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

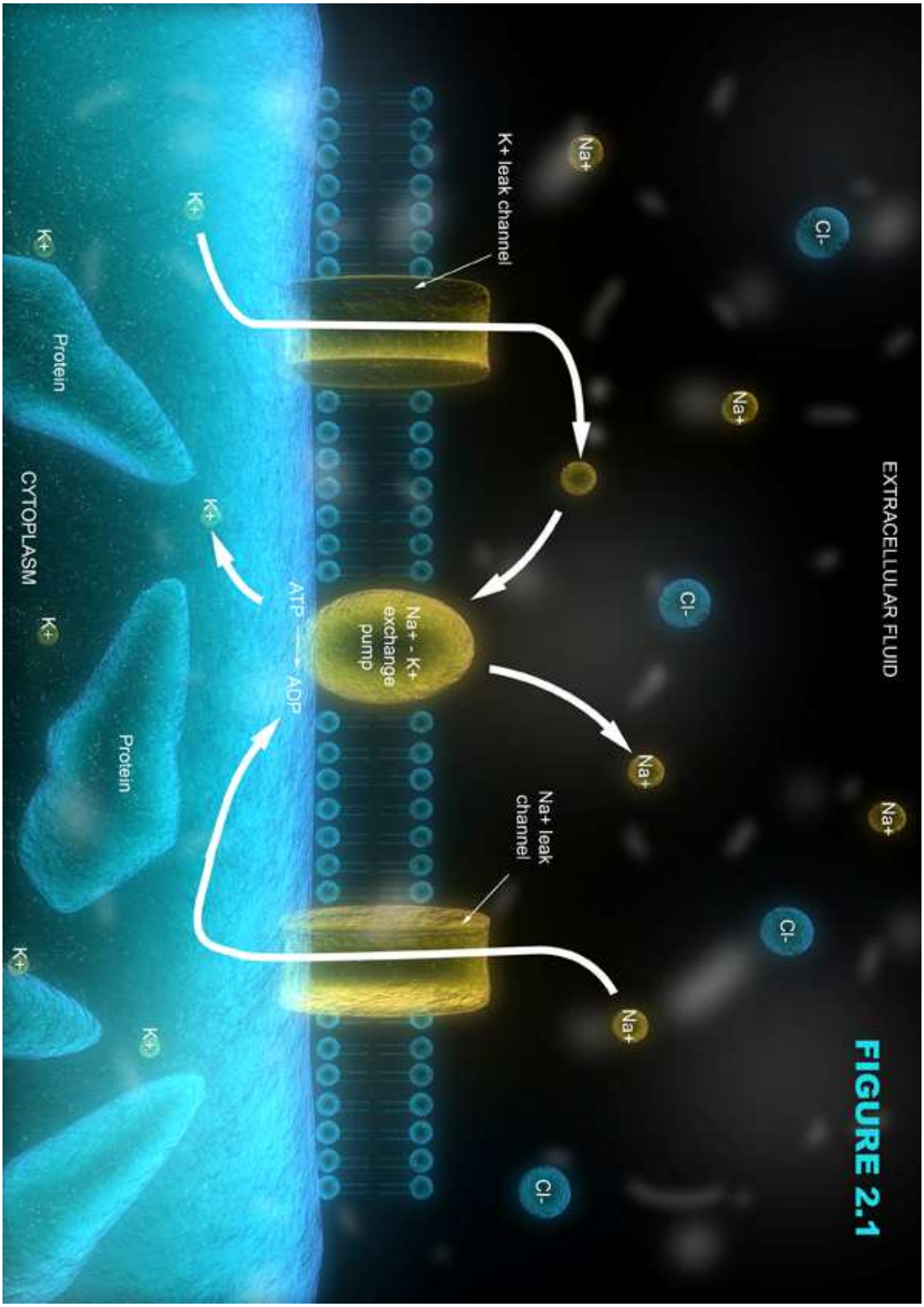
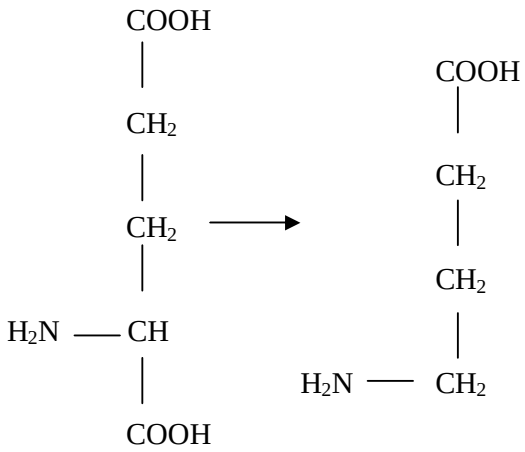

EXTRACELLULAR FLUID
Na+
Cl-
Na+
Cl-
Na+
Cl-
Na+
Cl-
K+ leak channel
Na+ - K+ exchange pump
Na+ leak channel
ATP
ADP
Protein
Protein
CYTOPLASM
K+
K+
K+
K+
K+
K+

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



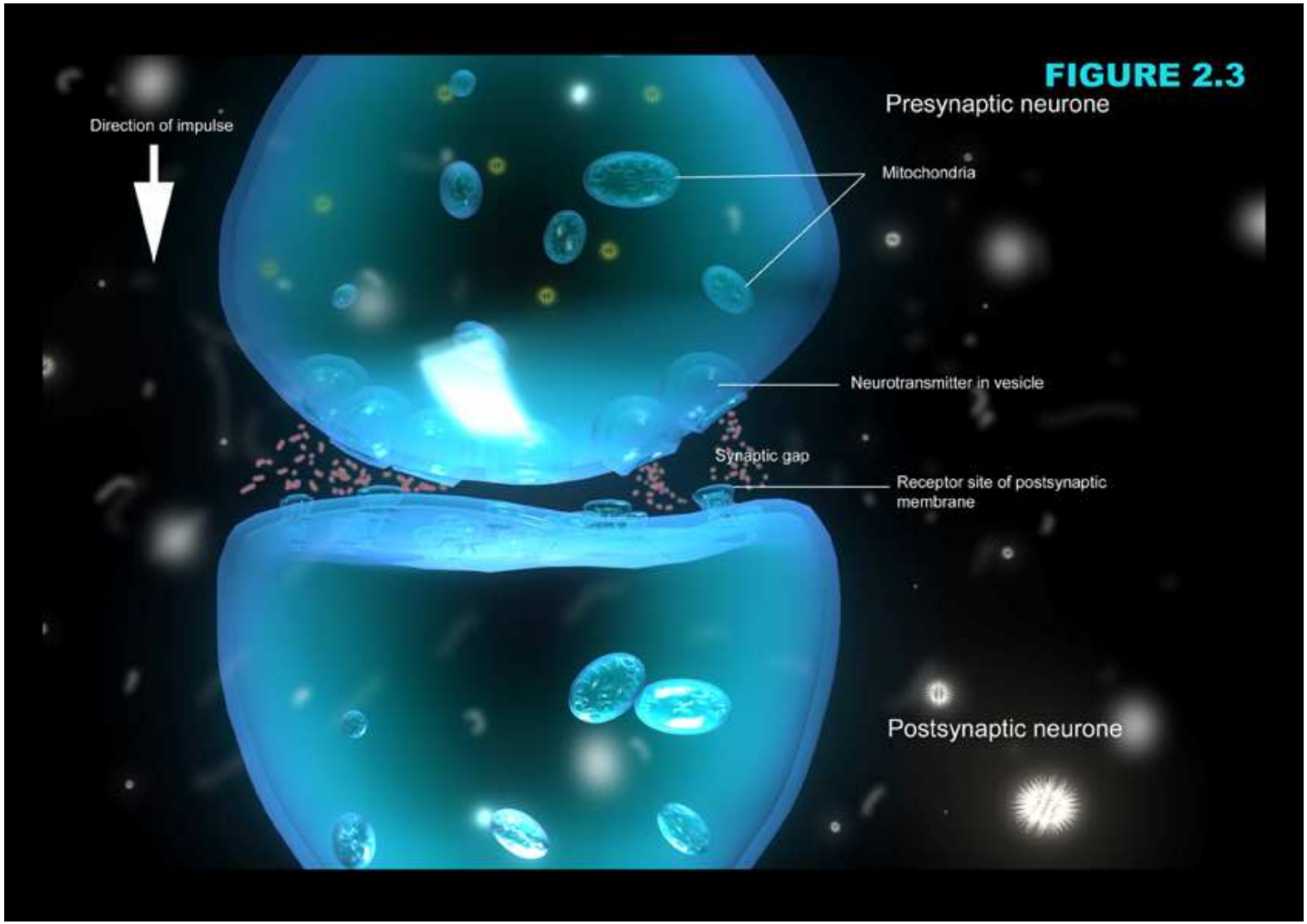
Glutamate GABA

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



(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

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

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

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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