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:
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 hypothalamic-pituitary-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 serotoninergic 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 serotoninergic 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.
- Hyperserotonemia in autism may also involve atypical metabolism of the metabolic serotonin precursor tryptophan as a potential mechanism for alterations in serotonin availability.
- 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).
• 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:
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.

5.8 LIST OF REFERENCES


Sapolsky, R.M. 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry*, 57:925-935.


