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:

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.

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.

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

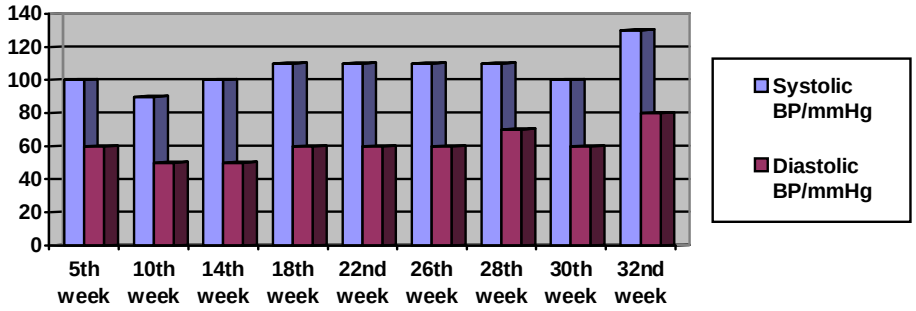

Table 4.1 Table for summarizing maternal blood-pressure values (units mmHg) during period of pregnancy

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.

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.

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-

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:

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

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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