foetal blood brain barrier, with toxic effects on foetal development (Edwards et al 1993:355). By allowing the high glucocorticoid concentrations to cross the placenta, the foetal HPA feedback system that regulates the foetal adrenal output may be overwhelmed. Not only will this have an immediate effect on development, but it may also result in long term ‘resetting’ of the foetal HPA axis, which may persist into adulthood, because the normal negative feedback system that regulates normal homeostasis is permanently altered (Edwards et al 1993:355).

As the foetal HPA axis regulates the response of the foetus to acute episodes of intrauterine stress and is central to other processes such as organ maturation, growth, neural programming, myelination and cardio-vascular regulation, any disturbance is likely to affect a wide range of foetal systems (Phillips et al 2000:1301). In the foetus,

glucocorticoids inhibit tissue expansion and growth. Foetal plasma cortisol concentrations are low until late gestation, when the HPA axis is activated, producing increased secretion of cortisol from the foetal adrenal gland and a progressive increase in cortisol concentrations, consequently there is a marked cortisol surge prior to delivery (Challis et al 2001:135). The rate of foetal growth normally decreases towards term delivery, which may be linked to the increase in plasma cortisol that occurs at this time, because high foetal glucocorticoid concentrations reduce foetal size. Inappropriate activation of the HPA axis or movement of maternal glucocorticoid across the placenta could therefore increase foetal glucocorticoid concentrations, thus influencing foetal growth. Glucocorticoids act as transcription factors with wide-ranging effects during development. Many genes are activated by glucocorticoids, and any disruption in HPA axis functioning could have subtle or overt effects on development of many tissues in the cardiovascular, pulmonary, renal and central nervous systems (Byrne 2001:153).

In addition, prenatal glucocorticoid exposure permanently programmes several central nervous system functions such as dopamine and serotonin sensitivity, as well as hippocampal formation. Prenatal exposure to glucocorticoids was associated with restricted foetal growth, and delayed myelination of the central nervous system. Barbazanges and coworkers (1996:3943) found that prenatal stress in the final third of gestation caused decreased expression of hippocampal mineralocorticoids receptors, but not glucocorticoid receptors. In humans, prenatal stress has been reported to induce mental retardation and sleep disturbances (Shell 1981:63-70; Barbazanges et al 1996:3943).

Exposure to excessive levels of glucocorticoids during pregnancy is detrimental in terms of brain structure (Uno et al 1994:336), and might even alter postnatal cortisol concentrations (Sapolsky 1996:294-304). Most evidence cited are now showing reduced birth weight, head circumference and more severe chronic lung disease (French et al 1999:114-121) among children who were treated with multi-dose glucocorticoids in utero. In addition to structural central nervous system changes due to high levels of glucocorticoids, the neuroendocrine system also seems to be disrupted. Research findings implicate both central nervous system and neuroendocrinological alterations in the pathogenesis of autism (Bertram & Hanson 2002:459-467).

The preceding literature study suggests that over exposure to glucocorticoids during gestation might affect the foetus adversely. Glucocorticoids act as transcription factors with wide-ranging effects on the foetus during gestation. Many genes are activated by glucocorticoids, implicating that organogenesis might be partly controlled by glucocorticoids, with specific focus on foetal central nervous system development (Perrotta et al 2003; Ross et al 2000; Maden 2001; Colbert 2002). In addition, the balanced supply of glucocorticoids to the foetus is essential, especially during the latter stages of gestation, due to the involvement of glucocorticoids in neural growth and cellular differentiation (Zachman 1995; Debier & Larondelle 2005), but an excess of glucocorticoids might alter central nervous system formation and adversely impact on post-natal sensorimotor learning and cognitive functioning. Disproportionate levels of glucocorticoids have also been implicated in disrupted programmed apoptosis, with specific focus on hippocampal, cerebellar and limbic formation. Structural differences, e.g., densely packed areas and lesser dendritic growth in other areas, are implicated in autistic disorder, and the cerebellum is of particular interest, due to involvement in almost all forms of motor learning and vestibular functioning, which areas once again are implicated in autistic disorder. The cerebellum consists of the cerebellar hemispheres, vermis and the flocculi, and forms part of the motor system that participates in post-natal sensorimotor functioning. The flocculonodular lobe receives projections from the vestibular system (the sensory receptors in the middle ear) and takes part in the control of balance and eye movements (Kolb & Whishaw 2003:217). Lesions to the midline areas of the cerebellum might disrupt balance, eye movements, upright posture and walking, but do not substantially disrupt other movements such as reaching, grasping and using the fingers (Kolb & Whishaw 2003:217). It is thus suggested that attainment of developmental milestones, particularly crawling and walking, might be delayed due to over exposure to glucocorticoids during gestation. Sensorimotor learning during the early post-natal developmental years might thus be adversely affected.

Considering the preceding literature on the adverse effects of glucocorticoids during gestation, as well as the structural and functional brain alterations that result from over-exposure, it is suggested that prenatal stress might play a significant role in the pathogenesis of autism. The various impairments associated with autism also suggest that excess levels of glucocorticoids might be implicated, i.e., speech deviations such as pitch, intonation, rate, rhythm, odd hand movements and body posture, high threshold for pain,

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emotionality, abnormalities in sleep, deviant fear response, and so forth (APA 2000:75-77), because many of these behaviours are mediated by the brain stem, the cerebellum, the hippocampus, the limbic system and its relays.

1.5 DEFINITION OF KEY CONCEPTS

1.5.1 Prenatal

Wevell (1996:435) defines the concept *prenatal* as the period of gestation, relating to the duration of pregnancy, from conception to birth. The gestation period of humans is approximately 40 weeks.

1.5.2 Stress

The concept *stress* is defined as a transaction between a person and the environment that includes the person's appraisal of the challenges posed by the situation and available coping resources, along with the psychological responses to those perceived challenges (Bishop 1994:126). This type of stress thus refers to the subjective stress experienced by the mother during pregnancy. However, prenatal stress might also include foetal stress, e.g., strangling by the umbilical cord, placentae insufficiency, or abruptio placentae (Edwards et al 1993:355).

1.5.3 Pathogenesis

The prefix *patho* comes from Greek and means 'deviant' or 'pathological', while the concept *genesis* means 'origin' or 'beginning' (Wevell 1996:433). Within the context of this study the concept *pathogenesis* is indicative of the etiology of a certain disorder, namely autistic disorder (APA 2000:75).

1.5.4 Autism

Autism is defined as a developmental disorder first evident in childhood, and characterised by serious functional impairment pertaining to socialisation, communication and imagery (APA 2000:75; Panksepp 1998:276; Trevarthen 2000:4; Kates et al 2004:539). "The

median rate of Autistic Disorder in epidemiological studies is 5 cases per 10 000 individuals, with reported rates ranging from 2 to 20 cases per 10 000 individuals" (APA 2000:73). The onset of this disorder is prior to age 3 years, and there is an increased risk of Autistic Disorder among siblings of individuals with the disorder, with approximately 5% of siblings also exhibiting the condition (APA 2000:73; Kaplan & Sadock 1998:1182).

1.5.5 Neurobiological

The concept *neurobiological* refers to the study of "… a diversity of coherently operating brain systems which can generate psychologically meaningful classes of adaptive behavioural tendencies" (Panksepp 1998:12). In addition, the concept *neurobiological* also refers to the study of neural pathways and related electrophysiological and neurochemical activities. One of the best examples of neurochemistry comes from research findings implicating the role of serotonin in controlling human mood, including aggression and depression (Young 1996:313).

1.5.6 Developmental disorder

A developmental disorder manifests itself during the early developmental years, and often persists as the person grows older, e.g., Autistic Disorder. The concept 'early developmental disorder' or 'childhood disorder' therefore seems to be a misnomer, because these conditions are relatively permanent in nature and the duration thereof persists through adulthood (Barlow & Durand 2002:455). The majority of developmental disorders are by nature not unique to early childhood (Barlow & Durand 2002:455). However, Educational Psychologists take special interest in these developmental disorders, since significant focus is placed on the social, affective and cognitive development of children diagnosed with these disorders. These disorders are of clinical significance within the field of Educational Psychology, since the child's normal development is affected by the condition, implicating compromised mastery of developmental milestone. When the full range of diagnostic criteria only manifests during adulthood, the mastery of basic developmental skills is not compromised; yet, adult psychopathology might contribute to sensory, motor and cognitive impairment or deterioration. In addition to non-mastery of certain developmental milestones, developmental disorders usually first diagnosed in

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infancy or childhood also affect family life, educational needs, education planning and provision (Naudé 2005).

1.5.7 Dizogotic

Dizygotic twins develop from two different ova that were fertilized at the same time. Consequently, these dizygotic siblings share no more genetic similarities than normal siblings (Plug, Meyer, Louw & Gouws 1987:67).

1.6 PROBLEM STATEMENT

Based upon the rationale of this research project and the conceptualisation of the topic of interest, the research problem can be formulated as follows:

In what unique ways does prenatal stress contribute to the pathogenesis of autism as a neurobiological developmental disorder?

1.6.1 Sub questions

- Did the mother of the dizygotic twins experience significant stress during the period of gestation?
- What blood plasma differences can be observed among the dizygotic twins at hand of blood sampling?
- Does HPA activity manifest differently among this pair of dizogotic siblings?
- How does elevation of glucocorticoids disrupt programmed foetal development?
- How do blood plasma differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins?
- Does the MR image of the sibling diagnosed with autism differ in respect of structural brain development from what is normally expected?
- To which periods of prenatal development can these structural differences be related?
- How do these structural differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins?

1.6.2 Research hypothesis

The research hypothesis can be formulated as follows:

Elevation of glucocorticoids due to prenatal stress disrupts programmed foetal development and contributes to the pathogenesis of autism as a neurobiological developmental disorder.

1.7 PURPOSE OF THE STUDY

The purpose of this research project is to explore at hand of dizygotic twin study the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder.

1.8 THEORETICAL FRAMEWORK AND PARADIGMATIC PERSPECTIVE

This research design is firmly embedded in a positivistic paradigm. Positivism is based upon the utilization of research methods and practices derived from the natural sciences and application of these to the social sciences. Research findings are interpreted in terms of quantifiable units, and deviations are reported as significant deviations at 0.001 or 0.005 levels or reliability (Cohen, Lui, Schutz et al 2003:8). Data is viewed to be linear and objective in nature, as well as relatively free from researcher contamination or bias. Reality is perceived as external and scientific data is objective and quantifiable, follows the medical approach, experimental in nature and seen as irrefutable or refutable.

In addition, this research project is firmly embedded in the cognitive science. Eysenck (2001:7) defines the cognitive science as follows: 'Cognitive scientists develop computational models to understand human cognition. A good computational model … allows us to predict behaviour in new situations. This is a clear advantage over many previous theories in cognitive psychology, which were expressed so vaguely that it was not clear exactly what predictions were supposed to follow from them.'

However, the cognitive science to a great extent lacks interpretation of the neurobiological systems that underlie affective human behaviour. Affective neuroscience developed from

the cognitive science and seeks to provide conceptual bridges that can link our understanding of basic neural circuits for the emotions with straightforward *cognitive* and p*sychological* views of the human mind and, most importantly, developmental disorders first evident in infancy and early childhood. Affective neuroscience is deeply rooted within *physiological psychology*, *behavioural biology*, and *behavioural neuroscience*, and provides the modernized umbrella label for various physiological, affective, and cognitive psychological approaches to disorders (Panksepp 1998:9). This interdisciplinary approach strongly draws upon our introspective-linguistic access to our subjective feelings. "Because of this small psychological window, and because the key emotional circuits are conserved in the human brain, the two can be linked in such a way that we can finally understand the neurobiological underpinnings of our human emotions" (Panksepp 1998:304). Thus, our introspective access to emotions supplements "hard" scientific data, resulting in an in-depth psychological understanding of developmental disorders. Panksepp's (1998) summary of the major premises of affective neuroscience is captured in table 1.1 below.

Table 1.1 The major premises of affective neuroscience (Panksepp 1998:14-15)

"Emotional processes … play a key role in the causal chain of events that control the actions of humans. They provide various types of natural internal values upon which many complex behavioural choices in humans are based. However, such internal feelings are not simply mental events; rather, they arise from neurobiological events. In other words, emotional states arise from material events at the neural level." "Emotional feelings not only sustain certain unconditioned behavioural tendencies but also help guide new behaviours by providing simple value-coding mechanisms that provide self-referential salience, thereby allowing humans to categorize world events efficiently so as to control future behaviours."
"A series of basic emotional processes arises from distinct neurobiological systems and everyday emotional concepts such as anger, fear, joy, and loneliness are not merely the arbitrary taxonomic inventions of noncritical thinkers. These brain systems have several common characteristics. The core function of emotional systems is to coordinate many types of behavioural and physiological processes in the brain and body."
"When such neural activities continue at low levels for extended periods of time, they generate moods and, ultimately, such personality dimensions as the differential tendency to be happy, irritable, fearful, or melancholy. These systems help create a substantial portion of what is traditionally considered universal "human nature".
"A complete study of emotional systems is also essential for understanding the many psychiatric disturbances that assail humans – schizophrenia, autism, mania, depression, anxiety, panic, obsessive-compulsive disorders, post-traumatic stress disorders, neuroses, and other vexations of the human spirit."
"We will not understand the underlying neurodynamics of emotional systems without a great deal of

concurrent brain research."

Affective neuroscience forms the paradigmatic conceptual bridge needed for this research project, since this approach can yield clear empirical predictions in both directions - from neuroscience to social science and vice versa, and it serves as an intellectual highway for productive interaction between the psychosocial and neurobiological sciences (Panksepp 1998:304). This paradigmatic conceptual bridge might facilitate an in-depth understanding of autism as a neurobiological developmental disorder.

1.9 RESEARCH DESIGN AND METHODOLOGY

This research project represents quantitative research. Quantitative research is deductive in nature and the research hypothesis, which flows from the research problem, directs the scientific inquiry, leading to hypothesis-testing. This approach deals with relations, correlations and covariance of variables. Pertaining to this research project, the mode of inquiry is non-experimental at hand of a single dizygotic twin study, which is *ex post facto* in nature i.e., 'after the fact'. Dizygotic twins come from different ova and have only about 50% of their genes in common, as do all first-degree relatives (Barlow & Durand 2002:104). The advantage of including dizygotic twins is that all prenatal variables are uniform and constant, therefore this research design provides for experimental control which heightens validity of the results because future studies can be replicated at hand of similar dizygotic twins. Replicating findings usually convince researchers that the findings cannot merely be ascribed to coincidence (Cohen et al 2000:181). "The strength of a research program is in its ability to replicate findings in different ways to build confidence in the results" (Barlow & Durand 2002:104). A stress survey will be utilized, which is also non-experimental in nature.

Research involves establishing a hypothesis that is then tested. In abnormal psychology, research focuses on hypotheses meant to explain the nature and causes of disorders (Barlow & Durand 2002:110).

The following data generating strategies will be employed: intake interviews coupled with a diagnostic stress inventory, retrieval of diagnostic records where applicable, administration of the 16-PF Questionnaire, MR-imaging, and blood plasma sampling.

Both the mother and father of the dizygotic twins will be required to complete the diagnostic stress inventory, because they might have different perspectives on the significance of various stressors that were endured during pregnancy.

Magnetic resonance imaging (MR-imaging) of the sibling diagnosed with autistic disorder will be done in order to identify whether structural brain development was altered, compared to what is normally expected. These structural differences will be interpreted in light of prenatal neural programmed neural development, the impact of endocrine system changes on foetal central nervous system development, and the consequent manifestations of autism. MR-imaging is a procedure using radio signals generated in a strong magnetic field and passed through body tissue to produce detailed, even layered, images of brain structures, which are useful in detecting very small brain lesions (Eysenck 2001:9).

Blood plasma sampling of both siblings will complement the research hypothesis and allow exploration of the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder, since endocrine system changes might contribute to disruption of programmed neural development, and these plasma differences between siblings might continue to be present postnatally. The different modes of inquiry and research design that will be followed are presented in table 1.2 below.

Table 1.2 Different modes of inquiry and research design

	QUANTITATIVE		QUALITATIVE	
	Experimental	**Non-experimental**	**Interactive**	**Non-interactive**
Research design	True experimental	Descriptive	Ethnographic	Concept analysis
	Quasi-experimental	Comparative	Phenomenological	Historical analysis
	Single-subject	Correlational	Case study	
		Survey	Grounded theory	
		Ex post facto	Critical studies	

(Adapted from: McMillan & Schumacher 1997: 31.)

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1.9.1 Data analysis and Interpretation

The data will be quantified and interpreted along linear lines of thought, i.e., according to specific conceptual schemes and parameters of pre- and postnatal endocrinological and central nervous system development. Where applicable, significance of deviations will be calculated at the 0.001 and 0.005 levels of reliability.

In addition to my appointed supervisor and co-supervisors from the Faculty of Health Sciences, additional external expertise will be provided by dr. Becker, independent statistical analyst employed by the Medical Research Board, towards the statistical calculations. Du Buisson, Bruinette & Kramer (Incorporated) Pathologists will assist in analysing the blood plasma samples. Dr. Van Rensburg, radiologist, will provide MR-imaging services at the MR-centre at Willows Hospital.

1.10 ETHICAL CONSIDERATIONS

Planning a research project involves much more than selecting the appropriate design – it also includes ethical considerations. The ethics of doing research in developmental disorders deserve special attention and a child-sensitive approach. It is of utmost importance that informed consent is obtained prior to commencement of the research project. Informed consent involves a research participant's formal agreement to cooperate in a study following full disclosure of the nature of the research and the participant's role in it (Simon in Hales, Yudofsky & Talbott 1999:1493-1534). The basic components of informed consent are competence, voluntarism, full information, and comprehension on the part of the research participant (Imber et al 1996:137-146). In other words, research participants must be capable of consenting to participation in the research, they must volunteer or not be coerced into participating, they must have all the information they need to make the decision, and they must understand what their participation will involve. Children and individuals with cognitive impairments, for example, often do not fully appreciate what will occur during research, nor will they understand their role or their rights as participants.

Considering the preceding principles, certain general protections will be implemented to ensure that these concerns are properly addressed. First, approval of the research project by the review board of the Department of Educational Psychology was sought before commencement of the study. The purpose of seeking approval was to ensure that the research procedures provide for the adequate protection of the research participants' rights, welfare and dignity, in keeping with the Ethical Principles of Psychologists (APA 1992:1597-1611). To safeguard those who participate in psychological research and to clarify the responsibilities of researchers, these ethical principles include general guidelines for conducting research, which will be adhered to. This means that participants will be protected from both physical and psychological harm. These principles also emphasize the researcher's responsibility for the research participants' welfare, because the researcher ultimately must ensure that the welfare of the research participants is given priority over any other consideration, including research design.

In addition to the principles of informed consent, protection against potential harm, and the right to confidentiality, the Society for Research in Child Development (1990) has endorsed ethical guidelines for research that address some of the issues unique to research with children. Not only do these guidelines call for confidentiality, protection from harm, and debriefing, but they also require informed consent from children's caregivers and from the children themselves if they are age seven and older. These guidelines specify that the research must be explained to children in language they can understand so they can decide whether they wish to participate.

Many other ethical issues extend beyond protection of the participants, including the proper way to give credit to other researchers and co-workers. These concerns will be adhered to, as depicted in the ethical principles of the Faculty of Education, University of Pretoria (2003) attached to this dissertation as Appendix C.

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1.11 CHAPTER PLANNING

Chapter one consists of the orientational introduction and actualisation of the research problem, the problem statement, the research hypothesis, as well as the research methodology and research design. Ethical considerations are also included.

Chapter two describes the relevance of a selection of hormones that are implicated in the pathogenesis and/or manifestation of autism, as well as how these hormones link to the different stages of programmed foetal development.

Chapter three describes the relevance of structural brain differences found among individuals diagnosed with autistic disorder, as well as how these differences link to different stages of programmed foetal development.

Chapter four describes the empirical research and related findings. Based upon the findings, the research hypothesis will be accepted or rejected.

Chapter five gives an overview of the research findings, as well as deductions and conclusions derived at. Relevant recommendations will be made, and shortcomings inherent to the design will be pointed out.

1.12 SYNOPSIS

In Chapter One the theoretical framework and paradigmatic perspective, as well as the research design and methodology directing this research project were outlined. The actuality of the research project, i.e., the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder, was emphasized. An analysis of the research problem was done and ambiguous concepts were defined. The research project was clearly demarcated at hand of relevant problem statements. **Chapter Two** describes the relevance of a selection of hormones that are implicated in the pathogenesis and/or manifestation of autism, as well as how these hormones link to the different stages of programmed foetal development.

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CHAPTER 2

BIOCHEMICALS IMPLICATED IN PROGRAMMED FOETAL DEVELOPMENT

2.1 INTRODUCTION

In chapter one various researchers' findings were cited, implicating the role of biochemicals in the pathogenesis of psychiatric disorders, more specifically the role that certain stress hormones and neurotransmitters play in programmed foetal development. This chapter describes the relevance of cortisol, digoxin and serotonin as implicated in the pathogenesis and/or manifestation of autism, and the link with the different stages of programmed foetal development, specifically the interactivity with the hipothalamic-pituary-adrenal axis (HPA axis) (Kurup & Kurup 2003:1537-1559). These biochemicals are significantly implicated in programmed foetal development, postnatal cortical behaviour, postnatal learning, as well as in functional impairment of socialization, communication and imagery associated with autism (APA 2000:75). In keeping with Panksepp's (1998:20) neurodynamic explanation of psychological phenomena, it is proposed that a study of autism necessitates an understanding of the intrinsic contribution of biochemicals to the pathogenesis of autism as a neurobiological developmental disorder. In order to understand these neurochemical maps of the brain, it is necessary to first discuss neurotransmitter and receptor synthesis, as well as neurochemical coding.

2.2 NEUROTRANSMITTER, RECEPTOR SYNTHESIS AND NEUROCHEMICAL CODING

Kolb and Whishaw (2003:Glossary) describe the concept *neurotransmitter* as a chemical that is released from a synapse in response to an action potential and acts on postsynaptic receptors to change the resting potential of the receiving cell; it thus transmits information chemically from one neuron to another. The term *neurotransmitter* also includes chemicals that have little effect on membrane voltage, but instead induce effects such as changing the structure of a synapse. Herlenius and Lagercrantz (2004:8) simplified the

concept by defining neurotransmitters as chemicals released from neurons that act on specific receptors. According to Barlow and Durand (2002:42) neurons that are sensitive to one type of neurotransmitter cluster together and form circuits from one part of the brain to another. Kolb and Whishaw (2003:Glossary) describe the concept *receptor* as a protein on a cell membrane to which another molecule can attach. There are two general classes of receptors: ionotropic receptors and metabotropic receptors, each producing a different effect on the postsynaptic membrane.

In order to fully appreciate the definitions presented in the preceding paragraph, one has to understand the structure and electrical activity of neurons, as well as communication between neurons. Neurons are involved in the manufacture of protein molecules. The chromosomes of the nucleus contain genes, and each gene contains the code for one polypeptide chain. The DNA of a gene is *transcribed* into mRNA (i.e., messenger RNA), which then carries the code for the polypeptide to a ribosome. The code contained in the mRNA is *translated* on the ribosome into a series of amino acids connected by peptide bonds. These long chains of amino acids are further manipulated to form different proteins. Specific types of proteins involved in the formation of channels, gates, and pumps that regulate the flow of ions across the cell membrane are embedded in the neuron's membrane (Panksepp 1998:102).

Neurons carry an electrical charge across their membranes, called the resting potential. This charge is produced by unequal concentrations of ions across the membrane, as illustrated in figure 2.1 (*page inserted*). Figure 2.1 illustrates that these unequal concentrations of ions across the membrane is maintained and regulated by membrane ion channels, gates and pumps. When the gates on the membrane open briefly, ion efflux or influx can take place, thereby changing the membrane's charge, known as a graded potential. If a graded potential is sufficient to change the membrane's charge to the threshold at which voltage-sensitive sodium and potassium channels open, an action potential is produced. The voltage change of an action potential on one part of the membrane is sufficiently large to open adjacent voltage-sensitive channels, thus propagating the action potential along the membrane. The propagated action potential is called a nerve impulse. On myelinated axons, the action potential can be propagated only at the nodes between glial cells, and this form of propagation, called saltatory conduction, is especially rapid. These functions underlie the way in which cells communicate with one

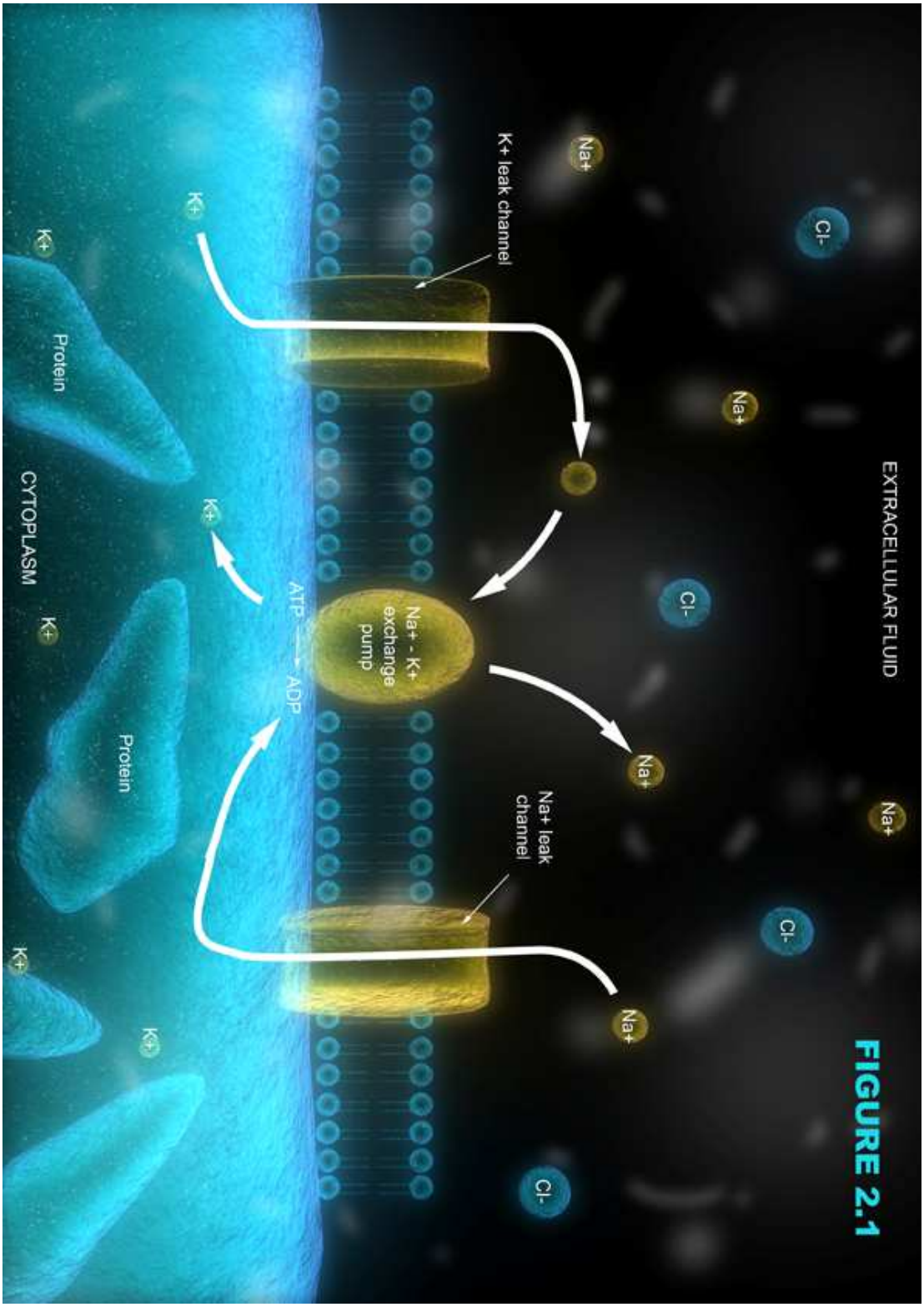
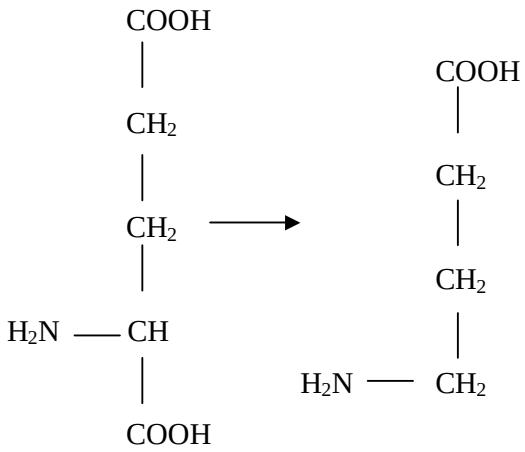

EXTRACELLULAR FLUID
Na+
Cl-
Na+
Cl-
Na+
Cl-
Na+
Cl-
K+ leak channel
Na+ - K+ exchange pump
Na+ leak channel
ATP
ADP
Protein
Protein
CYTOPLASM
K+
K+
K+
K+
K+
K+

FIGURE 2.1

another and how they contribute to behaviour (Panksepp 1998; Kandel, Schwartz & Jessell 2000; Kolb & Whishaw 2003).

Furthermore, the anabolism (manufacture) and catabolism (destruction) of protein molecules in the human body are under the control of a multitude of enzymes, each enzyme having its own function in cell activity and in the biochemical transactions that allow communication between neurons (Panksepp 1998:98). Enzymes promote the biochemical transactions involved in the construction of synaptic neurotransmitters and neuromodulators, for example cleavage enzymes that are responsible for clipping neuropeptides from larger "mother proteins", specific anabolic enzymes that assist in joining larger molecules, and the formation of enzymatically modified molecules such dopamine, noradrenalin and serotonin. This latter modification is explained by Kolb and Whishaw (2003:109) and illustrated in figure 2.1 below.

Figure 2.1 The removal of the carboxyl (COOH) group from glutamate produces GABA



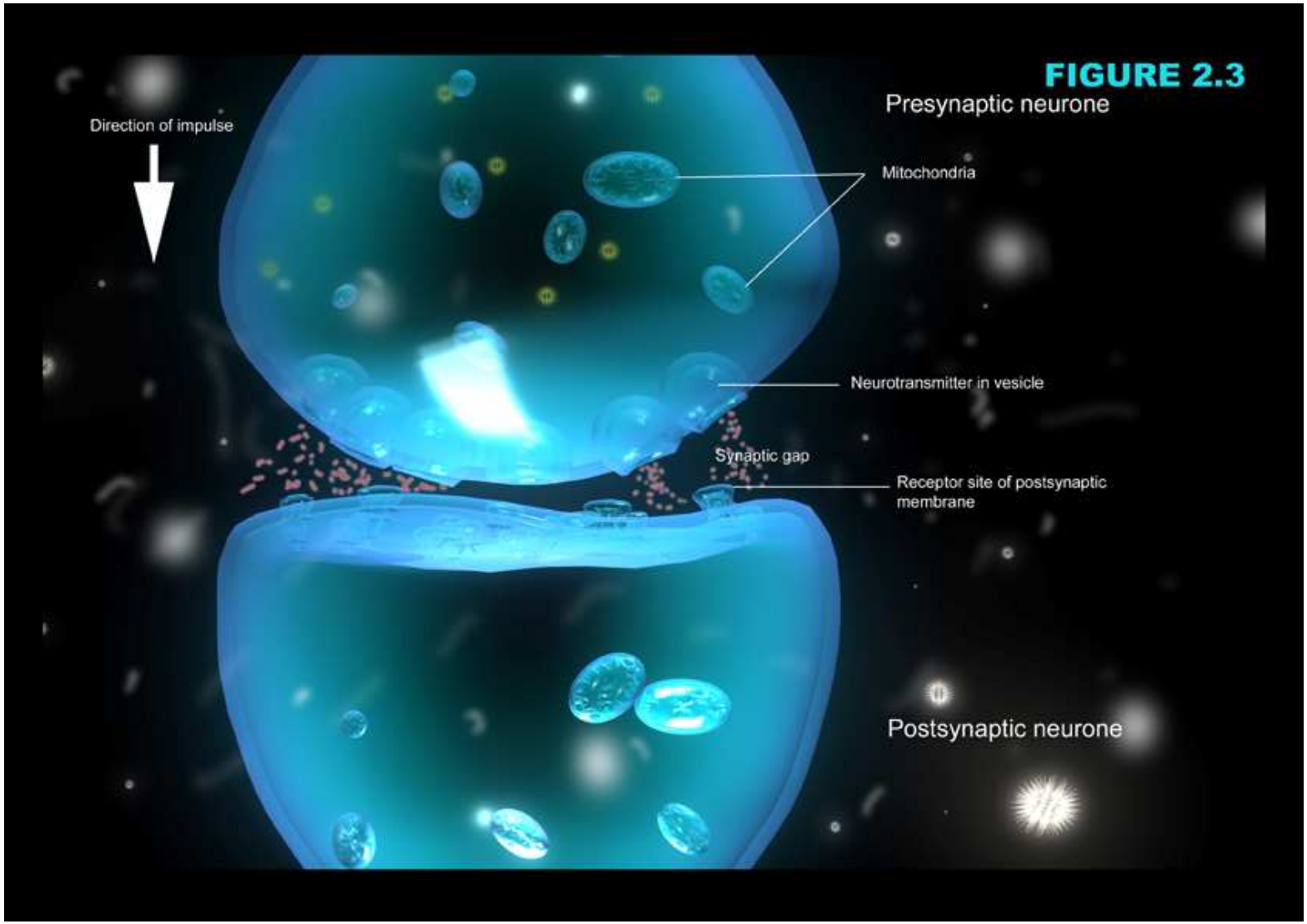
Glutamate GABA

Figure 2.1 illustrates how glutamate (an excitatory neurotransmitter in the brain) and γ-aminobutyric acid (GABA) (an inhibitory neurotransmitter in the brain) are chemically related by the removal or the carboxyl (COOH) group from glutamate to produce GABA. Because these molecules are differently shaped, they can bind to different receptors. According to Panksepp (1998:99) various neuromodulators are short proteins called

neuropeptides, which are produced form larger proteins by specific cleavage enzymes, while specific anabolic enzymes assist in the production of the synaptic transmitter acetylcholine (ACh) when larger molecular fragments from various brain chemicals are joined. Many other transmitters are amino acids that have been enzymatically modified, for example by addition of a hydroxyl (OH) group or the removal of a carboxyl (COOH) group (Kolb & Whishaw 2003:109). This is how the neurotransmitters dopamine, noradrenalin, and serotonin are manufactured (Panksepp 1998:99).

All brain transmitters require specific anabolic enzymes for their construction, and a multitude of catabolic enzymes to ensure that transmitters are inactivated soon after they have conveyed their messages to receptors. Most transmitters are destroyed soon after release by specific catabolic enzymes, or by reuptake processes, which remove the transmitter from the synaptic cleft into the same neuron (Barlow & Durand 2002:42; Panksepp 1998:99). This process is illustrated in figure 2.3 *(page inserted)*.

As illustrated in figure 2.3, neurotransmitters are packaged in vesicles in presynaptic endings. This is to ensure that neurotransmitters are protected from catabolic enzymes, and to allow synapses to dump a substantial number of neurotransmitter molecules into the synaptic cleft at one time. This process of neurotransmitter release is initiated by calcium entry into the presynaptic terminal as a result of arriving action potentials. The released neurotransmitters bind to postsynaptic as well as presynaptic receptors, thereby inducing complex cascades of intracellular events that modify the electrical activities of the receiving cells (Panksepp 1998:100). Synaptic acitivity can be terminated by a variety of mechanisms, including active *enzymatic degradation* of neurotransmitters, specific presynaptic *reuptake or transporter mechanisms* that extract neurotransmitters from the synaptic cleft and return them into the presynaptic ending, where they can be either degraded or recycled into vesicles, or *passive dissipation* (i.e., diffusion) and slow degradation, which appears to be the most common form of degradation for the many peptide neuromodulators that help create motivational and emotional specificity within the brain (Panksepp 1998:100). Occasionally, medications called serotonin reuptake inhibitors such as *Fluoxetine*, *Sertraline* and *Paroxetine* are prescribed for some individuals with autism. Serotonin reuptake inhibitors keep serotonin in the brain longer so that its function as a neurotransmitter is further enhanced (Cuccaro, Wright, Abramson, Marstellar & Valentine 1993:96). Some pregnant women do take selective serotonin reuptake inhibitor



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(SSRI) antidepressants, but animal and human studies are inconclusive regarding eventual adverse effects on central nervous system development at therapeutic doses, even though high doses may cause anatomical and behavioral changes (Simons, Gogineni, Iodi Carstens & Carstens 2002:139). Based upon their composition, neurotransmitters can be classified into three groups, namely small molecule transmitters, peptide transmitters (also known as neuropeptides), and transmitter gases (Kolb & Whishaw 2003:108). These different groups are briefly discussed in the following paragraphs.

2.2.1 Small molecule neurotransmitters

Small molecule neurotransmitters are synthesized for use in the axon terminals. As previously illustrated in figure 2.3, once a small-molecule transmitter is released from an axon terminal, it can be quickly replaced at the presynaptic membrane (Kolb & Whishaw 2003:108). Some of the small-molecule neurotransmitters are summarized in table 2.1 below.

Table 2.1 Small-molecule neurotransmitters

Transmitter	**Abbreviation**
Acetylcholine	Ach
Amines	
Dopamine	DA
Noradrenalin (Norepinephrine)	NE
Adrenalin (Epinephrine)	EP
Serotonin	5-HT
Amino Acids	
Glutamate	Glu
γ-Aminobutyric acid	GABA
Glycine	Gly
Histamine	H

Source: Adapted from Kolb & Whishaw (2003:108).

For purposes of blood sampling that follows in chapter four, the focus is on serotonin (5-hydroxytryptamine or 5-HT). There are approximately six major circuits of serotonin

spreading from the midbrain (Barlow & Durand 2002:43). These circuits extend through the brain and many of them end up in the cortex, thereby playing a significant influence on behaviour and information processing. However, there are at least fifteen different receptors in the serotonin system, therefore the effects of serotonin differ slightly depending on the involvement of a specific type or subtype receptor (Owens, Mulchahey, Stout & Plotsky 1997:210). Research findings demonstrate that lesions that interrupt serotonin circuits seem to impair the ability to ignore irrelevant external cues, making the individual overreative (Barlow & Durand 2002:46). Elevated serotonin levels are implicated in autism, suggesting impaired thought and impulse control among individuals diagnosed with autism.

2.2.2 Peptide neurotransmitters

There are more than 50 known short chain amino acids that are grouped into families of peptide neurotransmitters (Kolb & Whishaw 2003:109). These families are summarized in table 2.2 below.

Table 2.2 Peptide neurotransmitters

Family	*Example*
Opioids	Enkephaline, dynorphin.
Neurohypophyseals	Vasopressin, oxytocin.
Secretins	Gastric inhibitory peptide, growth-hormone-releasing peptide.
Insulins	Insulin, insulin growth factors.
Gastrins	Gastrin, cholcystokinin.
Somatostatins	Pancreatic polypeptides.

Source: Adapted from Kolb & Whishaw (2003:109).

Peptide neurotransmitters are produced directly from instructions contained in the cell's DNA. In some neurons these peptide neurotransmitters are produced in axon terminals. Transporter proteins in the cell membrane absorb the required precursor chemicals from the blood supply, while the mitochondria in the axon terminals provide the energy for the

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synthesis of these neurotransmitters from their precursor chemicals. Most neurotransmitters, however, are manufactured in the cell body according to instructions contained in the neuron's DNA. These neurotransmitters are then packaged in membranes on the Golgi bodies and transported on microtubules to axon terminals (Kolb & Whishaw 2003:110). There is also evidence that mRNA is transported to the synapse, where it serves as the message for the manufacture of a neurotransmitter within the synapse, rather than in the ribosomes surrounding the nucleus. This process of synthesis and transport is relatively slow compared with small-molecule neurotransmitters; therefore peptide neurotransmitters cannot be replaced quickly once used. In addition, peptide neurotransmitters do not bind to ion channels and therefore have no direct effect on the voltage of the postsynaptic membrane, but rather activate receptors that indirectly influence cell structure and function (Barlow & Durand 2002:280).

Peptides play a specific role in nervous system functioning, e.g., peptides serve as hormones such as glucocorticoids that are elevated in response to stress, peptides serve as neurotransmitters such as oxytocin, vasopressin, β-endorphin, Met-enkephalin and Leu-enkephalin involved in social processes and feelings, social memory, as well as pain and pleasure regulation (Panksepp 1998:101). Some of these emotions and behaviours are defiant in individuals diagnosed with autism, suggesting altered hormonal and peptide neurotransmitter changes.

2.2.3 Transmitter gases

Nitric oxide (NO) and carbon monoxide (CO) are soluble neurotransmitter gases, which are synthesized as needed. Nitric oxide serves as a messenger in many parts of the body, i.e., it controls the muscles in intestinal walls, and it dilates blood vessels in brain regions that are in active use (Kolb & Whishaw 2003:110). However, for purposes of blood sampling done in chapter four, no particular attention is paid to these two transmitter gases.

2.3 DEVELOPMENT OF NEUROTRANSMITTER SYSTEMS DURING CRITICAL PERIODS

As evidenced by the contents of the preceding paragraphs, neurotransmitters are primarily responsible for the neuronal communication mediated by the numerous synapses, although

there are also electrical synapses. Neurotransmitter expression can be high during certain stages of development, known as susceptible developmental time windows, yet may persist in only a few synapses afterwards (Parnavelas & Cavanagh 1988:92-93). Accurate timing and spacing of developmental time windows are essential for precise programmed development to take place, since these neurotransmitters and modulators affect formation of synaptic contacts, maturation of synapses, and structural refinement of connectivity by regulating electrical activity, excitability, and release of neurotrophins (Zhang & Poo 2001:1207-14). Particularly at birth, a vast number of neurotransmitters and transcriptional factors are activated, yet critical periods do not terminate suddenly but rather tapers off gradually. Expression of neurotransmitters and receptor subtypes, i.e., ionotropic and metabotropic receptors (refer to paragraph 2.2), are critical for the development of synapses and formation of neuronal networks underlying behavior in the foetus as well as in the growing child and adult human.

The concept of foetal and neonatal programming also includes the development of neurotransmitters and neuromodulators, i.e., an early stimulus or insult at a critical period can result in long-term structural and functional changes in the central nervous system (Sayer, Cooper & Barker 1997: F162-F164). In keeping with the concept of foetal and neonatal programming, Herlenius and Lagercrantz (2004:11) postulated that prenatal or perinatal stress could disturb the programmed timetable regulating expression of neurotransmitters and neuromodulators, as well as their receptors. Disruption of the normal timing or intensity of neurotransmitter signaling can lead to permanent changes in proliferation differentiation and growth of their target cells during critical phases of development of the nervous system, thereby possibly providing the underlying mechanisms for neurobehavioral or neurophysiological abnormalities associated with developmental exposure to neuroactive drugs and environmental toxins (Herlenius & Langercrantz 2004: 11).

In addition, research findings support the importance and early role for neurotransmitter signaling before synaptogenesis (Verhage et al 2000:864-69), because neurotransmitters and synaptic activity are necessary for survival of synaptic contacts. Without vesicle release of neurotransmitters, neurons undergo apoptosis after formation of synapses, since their maintenance depends on neurotransmitter secretion (Demarque et al 2002:1051-61; Owens & Kriegstein 2002:989). Markers for neurotransmitters and neuromodulators

during CNS development generally appear first in the caudal and phylogenetically older part of the brain probably due to earlier neurogenesis (Semba 1992:33-62). Roder (2000:56) implicated altered brainstem development in the pathogenesis of autism. This researcher found that among individuals diagnosed with autism an area of the brainstem in the caudal part of the pons is small, and that several nuclei in this area, including the facial nucleus that controls facial musculature, are small or missing. Thus, when these structures are smaller and/or missing, it follows that the four ascending activating systems, i.e., the cholinergic, dopaminergic, noradrenergic, and serotonergic systems will also be adversely affected, since these four ascending activating systems are similarly organized in that the cell bodies of their neurons are clustered together in only a few nuclei located in or near the brainstem, whereas the axons of the cells are widely distributed in the forebrain, brainstem, and spinal cord (Kolb & Whishaw 2003:114). In addition, there is accumulating evidence that individuals diagnosed with autism have consistent abnormalities in the cell density of the amygdala (Courchesne 1997:269), and Baron-Cohen (1995) theorized that the extreme abnormalities in social cognition and behaviour in autism result from abnormalities in the amygdala-prefrontal circuit. Early brain development and neurogenesis will be discussed in the next chapter of this research report.

Since the focus of this research project is on the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder, the focus of this literature review now shifts to the role that specific hormones and neurotransmitters play in autism expression.

2.4 THE RELEVANCE OF THE HIPOTHALAMIC-PITUARY-ADRENAL (HPA) AXIS IN THE PATHOGENESIS OF AUTISM

The physiological effects of stress on prenatal development were already attended to in chapter one. It was noted that the endocrine system increases with stress, primarily through activation of the HPA axis. Attention has focused on the endocrine system's neuromodulators or neuropeptides that are secreted in reaction to stress, thereby affecting the nervous system, since these stress hormones are donated directly into the bloodstream (Owens et al 1997:210-257). These neuromodulating hormones act very much like

neurotransmitters in carrying the brain's messages to various parts of the body (Barlow & Durand 2000:280). Corticotrophin releasing factor (CFR) is secreted by the hypothalamus and stimulates the pituitary gland. Further down the HPA axis the pituitary gland activates the adrenal gland, and the stress hormones adrenaline, noradrenaline and cortisol are secreted to complete the feedback loop between the limbic system and the various parts of the HPA axis (Panksepp 1998:119). The stress response, via sympathetic efferents, activates the release of adrenaline (epinephrine) and noradrenaline (norepinephrine) from the adrenal medulla. Up to 15 discrete noradrenaline and dopamine cell groups, designated A1 to A17 have been discovered, scattered from the lower to upper reaches of the hypothalamus, with A16 being in the olfactory bulbs and A17 in the retina (which is still part of the central nervous system). The lower cell groups (A1 to A7) contain noradrenaline and all the higher ones contain dopamine (Panksepp 1998:109). It thus follows that if the involvement of glucocorticoids in the pathogenesis of autism is investigated, the involvement of adrenaline and noradrenaline should similarly be looked into. Therefore, the contribution of adrenaline and noradrenaline to the pathogenesis and expression of autism is now discussed in more depth.

2.4.1 Adrenaline

The existence of adrenaline in the brain was not accepted until the adrenaline-synthesizing enzyme phenyl-ethanolamine-N-methyl transferase (PNMT) was detected by immunohistochemical methods (Panksepp 1998:119). This enzyme was localized in the lower brainstem fused with noradrenergic neurons (Herlenius & Lagercrantz 2004:8). Adrenaline in the brain is probably involved in neuroendocrine and blood pressure control. In addition, adrenaline has inhibitory actions on the locus coeruleus and brainstem respiratory rhythm. Research findings by Foster (1992:115) demonstrated that PNMT occurs predominantly before birth in the rat CNS, while there is a decline in PNMT-containing structures after birth, implicating similar prenatal neuroendocrine involvement in humans.

2.4.2 Noradrenaline

Compared with dopamine systems, which restrict their outputs to the reptilian brain (i.e., the basal ganglia) and frontal cortex, the projections of the caudally situated noradrenaline

systems are more widespread. The cell bodies of the noradrenergic neurons are concentrated in the brain stem, particularly in the locus coeruleus within the caudal pons (Kolb & Whishaw 2003:114). Five major noradrenergic tracts originate from the locus coeruleus that disperse through the whole brain. There are also clusters of noradrenergic cell bodies in the nucleus tractus solitarius, and in the lateral ventral tegmental field (Herlenius & Lagercrantz 2004:9). Fibers from these nuclei intermingle with those from the locus coeruleus. The A6 noradrenaline cell group, better known as the locus coeruleus, controls higher brain activity via the *dorsal noradrenaline pathway*. This group sends inputs to the cortex, hypothalamus, cerebellum, lower brain stem, and spinal cord, thereby exerting control over cortical arousal and attention, fear and anxiety, and learning and memory. The *ventral noradrenaline pathway* infiltrates the hypothalamus and the limbic system (Panksepp 1998:101). In the following paragraphs noradrenaline involvement in prenatal, perinatal and postnatal development is investigated.

Noradrenergic neurons appear at an early stage in the development of the central nervous system. Sundstrom, Kolare, Souverbie and coworkers (1993:2) reported noradrenergic neuronal development at the 12^{th} to 14^{th} day of gestation in the rat and within five to six weeks in the human, suggesting that noradrenaline is essential for normal brain development (Herlenius & Lagercrantz 2004:9). In addition, the noradrenergic system regulates the development of the Cajal-Retzius cells that are the first neurons to be formed in the cortex (Herlenius & Lagercrantz 2004:10). Naqui, Harris, Thomaidou and Parnavelas (1999:75-82) investigated noradrenergic system influences on Cajal-Retzius cells in the developing cerebral cortex and reported that Cajal-Rentzius cells are instrumental in neuronal migration and laminar formation. These researchers furthermore reported that alpha 2A receptors are expressed by migrating neurons in the intermediate zone, characterized by radial alignment and in spindle-like shape, and in close association with radial glia. Wang and Lidow (1997:493-507) demonstrated that radial glia participate in key steps of brain development and cortical neurogenesis, while two independent studies demonstrated glia participation in migration (Noctor, Flint, Weissman, Dammerman & Kriegstein 2001: 714– 720; Noctor, Martinez-Cerdeno, Ivic, & Kriegstein 2004:136-144). Thus, adrenergic transmission may be involved in regulating the generation, migration, and maturation of cerebral cortical cells. Herlenius and Lagercrantz (2004:10) reported that administration of 6-OH-dopamine prevents programmed cell death of these neurons and delays the formation of cortical layers. Lesioning of the noradrenergic projections or

blocking of neurotransmission with receptor antagonist prevents astrogliosis and glial cell proliferation.

Berger-Sweeney and Hohmann (1997:121-142) investigated behavioral consequences of abnormal cortical development and reported that depleted noradrenaline during the perinatal period results in subtle dendritic changes and possibly also alterations in cortical differentiation. Thomas and coworkers in 1995 investigated the role of noradrenaline through targeted disruption of the dopamine h-hydroxylase (DBH) gene in mice and demonstrated that disruption resulted in foetal death, probably due to cardiovascular failure (Thomas, Matsumoto & Palmiter 1995: 643–646). Only about 5% of the homozygotic mice survived until adulthood, presumably due to some placental transfer of noradrenaline. Most of the mice could be rescued to birth by providing them with dihydroxyphenylserine (DDPS), a precursor that can be converted to noradrenaline in the absence of DBH. These mice had a reduced ability of acquisition and retention for some tasks.

In a follow-up study Thomas and coworkers in 1997 investigated the role of noradrenaline in perinatal maternal bonding among mice. They observed that depleted noradrenaline resulted in deficient ability among female mice to take care of their offspring. Thus, there seems to be a critical window during early development when noradrenaline is involved in forming the pathways responsible for maternal bonding (Thomas & Palmiter 1997: 583–592). In keeping with these observations Insel and Young in 2001 investigated the neurobiology of attachment and reported noradrenaline to be involved in the olfactory learning of the newborn, which is of importance for maternal recognition (Insel & Young 2001: 129).

During postnatal development noradrenaline plays an important role in regulating attention, since noradrenergic cells are exquisitely sensitive to environmental stimuli, especially powerful emotional events (Panksepp 1998:110). With low noradrenaline activity individuals tend to perseverate on a task despite changes in stimulus contingencies because of attention deficits. Such individuals are prone to act impulsively rather than deliberately. According to Panksepp (1998:111) noradrenaline dampens the background “noise” or cortical neural activity irrelevant to a given task. This makes the influence of specific incoming signals more prominent in the cortex, namely the ratio of the signal to

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background noise is increased. Thus, it is suspected that with high noradrenaline activity, individuals can better process information that already has access to the cortex.

2.5 THE RELEVANCE OF SERUM CORTISOL LEVELS IN THE PATHOGENESIS OF AUTISM

The relevance of serum cortisol levels in programmed neural development was already described in paragraph 1.4 of chapter one. To supplement paragraph 1.4 some additional research findings are overviewed.

Chronic high endogenous corticosteroid levels can be induced by stress to the mother before birth, or to the child postpartum. About two decades ago scientists started to investigate the link between glucocorticoids and programmed neural development. Kurosawa, Kageyama & John et al (1980:213) investigated the effect of neonatal hydrocortisone treatment on brain monoamines in developing rats. These researchers reported that hydrocortisone enhances the maturation of the monoaminergic systems in the brain. In keeping with these findings Diaz, Fuxe and Ogren (1997:129) reported that administration of glucocorticosteroids to the rat fetus induces alterations of dopamine receptor responses, which affects the spontaneous motor control. El-Khodor and Boksa (2002:201-206) demonstrated that birth insult and stress interact to alter dopamine transporter binding in the rodent brain and ascribed hyper locomotion among rodents to altered dopamine transporter binding. Enduring stress during gestation can alter healthy immune system functioning, thereby affecting cytokines and indirectly the development of monoaminergic circuits in the foetal brain (Jarskog, Xiao & Wilkie et al 1997:711). Teratogenic effects of chronic prenatal exposure to glucocorticoids can alter the monoamine turnover in the locus coeruleus and nucleus tractus solitarius (Peyronnet, Dalmaz & Ehrstrom et al 2002:858). In addition, enduring stress appear to affect ascending serotonergic projections into the hippocampus and long-lasting increase in glucocorticoid receptors (Sapolsky 1997:1620). These reciprocal changes are implicated in a permanently altered HPA axis and consequently in the pathogenesis of autism as a developmental disorder.

In addition to adverse effects of prenatal exposure to elevated levels of glucocorticoids discussed in paragraph 1.4 before, it has also been shown to have deleterious effects on

programmed neural development, i.e., inhibition of neural stem cells, neurogenesis, and migration leading to irreversible decrease in brain weight in certain cortical areas (Edwards & Burnham 2001:433; Challis, Sloboda & Matthews et al 2001:135).

2.6 THE RELEVANCE OF SERUM DIGOXIN LEVELS IN THE PATHOGENESIS OF AUTISM

Kurup and Kurup (2003:1537) implicated elevated serum digoxin levels in schizophrenia, bipolar mood disorder and autism, resulting in increased serotonin in the plasma of patients with autism, while dopamine and noradrenaline are decreased. An increase in endogenous digoxin inhibits membrane Na + -K + ATPase, which causes an increase in intracellular calcium. This increase in intracellular calcium inhibits the functional availability of magnesium, because magnesium is displaced from its binding sites. Kurup and Kurup (2003:1539) propose that low intracellular magnesium and high intracellular calcium consequent to Na + -K + ATPase inhibition appear to be crucial to the pathophysiology of autism. This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation which along with low magnesium can cause further inhibition of Na + -K +ATPase, since ATP-magnesium complex is the actual substrate for this reaction. Cytosolic-free calcium is normally buffered by two mechanisms: ATP-dependent calcium extrusion from cell and ATP-dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus progressive inhibition of Na + -K + ATPase activity first triggered by digoxin. Hisaka (1990:621) established that digoxin also influences the transport of amino acids and various neurotransmitters across cellular membranes, suggesting that a decrease in membrane Na + -K + ATPase activity in autism might be caused by the reduction of the hyperpolarizing neurotransmitters dopamine and noradrenaline and the increase of the depolarizing neuro-active compound serotonin.

Serum digoxin levels in autism is very important, because digoxin, a membrane Na + -K + ATPase inhibitor, is thought to be involved in the regulation of conscious perception (Kurup & Kurup 2003:1557), including perceptual binding, focused attention, and short-term memory. The hypothalamus is connected to the thalamus by the mamillothalamic tract and it is hypothesized that digoxin may play a role in regulating these synapses. There are two-way connections between the cerebral cortex and the thalamic nucleus, and

between the cerebral cortex and hypothalamus, and it is suggested that digoxin also regulate these synapses, thereby mediating conscious perception through the hypothalamus-thalamus-cerebral cortex reverberatory circuit (Kinney & Samuels 1994:458). Individuals with autism may present with a range of behavioural symptoms, including short attention span and odd responses to sensory stimuli, e.g., oversensitivity to sounds, tactile perception, and exaggerated reactions to light or odors (APA 2000:72). It seems as if these individuals find it difficult to screen out various stimuli and to focus on one piece of information. Increased secretion of digoxin produces a hyperconscious state with increased focused attention, perceptual binding, and short-term memory. There are connections between the hypothalamus and cerebral cortex and digoxin may serve as a neurotransmitter for these synapses (Kurup & Kurup 2003:1539). Hypothalamic digoxin can thus integrate multiple brain functions. Digoxin can regulate neuronal transmission and conscious perception in the brain by its effect on neutral amino acid and neurotransmitter transport. Digoxin can also play a role in endocrine integration (Greenamyre & Poter 1994:S7–S13). The hypothalamic hormone secretion is regulated by the biogenic amines noradrenaline, dopamine, and serotonin. Digoxin, by regulating the release and uptake of these neurotransmitters, can control hypothalamic hormone secretion, thereby influencing HPA axis functionality. These observations are in keeping with research findings implicating glucocorticoids in disrupted neural development, i.e., brain stem, cerebellum, hippocampal and limbic pathway abnormalities among individuals with autistic disorder (Bauman & Kemper 1995:1-26; Sapolsky 2000:925-935).

In addition, Sapolsky (2000:925-935) found that programmed apoptosis is affected by elevated levels of glucocorticoids, which concept relates closely to the pathogenesis of autism (Kalat 2001:346). Not only will elevated levels of glucocorticoids have an immediate effect on neural development, but it may also result in long term ‘resetting’ of the foetal HPA axis, which may persist into adulthood, because the normal negative feedback system that regulates normal homeostasis is permanently altered (Edwards et al 1993:355). In addition, prenatal glucocorticoid exposure permanently programmes several central nervous system functions such as serotonin sensitivity (Cuccaro et al 1993:95). Serotonin is known to play a role in brain development prior to the time it assumes its role as a neurotransmitter in the mature brain. Serotonin regulates both the development of serotonergic neurons (termed autoregulation of development) and the development of target tissues. In both cases, the astroglial-derived protein, S-100_ plays a role. Disruption

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of serotonergic development can leave permanent alterations in brain function and behavior. This may be the case in such human developmental illnesses as autism (Whitaker-Azmitia 2001:479–485), which will now be reviewed in the following paragraphs.

2.7 THE RELEVANCE OF SERUM SEROTONIN LEVELS IN THE PATHOGENESIS OF AUTISM

Serotonin, like other monoamine neurotransmitters, has been shown to play a role in regulating brain development prior to the time it assumes its role as a neurotransmitter in the mature brain (Chubakov, Gromova, Konovalov, Sarkisova & Chumasov 1986:285; Chubakov, Tsyganova & Sarkisova 1993:271; Lauder 1990:297; Turlejski 1996:619; Whitaker-Azmitia 1991:553; Whitaker-Azmitia, Druse, Walker & Lauder 1996:19).

Serotonin (5-HT) is a chemical that functions as a neurotransmitter in the brain. This neurotransmitter is concentrated in the raphe nucleus of the brain, and it is also present in certain blood cells called *platelets*. Serotonin is of interest to autism researchers because some individuals with autism have consistently been found to have high levels of blood plasma serotonin (Cuccaro et al 1993:95).

Serotonin and serotonergic neurons are localized in the midbrain, the pineal gland, the substantia nigra, the hypothalamus, and the raphe nuclei of the brain stem (Herlenius & Lagercrantz 2004:18). The 5-HT neurons have widespread projections making it possible to coordinate complex sensory and motor behavioural conditions. There are a multitude of heterogeneous 5-HT receptors - in fact more than 15 molecularly 5-HT receptors were identified. The majority of the 5-HT receptors belong to the G-protein receptor family (Hoyer, Hannon & Martin 2002: 533– 554), involved in enhancing motor neuron excitability (Herlenius & Lagercrantz 2004:18). Boutrel, Franc & Hen et al (1999:3204-3212) investigated the key role of 5-HT1B receptors in the regulation of paradoxical sleep and reported that serotonin is involved in inducing sleep, sensory perception, temperature regulation, and control of mood, therefore serotoninergic activity was found to be highest during waking and arousal, and absent during active or rapid-eye-movement sleep.

In addition, serotonin has been reported to affect neuronal proliferation, differentiation, migration, and synpatogenesis (Gaspar, Cases & Maroteaux 2003:1002). In the mammalian brain, all of the monoamine neurotransmitter systems are present relatively early, but in particular, serotonin is likely present the earliest in the most terminal regions (Whitaker-Azmitia 2001:479). These early appearances of serotonergic neurons with their wide distribution of terminals play a crucial role in programmed neurogenesis, synaptogenesis and apoptosis. In addition to its role in regulating maturation of terminal areas, serotonin can set its own terminal density - a phenomenon Whitaker-Azmitia (2001:480) has termed autoregulation of development. According to Gaspar et al (2003:1002) serotonin can already be detected in the fertilized egg and is involved in early morphogenesis of the heart, the craniofacial epithelia, and other structures. They demonstrated specific craniofacial malformations in embryos if cultured in the presence of serotonin uptake inhibitors or receptor ligands.

Serotonergic cells in the raphne are among the earliest to be generated in the brain (Gaspar et al 2003:1003). After their generation in the raphe, they start to project diffusely into the spinal cord and the cortex. Serotonergic cells emerge during the fifth to the twelfth gestational week in the human foetus. These serotonergic cells send axons to the forebrain and may be of importance in the differentiation of neuronal progenitors (Gaspar et al 2003:1003). Excess of serotonin prevents the normal development of the somatosensory cortex, which has been demonstrated in monoamine oxidase knockout mice. In keeping with Gaspar and colleagues' research findings, Cases, Vitalis, Seif and coworkers (1996:297–307) have also demonstrated that excess of serotonin prevents the normal development of the somatosensory cortex in knockout mice. They reported that at birth, serotonergic-containing axons penetrate all cortical layers, but then decline markedly after about three weeks. Depletion of serotonin after birth seems to have little effect on cortical development in mice; however, a transient uptake and storage of serotonin in developing thalamic neurons occur during formation of somatosensory cortex. Lebrand, Cases, Adelbrecht and coworkers (1996:823) investigated transient 5-HT uptake and storage in developing thalamic neurons, and concluded that transient 5-HT uptake and storage is due to the temporary expression of the high affinity serotonin transporter (SERT) during formation of the somatosensory cortex. Gaspar et al (2003:301) postulated that this 5-HT uptake and possibly the use of 5-HT as a 'borrowed transmitter' seem necessary for the normal neural development and the fine-tuning of cortical sensory maps during critical

developmental periods in rodents. Human foetuses have a similar restricted time period of SERT expression (gestational week 12– 14) when thalamocortical fiber tracts develop and fine-tuning of cortical sensory maps occurs (Verney, Lebrand & Gaspar 2002:87). The foetal human brain, especially the cortex and hippocampus, exhibits a prenatal peak in the density of serotonin 5-HT1A receptors during gestational weeks 16 to 22 (Bar-Peled, Gross-Isseroff, Ben-Hur, et al 1991:173). These researchers reported that activation of the 5-HT1A receptor is associated with increased neurogenesis, neural differentiation, and dendritic maturation in the hippocampus. It thus follows that serotonin concentration must be neither too high nor too low during the critical period of synaptogenesis and formation of cortical connections. Disruptions of the serotonergic pathways due to excess or inadequate activation of specific 5-HT receptors during development are thus implicated in the pathogenesis of autism (Gaspar et al 2003). Gaspar and colleagues' observations in this regard are in support of previous findings related to autism and hyperserotonism. Chugani (2002:16; 2004:112) on two occasions reported that the pathogenesis of autism might be related to hyperserotonism during foetal life but also with hyperserotonism postnatally. Herlenius and Lagercrantz (2004:18) reported that serotonin is temporarily synthesized in high levels in young children. Although this elevated serotonin supply declines in normal children, it does not decline in autistic children.

Serotonergic abnormalities have been reported in autism, specifically hyperserotonemia, as well as elevated blood serotonin in the first-degree relatives of children with autism (Leventhal et al 1990, Piven & Palmer 1999, Leboyer et al 1999, Chugani 2004). Chugani (2004:112) furthermore proposed that serotonergic abnormalities during prenatal and early postnatal development might lead to reciprocal changes in thalamocortical connectivity, which results in a certain predisposition for autism. As indicated in the preceding paragraphs, this might result in altered programmed neural development and 'resetting' of the foetal HPA axis, because the normal negative feedback system that regulates normal homeostasis is permanently altered (Edwards et al 1993:355).

Hhyperserotonemia in autism may also involve atypical metabolism of the metabolic serotonin precursor tryptophan as a potential mechanism for alterations in serotonin availability. In addition Nabi and coworkers in 2004 established a susceptibility mutation in a promoter variant of the tryptophan 2,3-dioxygenase gene that might impact serotonin metabolism in autism (Nabi, Serajee, Chugani, Zhong & Huq 2004:63-68). Tryptophan

2,3-dioxygenase is a rate-limiting enzyme in the metabolism of tryptophan by the kynurenine pathway. Tryptophan 2,3-dioxygenase, as well as the tryptophan catalyst indoleamine 2,3-dioxygenase, is expressed in the placenta and have a role in the prevention of allogeneic rejection of the foetus (Munn, Zhou, Attwood & Bondarev et al 1998:1191-1193; Suzuki, Tone, Takikawa & Kubo et al 2001:425-429). According to Chugani (2004:112) a mutation that results in decreased activity of this enzyme could decrease the metabolism of tryptophan through the kynurenine pathway, causing a shift toward increased levels of serotonin as noted in autism.

The imipramine-sensitive serotonin transporter is highly expressed in the human placental brush-border membranes and may mediate transport of serotonin from the maternal circulation to the developing foetus (Balkovetz et al 1989:2195-2198; Chugani 2004:112), therefore placental serotonin transporter expression might constitute a risk factor for autism (Persico, Militerni, Bravaccio & Schneider et al 2000:123-127; Anderson, Gutknecht, Cohen & Brailly-Tabard et al 2002:831-836; Persico, Pascucci, Puglisi-Allegra & Militerni et al 2002:795-800; Betancur, Corbex, Spielewoy & Phillippe et al 2002:67-71). According to Chugani (2004:113) alterations of serotonin (or tryptophan) metabolism and/or transport during prenatal development may regulate key steps during cortical development, based upon Janusonis and coworkers' findings that serotonergic fibres innervate Cajal Retzius cells, which are necessary for cortical column development (Janusonis, Gluncic & Rakic 2004:1652-1659). Janusonis and coworkers reported that treatment with the serotonin agonist 5-methoxytryptamine during foetal development led to alterations in brain reelin levels (a glycoprotein produced by Cajal Retzius cells) and abnormalities of presubicular cortical column development, which concept is addressed in chapter three of this research report. Abnormal levels of reelin and dysregulation of reelin and Bcl-2 proteins in the cerebellum (Fatemi, Stary, Halt & Realmuto 2001:529), as well as an increased number of minicolumns with fewer cells per column or greater cell dispersion were reported in human autism autopsy brain tissue (Casanova, Buxhoeveden & Brown 2002:692; Casanova, Buxhoeveden, Switala & Roy 2002:428).

Cuccaro and colleagues investigated the link between blood plasma serotonin levels and the verbal ability of individuals with autism and their immediate relatives, administering the Wechsler scales. These researchers found that individuals with high blood plasma serotonin levels had lower verbal ability scores. However, other measurements of

intellectual abilities were not changed, including visual-spatial ability and memory (Cuccaro et al 1993:99).

To determine whether there are serotonergic abnormalities in the brains of children with autism, Chugani and coworkers (1998:33-43) evaluated human brain serotonin synthesis capacity in vivo with positron emission tomography (PET), using the tryptophan analog alpha-[C-11]methyl-L-tryptophan (AMT) as a tracer. These researchers (Chugani et al 1999:287-295) attributed developmental changes to altered brain serotonin synthesis capacity in autistic children (1999:287-295) and they reported on two fundamentally different types of serotonergic abnormalities, namely a difference in the change with age in *whole brain* serotonin synthesis, and *focal* abnormalities in brain serotonin synthesis. Following this evaluation, it was established that for non-autistic children serotonin synthesis capacity was >200% of adult values until the age of five years and then declined towards adult values. In autistic children, serotonin synthesis capacity increased gradually between the ages of two years and 15 years to values 1.5 times the adult normal values, thereby implicating that at a given early age less than five years, the serotonin synthesis capacity in an autistic child is much lower than that in a non-autistic child. These findings thus illustrated that in the human brain there is a period of high brain serotonin synthesis capacity during early childhood and that this developmental process is disrupted in autistic children. With regards to focal abnormalities in brain serotonin synthesis, Chugani et al (1999:287-295) established asymmetrical uptake of AMT in frontal cortex, thalamus, and cerebellum among children with autism. In a follow-up report Chugani (2004:113) reported that autistic children with left cortical AMT decreases showed a higher prevalence of severe language impairment, whereas those with right cortical decreases showed a higher prevalence of left- and mixed-handedness. Based upon the research findings of Hutsler (2003:226-242) and Hutsler and Galuske (2003:429-435) significant differences were observed in minicolumn organization between the left and right sides in the normal human brain, as well as asymmetry in the size of the pyramidal cells constituting the minicolumns, with a greater number of large pyramidal cells in the left hemisphere than in the right hemisphere. These differences suggest a profound impact on how various autistic traits are expressed, such as language, because pyramidal cells in the left hemisphere contact fewer adjacent minicolumns than pyramidal cells in the right hemisphere, with a specific locus in the posterior language cortex on the left. Conversely, the distance

between macrocolumns are 20% greater on left than on the right in Brodmann area 22 (Galuske et al 2000:1946).

How do these alterations link with serotonin? The serotonin transporter is transiently expressed by glutamatergic thalamocortical afferents during the first two postnatal weeks in rodents (Bennett-Clarke, Chiaia & Rhoades 1996:301-306; Lebrand et al 1996:823-835). Postnatal serotonin levels regulate the size of cortical barrel macrocolumns – too little serotonin leads to smaller barrel macrocolumns and too much serotonin leads to larger barrels (Chugani 2004:113). Cases and coworkers (1996:297-307) reported on research findings indicating that increased brain serotonin levels during this critical postnatal weeks led to increased peripheral arborization of thalamocortical axons, resulting in blurring of the boundaries of the cortical barrels. In addition, Chugani (2004:113) proposed that the presence of smaller, more closely spaced minicolumns in the brains of autistic children might trigger compensatory changes in cortical serotonin synthesis in the early postnatal period when serotonin regulates formation of thalamocortical afferents. Rosen, Burstein and Galaburda (2000:423) reported that thalamocortical afferent fibers contain serotonin during development due to transient expression of the serotonin transporter, therefore Chugani (2004:114) inferred that these fibers continue to express the transporter in tissue with cortical dysplasia rather than down-regulating the serotonin transporter with brain development. Such a developmental abnormality seems to be consistent with increased immunoreactivity of fine fibers (presumably thalamocortical) as reported earlier by Trottier and coworkers (1996:25).

From what is known about serotonin and development, it is likely that high levels of serotonin during development would cause a loss of serotonin terminals. This may be what occurs in autistic children, and there is some clinical data that suggests this. Firstly, there is evidence that autistic children respond to serotonin-enhancing drugs (Buitelaar & Willemsen-Swinkels 2000:97; McDougle, Kresch & Posey 2000:427; Posey & McDougle 2000:45). Secondly, an alarming rate of autism is reported in children exposed *in utero* to drugs known to alter serotonin, including cocaine (Davis, Fennoy & Laraque et al 1992:315; Kramer, Azmitia & Whitaker-Azmitia 1994:142) and alcohol (Nanson 1992:558). Thirdly, recent evidence suggests hypersensitivity of the serotonin 5-HT1D autoreceptor (Novotny, Hollander & Allen et al 2000:173), which may be related to repetitive behaviors (Hollander, Novotny & Allen et al 2000:163). Finally, a PET study

using a radiolabeled form of a serotonin precursor found decreased serotonin synthesis in cortex and thalamus of autistic individuals, although an increase was found in the dentate nucleus (Chugani, Muzik & Rothermel et al 1997:666). This loss of serotonin terminals could then lead to altered developmental processes in target areas

Chugani (2004:115) concluded that serotonergic abnormalities are associated with abnormalities of cortical development and thalamocortical connectivity as abnormal serotonin transport or synthesis during brain development may directly affect formation of intracortical and thalamocortical circuitry. The relative balance of tryptophan metabolism, regulated by the serotonin and kynurenine pathways might therefore be important in the pathogenesis of autism and these serotonergic abnormalities may at least partially explain characteristic expression of autism.

2.8 SYNOPSIS

In this chapter the contribution of biochemicals to the pathogenesis of autism was described. According to research findings glucocorticoids, digoxin and serotonin might play an important and unique role in foetal cortical development.

Chapter four describes the empirical research and related findings. Based upon the findings, the research hypothesis will be accepted or rejected.

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CHAPTER 3

NEUROANATOMICAL OBSERVATIONS OF THE BRAIN IN AUTISM

3.1 INTRODUCTION

This chapter first describes normal brain development, followed by neuroanatomical observations of the brain in autism. Steffenburg (1991:495) found that almost 90 percent of a sample of autistic children had evidence of a brain abnormality. Recently it has been reported that a substantial proportion of cases are associated with megalocephaly, an abnormal enlargement of the head (Courchesne 2004:106). Various other anatomical sites in the brain have been hypothesized as the primary source of pathology, such as enlarged brain size, reductions in the area of the corpus callosum, and abnormalities of the cerebellum and the medial temporal lobe structure, therefore the relevance of structural brain differences found among individuals diagnosed with autistic disorder, as well as how these differences link to different stages of programmed foetal development are reviewed in this chapter. Because this research focuses on prenatal stress and autism, findings related to brain abnormalities as well as altered neural programming due to prenatal stress will complement the review.

3.2 NORMAL BRAIN DEVELOPMENT

The brain develops through a series of overlapping stages (Teicher et al 2002:397). Embryonic and foetal stages of development of the human brain are characterised by a series of changes that take place in a relatively fixed sequence, i.e., cell birth (neurogenesis and gliogenesis), cell migration, cell differentiation, cell maturation (dendritic and axonal growth), synaptogenesis (formation of synapses), programmed cell death (apoptosis) and synaptic pruning, and myelogenesis (formation of myelin) (Kolb & Whishaw 2003:610). A[1]t the time that an egg is fertilized by a sperm, a human embryo consists of just a single cell, which starts to divide, and by the 14th day the embryo consists of several sheets of

[1]The conversion of DNA to RNA is called transcription. The conversion of RNA to proteins is called translation (Panksepp 1998:98).

cells with a raised middle area, representing the primitive body. At about 18 days after conception the human brain starts to develop from the neuroectoderm, a placode of cells that are induced to differentiate from the surrounding ectoderm. This differentiation is induced by factors such as retinoids and follistatin (Clark 2002). Retinoic acid can alter the pattern of transcriptional factors in neuroepithelial cells, explaining some of the craniofacial defects seen in retinoid embryopathy (Clark 2002:3). Almost all steps in organogenesis are controlled by retinoic acids, thus suggesting that retinol is necessary for normal development of embryonic tissues, neuronal growth and cellular differentiation (Zachman 1995:1634S; Perrotta et al 2003:457; Debier & Larondelle 2005:153).[2] Retinal lesions include atrophy and gliosis of the ganglion cell layer and the nerve fibre layer (Van der Lugt & Prozesky 1989:99). At three weeks after conception the human embryo has a primitive brain, essentially consisting of a sheet of cells (neural plate), which rolls up to form a structure called the neural tube (Kolb & Whishaw 2003:611). The neural tube forms in axial fusion, with closure occurring in a simultaneous caudal and cranial progression at 22 days of gestation (Clark 2002:2). This event practically represents the start of the central nervous system (CNS). Even before the neural tube is seen on gestational day 21, critical events in the formation of the CNS have taken place. Gastrulation establishes a midline, axes for dorsal-ventral and anterior-posterior orientation, and symmetry. The notochord and somites develop during this phase to induce the ectoderm to form the neural plate and to establish segmental organization. During this process of neurulation, the cranial neuropore of the neural tube closes by 24 days of gestation and serves as the foundation for further brain development. The caudal neuropore of the neural tube closes by gestational day 26 and serves as the foundation for further spinal cord development (Schmid & Rotenberg 2005:4).

The body and nervous system change rapidly during the next three weeks of gestation. After neurulation described in the preceding paragraph, subsequent processes such as cell migration and differentiation, dendritic and axonal growth, synaptogenesis, programmed apoptosis and myelogenesis start after gestational day 28, but some of these processes continue postnatally, for example glial and synapse formation that continue to vigorously until approximately three years of age (Schmid & Rotenberg 2005:4). By seven weeks of gestation (49 days), the embryo starts to resemble a miniature person, and by about 100

days after conception, the brain looks distinctly human; however, it does not begin to form gyri and sulci until about seven months. At full term, i.e., 40 weeks, the brain grossly resembles the adult brain, though its cellular structure is different (Kolb & Whishaw 2003:611).

This programmed prenatal cortical development may be disrupted by deficits in the genetic program, intrauterine trauma, the influence of toxic agents, or other factors that may lead to the pathogenesis of developmental disorders in childhood. It is postulated that specific toxins may have mechanisms to exploit certain periods during development, as illustrated in **table 3.1** below (Schmid & Rotenberg 2005:5).

As depicted in table 3.1, neural proliferation is vulnerable to ethanol, organophosphates, and MeHg disruption. It follows that if proliferation is altered, migration may also be altered, leading to ectopic tissues. Cell differentiation may be changed and or interrupted by ethanol, nicotine, MeHG, and lead. Some of the same agents, ethanol, lead, MeHg, parathion, permethrin, di-isopropyl fluorophosphates and PCB compounds are involved in altering synaptogenesis. These insults may continue to disrupt programmed cortical development for years (Schmid & Rotenberg 2005; Courchesne 2004; Levitt 2003; Nicolson & Szatmari 2003; Dawson et al 2002; Clark 2002; Sparks et al 2002; Schultz & Klin 2002; Teicher et al 2002). Myelination peaks during the third trimester in humans and continues into the young adult years, accounting for the developing brain's longer period of vulnerability (Coleman 1994:107). Myelination disturbances have been linked to malnutrition, iron deficiency, alcohol, and lead exposures (Schmid & Rotenberg 2005:5). In addition, programmed cell death or apoptosis might also be disrupted by toxic exposures through a shift in the balance of neurotrophic signals, resulting in an increase or decrease in the number of cells. Ethanol, lead, MeHg, and PCBs have been implicated in altered cell numbers (Schmid & Rotenberg 2005:5). Each of these developmental processes is now discussed in more detail.

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Table 3.1 Potential neurotoxic agents and their teratogenic windows

Age	Process in development	Potential neurotoxic agents	Altered outcomes
0 to 4 weeks of gestation	Gastrulation-notochord and somite formation.	Retinoic acid.	Disordered polarity, malformations of the hindbrain and spinal cord.
4 weeks of gestation	Neurogenesis in spinal cord and hindbrain.	Hot tubs; Folic acid antagonists.	Anencephaly, hydrocephaly.
28 to 35 weeks of gestation	Migration.	Ionizing radiation; MeHg.	Ectopia, Cerebral palsy, Learning disorders.
Middle-late pregnancy	Neuron proliferation and synaptogenesis.	Lead; PCBs; MeHg.	Neurobehavioral deficits.
Third trimester	Neurogenesis in cerebellum, hippocampus cell migration, myelination, synaptogenesis.	Pesticides.	Multiple: poor motor control, emotional lability, cognitive deficits and delays.
Infant to 3 years of age	Development of executive functions in the prefrontal cortex.	Lead (postnatal); Alcohol (prenatal); Cigarettes (prenatal).	Behavioral impairments, possible increased criminality.
4 to 17 years of age	Increase in fiber tracts of motor and speech functions; ability to build on previous learning; improved sensory function, specifically auditory.	Organophosphates; Lead, PCBs; MeHg; Lead.	Poor axonal outgrowth. Lowered IQ. Impaired concentration.

Source: Schmid & Rotenburg 2005:5

3.2.1 Neurogenesis and gliogenesis

The genesis of glia cells begins at the time of neuron genesis (early gestation), and the glia continue to differentiate and proliferate long after the migration of neural cells is complete (Teicher et al 2002:398; Clark 2002:5). The cells lining the neural tube are known as neural stem cells due to these cells' capacity for self-renewal. When a stem cell divides, two stem cells are produced, of which one dies and the remaining one continues to divide

again – a process that is repeated throughout an individual's lifespan. In an adult, neural stem cells line the ventricles to form the ventricular zone, and they also generate progenitor (precursor) cells. These progenitor cells can also divide, but they eventually produce nondividing neuroblasts and glioblasts that mature into neurons and glia. Neurogenesis (production of new neurons) ceases in most brain regions at birth, although stem cells continue to generate neurons and glia into adulthood, even in an aging brain, at least within the olfactory bulb and the hippocampal dentate gyrus throughout life (Eriksson, Perfilieva & Bjork-Eriksson 1998:1313; Teicher et al 2002:398; Kolb & Whishaw 2003:612). This cell division is programmed, resulting in the appropriate number of cells for the future cortex. Abnormalities in the number of proliferative units or in the total number of divisions can lead to disorders of the brain manifested by abnormal brain size and, therefore, an unusaually small or large head circumference (Clark 2002:5). From birth to five years of age, the brain triples in mass. Much of the gain in brain size stems from the vigorous myelination of fiber tracts (Teicher et al 2002:398).

3.2.2 Cell migration and differentiation

Cell migration starts shortly after the first neurons are generated. Clark (2002:7) provided the following simplified discription of cell differentiation and migration. At the completion of general neurogenesis, cell differentiation begins, the process in which neuroblasts become specific types of neurons. At the time of neuronal differentiation the neural tube consists of four consecutive layers: (a) the innermost layer is called the ventricular zone and gives rise to neurons and all of the glia of the CNS; (b) the adjacent more superficial layer is the subventricular zone, which is the staging area from which postmitotic neurons begin to differentiate and to migrate; (c) the adjacent intermediate zone is destined to become the cortical plate and the future cerebral cortex; and (d) the marginal zone, which is the outermost zone and which is composed of the cytoplasmic extensions of ventricular neuroblasts, corticopetal fibers, and the terminal processes of radial glia (which, at this time, are completely spanning the neural tube). Differentiation of neuroepithelial cells begins in the subventricular layer at approximately gestational day 26. The older, larger pyramidal cells are the first cells to be born and probably differentiate early to act as targets in the migration of the nervous system (Clark 2002:7). Disorders such as tuberous sclerosis, cortical dysplasia, cortical migration abnormalities, cortical

dysgenesis and the development of giant-cell astrocytomas might be due to "faulty" neuronal differentiation (Clark 2002:8).

The genesis of neuroblasts predestined to construct the cerebral cortex largely reach completion at about 4½ months of gestation, whereas cell migration to various regions continues for a number of months, even postnatally, with some regions not completing cell migration until about eight months after birth (Kolb & Whishaw 2003:613). At the most rostral end of the neural tube in the 40-to-41-day-old foetus, the first mature neurons, Cajal-Retzius cells, begin to migrate to the cortical surface. Radial glial fibers extend from the ventricular zone to the cortical surface, and neurons migrate along the radial glial fibers, which take them from the protomap in the ventricular zone to the corresponding region in the cortex (Kolb & Whishaw 2003:613). These migrating neurons accomplish this task by attaching to and migrating along radial glial in a process known as *radial migration* or by *somal translocation* in a neuronal process (Clark 2002:7). In the process of migration, the deepest layer of the cortical plate migrates and deposits before the other layers. Therefore, the first neurons to arrive at the future cortex are layer VI neurons. More superficial layers of cortex then are formed - the neurons of layer V migrate and pass the neurons of layer VI; the same process occurs for layers IV, II, and I. The cortex therefore is formed in an inside-out fashion (Clark 2002:7). Although most cortical neurons follow the radial glial fibers, a small number of them appear to migrate by following some type of chemical signal (Kolb & Whishaw 2003:613). Migration can stop prematurely, leaving a group of cells that belong in an outer layer scattered among inner layers of cells.

Disorders of migration can be identified by means of MR-imaging, and the most common effect of faulty migration in humans are disorders such as dyslexia or epilepsy (Kolb & Whishaw 2003:614). Some relatively unknown migration disorders are lissencephaly spectrum disorders (smooth brain), which refers to the external appearance of the cerebral cortex in which a neuronal migration aberration leads to a relatively smooth cortical surface, for example classic lissencephaly, isolated lissencephaly, X-linked lissencephaly, lissencephaly with cerebellar hypoplasia, cobblestone lissencephaly, polymicrogyria, and heterotopias (Clark 2002:8-16).

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3.2.3 Cell maturation (dendritic and axonal growth)

After neurons have migrated to their final destinations and differentiated into specific neuron types, they begin the process of growing dendrites to provide the surface area for synapses with other cells (Kolb & Whishaw 2003:614). This process is illustrated in figure 3.1 *(page inserted)*.

As illustrated in figure 3.1 dendritic development involves dendritic arborization (or branching), and the growth of dendritic spines. Arborization commences with dendrites that start to protrude from the cell body and then develop into increasingly complex extensions that resemble the branches of a tree. These dendritic branches then start to form spines, on which most dendritic synapses take place (Kolb & Whishaw 2003:614). Although dendritic development begins prenatally in humans,there is a marked expansion of axonal and dendritic arborizations and a rapid increase in synaptic contacts during the postnatal phase and during childhood. This process mirrors the earlier overproduction and elimination of neurons (Teicher 2002:398). Axons develop at the rate of a millimeter per day, whereas dendritic growth happens at a slow rate, measurable in micrometers per day. This disparate development allows the developing axon to contact the target cell before the dendrites of that cell are completely formed, thereby enabling the axon to play a role in dendritic differentiation (Kolb & Whishaw 2003:615). The formation of these neural pathways can be disrupted by perinatal lesions, while early postnatal lesions can result in the axon failing to reach its target. Axonal development can also be adversely affected by neurotoxins, malnutrition, lesioned target cells, and genetic mutations (Kolb & Whishaw 2003:615; Courchesne 2004:106; Schmid & Rotenberg 2005:5).

A series of complex peptide molecules have been indentified that govern the maturation and development of specific neural systems such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), epidermal growth factors, fibroblast growth factors, glial-derived growth factors, insulin-like growth factors, and many others (Panksepp 1998:114). These molecules control specific growth processes in the brain, and they can also protect neurons against various forms of toxicity, for example BDNF and NGF can protect cerebellar granule cells and Purkinje cells (Tatter, Galpern & Isacson 1995:286-297). These are the types of neurons known to be deficient within cerebellar tissues of many autistic children (Panksepp 1998:114; Bailey, Luthert, Harding et al 1998:880-905).

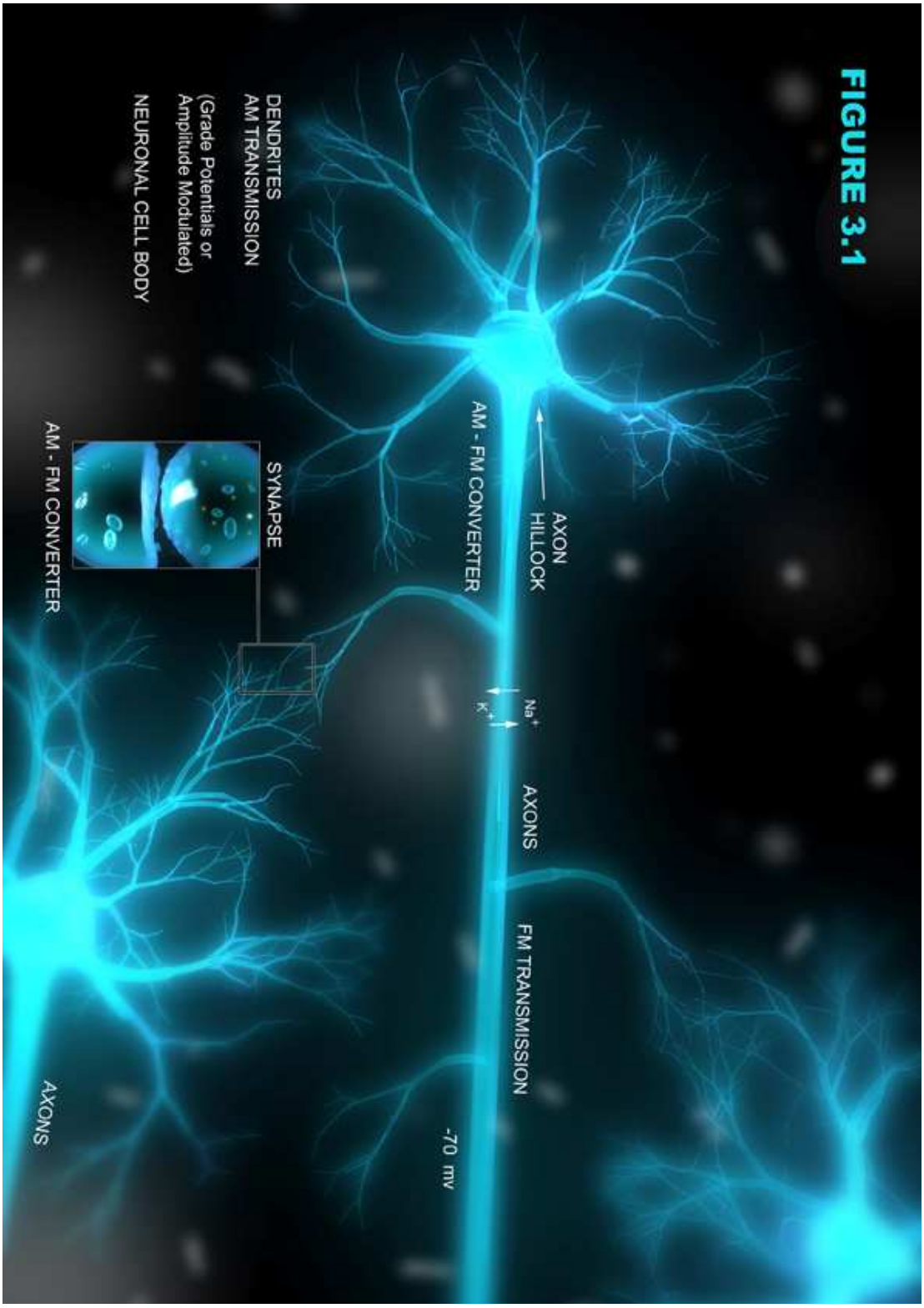
FIGURE 3.1
AXON HILLOCK
AM - FM CONVERTER
DENDRITES
AM TRANSMISSION
(Grade Potentials or Amplitude Modulated)
NEURONAL CELL BODY
SYNAPSE
AM - FM CONVERTER
Na+
K+
AXONS
FM TRANSMISSION
-70 mv
AXONS

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3.2.4 Synaptogenesis, programmed cell death and synaptic pruning

Teicher and coworkers (2002:399) reported on *neuronal modification by selective depletion*, which refers to the genetically programmed overproduction of synapses. According to these researchers programmed synapse formation takes place according to distinct phases, and the final configuration of the circuitry occurs by elimination of synapses based on cell interactions, i.e., programmed cell death and synaptic pruning, also known as apoptosis, which process is illustrated in figure 3.2 *(page inserted)*.

As illustrated in figure 3.2, synaptogenesis in humans starts during early embryonic life and this phase is characterized by the generation of low-density synapses. The next phase of synapse formation starts before birth, continues until nearly two years of age, and is characterized by a rapid growth in the number of synapses, i.e., this phase peaks at about 40 000 synapses per second. During the next phase synaptogenesis initially reaches a plateau in numbers, followed by a rapid elimination of synapses, i.e., synaptic pruning (apoptosis) (Kolb & Whishaw 2003:616). Because there is a substantial overproduction of synapses, receptors, dendrites and axons, some are pruned back during the transition into adulthood without cell death (Teicher et al 2002:398). Synaptic pruning peaks during puberty, and the number of synapses may be pruned at a rate of 100 000 per second to 50% of the number present at age two. The final phase is characterized by a plateau in synapse number through middle age, followed by a slow but steady decline in the density of synapses with advancing age. There is a rapid drop during senescence before death (Kolb & Whishaw 2003:616).

Some of these synapses are experience dependent (Teicher et al 2002:399). Schmid and Rotenberg (2005:5) reported abnormal patterns of apoptosis after neurotoxic exposure. The teratogenic effect of exposure to neurotoxic agents can lead to a shift in the balance of neurotrophic signals, resulting in an increase or decrease in the number of cells. Overproduction and elimination specifically affects excitatory synapses and the density of glutamate, dopamine, and neurotensin receptors. The rate and degree of pruning vary from region to region (Teicher 2002:398).

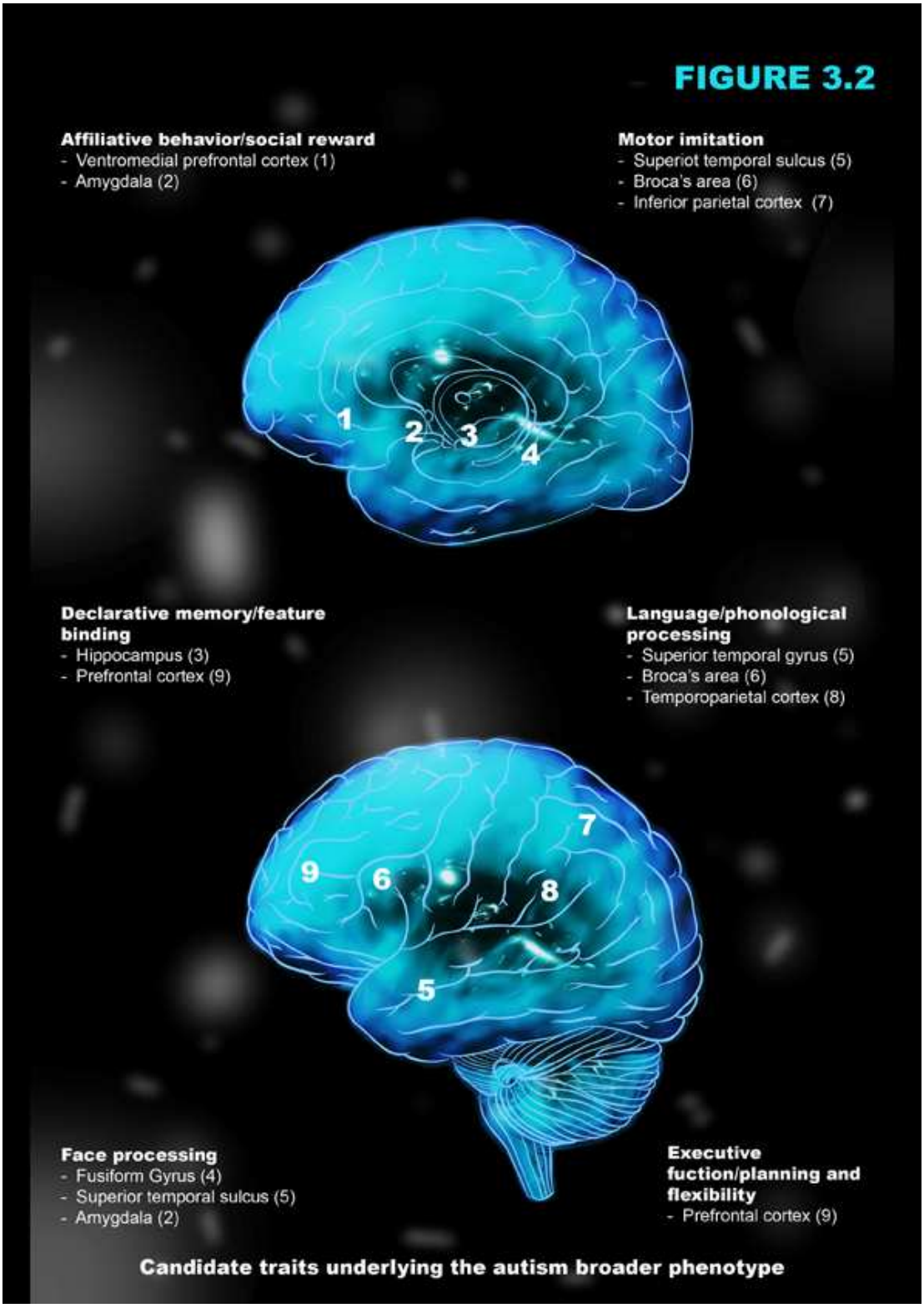
FIGURE 3.2
Affiliative behavior/social reward
- Ventromedial prefrontal cortex (1)
- Amygdala (2)
Motor imitation
- Superiot temporal sulcus (5)
- Broca's area (6)
- Inferior parietal cortex (7)
1
2
3
4
Declarative memory/feature binding
- Hippocampus (3)
- Prefrontal cortex (9)
Language/phonological processing
- Superior temporal gyrus (5)
- Broca's area (6)
- Temporoparietal cortex (8)
7
9
6
8
5
Face processing
- Fusiform Gyrus (4)
- Superior temporal sulcus (5)
- Amygdala (2)
Executive fuction/planning and flexibility
- Prefrontal cortex (9)
Candidate traits underlying the autism broader phenotype

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3.2.5 Myelogenesis

Myelination begins prenatally and continues in the CNS through childhood and into adulthood. Myelination markedly increases the speed of information exchange (Teicher 2002:398). The vestibular system is primarily myelinated prenatally, whereas association cortices continue to be myelinated into the second decade (adolescence) (Schmid & Rotenberg 2005:1). Myelination and axonal growth are substantial throughout childhood, and the myelination rate varies markedly between brain regions, for example, frontal grey matter volumes increase 20% between early childhood and the end of childhood (Carper et al 2002:1038). Cerebral white matter volumes increase 59% between two to three and sixteen years of age (Courchesne et al 2001:245). Maximum brain volumes are not reached until about 10 to 12 years of age (Courchesne 2004:109), while cerebral white matter volume continues to increase through middle age (Courchesne et al 2000:672), and the corpus callosum continues to grow throughout childhood and into adulthood (Pujol, Vendrell, Junquae, et al 1993:71). Critical motor systems myelinate at an early age, whereas the process is quite protracted in the prefrontal cortex, and gender also affects myelination rate in some regions, such as the corpus callosum and the hippocampus (Teicher 2002:398).

According to recent MRI studies, this slow and differential maturation of the brain does not happen in autism – there is rather a relatively brief period of overgrowth, followed by reduced or arrested growth (Courchesne 2004:109). Oversized brain structures in the two-to-three-year-old autistic brain stop growing at an accelerated rate (Courchesne et al 2001:251), consequently the volume of typically developing children eventually "catches up" to the autistic brain (Courchesne 2004:109), i.e., volumetric differences between the autistic and the typically developing brain disappear almost completely during adolescence and adulthood. The only exception is the amygdala, which remains larger in autism compared to a typically developing brain (Aylward, Minshew, Field, Sparks & Singh 2002:176). Courchesne (2004) conducted MRI studies and demonstrated that maximum brain size in autism is reached by about three to five years of age – about six to ten years earlier than in typically developing children.

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3.3 STRUCTURAL ABNORMALITIES OF THE BRAIN IN AUTISM

Research findings reported by Courchesne (2004), Nicolson and Szatmari (2003), Levitt (2003), Sparks, Friedman, Shaw and coworkers (2002), Schultz and Klin (2002), Dawson et al (2002), and Herman (1996) all demonstrated abnormal developmental processes early in the clinical course of autism, particularly implicating increased cerebellar volume, as well as bilateral enlargement of amygdalae and hippocampi due to early overgrowth followed by premature arrest of growth. Structural abnormalities and candidate traits underlying the autism broader phenotype are illustrated in figure 3.3 *(page inserted).*

Referring to figure 3.3, certain structural and functional studies have detected a variety of brain abnormalities in autism suggesting the underdevelopment of the neocortical neural networks, including the circuitry of the frontal systems (Hardan et al 2000:1033; Minshew 1996:205; Bauman & Kemper 1994:54; Zilbovicius, Garreau, Samson, et al 1995:248; Minshew, Luna & Sweeney 1999:917). Reduced metabolic correlations have been reported in a group of mostly high-functioning individuals with autism involving the frontal and parietal cortex and subcortical structures (Bauman & Kemper 1994). A PET study of blood flow in preschool autistic children revealed evidence of delayed maturation of the frontal lobes (Zilbovicius et al 1995:251). Recently, a study of saccadic eye movements in high-functioning individuals with autism reported significant abnormalities in volitional saccades subserved by the circuitry of frontal systems (Minshew et al 1999:921).

3.3.1 Enlarged brain size in autism

In his seminal paper describing autism, Kanner (1943) cited in Panksepp (1998:276) noted that five of his original eleven patients appeared to have relatively large heads. Over the past ten years, MRI studies have consistently found elevated brain volume, and estimates suggest that up to 20% of individuals with autism may have head circumferences above the 97th percentile (Nicolson & Szatmari 2003:531). Elevated brain volume and enlarged head circumference are consequently discussed in more detail.

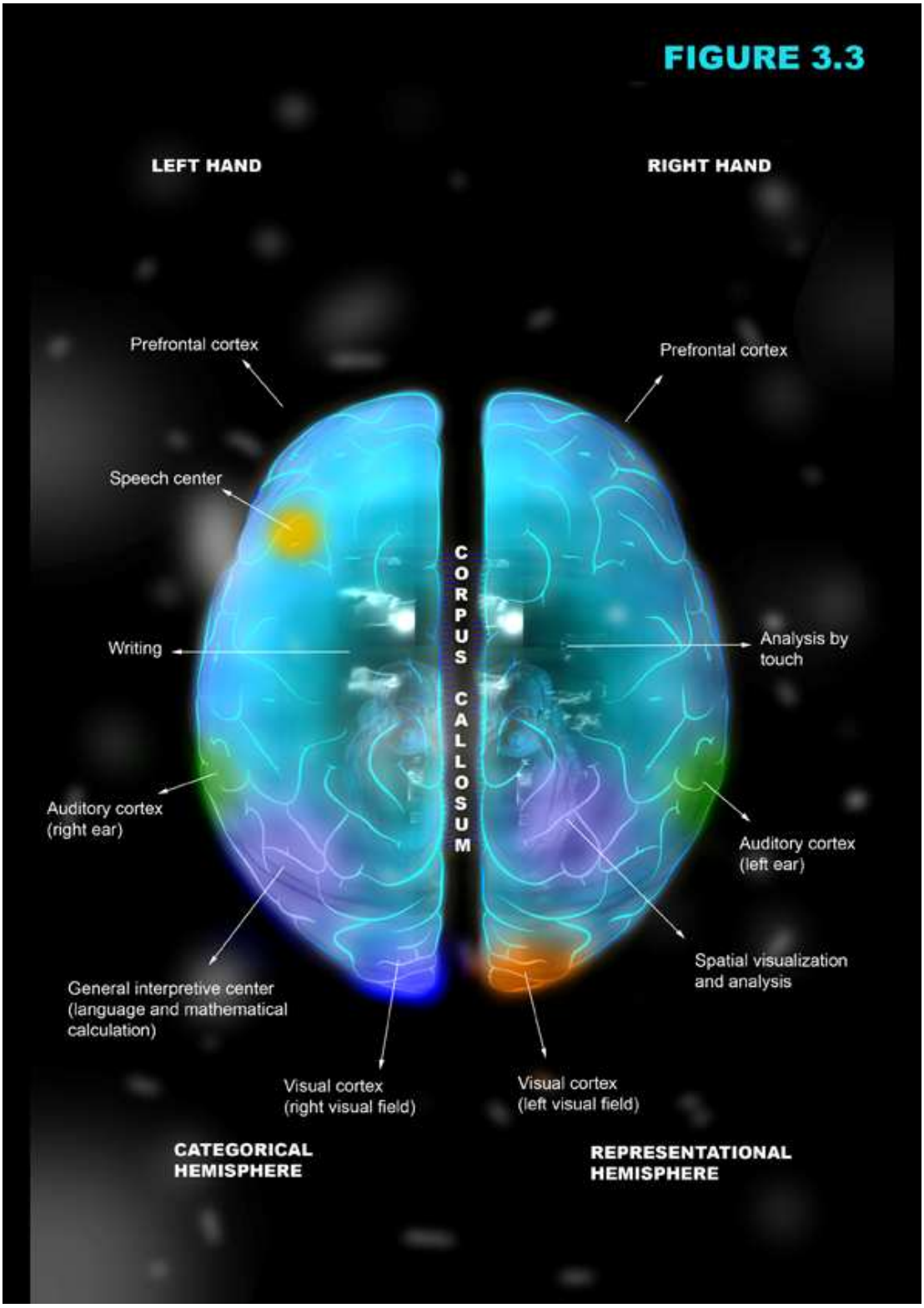
FIGURE 3.3
LEFT HAND
RIGHT HAND
Prefrontal cortex
Prefrontal cortex
Speech center
CORPUS CALLOSUM
Writing
Analysis by touch
Auditory cortex (right ear)
Auditory cortex (left ear)
Spatial visualization and analysis
General interpretive center (language and mathematical calculation)
Visual cortex (right visual field)
Visual cortex (left visual field)
CATEGORICAL HEMISPHERE
REPRESENTATIONAL HEMISPHERE

3.3.1.1 Elevated cerebral volumes

Children diagnosed with autism were found to have an overall 9.8% increase in cerebral volumes compared to typically developing children and an overall 12.5% increase compared to developmentally delayed children (Sparks et al 2002:10). Piven and coworkers (1997:546-555) reported increased brain volume in 22 male subjects with autism, compared with 20 male subjects without the disorder. In a follow-up study with an overlapping sample, Piven and coworkers (1998:105-110) again reported increased brain volume in patients with autism, with a regional subanalysis indicating that the volumetric elevation was most pronounced in posterior brain regions and that frontal regions did not differ between the groups. Elevated brain volume appears to be unique to autism, as most neurodevelopmental disorders and mental retardation are associated with a reduced brain volume (Nicolson & Szatmari 2003:531). Research findings published by Courchesne (2004:106-111) suggest that elevated brain volume in autism is associated with accelerated brain growth early in development, followed by arrested brain growth.

Three different MRI studies demonstrated that head circumference in autistic infants beyond normal head circumference of typically developing infants can be associated with abnormally large brain volumes. In the first MRI study of brain size in autistic toddlers, Courchesne et al (2001:245-254) reported that 90% of autistic subjects had brain volumes exceeding typically developing average by 10%. In keeping with these findings, Sparks et al (2002:184-192) also found brain volumes of autistic three-to-four-year-olds to be about 10% larger than typically developing controls. In 2004 Piven produced similar research results in support of the previous two studies. In a recent MRI study of two-to-five-year-olds Courchesne et al (2004:489) found that both girls and boys with autism had significantly enlarged whole-brain volume. At age two-to-four years a 10% greater brain volume represents a 1.5 centimeter greater head circumference in children with autism as compared to typically developing controls (Courchesne, Carper & Akshoomoff 2003:337-344; Bartholomeusz, Courchesne & Karns 2002:239-241).

3.3.1.2 Enlarged head circumference

Courchesne et al (2004:489-496) reported results from a longitudinal study of changes in head circumference during the first two years of life in a sample of children diagnosed with autism. As compared to the head circumference norms from the Center for Disease Control and Prevention (CDC), the head circumference of autistic children was at the 25th percentile at birth and then increased rapidly to the 84th percentile by six to fourteen months of age. This period of abnormally accelerated head circumference increase is thought to be largely concluded by the end of the second year of life. These researchers found that by 15 to 28 months, headcircumference was only two percentile points higher than that at 6 to 14 months. In a follow-up paper Courchesne (2004:107) concluded that the process of abnormally accelerated brain growth seems to be finished off during the second year of life; however, regional differences in overgrowth in autism were reported, based on the earlier research conducted by Courchesne and coworkers (2001:245-254). This earlier study already demonstrated overall brain enlargement due to significant increases in cerebral white matter by 18%, in cerebral grey matter by 12%, and in cerebellar white matter by 39%. Cerebellar grey matter was found not to be affected significantly. Carper and coworkers (2002:1038-1051) implicated early hyperplasia involvement in autism. According to these researchers abnormalities were found within the cerebrum, dorsolateral and medial frontal regions among two-to-four-year-old children diagnosed with autism. In addition, temporal grey matter and parietal white matter volumes were significantly enlarged. However, the occipital lobes were not significantly different from that of typically developing controls. Consistent with research findings showing regional differences in overgrowth among children with autism, Levitt and coworkers (2003:728) reported MRI results that demonstrated anterior and superior shifting of several sulci, with the greatest deviations seen in the superior frontal, inferior frontal, and superior temporal sulci and the lateral fissure.

3.3.2 Reduction in the area of the corpus callosum

The various regions of the neocortex are interconnected by three types of axonal projections, i.e., relatively short connections between one part of a lobe and another, longer connections between one lobe and another, and interhemispheric connections (commissures) between one hemisphere and another (Kolb & Whishaw 2003:67). The different areas of the brain are illustrated in figure 3.3 *(page inserted)*. As depicted in figure 3.3 the two interhemispheric commissures are the corpus callosum and the anterior

commissure. Research conducted by Bauman and Kemper (1995:1-26) provided evidence of a brain disconnection syndrome in autism, especially between cerebellar and limbic zones with other higher brain areas. In keeping with a brain disconnection syndrome, neural systems that should be working in close unison appear not to have developed normal synaptic interchange in various brain areas that control socialization, communication, and imagination in autism (Panksepp 1998:113).

All studies within the past decade have reported reductions in the area of the corpus callosum in autism (reviewed by Nicholson & Szatmari 2003:532). The corpus callosum is topographically organized and matures throughout childhood into young adulthood. Gender differences, testosterone levels, and handedness have been reported to affect corpus callosum anatomy (Witelson 1989:799; Moffat, Hampson, Hickett, et al 1997:297). Studies of autistic subjects with a wide range of functioning have documented quantitative abnormalities of the corpus callosum and reported the presence of an overall size reduction (Bauman & Kemper 1994; Egaas, Courchesne & Saitoh 1995; Piven, Bailey, Ranson & Arndt 1997). A smaller body and posterior subregions of the corpus callosum were also observed in two different studies examining individuals with autism with and without mental retardation (Egaas et al 1995; Piven et al 1997). Higher-level cognitive functions, such as language and linguistic processes and the ability to represent the action of others, depend upon hemispheric specialization, as the callosal pathways are involved in integrating these processes. Autism is often associated with abnormal motor and language lateralization, including left- and mixed-handedness and an unusual pattern of cerebral dominance for language (Kolb & Whishaw 2003:657). These findings implicate cerebral asymmetry and disrupted callosal pathway involvement in autism, particularly when one considers the importance of cerebral asymmetry in functions such as language that are impaired in autism.

Recent neuroanatomical studies of the corpus callosum that represent an index of neural connectivity between brain regions, provide impetus for investigating its role in autism. Hardan, Minshew & Keshavan (2000:1033-1036) measured the size of the seven subregions of the corpus callosum on MRI scans from 22 non–mentally retarded autistic subjects and 22 individually matched controls. These researchers reported smaller anterior subregions in the autistic group. In a subsample, measurements were adjusted for intracranial, total brain, and white matter volumes and the differences between groups

remained significant. No differences were found in the other subregions. This observation is consistent with the frontal lobe dysfunction reported in autism previously.

Hardan et al (2000:1035) reported a decrease in the size of the anterior regions of the corpus callosum, and a strong trend toward a decrease in the overall size of the corpus callosum. The greatest reduction in corpus callosum area in the autistic participants was found in the genu (region 2), involving the projections from prefrontal cortex. This is consistent with the cognitive, neurophysiological, and behavioural evidence of frontal lobe dysfunction reported recently in the literature (Bauman & Kemper 1994; Minshew et al 1999:920). There have been reports of deficits in executive function (Bauman & Kemper 1994), spatial working memory (Bauman & Kemper 1994), and the capacity for suppressing context-inappropriate responses in autism (Minshew et al 1999:921). Interestingly, the orbitofrontal cortex, which projects through the rostrum (region 1), has not been investigated in autism but is thought to play a significant role in the ritualistic behaviour of obsessive-compulsive disorder and may make a similar contribution to such behaviour in autism (Rosenberg, Keshavan & O'Hearn, et al 1997:824). Furthermore, Hardan and coworkers' findings of decreased size of the anterior regions of the corpus callosum may reflect the regional enlargement of parietal, temporal, and occipital but not the frontal regions (Piven, Arndt, Bailey & Andreasen 1996:530). This discrepancy may also reflect an increase in intrahemispheric connectivity and a decrease in the interhemispheric one. The reduction in the total cross-sectional area of the corpus callosum observed relative to total brain volume may indicate a decrease in interhemispheric connectivity, in keeping with Kolb and Whishaw's observations (Kolb & Whishaw 2003:67).

3.3.3 Abnormal patterns of cerebellar development

Sparks and coworkers (2002:10) observed that cerebellar volume in four-to-five-year-old children diagnosed with autism was increased compared with typically developing children, although some researchers reported normal or reduced cerebellar volumes in older populations (Courchesne, Townsen & Saitoh 1994:214-223; Piven, Saliba & Bailey 1997:546-555; Courchesne, Karns, Davis et al 2001:245-254).

Marked decreases in the number of Purkinje cells and granule cells throughout the cerebellar hemispheres were observed (Tatter et al 1995:286-297; Panksepp 1998:114; Bailey et al 1998:880-905). The most significant cell decrease was found in the posterior inferior neocerebellar cortex and adjacent archicerebellar cortex. Atrophy of the neocerebellar cortex was noted in the biventral, gracile, tonsillar, and inferior semilunar lobules, as well as abnormalities in the emboliform, fastigial, and globose nuclei in the roof of the cerebellum. Therefore, the normal circuitry of the cerebellum does not develop, and the deep cerebellar nuclei and olivary nucleus show a reduction in cell size and number (Herman 1996:5). Some changes were observed in the neurons of the deep cerebellar nuclei of autistic subjects, with younger subjects having abnormally large neurons and older subjects having abnormally small neurons in these nuclei (Nicholson & Szatmari 2003:533). These findings suggest that the cerebellar abnormalities occurred at or prior to 30 weeks gestation, suggesting atypical brain development in children diagnosed with autism.

Allen and Courchesne (2003:272-273) used fMRI to explore cerebellar function in autism at hand of eight autistic patients and eight control subjects. Relative to controls, the autism patients demonstrated increased cerebellar activation during a motor task and less cerebellar activation during an attentional task, suggesting that abnormal cerebellar development might have different implications for motor and attentional functioning.

The results of several autism studies reviewed by Bauman and Kemper (1994) suggest that various brain abnormalities, particularly temporal and cerebellar abnormalities, might correlate with the degree of impairment displayed in autism. Temporal lobe abnormalities are implicated in compromised explicit memories (i.e., memories for daily events), whereas cerebellar abnormalities are implicated in implicit memory (i.e., skills and conditioned responses) (Kolb & Whishaw 2003:658).

Roder (2000:56-63) found that an area of the brainstem in the caudal part of the pons is small in autistic subjects and that several nuclei in this area, including the facial nucleus, which controls facial musculature, are small or missing. In addition, many children with autism have subtle facial abnormalities that may be due to abnormalities of the facial nerve (Kolb & Whishaw 2003:658). The preceding findings suggest a strong biological basis in the pathogenesis of autism.

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3.3.4 Abnormalities of the medial temporal lobe structures

Bauman and Kemper (1994:119-145) examined neuropathology among patients ranging in age from 9 to 29 years and diagnosed with autistic disorder. These researchers reported subtle alterations in the size of neurons and the complexity of their processes were confined to the limbic system and cerebellum. In the limbic system, the hippocampal complex, subiculum, entorhinal cortex, amygdala, mamillary body, anterior cingulate gyrus, and septal area are connected by neuronal circuits. In comparison with the brains of control subjects, the autistic brains showed reduced neuronal cell size and increased cell-packing density in these areas.

Bailey et al (1998:885) reported that neurons in parts of the limbic system of autistic patients, particularly in the hippocampus and amygdala, were unusually small and densely packed, pointing to deficient maturation in these areas. Sparks and coworkers (2002) confirmed bilateral enlargement both of the amygdalae and hippocampi in four-to-five-year-old children with autism compared with typically developing children. This notion was also observed among adults diagnosed with autism (Howard, Cowell, Boucher et al 2000:2931-2935). Bauman and Kemper (1994:125) also observed that CA1 and CA4 pyramidal cells in the hippocampus showed decreased complexity and extent of dendritic arbors, characteristic of an immature brain, suggesting constraint of normal development in these structures in autism.

Howard et al (2000) hypothesized that bilateral enlargement of the amygdala reflected incomplete neuronal pruning in early development. Pertinent to this observation, postmortem findings from adults with autism revealed increased cell packing density of the amygdala (Bauman & Kemper 1994:119). Other investigators reported normal or reduced size of these structures in samples of children and adults with autism (Pierce, Muller, Ambrose et al 2001:2059; Haznedar, Buchsbaum, Wei et al 2001:157; Aylward, Minshew, Goldstein et al 1999:2145; Piven, Bailey, Ranson et al 1998:105).

These observed differences might be ascribed to age differences among subjects included in different samples. Sparks and coworkers (2002) concluded that observed size differences of amygdalae and hippocampi in four-to-five-year-old children with autism (compared to adolescent or adult size) might be ascribed to arrested development or increased apoptosis of these structures over time, resulting in a reduction of size. Functional studies using fMRI consistently found abnormalities in activation of the amygdala and the fusiform gyrus, which contains the *fusiform face area*, so called because of its involvement in facial processing and social cognition. These fMRI studies demonstrated limited or no activation of the amygdala and the related fusiform gyrus in autism, and it appeared that autistic patients use brain regions not typically associated with facial processing. It was suggested that autistic patients were performing the task of facial processing, but that they were using alternate and idiosyncratic regions to do so, including regions more typically used for object perception (Nicolson & Szatmari 2003:533). These fMRI studies demonstrated that patients with autism do not use brain regions typically involved in social cognition in the same way that control subjects do (Nicolson & Szatmari 2003:533).

Individuals diagnosed with autism consistently fail theory-of-mind tasks, and Baron-Cohen (1995) theorized that the extreme abnormalities in social cognition in autism result from an abnormality in an amygdaloid-prefrontal circuit. The prefrontal cortex, the amygdala, the superior temporal sulcus and the insular cortex form part of the neural network underlying social cognition (Kolb & Whishaw 2003:602). Panksepp (1998:272) theorizes that specific regions such as the cingulate gyrus, septal area, bed nucleus of the stria terminalis, preoptic area, dorsomedial thalamus and the periaqueductal grey (PAG) all play an important role in social cognition and social bonding.

The limbic system, particularly the amygdala, plays a crucial role in behavioural responses to emotional stimuli and in emotional learning (Du Preez, Naudé & Pretorius 2004:27; Naudé, Pretorius, Van Schoor & Becker 2005:47; Pretorius, Naudé & Pretorius 2005:310). Autistic children have too many densely packed small neurons within parts of the limbic system (Bauman & Kemper 1994:119), suggesting that selective cell death (or apoptosis) has not progressed normally (Margolis, Chuand & Post 1994:946). This also means that neurons do not interconnect with the rest of the brain as well as in typically developing children, which suggest that a biochemical program for neuronal development has

malfunctioned (Panksepp 1998:276). Amygdalar damage impairs recognition of emotional faces (Adolphs, Tranel, Damasio et al 1994:669) and has been implicated in an impaired ability to link visual perception of emotionally relevant stimuli among individuals with autism (Adolphs, Sears & Piven 2001:232).

From the preceding discussion it is clear that human brain development follows a programmed continuum, and programmed development might be disrupted at any stage along this developmental continuum. Considerable progress has been made in unravelling the nature of the stress response and in understanding the neurobiological and neuroendocrine underpinnings of prenatal and postnatal stress on programmed cortical development.

The impact of prenatal stress on cortical development will be discussed in the following paragraphs.

3.4 THE IMPACT OF PRENATAL STRESS ON CORTICAL DEVELOPMENT AND AGENESIS

The concept *agenesis* refers to developmental failure in certain cortical regions (Kolb & Whishaw 2003:Glossary). Age is an important determinant of the effects of early lesions and general cortical development. Three critical age divisions have been identified: gestational period up until before one year of age, between one and five years, and older than five years (Kolb & Whishaw 2003:626). Kates, Burnette, Eliez et al (2004:539-546) postulated that agenesis might occur due to prenatal, perinatal or postnatal environmental events, which might include prenatal trauma in the form of reduced blood flow or oxygen and exposure to toxins and elevated levels of glucocorticoids due to prenatal and postnatal stress. In psychobiology the concept *stress* is anything that activates the pituitary-adrenal system (the ACTH-cortisol axis). Everything that is typically considered to be a stressor in humans generates this brain response (Suchecki, Nelson, Oers & Levine 1995:169).

Teicher and coworkers (2002:399) have postulated a cascade model for explaining the neurobiological effects of prenatal stress on programmed cortical development. Their cascade model is built on five fundamental premises. First, exposure to stress early in life activates stress-response systems and fundamentally alters their molecular organization to

modify their sensitivity and response bias. Second, exposure of the developing brain to stress hormones affects myelination, neural morphology, neurogenesis, and synaptogenesis. Third, different brain regions differ in their sensitivity, which depends, in part, upon genetics, gender, timing, rate of development, and desity of glucocorticoid receptors. Fourth, there are enduring functional consequences that include attenuated left hemisphere development, decreased right/left hemisphere integration, increased electrical irritability within limbic system circuits, and diminished functional activity of the cerebellar vermis. Fifth, there are associated neuropsychological consequences and vulnerabilities, which lead to enhanced risk for the pathogenesis of autism. In the following paragraphs the hipotalamic-pituitary-adrenal stress response (also known as the HPA-axis) will be discussed.

3.4.1 The Hipotalamic-Pituitary-Adrenal Stress Response

A variety of neuroemotional influences converge on cells of the paraventricular nucleus (PVN) of the hypothalamus, which contain corticotrophin releasing factor (CRF) (Suchecki et al 1995:172). The stress response thus consists of an alarm phase (the pituitary-adrenal stress response system), as well as a resistance phase (the sympathoadrenal stress response system), depicted in figure 3.4 *(page inserted)*.

As illustrated in figure 3.4, the piruitary-adrenal response is instigated by CRF from the PVN of the hypothalamus, which via axons descending toward the pituitary, can trigger ACTH release from the pituitary (Panksepp 1998:118). ACTH, which is released into the bloodstream, seeks out target tissue in the adrenal cortex, where it triggers the release of cortisol. Cortisol helps promote energy untilization in the body, and obviously more bodily resources need to be used in all stressful situations. This perpheral system is aroused in response to essentially all emotional stressors. The central CRF pathways within the brain help organize and coordinate various negative emotional responses.

Cortisol also feeds back onto brain tissue, where there are specific receptors for the steroid hormone, especially in the hippocampus (which controls cognitive processing), as well as on the CRF neurons of the PVN. Cortisol normally exerts an inhibitory effect on the PVN cells and thereby reulates the intensity of the stress response. This HPA axis may be permanently altered, resulting in this self-regulatory, negative feedback mechanism to no

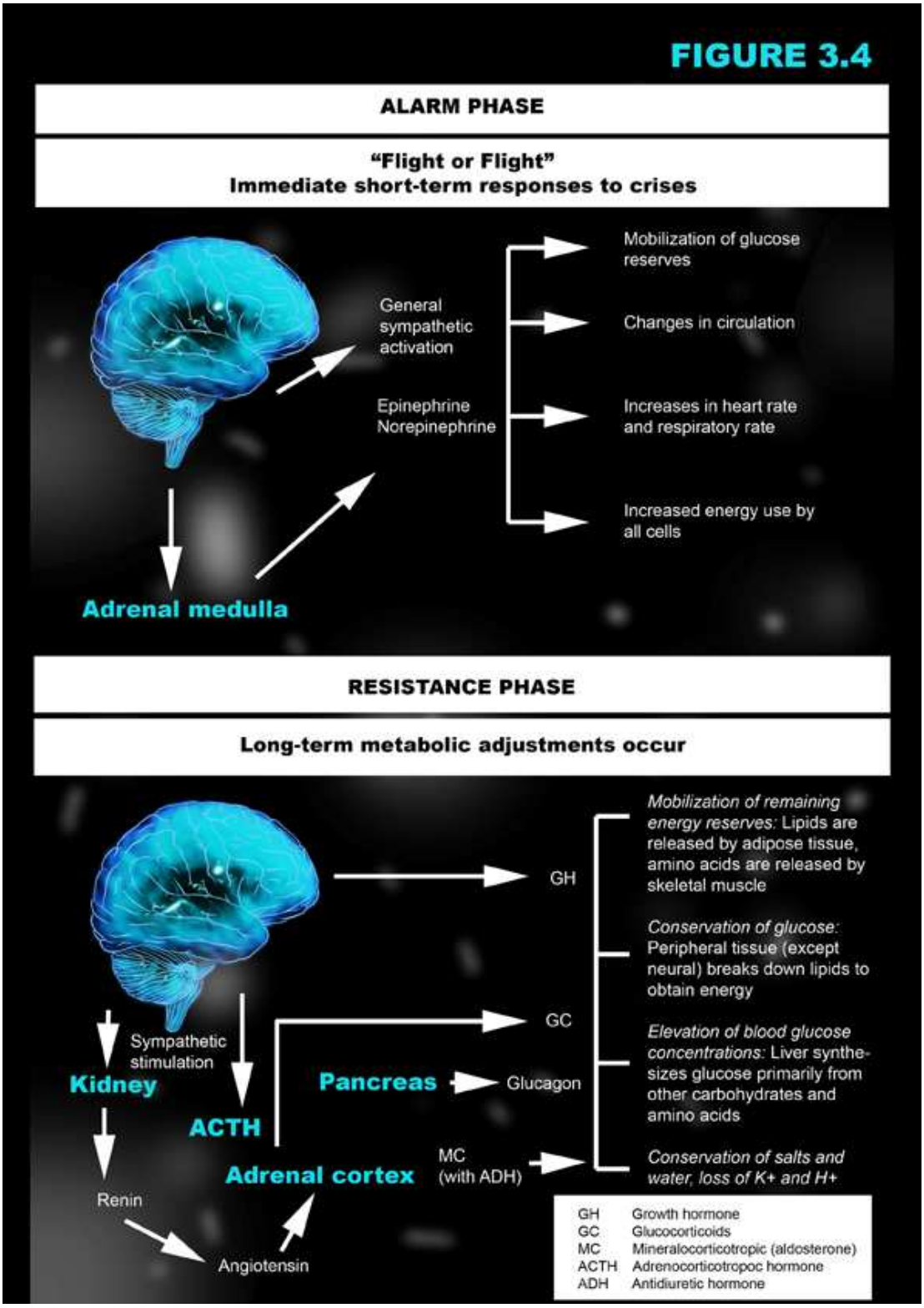
FIGURE 3.4
ALARM PHASE
"Flight or Flight"
Immediate short-term responses to crises
General sympathetic activation
Epinephrine
Norepinephrine
Mobilization of glucose reserves
Changes in circulation
Increases in heart rate and respiratory rate
Increased energy use by all cells
Adrenal medulla
RESISTANCE PHASE
Long-term metabolic adjustments occur
GH
GC
Sympathetic stimulation
Kidney
Pancreas
Glucagon
ACTH
Adrenal cortex
MC (with ADH)
Renin
Angiotensin
Mobilization of remaining energy reserves: Lipids are released by adipose tissue, amino acids are released by skeletal muscle
Conservation of glucose: Peripheral tissue (except neural) breaks down lipids to obtain energy
Elevation of blood glucose concentrations: Liver synthesizes glucose primarily from other carbohydrates and amino acids
Conservation of salts and water, loss of K+ and H+
GH Growth hormone
GC Glucocorticoids
MC Mineralocorticotropic (aldosterone)
ACTH Adrenocorticotropoc hormone
ADH Antidiuretic hormone

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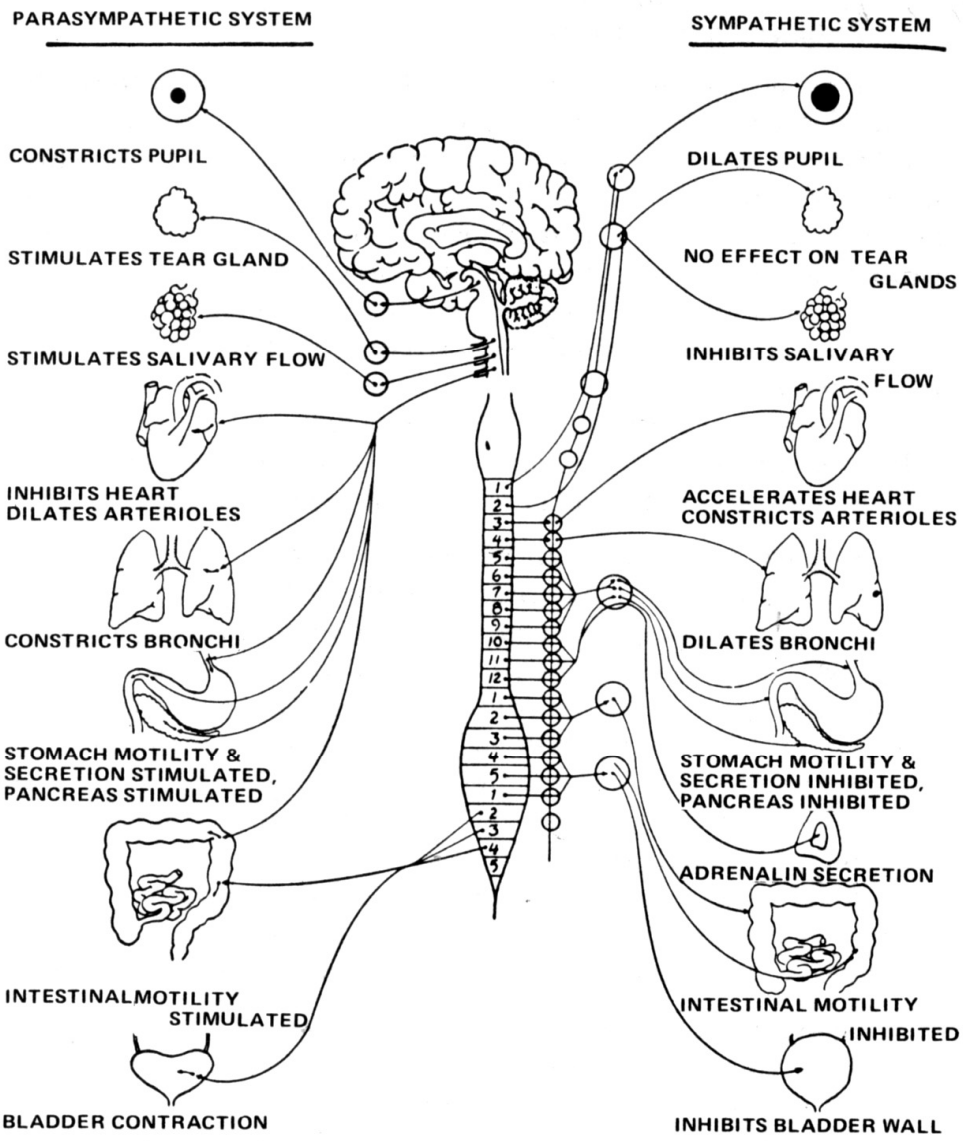
longer operate properly, consequently stress responses do not diminish normally once a stressful episode is over.

The feedback of cortisol onto hippocampal tussues also modifies cognitive abilities relevant to stress. It is postulated that cortisol might help promote cognitive strategies to cope with stressors (Panksepp 1998:118). This feedback mechanism is also subject to imbalances. The neurons that contain the cortisol receptors can tolerate only so much stimulation. If cortisol secretion is sustained at excessive levels, the metabolic resources of hippocampal neurons become depleted and die prematurely (McCubbin, Kaufmann & Nemeroff 1991; Sprott, Huber, Warner & Williams 1993; Friedman, Charney & Deutch 1995). Since brain cells are not replaced, this mechanism might impact on programmed foetal cortical development (Panksepp 1998:119).

A second major limb of the stress response is via a neural pathway arising from the hypthalamus and descending to the spinal cord, which via sympathetic efferents, activates the release of adrenaline (epinephrine) and noradrenaline (norepinephrine) from the adrenal medulla, as depicted in figure 3.1 below.

Adrenaline (epinephrine) and noradrenaline (norepinephrine) from the adrenal medulla help to break down liver glycogen rapidly and make abundant blood sugar available for the stressed individual. Practically all visceral organs and many other brain and immune responses are recruited during stress (Friedman et al 1995). These visceral, or enteric, nervous system is critical for elaborating organ responses during stress (McCubbin et al 1991) and consists of an endogenous plexus of nerves that line the gastrointetinal system and other organs; they are rich in various neuropeptides, which have some influence back into the brain via afferent neural and humoral routes. The brain itself contains many similar neural systems spread throughout the limbic system and related brain areas that govern the central integration of emotional responsivity, and autism might arise from overtaxed emotional responses prenatally (Panksepp 1998:119).

Figure 3.1 The anatomical and functional differences between the sympathetic and parasympathetic nervous systems



Source: Bruce, R.L. (1977:77)

3.4.2 The link between glucocorticoids and the pathogenesis of autism

There appears to be a link between the pathogenesis of autism and prenatal endogenous and exogenous glucocorticoids as well as endogenous opiates (Panksepp 1998). Research

findings implicate elevated levels of cortisol in the pathogenesis of autism (Beversdorf et al 2004), resulting in disrupted neural development during the second trimester of gestation when the foetal brain stem, cerebellum and limbic pathways must be generated (Bauman & Kemper 1995:1-26). In keeping with these findings, Beversdorf in 2004 pointed out a significant relation between prenatal stress and the development of autism (Beversdorf 2004). The neurobiological impact of stress prior to the 28th week of gestation might produce structural neural changes, specifically regarding the cerebellum, the brain stem and limbic pathways, including the hippocampal area (Sapolsky 2000:925-935). Sapolsky (2000) found that programmed apoptosis is affected due to the neurobiological impact of stress on foetal development, which concept relates closely to the pathogenesis of autism. Sapolsky (2000:925-935) established that increased levels of cortisol in response to chronic stress (maternal or foetal) might kill nerve cells in the hippocampus. If hippocampal activity is thus compromised, excessive cortisol is secreted and, over time, the ability to turn off the stress response decreases, which leads to further atrophy of the hippocampus. These findings indicate that chronic stress leading to chronic secretion of cortisol may have long-lasting effects on physical functioning, including brain damage. Programmed apoptosis may be grossly interfered with, especially within the areas of the hippocampus and the cerebellum. MR-imaging confirmed structural differences of the cerebellum, the brain stem and limbic system associated with autism (Beversdorf 2004), and these structural differences were further associated with elevated levels of glucocorticoids and endogenous opiates during gestation (Bertram & Hanson 2002:459–467). Elevated glucocorticoids inhibit foetal growth and are associated with altered programmed foetal cortical development (Bertram & Hanson 2002:460).

In addition, fMRI studies demonstrated different patterns of limbic and paralimbic structure activation due to an excess of glucocorticoids. Of theoretical importance are findings of failure to activate the anterior cingulate, as well as amygdala activation during symptom provocation studies, in keeping with similar observations that were made in autistic disorder (Panksepp 1998). Villarreal and King (2001) suggested that anterior cingulate dysfunction produces failure to inhibit amygdala activation and/or an intrinsic lower threshold of amygdala response to fearful stimuli. These observations are in keeping with research findings implicating disrupted neural development, i.e., brain stem, cerebellum, hippocampal and limbic pathway abnormalities among individuals with autistic disorder (Bauman & Kemper 1995:1-26; Sapolsky 2000:925-935). In addition,

Sapolsky (2000:925-935) found that programmed apoptosis is affected due to the neurobiology of stress, which concept relates closely to the pathogenesis of autism (Kalat 2001:346).

The preceding review of research findings demonstrate that various factors may contribute to disrupted programmed neurogenesis, and because this research focuses on the role that prenatal stress plays in the pathogenesis of autism, it follows that the critical age at which developmental failure occurs due to prenatal stress will determine the effects thereof. Exposure to excessive levels of glucocorticoids prenatally results in arrested programmed development in certain cortical regions, as summarized in table 3.3 at the end of this discussion. These regions are particularly implicated in the pathogenesis of autism, and abnormalities are closely linked to expression of autistic behaviour, as discussed in the following paragraphs.

3.4.3 Autistic expression in handwriting

Lesions to the lateral parts of the cerebellum disrupt arm, hand and finger movements, because of cerebellar involvement in the timing and accuracy of movements, which are also implicated in autistic disorder (APA 2000:75). According to Thatch and co-workers (1992:429) the primary role of the cerebellum is to help make fine adjustments needed to keep movements accurate. In addition to an inability to maintain movement accuracy, the child's error restoration might also be impaired. Traditionally these types of difficulties were ascribed to inadequate fine motor control. However, writing and drawing partially depend on movement-to-movement learning and adjustments that are made by the cerebellum (Kolb & Whishaw 2003:218). These authors explain error detection and restoration involved in handwriting as follows: Suppose a child performs a specific hand skill involved in letter formation, but finds that the shape is entirely incorrect. The child's next attempt is aimed at correcting the original error, resulting in two different versions of the same manoeuvre, i.e., the movement that the child intended to make, and the actual movement as recorded by sensory receptors in the fingers, arm and shoulder. If the first attempt at letter formation is successful, the child does not need to correct the next attempt; however, if the first attempt is incorrect, an adjustment is required. "One way in which the adjustment might be accomplished is through the feedback circuit that allows the cerebellum to correct movements. The cerebellum receives information about the

instructions sent to the motor neurons by the inferior olivary nucleus. It receives information about the actual movement through the spinocerebellar tract. By comparing the message for the intended movement with the movement that was actually performed, the cerebellum can send an error message to the cortex to improve the accuracy of the subsequent movement" (Kolb & Whishaw 2003:219). In keeping with this explanation, the cerebellum uses information about the intended movement as well as the actual movement to calculate the error and projects to the cortex how to correct the movement. Information about this correction is incorporated into the child's next attempt at letter formation. The rhythm involved in handwriting might also be disrupted.

3.4.4 Autistic expression in auditory and spatial functioning

Excessive exposure to glucocorticoids during gestation may also result in auditory and spatial deficits, because vestibular-temporal system abnormalities are implicated (Hendrickx & Hummler 1992; Emmanouil-Nikoloussi et al 2000). The receptors in the inner ear detect differences in air pressure as changes in pitch, loudness, and timbre, and these differences in pressure are conveyed from the inner ear to the brain as action potentials. These action potentials are interpreted in areas of the cortex in the temporal lobe as sounds, language, and music. The auditory system is composed of tonotopic maps, and it locates sound in space by comparing the time of the sound's arrival at each ear, which is subject to the perception of the space around the body (Kolb & Whishaw 2003:187). In autism this perception of space around the body seems to be compromised. In addition, when perception of timing and length of an auditory stimulus is compromised, post-natal sound location might be compromised as well, which is especially significant in autism. The inner ear also contains the receptor system that mediates static and dynamic balance, and once again, these functions seem to be compromised in autism. Apart from cerebellar involvement in eye movement control, the pathways projecting from the balance receptors to nuclei in the brainstem also aid in controlling eye movements (Kolb & Whishaw 2003:188), and therefore eye muscle control might also be impaired.

In addition, a child who suffers from disorders of the cerebellum might suffer a loss of timing, both in movement and in perception (finger tapping, judging rhythm and the length of an auditory stimulus) (Kolb & Whishaw 2003:217). This might manifest as poor time perception, e.g., embedded rhythm associated with speech sounds involved in language

acquisition, awareness of syllables in words, difficulty memorizing songs and poems, and these might easily be mistaken for temporal lobe lesions. Timing, rhythm and length of an auditory stimulus are closely linked to accurate perception of speech. Language is spoken at a rate of up to 12 phonemes per second, and one can understand speech at a rate of 50 to 60 sounds per second (Werker & Tees 1992:377). Eysenck (2001:243) asserts, "speech typically consists of a continuously changing pattern of sound with few periods of silence." When the perception of the length of sounds and silences are disrupted, the child might find it difficult to decide how the continuous stream of sound should be divided up into words, in keeping with Eysenck's proposed "segmentation problem" (Eysenck 2001:243). In addition, the child might have difficulty detecting the prosodic patterns of speech necessary for working out syntactic or grammatical structures. It is further suggested that synchronisation of visual and auditory information is closely related to the perception of timing, rhythm and length of auditory stimuli mediated by the cerebellum, and lip-reading might thus be compromised. Eysenck (2001:246) asserts that even individuals with normal hearing make use of visual information from lip movements to make sense of speech sounds. However, when perception of timing, rhythm and length of auditory stimuli is impaired, this might lead to the so-called McGurk effect (McGurk & MacDonald 1976:746-748). This McGurk effect might easily be misdiagnosed as auditory discrimination difficulties.

3.4.5 Autistic expression in attentional and emotional processes

The cerebellum was long believed to simply control motor coordination, but it is now known to contribute to attentional and emotional processes as well (Panksepp 1998:204; Heath, Llewellyn & Rouchell 1980:254-256). Along with the ganglion cells that play a part in vision, there are some other specialized ganglion cells that form the retinohypothalamic tract to the suprachiasmatic nucleus, which play a role in regulating circadian rhythms (Barlow & Durand 2002:203), implicated in sleep rhythm disturbances. The suprachiasmatic nucleus (SCN) might be viewed as the "major pacemaker for the daily clock" (Panksepp 1998:130), and is situated in the hypothalamus above the optic chiasm. The multiple output pathways from the SCN practically control all behavioural rhythms, from feeding and sleep to arousal and cortical energy balance regulation (Panksepp 1998:174). In addition, circadian rhythms are thought to have some relationship to mood and emotional processes (Barlow & Durand 2002:203). It thus follows that

lesions to the cerebellum and the SCN might result in defective arousal regulation, circadian rhythms, and mood irregularities, which are all implicated in autistic disorder.

Table 3.2 Possible sensorimotor learning deficits due to excess glucocorticoids during gestation (Naudé, Marx, Pretorius & Hislop-Esterhuyzen 2006 (in press)

Affected area	Sensorimotor deficits
Cerebellum	All forms of motor learning and vestibular functioning; Compromised movement-to-movement learning such as handwriting.
Flocculonodular lobe	Poor static and dynamic balance; Eye movement deficits.
Midline areas of the cerebellum	Disrupted balance, eye movements, upright posture and walking.
Lateral parts of the cerebellum	Disrupted arm, hand and finger movements; Compromised timing and accuracy of movements; Inability to detect and restore errors of movement.
Ganglion cell layer	Deficits in encoding of visual information; Faulty saccades and fixations; Compromised reading fluency and comprehension; Segmentation problems; Difficulty detecting the prosodic patterns of speech; Difficulty detecting syntactic or grammatical structures of speech; Faulty lip-reading; McGurk effect.
Suprachiasmatic nucleus (SCN)	Cortical energy balance dysregulation; Disturbed circadian rhythms; Altered mood and emotional processes; Defective arousal regulation; Altered attentional processes.

It is thus concluded that the supply of glucocorticoids should be carefully monitored during gestation to ensure that the developing fetus is exposed to neither too little nor too much, because either condition can disrupt programmed development.

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3.5 SYNOPSIS

Considering the preceding literature on the adverse effects of glucocorticoids during gestation, as well as the structural and functional brain alterations that result from over-exposure, it is suggested that prenatal stress might play a significant role in the pathogenesis of autism. The various impairments associated with autism also suggest that excess levels of glucocorticoids might be implicated, i.e., speech deviations such as pitch, intonation, rate, rhythm, odd hand movements and body posture, high threshold for pain, emotionality, abnormalities in sleep, deviant fear response, and so forth (APA 2000:75-77), because many of these behaviours are mediated by the brain stem, the cerebellum, the hippocampus, the limbic system and its relays.

In the following chapter the data of the empirical investigation will be discussed and analysed.

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CHAPTER 4

THE EMPIRICAL STUDY

4.1 INTRODUCTION

In the preceding chapters autistic disorder was described as a neurobiological developmental disorder, and particular attention was paid to specific biochemicals and structural brain differences implicated in autistic disorder. This chapter describes the empirical research and related findings. The focus of this research project is to explore the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder at hand of a dizygotic twin study, where only one of the pair meets the criteria for autistic disorder (APA 2000:75). Based upon the findings, the research hypothesis will be accepted or rejected.

The reason why a dizogotic twin study was decided upon is to explore whether HPA activity manifests differently among a pair of dizogotic siblings, since it is expected that both dizygotic foetuses were exposed to elevated glucocorticoids during gestation, yet only one of the siblings was affected and met the diagnostic criteria for autism. In addition, a dizygotic twin study offers the ideal research control, since all significant variables are uniform and constant.

The rationale of the study, as well as the theoretical framework and paradigmatic perspective were already discussed in chapter one. In recollection of what was presented in chapter one, some aspects of the research project such as the problem statement, the purpose of the study, the research design and the methodology are briefly presented below again, before the empirical results are offered and discussed.

4.2 PROBLEM STATEMENT

Based upon the rationale of this research project the research problem can be formulated as follows:

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In what unique ways does prenatal stress contribute to the pathogenesis of autism as a neurobiological developmental disorder?

4.2.1 Sub questions

- Did the mother of the dizygotic twins experience significant stress during the period of gestation?
- What blood plasma differences can be observed among the dizygotic twins at hand of blood sampling?
- Does HPA activity manifest differently among this pair of dizogotic siblings?
- How does elevation of glucocorticoids disrupt programmed foetal development?
- How do blood plasma differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins?
- Does the MR image of the sibling diagnosed with autism differ in respect of structural brain development from what is normally expected?
- To which periods of prenatal development can these structural differences be related?
- How do these structural differences account for sensory, motor, cognitive, and affective behavioural differences among the dizygotic twins?

4.2.2 Research hypothesis

The research hypothesis can be formulated as follows:

Elevation of glucocorticoids due to prenatal stress disrupts programmed foetal development and contributes to the pathogenesis of autism as a neurobiological developmental disorder.

4.3 PURPOSE OF THE STUDY

The purpose of this research project is to explore at hand of dizygotic twin study the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder.

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4.4 METHODS, MATERIALS AND PROCEDURES

This research project comprises of a single case study of dizygotic twin siblings, a boy and a girl, currently 4 years 7 months of age. The boy was diagnosed with infantile autism, and is the subject of this research project. A pediatrician and at least two psychologists had made the diagnosis of infantile autism independently, and clinical descriptions met the diagnostic criteria set out in the DSM-IV-TR (APA 2000). The girl showed normal development and served as the control for comparing different developmental values.

Information was obtained from case history data, which included a detailed interview concerning social background, prenatal, perinatal and developmental histories, clinical, neurological and psychological assessment, and blood plasma pathology reports.

The following data generating strategies were employed: a diagnostic stress inventory was administered to complement the intake interviews with the parents, and obstetric and developmental records of the mother and both siblings were retrieved. Both the mother and father of the dizygotic twins were required to complete the diagnostic stress inventory, because they might have had different perspectives on the significance of various stressors that were endured during pregnancy. In addition, the *16-Personality Factor Questionnaire* was administered with the mother, since it is thought that personality might influence the way that stressors are handled.

Magnetic resonance imaging (MR-imaging) of the dizygotic sibling diagnosed with autistic disorder was obtained in order to identify whether structural brain development was altered, compared to what is normally expected. These structural differences were interpreted in light of programmed neural development, the impact of endocrine system changes on foetal central nervous system development, and the consequent expression of autism.

Blood plasma pathology analyses were obtained, using blood plasma samples of both dizygotic siblings. Blood plasma pathology reports complemented the enquiry into the pathogenesis of autism as a neurobiological developmental disorder, since endocrine system changes might contribute to disruption of programmed neural development, and these plasma differences between siblings might continue to be present postnatally.

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4.5 RESULTS OF THE CASE STUDY

An outline of the clinical data of the mother as well as the obstetric and developmental data of the dizygotic twins are presented in the following paragraphs:

4.5.1 Maternal clinical data

The maternal clinical history is dealt with under the headings gestational period, blood-pressure readings, blood plasma pathology reports, and recorded stressors.

4.5.1.1 Gestational period

At the time of pregnancy the mother was 31 years old, with no prior family history of autism or other psychiatric disorders. No medical conditions were diagnosed prior to conception, although the mother reported two previous miscarriages and exposure to Rubella prior to conception. The mother first consulted the gynaecologist at five weeks of gestation. Folic acid, multivitamins, calcium, magnesium and *Asic* (Pharmaceutical Enterprises Pty Ltd) were prescribed during pregnancy. *Asic* was administered to treat nausea during early pregnancy, and the active ingredients are dicyclomine HCI 10 mg, doxylamine succinate 10 mg, and Vitamin B_6 50 mg (Mims Desk Reference 2002:1321). The prescribed dosage is two tablets at bedtime. *Asic* is a schedule 2 drug and the manufacturer published anticholinergic and central nervous system (CNS) interaction as side effects of *Asic* administration (Mims Desk Reference 2002:1321).

4.5.1.2 Blood-pressure readings

During the course of the pregnancy maternal blood pressure readings (BP/mmHg) were recorded at monthly intervals, followed by two-weekly recordings during the third trimester. Fluctuations in blood-pressure recordings are visually represented in figure 4.1 and summarized in table 4.1. These values were then compared with the World Health Organization's normal blood-pressure values (MicroLife Group Africa 2004:3), as depicted in table 4.2 below.

Figure 4.1 Table for tabulating maternal blood-pressure readings (BP/mmHg) during period of pregnancy

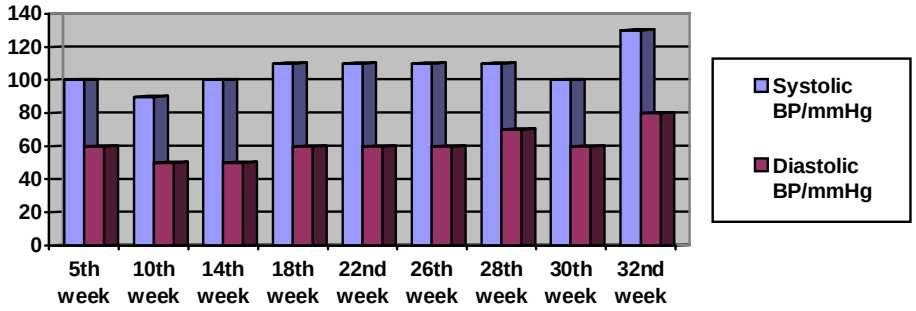

Table 4.1 Table for summarizing maternal blood-pressure values (units mmHg) during period of pregnancy

Week of gestation	Systolic Blood-pressure	Diastolic Blood-pressure	Measures
5th week	100 mm/Hg	60 mm/Hg	
10th week	90 mm/Hg	50 mm/Hg	
14th week	100 mm/Hg	50 mm/Hg	
18th week	110 mm/Hg	60 mm/Hg	
22nd week	110 mm/Hg	60 mm/Hg	
26th week	110 mm/Hg	60 mm/Hg	
28th week	110 mm/Hg	70 mm/Hg	
30th week	100 mm/Hg	60 mm/Hg	
32nd week	130 mm/Hg	80 mm/Hg	Caesarean section done in 32nd week

Table 4.2 Table for classifying blood-pressure values (units mmHG) according to World Health Organization

Range	**Systolic Blood-pressure**	**Diastolic Blood-pressure**	**Measures**
Hypotension	Lower than 100	Lower than 60	Consult with doctor
Normal range	Between 100 and 140	Between 60 and 90	Self-monitoring
Mild hypertension	Between 140 and 160	Between 90 and 100	Consult with doctor
Moderately serious hypertension	Between 160 and 180	Between 100 and 110	Consult with doctor
Serious hypertension	Higher than 180	Higher than 110	Consult with doctor immediately

The interpretation of the preceding data is that the mother's initial blood-pressure readings were generally within the normal range when compared with the World Health Organization's normal blood-pressure values (MicroLife Group Africa 2004:3). However, blood-pressure readings during the 10th and 14th week of gestation suggested a tendency towards hypotension, thereafter the blood-pressure readings stabilized within the normal range, with a sudden upsurge of blood-pressure during the 32nd week of gestation.

Hypotension during the 10th and 14th week of gestation is suggested by blood-pressure values that are too low, i.e., systolic values under 100mmHg and/or diastolic values under 60mmHg. Hypotension might play a significant role in the ability of the placenta to maintain the foetus, i.e., provision of nutrients and removal of toxins, since proper development of the placental vascular system is essential to nutrient and gas exchange between mother and developing embryo (Edwards, Coulter, Symonds & McMillen 2001:938; Huxley, Sheill & Law 2000:815; Levitt, Lindsay, Holmes & Seckl 1996:412). These researchers have demonstrated that poor intra-uterine growth is associated with a reduced intra-uterine nutrient supply, which perturbs foetal growth and, concomitantly, alters or programmes the structure and function of developing systems. In addition, a reduced foetal nutrient supply might be a consequence of poor placental function, and an outcome of a sub-optimal placental nutrient supply is exposure of the foetus to excess glucocorticoids, which act to restrict foetal growth and to programme permanent changes in the neural, cardiovascular, endocrine and metabolic systems (Edwards, Simonetta &

Owens et al 1999:897; Edwards, Symonds & Warnes 2001:1778; Edwards & McMillen 2001:561; Robinson, Owens & Owens 1994:83; Phillips, Simonetta & Owens et al 1996: 861; Hoet & Hanson 1999:617; Tangalakis, Lumbers & Moritz et al 1992:709; Wood, Cheung & Brace 1987:904; Unno, Wong & Jenkins 1999:248).

4.5.1.3 Blood plasma pathology reports

Maternal blood plasma samples were collected and analized during the 6th week of gestation. The pathology reports were compared with normal range values, as depicted in table 4.3 below.

Table 4.3 Results of blood plasma analyses for Rubella antibodies during the 6th week of gestation

Test	Description	Result	Range
Rubella antibodies			
=> Elisa IGM	High	1.20	0.00-0.59 INDEX
=> Elisa IGG	High	132.40	0.00-4.90 IU/mL

Interpretation of Rubella antibodies test results

IGE		***IGM***
0 – 4.90	Negative	0 – 0.59
5.00 – 9.90	Undecided	0.60 – 0.79
> 10.00	Positive	> 0.80

Low/High = highly abnormal results

Values provided by Du Buisson, Bruinette & Kramer (Incorporated) Pathologists

Highly abnormal Rubella antibodies results showed up and in order to exclude the possibility of acute Rubella viral infection follow-up blood samples were analyzed within ten days, i.e., approximately during the 7th week of gestation. These laboratory results were compared with normal range values, as depicted in table 4.4 below.

Table 4.4 Results of blood plasma analyses for Rubella antibodies during the 7th week of gestation

Test	Description	Result	Range
Rubella antibodies			
=> Elisa IGM	High	1.04	0.00-0.59 INDEX
=> Elisa IGG	High	250.80	0.00-4.90 IU/mL

Interpretation of Rubella antibodies test results

IGE		***IGM***
0 – 4.90	Negative	0 – 0.59
5.00 – 9.90	Undecided	0.60 – 0.79
> 10.00	Positive	> 0.80

Low/High = highly abnormal results

Values provided by Du Buisson, Bruinette & Kramer (Incorporated) Pathologists

Highly abnormal Rubella antibodies results showed up in both the previous two blood plasma analyses, and in order to exclude the possibility of a recent Rubella viral infection an additional Rubella affinity index was done during the 8th week of gestation. These results are reflected in table 4.5 below.

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Table 4.5 Results of the Rubella affinity index during the 8th week of gestation

Test	Description	Result	Range
Rubella affinity index		84.0	30.00 – 100.00 INDEX
Treponema Pallidum antibodies	Negative	0	0 – 2 NEGATIVE
=> Elisa IGM		0.18	0 – 1.20 NEGATIVE
=> Elisa IGG		0.04	0 – 0.89 NEGATIVE
Interpretation of Rubella affinity index			
An affinity index of less than 30% is indicative of a possible recent Rubella viral infection			

Values provided by Du Buisson, Bruinette & Kramer (Incorporated) Pathologists

The interpretation of the preceding blood plasma pathology results is that highly abnormal Rubella antibodies were present during early gestation, but that recent Rubella viral infection was absent. Neurological sequelae following MMR[3] are widely reported. Elevated titers of anti-measles antibodies in autistic children could signify a chronic activation of the immune system against this neurotropic virus, which may play a role in the pathogenic sequences of events leading to autism. Vaccination during pregnancy and risk for autism was implicated by Yazbak (1999). Yazbak describes six mothers who received live virus vaccines and one received a Hepatitis B vaccine during pregnancy after having received an MMR booster five months prior to conception. All the children who resulted from these pregnancies have had developmental problems, six out seven (85%) were diagnosed with autism, and the seventh seems to exhibit symptoms often associated with autistic spectrum disorders. However, research findings implicating Rubella in the pathogenesis of autism are not conclusive and need to be further investigated.

[3] MMR: Mumps, Measels and Rubella vacine.

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4.5.1.4 Perinatal period

In light of the elevated blood-pressure (refer to figure 4.1), maternal blood plasma samples were collected and analysed during the 32nd week of gestation. The laboratory results were compared with normal range values, as depicted in table 4.6 below.

Table 4.6 Results of maternal blood plasma analyses during the 32nd week of gestation

Test	Description	Result	Range
Full blood count			
=> HB count (Haemoglobin count)		12.1	12.0 - 16.0 g/dL
=> Red cell count	Low	3.91	4.00 – 5.00 10^12/L
=> Haematocrit (Anemia)		36.3	36 – 46 %
=> MCV (Mean corpuscular volume)		92.9	80 – 100 fL
=> MCH (Mean corpuscular haemoglobin)		31.1	27 – 32 pg
=> MCHC (Mean corpuscular haemoglobin concentration)		33.4	32.0 – 35.0 g/dL
=> RDW (Red cell distribution width)		12.1	11.7 – 13.6 %
White cell differential count			
=> White cell count	High	11.7	4.0 – 10.0 10^9/L
=> Neutrophil %	High	92.2	%
=>Neutrophil absolute	High	10.80	1.90 – 7.40 10^9/L
=> Lymphocyte %	Low	5.7	%
=> Lympho absolute	Low	0.70	1.00 – 4.50 10^9/L
=> Monocyte %		2.0	%
=> Mono absolute		0.20	0.20 – 1.00 10^9/L
=> Eosinophil %		0.1	%
=> Eosino absolute		0.00	0.00 – 0.50 10^9/L
=> Basophil %		0.0	%
=> Baso absolute		0.00	0.00 – 0.10 10^9/L
=> Platelet count		175	140 – 450 10^9/L
=> ESR (sedimentation rate)		20	1 – 20 mm/hr

=> FBCC (Full blood count comments)	*Neutrophil leucocytes present – may reflect surgery, bleeding, tissue damage, bacterial infection, steroid therapy and pregnancy.* *Low/High = highly abnormal results*

Values provided by Du Buisson, Bruinette & Kramer (Incorporated) Pathologists

The preceding blood plasma pathology results indicate an abnormally low red blood cell count and the presence of Neutrophil leucocytes, which might reflect bleeding, tissue damage, bacterial infection, steroid therapy and pregnancy. Enduring stress might produce similar pathology results due to a compromised immune system, because the experience of stress affects cellular immunity, and is implicated in the immunobiology of autism.

Human immune function is mediated by the release of cytokines, nonantibody messenger molecules, from a variety of cells of the immune system, and from other cells, such as endothelial cells. There are Th1 and Th2 cytokines. Autoimmune and allergic diseases involve a shift in the balance of cytokines toward Th2. The autoimmune aspect of autism has been related to excessive Th2 cytokines resulting, in part, from vaccination (Rabin 1999:15).

Cytokines stimulate cellular release of specific compounds involved in the inflammatory response. Stress-induced activation of the sympathetic nervous system and the sympathetic-adrenal medullary and hypothalamic-pituitary adrenal axes lead to the release of cytokines (Rabin 1999:15). Enduring stress during gestation can alter healthy immune system functioning, thereby affecting cytokines and indirectly the development of monoaminergic circuits in the foetal brain (Jarskog, Xiao & Wilkie et al 1997:711). Teratogenic effects of chronic prenatal exposure to glucocorticoids can alter the monoamine turnover in the locus coeruleus and nucleus tractus solitarius (Peyronnet, Dalmaz & Ehrstrom et al 2002:858). In addition, enduring stress appear to affect ascending serotonergic projections into the hippocampus and long-lasting increase in glucocorticoid receptors (Sapolsky 1997:1620). These reciprocal changes are implicated in a permanently altered HPA axis and consequently in the pathogenesis of autism as a developmental disorder. Blocking the response of the sympathetic nervous system by pre-

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treating subjects in stressful experiments with adrenergic antagonists can reduce this release of cytokines and decrease the resulting inflammatory response (Bachen, Manuck & Cohen 1995:366; Benschop, Nieuwenhuis & Tromp et al 1994:762). Discrete areas of the brain (for example, the hypothalamus and the locus coeruleus) regulate the sympathetic nervous system and therefore the levels of circulating adrenergic stress hormones, thereby influencing the activity of the immune system (Wetmore & Nance 1991:113; Rassnick, Sved & Rabin 1994:6033). Adrenergic stress hormones alter the synthesis and release of cytokines by white blood cells (leukocytes) as implicated by the preceding blood plasma pathology results presented in table 4.6.

4.5.1.5 Recorded stressors

Three interviews were conducted with the parents, i.e., a joint interview with both parents present, and two individualized interviews with the mother and father separately. These self-reportings, together with the results of the *16-Personality Questionnaire*, were analyzed to construct a relatively reliable estimate of the stressors the mother was enduring at the time of conception, as well as during the postnatal period. The results are now reported under separate headings, namely social and occupational stressors, predisposition for stress due to personality structure, peri- and postnatal stressors.

a. Social and occupational stressors

- The maternal grandmother was diagnosed with breast cancer four weeks prior to conception;
- The mother switched jobs (from education to private sector) about seven weeks prior to conception;
- Starting a new career then, both parents reported extreme levels of occupational stress during pregnancy. In addition, her new full-time occupation required of her to drive very frequently almost everyday, which she experienced as very stressful;
- Both parents reported extreme stressors associated with the husband's occupation for the duration of the pregnancy.

The interpretation is that psychological stress inhibits many aspects of the immune response including innate immunity (e.g., natural killer cell lysis), T-cell responses, and antibody production (Rabin 1999:21). Cohen and co-workers administered a questionnaire for stressful life events, and low immunity subjects scored higher for stressful life events, they showed higher perceptions of stress, and more negative emotional experiences were associated with a greater likelihood of developing a clinical illness defined as cold symptoms concomitant with isolating an infectious virus or developing a fourfold increase in antibody titers (Cohen, Frank & Doyle et al 1998:214), in keeping with recorded stressors reported in paragraph 4.4.1.5 below and blood plasma pathology results reported in table 4.6.

In a second study by the same researchers, a life-stress interview replaced the questionnaire. This technique allowed the specification of the types of stressful events that increase risk. These included chronic events (lasting a month or longer), especially chronic social conflicts and underemployment or unemployment (Cohen et al 1998:220). Other plausible factors that might be the cause of both changes in stress and greater susceptibility to disease, such as age, sex, education, and personality characteristics including self-esteem and personal control, were unable to account for these results. The results demonstrated a relationship between psychological stress and susceptibility to compromised immune system.

Outside of proven clinical interventions, there is reason to think that certain changes in lifestyle might increase an individual's resistance to infectious diseases. These include broadening one's social involvements (e.g., joining social or spiritual groups, having a confidant, spending time with supportive friends) and being more careful to maintain healthful practices such as proper diet, exercise, and sleep, especially under stressful conditions (Cohen, Doyle, Skoner, Rabin & Gwaltney 1997:1940-1944).

b. Predisposition for stress tolerance due to personality structure

The 16-Personality Factor Questionnaire (16-PF) was administered with the mother, and the results are summarized in table 4.7 below:

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Table 4.7 Table for summarizing maternal 16-PF standardized sten scores

	LOW SCORE DESCRIPTION	1	2	3	4	5	6	7	8	9	10	HIGH SCORE DESCRIPTION	
MD	Low Motivational Distortion				•							High Motivational Distortion	MD
A	Reserved							•				Outgoing	A
B	Concrete thinking								•			Abstract thinking	B
C	Affected by feelings									•		Emotional stable	C
E	Submissiveness						•					Assertiveness	E
F	Desurgency (sober)					•						Surgency	F
G	Superego weakness				•							Superego strength	G
H	Shy					•						Adventurous	H
I	Tough-minded								•			Tender-minded	I
L	Trusting								•			Suspicious	L
M	Practical										•	Imaginative	M
N	Forthright			•								Shrewdness	N
O	Self-assured, placid						•					Guilt proneness, Apprehensive	O
Q1	Conservatism of temperament, tolerant of traditional difficulties			•								Radicalism, Experimenting, Analytical	Q1
Q2	Group-dependency					•						Self-sufficiency	Q2
Q3	Weak self-sentiment integration, lax					•						High strength of self-sentiment, controlled	Q3
Q4	Low ergic tension, relaxed					•						High ergic tension, tense, frustrated, driven	Q4

Considering the preceding 16-PF results (expressed as sten scores), the intension is not to provide an in-depth personality analysis, but to consider *only* those factors that might predispose adverse psychological reaction to enduring stressors.

As reflected in table 4.7, significantly high scores were achieved on factors I, L and M, while significantly low scores were achieved on factors N and Q1. These scores are now being considered individually.

Factor I measures the construct *feeling* versus *thinking* as contrasting modes of evaluating experiences (Cattell 1989:152). The right pole (factor I+; sten score 8) is called *premsia*, a condensation for "protected emotional sensitivity" (Cattell 1989:153). In a nutshell, I+ individuals rely on their empathetic understanding to make evaluations; they are compassionate and sensitive as well as attuned to their own vulnerability. Cattell (1989:155) proposes that factor I+ might relate to right hemispheric specialization, implicating that information is processed subjectively and emotionally. Heredity plays a significant role in factor I, and research findings demonstrated that genetics contributes 47% of the nature/nurture variance (Cattell 1989:156). Stress-related illnesses, particularly of the coronary vascular system, are associated with factor I+ scores (Cattell 1989:162).

Factor L measures the construct *alienation* versus *identification* in social orientations (Cattell 1989:169). "From a medical point of view, L+ scores are important indicators of proneness to stress, which shows most conspicuously by physical illness" (Cattell 1989:182). This researcher also noted that high L+ scores are especially implicated in the onset of severe depression.

Factor M measures the construct *intuiting* and *sensing* as contrasting perceptual modes (Cattell 1989:189), i.e., the temperamental proclivity to give either sensory data or ideational contents more immediate intensity. Once again factor M seems to relate to hemispheric specialization, and individuals scoring high on factor M (M+; *autia*) show a strong tendency to favour the use of the right hemisphere to respond emotionally and subjectively (like I+ scorers). "M+ scorers' perceptions are diffuse and draw heavily on subliminal information, and these qualities, too, seem right-brained" (Cattell 1989:192). Cattell (1989:199) furthermore reported a high incidence of M+ scores among three major clinical syndromes, namely substance abuse, schizophrenia, and major depression. In addition this researcher reported on a high incidence of job dissatisfaction as primary complaint among high M+ scorers, suggesting poor tolerance for stress.

Factor N measures the construct *self-presentation* in social situations, and although there is no research on the genetic basis for factor N, a combination of N- and M+ characteristics suggests "difficulty in dealing with social reality and in responding appropriately to interpersonal cues" (Cattell 1989:220).

Factor Q1 measures the construct *orientation towards change* (Cattell 1989:237). Low Q1 scores (Q- scores) signal that the individual is likely to find it difficult to change. In addition, Cattell (1989:253) reported finding Q1- scores in the profiles of individuals with conversion hysteria, psychosomatic disorders, and obsessive-compulsive disorders, suggesting poor tolerance for stress, avoidance of change, and elevated levels of subjective anxiety. Surprisingly, this poor tolerance for stress is not toned down by higher intelligence (factor B+ scores) or by ego-strength (factor C+ scores).

These results suggest a maternal predisposition for poor stress tolerance, in keeping with recorded stressors reported in paragraph 4.4.1.5 and post partum depression reported below. Poor stress tolerance is associated with elevated stress hormone levels, implicating lowered immunity as suggested by blood plasma pathology results.

c. Peri- and postnatal stressors

The mother met the full diagnostic criteria for post partum depression (APA 2000), for which condition she was treated.

4.5.2 Dizygotic twin obstetric and developmental data

In the 32nd week of gestation the mother presented with premature contractions and a Caesarean section was indicated. A female foetus of 2.1 kilograms and a male foetus of 1.98 kilograms were delivered, with an Apgar count 9/10 each. No birth defects were noted at the time of delivery.

At round about three years of age a paediatrician in private practice diagnosed the dizygotic twin boy with autistic developmental disorder, since applying the diagnostic criteria for autistic disorder set out in the DSM-IV-TR (APA 2000), the boy met the autistic triad, i.e., compromised socialization, communication, and imagination, with sparing of visual-motor abilities. Habitual toe walking was also noted. This diagnosis was confirmed by in-depth psychological assessment.

Following the paediatrician's diagnosis, an Educational Psychologist specializing in autism again assessed the boy. The *Childhood Autism Rating Scale* (CARS) (Schopler, Reichler

& Renner 1992) was administered and the diagnosis of autism confirmed. The *Griffith Scales of Mental Development* (Holford 2000) was administered and a General Quotient of 82 was calculated, which signified low-average intellectual functioning (APA 2000).

4.5.2.1 Magnetic resonance imaging (MR-imaging)

The subject is of slender physique, and presents with a head circumference of 51 centimetres, compared with his twin sister's head circumference of 49.5 centimetres, implicating enlarged head-size. No marked abnormalities showed up on the MR images; however subtle abnormalities were noted with regards to the temporal lobe, the lateral fissure, the superior temporal gyrus and sulcus, as well as the rostrum of the corpus callosum (Bosman 2005). These brain abnormalities were extensively described in chapter three of this research report and will therefore not be repeated in this discussion of findings, except that these abnormalities are implicated in the autistic triad, i.e., compromised socialization, communication, and imagination (APA 2000).

4.5.2.2 Blood plasma pathology reports

Blood plasma samples were analysed to determine digoxin, serotonin and cortisol levels in both the dizygotic twin subject and the dizogotic twin control. The results are presented in table 4.8 below.

Table 4.8 Table for summarizing dizygotic twins' blood plasma pathology reports

Test	Description	Result	Range
Subject (male; 4 years 7 months)			
=> Serotonin		263	90 - 385 ng/mL
=> Digoxin	undetectable		1.0 – 2.6 nmol/L
=> Cortisol		187	Nmol/L
Control (female; 4 years 7 months)			
=> Serotonin		242	80 – 450 ng/mL
=> Digoxin	undetectable		1.0 – 2.6 nmol/L
=> Cortisol		87	Nmol/L

Values provided by Du Buisson, Bruinette & Kramer (Incorporated) Pathologists

The preceding blood plasma pathology results point towards distinct variations in plasma profiles for the subject and the control. Although digoxin levels were undetectably low for both the subject and the control, the subject's serotonin and cortisol levels were significantly higher compared with the plasma profile of the control. These results are in keeping with recent research findings implicating elevated levels of serotonin and cortisol among individuals diagnosed with autism. Teratogenic effects of chronic prenatal exposure to glucocorticoids appear to affect ascending serotonergic projections into the hippocampus and long-lasting increase in glucocorticoid receptors (Sapolsky 1997:1620). These reciprocal changes are implicated in a permanently altered HPA axis and consequently in the pathogenesis of autism as a developmental disorder. In addition to adverse effects of prenatal exposure to elevated levels of glucocorticoids discussed in paragraph 1.4 before, it has also been shown to have deleterious effects on programmed neural development, i.e., inhibition of neural stem cells, neurogenesis, and migration leading to irreversible decrease in brain weight in certain cortical areas (Edwards & Burnham 2001:433; Challis, Sloboda & Matthews et al 2001:135).

Serotonergic abnormalities have been reported in autism, specifically hyperserotonemia, as well as elevated blood serotonin in the first-degree relatives of children with autism (Levinthal et al 1990, Piven & Palmer 1999, Leboyer et al 1999, Chugani 2004). Chugani (2004:112) furthermore proposed that serotonergic abnormalities during prenatal and early postnatal development might lead to reciprocal changes in thalamocortical connectivity, which results in a certain predisposition for autism. As indicated in the preceding paragraphs, this might result in altered programmed neural development and 'resetting' of the foetal HPA axis, because the normal negative feedback system that regulates normal homeostasis is permanently altered (Edwards et al 1993:355).

4.6 DISCUSSION

The problem statement directing this research project was formulated as follows: *In what unique ways does prenatal stress contribute to the pathogenesis of autism as a neurobiological developmental disorder?*

Experimental evidence from animal studies suggested that experimentally induced anxiety in pregnant animals affects the psychological and behavioural characteristics of the offspring (Harper & Williams 1974:342). These early research findings are supported by recent findings suggesting that prenatal stress might play a significant role in the pathogenesis of autism (Chugani 2004:112-116; Nicolson & Szatmari 2003:526-537; Schultz & Klin 2002:1-5; Coleman 1994:104-109).

The preceding findings suggest elevated maternal stress prior to conception, as well as during gestation, as recorded by the parents of the subject. Various protocols support subjectively experienced stressors, as implicated by abnormally elevated leucocytes identified by maternal blood sampling. In addition, abnormal levels of Rubella antibodies showed up in the maternal blood sample, implicating a possible viral infection and/or exposure prior to conception. The presence of neutrophil leucocytes might reflect bleeding, tissue damage, bacterial infection, steroid therapy and pregnancy; however, enduring stress might also produce similar pathology results due to a compromised immune system, because the experience of stress affects cellular immunity due to HPA-axis involvement in stress. Furthermore, elevated glucocorticoids might permanently change the foetal HPA-axis, resulting in elevated cortisol levels, even postnatally. Significant cortisol differences were noted in the blood plasma pathology of the subject and the control. Elevated cortisol levels might also result in hyperserotonemia. Although the subject's serotonin measure did not exceed range values, it did exceed the control measure. Furthermore, hyperserotonemia and elevated glucocorticoids are implicated in altered programmed neural development, as suggested by the subject's MR images. The brain regions implicated were demonstrated by previous research findings to result in the typical autistic triad (APA 2000), suggesting that elevated stress during gestation might play a significant role in the pathogenesis of autism.

In addition to preceding findings, the difference between the subject's birth weight of 1.98 kilograms and the control's birth weight of 2.1 kilograms suggests intra-uterine deprivation or sub-optimal placental nutrient supply (Edwards, Coulter, Symonds & McMillen 2001: 938). Epidemiological studies have highlighted the potential importance of foetal adaptations to a poor intra-uterine environment for longer-term health outcomes (Barker 1992:3; Huxley, Sheill & Law 2000:815). Edwards and colleagues (2001) proposed that the physiological, neuroendocrine and metabolic adaptations that enable the foetus to adapt

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to a period of intra-uterine deprivation might result in a permanent reprogramming of the developmental pattern of proliferation and differentiation events within key foetal tissue and organ systems and have pathological consequences in adult life. This view is based upon observations that sub-optimal placental or maternal nutrient supply results in exposure of the foetus to excess glucocorticoids, which act to restrict foetal growth and programmed development (Hoet & Hanson 1999:617). In addition, proper development of the placental vascular system is essential to nutrient and gas exchange between mother and the developing embryo. Philipp, Brede, Hadamek & Gessler et al (2002:311) demonstrated that $\alpha 2^{-}$ adrenoceptors, which are activated by adrenaline and noradrenalin, are important regulators of placental structure and function, supporting the current hypothesis that prenatal stress during certain critical gestational periods contributes to altered programmed development implicated in autistic disorder. The only exception was that digoxin does not seem to play a significant role in the pathogenesis of autistic disorder.

These findings, however, are not conclusive, since unexpected variables entered the protocols, e.g., the possibility of prior exposure to Rubella, resulting in the production of Rubella antibodies. In addition, *Asic* was administered to treat nausea during early pregnancy, and the active ingredients are dicyclomine HCI 10 mg, doxylamine succinate 10 mg, and Vitamin B_6 50 mg (Mims Desk Reference 2002:1321). *Asic* is a schedule 2 drug and the manufacturer published anticholinergic and central nervous system (CNS) interaction as side effects of *Asic* administration (Mims Desk Reference 2002:1321).

The sub-questions were theoretical in nature, and were answered by means of an in-depth literature study, and supplemented with empirical findings.

4.7 REFLECTIVE VALIDATION OF RESEARCH HYPOTHESIS

The research hypothesis was formulated as follows: *Elevation of glucocorticoids due to prenatal stress disrupts programmed foetal development and contributes to the pathogenesis of autism as a neurobiological developmental disorder.*

Reflecting on this research hypothesis, it was demonstrated at hand of a dizygotic twin study that prenatal stress might have significantly contributed to the pathogenesis of autism, despite unexpected variables that might have interfered with the protocols, such as

drug administration and Rubella antibodies. Therefore the research hypothesis is only provisionally accepted.

4.8 SYNOPSIS

In this chapter the empirical research findings were discussed. Blood plasma pathology results suggested a compromised immune system, which might be ascribed to elevated glucocorticoids and serotonin. The presence of elevated glucocorticoids might permanently alter foetal HPA axis, as suggested by elevated cortisol and serotonin levels in the subject, as well as various brain abnormalities. These findings thus suggest disrupted programmed foetal development as implicated in autistic disorder.

In the last chapter the research findings, conclusions and recommendations are discussed.

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CHAPTER 5

FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

By means of this research project the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder had been explored. Autism is characterised by serious functional impairment pertaining to socialisation, communication and imagery (Panksepp 1998:276; Trevarthen 2000: 4; Kates et al 2004:539).

Researchers are currently united in their view that autism is primarily a neurobiological developmental disorder (Bauman & Kemper 1994; Herman 1996; Panksepp 1998; Trevarthen 2000; Clark 2002; Courchesne 2002; Keller & Persico 2003; Schmidt & Rotenberg 2005), thereby ascribing altered programmed development to disrupted neural development that results in atypical formation of the foetal brain stem, cerebellum and limbic pathways (Bauman & Kemper 1995:1-26). In wrapping up this research project the most significant findings, conclusions and recommendations are summarized in this chapter.

5.2 OVERVIEW

This research project is based upon an in-depth literature study and substantiated by original empirical data at hand of a dizygotic twin study. The purpose of this research project was to explore at hand of a dizygotic twin study the contribution of prenatal stress to the pathogenesis of autism as a neurobiological developmental disorder. The research statement that directed this project was formulated as follows:

In what unique ways does prenatal stress contribute to the pathogenesis of autism as a neurobiological developmental disorder?

The research hypothesis was defined as follows:

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Elevation of glucocorticoids due to prenatal stress disrupts programmed foetal development and contributes to the pathogenesis of autism as a neurobiological developmental disorder.

5.3 FINDINGS

In response to the research statement and sub questions, the findings are summarized as follows:

5.3.1 Significant findings related to biochemicals implicated in programmed foetal development

- Cortisol, digoxin and serotonin are implicated in the pathogenesis and/or manifestation of autism, based upon a definite link with the different stages of programmed foetal development, specifically the interactivity with the hipothalamic-pituary-adrenal axis (HPA axis) (Kurup & Kurup 2003:1537-1559).
- It was established that neurotransmitter expression can be high during certain stages of development, known as susceptible developmental time windows, yet may persist in only a few synapses afterwards (Parnavelas & Cavanagh 1988:92-93). Accurate timing and spacing of developmental time windows are essential for precise programmed development to take place, since these neurotransmitters and modulators affect formation of synaptic contacts, maturation of synapses, and structural refinement of connectivity by regulating electrical activity, excitability, and release of neurotrophins (Zhang & Poo 2001:1207-14).
- An early stimulus or insult at a critical period can result in long-term structural and functional changes in the central nervous system (Sayer, Cooper & Barker 1997: F162-F164). Disruption of the normal timing or intensity of neurotransmitter signaling can lead to permanent changes in proliferation differentiation and growth of their target cells during critical phases of development of the nervous system.
- Without vesicle release of neurotransmitters, neurons undergo apoptosis after formation of synapses, since their maintenance depends on neurotransmitter secretion (Demarque et al 2002:1051-61; Owens & Kriegstein 2002:989).

- Teratogenic effects of chronic prenatal exposure to glucocorticoids can alter the monoamine turnover in the locus coeruleus and nucleus tractus solitarius (Peyronnet, Dalmaz & Ehrstrom et al 2002:858).
- Enduring stress appears to affect ascending serotonergic projections into the hippocampus and long-lasting increase in glucocorticoid receptors (Sapolsky 1997:1620). These reciprocal changes are implicated in a permanently altered HPA axis and consequently in the pathogenesis of autism as a developmental disorder.
- Elevated levels of glucocorticoids have deleterious effects on programmed neural development, i.e., inhibition of neural stem cells, neurogenesis, and migration leading to irreversible decrease in brain weight in certain cortical areas (Edwards & Burnham 2001:433; Challis, Sloboda & Matthews et al 2001:135).
- Elevated serum digoxin levels are implicated in the pathogenesis of autism, resulting in increased serotonin in the plasma of patients with autism, while dopamine and noradrenalin are decreased. An increase in endogenous digoxin inhibits membrane Na + -K + ATPase, which causes an increase in intracellular calcium. This increase in intracellular calcium inhibits the functional availability of magnesium, because magnesium is displaced from its binding sites.
- Serum digoxin levels in autism is very important, because digoxin, a membrane Na + -K + ATPase inhibitor, is thought to be involved in the regulation of conscious perception (Kurup & Kurup 2003:1557), including perceptual binding, focused attention, and short-term memory.
- Serotonin, like other monoamine neurotransmitters, has been shown to play a role in regulating brain development prior to the time it assumes its role as a neurotransmitter in the mature brain (Chubakov, Gromova, Konovalov, Sarkisova & Chumasov 1986:285; Chubakov, Tsyganova & Sarkisova 1993:271; Lauder 1990:297; Turlejski 1996:619; Whitaker-Azmitia 2001:553; Whitaker-Azmitia, Druse, Walker & Lauder 1996:19).
- Serotonin has been reported to affect neuronal proliferation, differentiation, migration, and synaptogenesis (Gaspar, Cases & Maroteaux 2003:1002). In the mammalian brain, all of the monoamine neurotransmitter systems are present relatively early, but in particular, serotonin is likely present the earliest in the most terminal regions (Whitaker-Azmitia 2001:479). These early appearances of serotonergic neurons with

their wide distribution of terminals play a crucial role in programmed neurogenesis, synaptogenesis and apoptosis.

- Excess of serotonin prevents the normal development of the somatosensory cortex, and prevents the normal development of the somatosensory cortex.
- It was found that serotonin concentration must be neither too high nor too low during the critical period of synaptogenesis and formation of cortical connections.
- Serotonergic abnormalities have been reported in autism, specifically hyperserotonemia, as well as elevated blood serotonin in the first-degree relatives of children with autism (Leventhal et al 1990, Piven & Palmer 1999, Leboyer et al 1999, Chugani 2004).
- Serotonergic abnormalities during prenatal and early postnatal development might lead to reciprocal changes in thalamocortical connectivity, which results in a certain predisposition for autism.
- Hhyperserotonemia in autism may also involve atypical metabolism of the metabolic serotonin precursor tryptophan as a potential mechanism for alterations in serotonin availability.
- The imipramine-sensitive serotonin transporter is highly expressed in the human placental brush-border membranes and may mediate transport of serotonin from the maternal circulation to the developing foetus (Balkovetz et al 1989:2195-2198; Chugani 2004:112), therefore placental serotonin transporter expression might constitute a risk factor for autism (Persico, Militerni, Bravaccio & Schneider et al 2000:123-127; Anderson, Gutknecht, Cohen & Brailly-Tabard et al 2002:831-836; Persico, Pascucci, Puglisi-Allegra & Militerni et al 2002:795-800; Betancur, Corbex, Spielewoy & Phillippe et al 2002:67-71).
- There might be a relation between high blood plasma serotonin levels and lower verbal ability scores.
- The relative balance of tryptophan metabolism, regulated by the serotonin and kynurenine pathways might significantly contribute to the pathogenesis of autism and these serotonergic abnormalities may at least partially explain characteristic expression of autism.

5.3.2 Significant findings related to neuroanatomical observations of the brain in autistic disorder

- Various anatomical sites in the brain have been hypothesized as the primary source of pathology in autistic disorder, such as enlarged brain size, reductions in the area of the corpus callosum, and abnormalities of the cerebellum and the medial temporal lobe structure.
- According to recent MRI studies, slow and differential maturation of the brain does not happen in autism – there is rather a relatively brief period of overgrowth, followed by reduced or arrested growth (Courchesne 2004:109).
- Research findings reported by Courchesne (2004), Nicolson and Szatmari (2003), Levitt (2003), Sparks, Friedman, Shaw and coworkers (2002), Schultz and Klin (2002), Dawson et al (2002), and Herman (1996) all demonstrated abnormal developmental processes early in the clinical course of autism, particularly implicating increased cerebellar volume, as well as bilateral enlargement of hippocampi due to early overgrowth followed by premature arrest of growth.
- Children diagnosed with autism were found to have an overall 9.8% increase in cerebral volumes compared to typically developing children and an overall 12.5% increase compared to developmentally delayed children (Sparks et al 2002:10). Elevated brain volume appears to be unique to autism, as most neurodevelopmental disorders and mental retardation are associated with a reduced brain volume (Nicolson & Szatmari 2003:531).
- Three different MRI studies demonstrated that head circumference in autistic infants beyond normal head circumference of typically developing infants can be associated with abnormally large brain volumes.
- Courchesne and coworkers (2001:245-254) demonstrated overall brain enlargement due to significant increases in cerebral white matter by 18%, in cerebral grey matter by 12%, and in cerebellar white matter by 39%. Cerebellar grey matter was found not to be affected significantly.
- Research conducted by Bauman and Kemper (1995:1-26) provided evidence of a brain disconnection syndrome in autism, especially between cerebellar and limbic zones with

other higher brain areas. All studies within the past decade have reported reductions in the area of the corpus callosum in autism.

- These findings implicate cerebral asymmetry and disrupted callosal pathway involvement in autism, particularly when one considers the importance of cerebral asymmetry in functions such as language that is impaired in autism.
- Sparks and coworkers (2002:10) observed that cerebellar volume in four-to-five-year-old children diagnosed with autism was increased compared with typically developing children, although some researchers reported normal or reduced cerebellar volumes in older populations.
- Marked decreases in the number of Purkinje cells and granule cells throughout the cerebellar hemispheres were observed (Tatter et al 1995:286-297; Panksepp 1998:114; Bailey et al 1998:880-905).
- The most significant cell decrease was found in the posterior inferior neocerebellar cortex and adjacent archicerebellar cortex. Atrophy of the neocerebellar cortex was noted in the biventral, gracile, tonsillar, and inferior semilunar lobules, as well as abnormalities in the emboliform, fastigial, and globose nuclei in the roof of the cerebellum. Therefore, the normal circuitry of the cerebellum does not develop, and the deep cerebellar nuclei and olivary nucleus show a reduction in cell size and number (Herman 1996:5).
- Some changes were observed in the neurons of the deep cerebellar nuclei of autistic subjects, with younger subjects having abnormally large neurons and older subjects having abnormally small neurons in these nuclei (Nicholson & Szatmari 2003:533).
- These findings suggest that the cerebellar abnormalities occurred at or prior to 30 weeks gestation, suggesting atypical brain development in children diagnosed with autism.
- The results of several autism studies reviewed by Bauman and Kemper (1994) suggest that various brain abnormalities, particularly temporal and cerebellar abnormalities might correlate with the degree of impairment displayed in autism.
- Temporal lobe abnormalities are implicated in compromised explicit memories (i.e., memories for daily events), whereas cerebellar abnormalities are implicated in implicit memory (i.e., skills and conditioned responses) (Kolb & Whishaw 2003:658).

- Roder (2000:56-63) found that an area of the brainstem in the caudal part of the pons is small in autistic subjects and that several nuclei in this area, including the facial nucleus, which controls facial musculature, are small or missing.
- Bauman and Kemper (1994:119-145) reported subtle alterations in the size of neurons and the complexity of their processes were confined to the limbic system and cerebellum.
- Bailey et al (1998:885) reported that neurons in parts of the limbic system of autistic patients, particularly in the hippocampus and amygdala, were unusually small and densely packed, pointing to deficient maturation in these areas.
- Howard et al (2000) hypothesized that bilateral enlargement of the amygdala reflected incomplete neuronal pruning in early development.
- Individuals diagnosed with autism consistently fail theory-of-mind tasks, and Baron-Cohen (1995) theorized that the extreme abnormalities in social cognition in autism result from an abnormality in an amygdaloid-prefrontal circuit.
- Panksepp (1998:272) theorizes that specific regions such as the cingulate gyrus, septal area, bed nucleus of the stria terminalis, preoptic area, dorsomedial thalamus and the periaqueductal grey (PAG) all play an important role in social cognition and social bonding.

5.3.3 Significant findings related to the impact of prenatal stress on cortical development and agenesis

- The concept *agenesis* refers to developmental failure in certain cortical regions (Kolb & Whishaw 2003:Glossary).
- Three critical age divisions have been identified: gestational period up until before one year of age, between one and five years, and older than five years (Kolb & Whishaw 2003:626). Kates, Burnette, Eliez et al (2004:539-546) postulated that agenesis might occur due to prenatal, perinatal or postnatal environmental events, which might include prenatal trauma in the form of reduced blood flow or oxygen and exposure to toxins and elevated levels of glucocorticoids due to prenatal and postnatal stress.
- Exposure to stress early in life activates stress-response systems and fundamentally alters their molecular organization to modify their sensitivity and response bias.

- Exposure of the developing brain to stress hormones affects myelination, neural morphology, neurogenesis, and synaptogenesis.
- Different brain regions differ in their sensitivity, which depends, in part, upon genetics, gender, timing, rate of development, and density of glucocorticoid receptors.
- There are enduring functional consequences that include attenuated left hemisphere development, decreased right/left hemisphere integration, increased electrical irritability within limbic system circuits, and diminished functional activity of the cerebellar vermis.
- There are associated neuropsychological consequences and vulnerabilities, which lead to enhanced risk for the pathogenesis of autism.
- Sapolsky (2000) found that programmed apoptosis is affected due to the neurobiological impact of stress on foetal development. Increased levels of cortisol in response to chronic stress (maternal or foetal) might kill nerve cells in the hippocampus. If hippocampal activity is thus compromised, excessive cortisol is secreted and, over time, the ability to turn off the stress response decreases, which leads to further atrophy of the hippocampus. These findings indicate that chronic stress leading to chronic secretion of cortisol may have long-lasting effects on physical functioning, including brain damage. Programmed apoptosis may be grossly interfered with, especially within the areas of the hippocampus and the cerebellum.
- Elevated glucocorticoids inhibit foetal growth and are associated with altered programmed foetal cortical development (Bertram & Hanson 2002:460).

5.3.4 Significant findings related to the dizygotic twin study

- Maternal hypotension during the 10th and 14th week of gestation was suggested by blood-pressure values that were too low, i.e., systolic values under 100mmHg and/or diastolic values under 60mmHg. Hypotension might play a significant role in the ability of the placenta to maintain the foetus, i.e., provision of nutrients and removal of toxins, since proper development of the placental vascular system is essential to nutrient and gas exchange between mother and developing embryo (Edwards, Coulter, Symonds & McMillen 2001:938; Huxley, Sheill & Law 2000:815; Levitt, Lindsay, Holmes & Seckl 1996:412).

- Poor intra-uterine growth is associated with a reduced intra-uterine nutrient supply, which perturbs foetal growth and, concomitantly, alters or programmes the structure and function of developing systems.
- A reduced foetal nutrient supply might be a consequence of poor placental function, and an outcome of a sub-optimal placental nutrient supply is exposure of the foetus to excess glucocorticoids, which act to restrict foetal growth and to programme permanent changes in the neural, cardiovascular, endocrine and metabolic systems.
- Blood plasma pathology results identified highly abnormal Rubella antibodies during early gestation, but recent Rubella viral infection was absent.
- Elevated titers could signify a chronic activation of the immune system against neurotrophic viral infection, which may play a role in the pathogenic sequences of events leading to autism.
- Maternal blood plasma pathology results indicated an abnormally low red blood cell count and the presence of neutrophil leucocytes.
- Human immune function is mediated by the release of cytokines (neutrophil leucocytes), nonantibody messenger molecules, from a variety of cells of the immune system, and from other cells, such as endothelial cells.
- Cytokines stimulate cellular release of specific compounds involved in the inflammatory response. Stress-induced activation of the sympathetic nervous system and the sympathetic-adrenal medullar and hypothalamic-pituitary adrenal axes lead to the release of cytokines (Rabin 1999:15). Enduring stress during gestation can alter healthy immune system functioning, thereby affecting cytokines and indirectly the development of monoaminergic circuits in the foetal brain.
- The results of the 16-PF Questionnaire suggested maternal predisposition for poor stress tolerance.
- The dizygotic twins' blood plasma pathology results pointed towards distinct variations in plasma profiles for the subject and the control. The subject's serotonin and cortisol levels were significantly higher compared with the plasma profile of the control. These results are in keeping with recent research findings implicating elevated levels of serotonin and cortisol among individuals diagnosed with autism. Teratogenic effects of chronic prenatal exposure to glucocorticoids appear to affect ascending serotonergic projections into the hippocampus and long-lasting increase in glucocorticoid receptors (Sapolsky 1997:1620).

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- Digoxin levels were undetectably low for both the subject and the control.

5.4 CONCLUSIONS

The following conclusions were arrived at:

- The preceding findings suggest elevated maternal stress prior to conception, as well as during gestation, as implicated by abnormally elevated leucocytes identified by maternal blood sampling.
- Abnormal levels of Rubella antibodies showed up in the maternal blood sample, implicating a possible viral infection and/or exposure prior to conception.
- Enduring stress might also produce elevated leucocytes, because the experience of stress affects cellular immunity due to HPA-axis involvement in stress.
- The subject's elevated glucocorticoids suggested a permanently changed foetal HPA-axis, resulting in postnatal elevated cortisol levels.
- Significant cortisol differences were noted in the blood plasma pathology of the subject and the control, suggesting that elevated cortisol resulted in hyperserotonemia.
- Hyperserotonemia and elevated glucocorticoids are therefore implicated in altered programmed neural development, as suggested by the subject's MR images.
- The difference between the subject's birth weight of 1.98 kilograms and the control's birth weight of 2.1 kilograms suggests intra-uterine deprivation or sub-optimal placental nutrient supply.
- The research hypothesis was provisionally accepted, since unexpected variables entered the protocols, which were outside the focus of this research project.

5.5 RECOMMENDATIONS

The following recommendations are made:

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5.5.1 Educational Psychological training

- A thorough understanding of neuropsychology should enhance basic training programmes to augment an understanding of childhood developmental disorders.
- Students of Educational Psychology should be familiarized with transdisciplinary research outcomes, including employment of research methodologies that were traditionally valued to be exclusive to the medical domain.
- Studies of the brain and behaviour in the first five years of life in autism are essential and will most likely unlock the door to earlier identification, more successful treatment, and understanding of the causes of autism.

5.5.2 Prenatal primary health care

- Prenatal primary health care regimes should include stress management programmes.
- Maternal blood plasma sampling should be routinely done as a means to monitor glucocorticoids and serotonin levels.

5.5.3 Further research

- The possible contribution of Rubella viral infection to the pathogenesis of autism should be further investigated.
- The relationship between the postnatal process of brain overgrowth and prenatal neural defects remain to be determined.
- The prenatal stress hypothesis involved in the pathogenesis of autism should be refined by further research.
- A reduced foetal nutrient supply might be a consequence of poor placental function, and an outcome of a sub-optimal placental nutrient supply is exposure of the foetus to excess glucocorticoids, which act to restrict foetal growth and to programme permanent changes in the neural, cardiovascular, endocrine and metabolic systems. The role of poor placental function and sub-optimal placental nutrient supply in the pathogenesis of autism should be further investigated.

- The link between hyperserotonemia and lowered verbal abilities among individuals diagnosed with autism should be investigated at hand of more representative samples.

5.6 LIMITATIONS TO THIS STUDY

These findings are not conclusive, since unexpected variables entered the protocols, e.g., the possibility of prior exposure to Rubella, resulting in the production of Rubella antibodies. In addition, *Asic* was administered to treat nausea during early pregnancy, and drug interaction with systems implicated in autism was not considered. This project was based on a limited sample, i.e., a dizygotic twin study.

5.7 CONCLUDING REMARK

It seems certain that the coming years will yield many exciting and important clues to the pathogenesis of autistic disorder. It is likely that future research will demonstrate that neuroimaging, conducted in the first two years of life, will provide valuable diagnostic and prognostic information. The combination of continually evolving methodological and technological advances will, hopefully, bring us closer to the goal of better understanding and earlier intervention in autism.

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ANNEXTURE B

Diagnostic Criteria for Autistic Disorder (DSM-IV-TR 2000)

A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3);

(1) qualitative impairment in social interaction, as manifested by at least two of the following:

(a) marked impairment in the use of multiple nonverbal behaviour such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction

(b) failure to develop peer relationships appropriate to developmental level

(c) a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest)

(d) lack of social or emotional reciprocity

(2) qualitative impairments in communication as manifested by at least one of the following:

(a) delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)

(b) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others

(c) stereotyped and repetitive use of language or idiosyncratic language

(d) lack of varied, spontaneous make-believe play social imitative play appropriate to developmental level

(3) restricted repetitive and stereotyped patterns of behaviour, interest, and activities, as manifested by at least one of the following:

(a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus

(b) apparently inflexible adherence to specific, non-functional routines or rituals

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(c) stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements)
(d) persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play

C. The disturbance is not better accounted for by Rett's disorder or childhood of disintegrative disorder.

Source: APA (2000:1210)

University of Pretoria etd – Claassen, M (2006)

ANNEXTURE C

ETHICS AND RESEARCH STATEMENT

FACULTY OF EDUCATION
UNIVERSITY OF PRETORIA

While research has produced many positive social and educational outcomes, it has also raised disturbing questions about the conduct of researchers with respect to ethics, values and community. The purpose of ethical review, therefore, is to ensure that human respondents participate in research freely and without unreasonable risk. Where there is some degree of risk, the process of ethical review has to ensure that the potential benefits outweigh the risk and that the participation of human respondents enjoys the full and informed consent of these respondents.

The broader goals of the ethical review of research proposals in the Faculty of Education are the following:

- to develop among students and researchers a high standard of ethics and ethical practice in the conceptualisation and conduct of educational research.
- to cultivate an ethical consciousness among scholars especially in research involving human respondents.
- to promote among researchers a respect for the human rights and dignity of human respondents in the research process.

The ethical review process is guided by the following principles common to research involving human respondents:

- the principle of *voluntary participation* in research, implying that the participants may withdraw from the research at any time.
- the principle of *informed consent,* meaning that research participants must at all times be fully informed about the research process and purposes, and must have given consent to their participation in the research.

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- the principle of *safety in participation;* put differently, that the human respondents must not be placed at risk or harm of any kind, e.g., research with young children.
- the principle of *privacy,* meaning that the *confidentiality* and *anonymity* of human respondents must be protected at all times.
- the principle of *trust,* which implies that human respondents will not be subjected to any acts of deception or betrayal in the research process or its published outcomes.

The process of ethical review is not intended to add bureaucratic burden to the research process. Rather, this process is intended to protect the researcher as well as the participating human respondents. At a higher level, the process is also intended to higher the quality of research in the Faculty of Education - where research is conceived not simply as a set of techniques, but as a well-considered, ethically grounded process that builds values such as trust, respect, empathy and dignity among both the researcher and the researched. In such a process, participants are treated as authentic 'respondents' in the research endeavour and not simply as 'objects' to be studied.

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MARLEEN CLAASSEN DATE