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LONG-TERM FOLLOW-UP OF PRENATALLY DEXAMETHASONE-TREATED CHILDREN AT RISK FOR CONGENITAL ADRENAL HYPERPLASIA

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To Kasper and Ellen

ABSTRACT

Congenital adrenal hyperplasia (CAH) is a disorder of steroid genesis affecting approximately 1:10 000 children and leading to increased levels of androgens during foetal life and subsequent virilization of external genitalia in affected girls. However, prenatal virilization can be eliminated by antenatal dexamethasone (DEX) treatment. To be fully effective, DEX treatment has to be started in the 6–7th postmenstrual week and continued until the results of the prenatal diagnosis are available at gestational week 11–12. This means that 7 out of 8 foetuses (boys and unaffected girls) are treated unnecessarily during early gestation. CAH-affected girls are treated to term.

We performed a long-term follow-up of children treated in Sweden during the years 1985–1995, and 26 of the 40 treated individuals participated in the study. The control group consisted of 35 sex- and age-matched healthy children.

In general, the DEX-treated children were as well adjusted as the controls (Studies I and II). There were no between-group differences in major cognitive measures such as IQ, learning and memory. Parents reported that the DEX-treated children performed just as well at school as the controls. However, in a test of verbal working memory (WM), significantly lower results were observed in CAH-unaffected short-term treated children. The CAH-affected children did not differ from the control group, probably owing to small sample size and, consequently, low power. The verbal WM was correlated with the children's self-perception of difficulties in scholastic ability, another measure in which CAH-unaffected children differed from the controls. In measures of temperament, psychopathology and well-being, parents reported generally as good health in the DEX-exposed group as in the control group. The only difference was an observed increase in sociability in DEX-exposed children. In the children's self-ratings, however, increased social anxiety was observed. This difference was significant in CAH-unaffected short-term-treated children.

In order to study gender role behaviour (Studies III and IV), we developed a new instrument, the Karolinska Inventory of Gender Role Behaviour (KI-GRB), which was evaluated in an additional group of 180 school-age children. The underlying dimensions of the inventory were described by the factor structure and the KI-GRB subscales were also associated with sex-specific cognition. In prenatally DEX-exposed, CAH-unaffected boys, more neutral behaviours were observed, while in girls no group differences emerged after controlling for site of residence. A similar pattern was found when CAH-affected children were included in the analyses

In summary, these studies indicate that prenatal DEX treatment of CAH may have negative effects on certain aspects of cognitive and affective development, as well as affect gender role behaviour.

LIST OF PUBLICATIONS IN THIS THESIS

I. **Hirvikoski, T**., Nordenstrom, A., Lindholm, T., Lindblad, F., Ritzen, E. M., Wedell, A., Lajic S. (2007). Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *Journal of Clinical Endocrinology and Metabolism*, 92(2), 542-548.

II. **Hirvikoski, T.**, Nordenstrom, A., Lindholm, T., Lindblad, F., Ritzen, E. M., Lajic, S. (2008). Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *European Journal of Endocrinology*, 159(3), 309-316.

III. **Hirvikoski, T.**, Lindholm, T., Lajic, S., & Nordenström, A. Gender role behavior in prenatally dexamethasone treated children at risk for congenital adrenal hyperplasia – a pilot study. *Acta Paediarica*, 2011 Mar 9. doi: 10.1111/j.1651-2227.2011.02260.x. [Epub ahead of print].

IV. **Hirvikoski, T.**, Lindholm, T., Nordenström, A., & Lajic, S. Sex-specific cognition, gender role behavior and sex role identification in 8–15 year-old children (*submitted to Archives of Sexual Behaviour, article in review*).

PUBLICATIONS NOT INCLUDED IN THIS THESIS

I. Lajic S, Nordenström A, **Hirvikoski T**. (2011). Long-term outcome of prenatal dexamethasone treatment of 21-hydroxylase deficiency. *Endocr Dev*, 20:96-105.

II. Lajic, S., Nordenström, A., & **Hirvikoski, T.** (2008). Long-term outcome of prenatal treatment of CAH,. In Flück CE, Miller WL (eds): Disorders of the Human Adrenal Cortex. *Endocr Dev*. Basel, Karger, vol 13, s. 82-98.

III. **Hirvikoski, T**., Waaler, E., Alfredsson, J., Pihlgren, C., Holmström, A., Johnson, A, Rück, J, Wiwe, C, Bothén, P, Nordström, A-L. (2011). Reduced ADHD symptoms in adults with ADHD after Skills Training group – results from a randomized controlled trial. *Behaviour Research and Therapy*, 14;49:175-85.

IV. **Hirvikoski, T.**, Olsson, E., Nordenström, A., Lindholm, T., Nordström, A-L., & Lajic, S. (2011). Deficient cardiovascular stress reactivity is associated with poorer cognitive performance in adults with ADHD (attention deficit hyperactivity disorder). *J Clin Exp Neuropsychol*, 33 (1), 63–73.

V. **Hirvikoski, T.**, Lindholm, T., Nordenström, A., Nordström, A-L., & Lajic, S. (2009). High self-perceived stress and many stressors, but normal diurnal cortisol rhythm in adults with ADHD (attention deficit hyperactivity disorder). *Hormones and Behaviour*, 55(3):418-24.

VI. Ginsberg, Y., **Hirvikoski, T.** Lindefors, N. (2010). Attention Deficit Hyperactivity Disorder (AD/HD) in male prison inmates is a prevalent, persistent and disabling disorder. *BMC Psychiatry*, 10: 112.

VII. Westerberg, H., **Hirvikoski, T.**, Forssberg, H. & Klingberg, T. (2004). Visuo-spatial working memory span: a sensitive measure of cognitive deficits in children with ADHD. *Child Neuropsychology*, 10; 155 – 161.

VIII. Westerberg, H., Jacobaeus, H., **Hirvikoski, T**., Clevberger, P., Östensson, M-L, Bartfai, A., Forssberg, H., & Klingberg, T. (2007). Computerized working memory training after stroke – A pilot study, *Brain Injury*; 21(1): 21–29.

IX. Sinai, C., **Hirvikoski, T.**, Dencker Vansvik, E., Nordström, A-L., Linder, J., Nordström, P., & Jokinen, J. (2009). Thyroid hormones and personality traits in attempted suicide. *Psychoneuroendocrinology*, 34(10):1526-32.

X. **Hirvikoski, T.**, & Jokinen, J. Personality traits in attempted and completed suicide. *European Journal of Psychiatry*, *in press*, doi: 10.1016/j.eurpsy.2011.04.004).

CONTENTS

LIST OF ABBREVIATIONS

11βHSD2	11β-hydroxysteroid dehydrogenase type 2
21OHD	21-hydroxylase deficiency
ACTH	Adrenocorticotropin hormone
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AQ	Autism quotient scale
BSRI	The Bem Sex Role Inventory
CAH	Congenital adrenal hyperplasia
CBCL	The Child Behaviour Check List
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CSRI	The Children's Sex Role Inventory
CYP21	The gene encoding 21-hydroxylase
CVS	Chorionic villus sampling
d	Cohen's *d*, an effect size index
DEX	Dexamethasone
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
EAS	The Emotionality-Activity-Sociability-Shyness Temperament Survey
ES	Effect size
fMRI	Functional magnetic resonance imaging
FSIQ	Full-scale intelligence quotient
GBG	The Geschwind-Behan-Galaburda theory
GC	Glucocorticoid
GR	Glucocorticoid receptor
GRB	Gender role behaviour
HPA	Hypothalamic-pituitary-adrenal
ICC	Intraclass correlation
IQ	Intelligence quotient
KI-GRB	Karolinska Inventory of Gender Role Behaviour
LPI	Inferior parietal lobule
M	Mean value
MR	Mineralocorticoid receptor
MRI	Magnetic resonance imaging
MRT	Mental rotation test
NA	Noradrenaline
N/A	Not applicable
NE	Norepinephrine
NEPSY	A Developmental Neuropsychological Assessment

NS	Non-significant
PCA	Principal component analysis
SAM	Sympathetic-adrenomedullar system
SASC-R	The Social Anxiety Scale for Children - Revised
SD	Standard deviation
SE	Standard error
SNS	Sympathetic nervous system
SPAI-C-P	The Social Phobia and Anxiety Inventory for Children – Parental Rating
SV	Simple virilizing CAH
SW	Salt-wasting CAH
VFT	Verbal fluency test
WISC	Wechsler Intelligence Scale for Children
WM	Working memory

1 INTRODUCTION

Glucocorticoids (GCs) affect a range of somatic and mental processes. The focus of this thesis is on how prenatal exposure to the synthetic GC dexamethasone (DEX) affects the function of the central nervous system, measured as cognitive performance, well-being, gender role behaviour and other aspects of behaviour. The clinical group studied consists of children at risk for congenital adrenal hyperplasia (CAH) exposed prenatally to DEX.

1.1 CONGENITAL ADRENAL HYPERPLASIA (CAH)

The diagnostic term congenital adrenal hyperplasia (CAH) applies to a family of inherited disorders of steroid genesis caused by an abnormality in one of the five necessary enzymatic steps in the conversion of cholesterol to cortisol. Cortisol (the endogenous GC), aldosterone (the salt-retaining hormone) and testosterone are all steroids derived from cholesterol, and many of the same enzymes are used for their synthesis in the adrenal cortex. Cortisol synthesis is normally regulated by a negative feedback loop in which high serum levels of cortisol inhibit the synthesis and/or release of corticotropin-releasing hormone (CRH) at the level of the hypothalamic paraventricular nucleus and adrenocorticotropin hormone (ACTH) from the pituitary, whereas low serum levels of cortisol stimulate the release of CRH and ACTH. This loop defines the hypothalamic-pituitary-adrenal axis (HPA axis).

In more than 95% of cases CAH is caused by a 21-hydroxylase deficiency (21OHD) due to mutations in the 21-hydroxylase gene (*CYP21A2*). The *CYP21A2* is located on the short arm of chromosome 6, and the disorder has an autosomal recessive mode of inheritance (Figure 1). Postnatally, individuals with CAH receive life-long medication with synthetic GC, such as prednisolone. Individuals with milder forms of CAH sometimes only need synthetic GC medication, while in more severe CAH, when the 21OHD enzyme block is more complete, fludrocortisone must also be taken in order to avoid salt loss.

CAH is a rare condition affecting approximately 1:10 000 children in Sweden. Thus each year, 10–15 children are diagnosed with CAH.

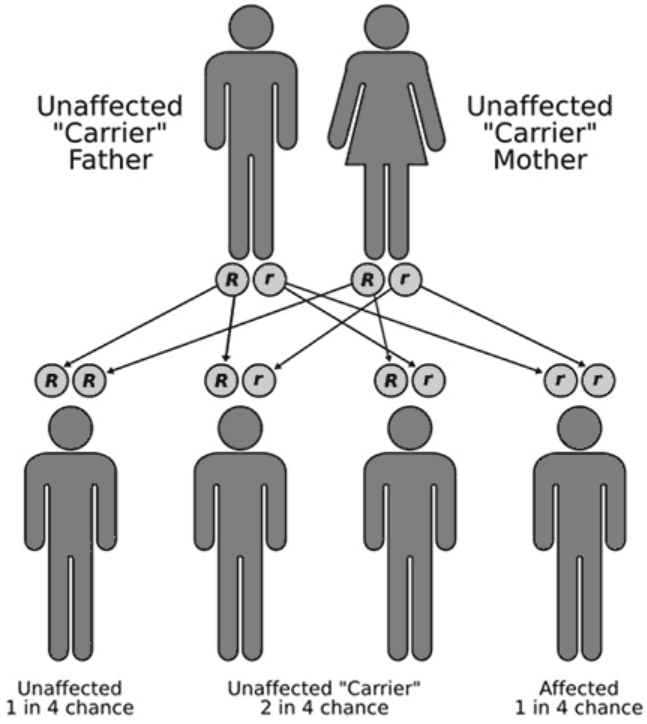


Figure 1. CAH has an autosomal recessive pattern of inheritance, meaning that one out of four children is affected with CAH in families with two unaffected "carrier" parents. Thus, one out of eight children is a CAH-affected girl.

The clinical symptoms of CAH range from mild disease (non-classical CAH, NC) that may present as late as in adult life in women with hirsutism and infertility to more severe prenatally virilizing forms without (simple virilizing CAH, SV) or with salt loss (salt-wasting CAH, SW). Three fourths of individuals with classical CAH are salt-wasting, i.e. have an aldosterone deficiency. The salt-wasting variant is characterized by a more complete block of the enzyme 21-hydrozylase, which generates higher androgen levels and is associated with increased genital virilization. Thus, depending on the type of CAH, the prenatal hormonal milieu and, consequently, degree of virilization can be predicted in future pregnancies (i.e. the genotype predicts the phenotype) (Lajic, Nordenstrom, Ritzen, & Wedell, 2004).

The newborn girl with virilizing CAH (SV or SW) can in the most severe cases be taken for a boy due to clitoromegaly, labioscrotal fusion and formation of a urogenital sinus. The virilization is due to increased production of androgen precursors (dehydroepiandrosterone DHEA/dehydroepiandrosterone sulphate DHEA-S, as well as androstenedione) in the adrenal cortex and their conversion to the potent androgens testosterone and dihydrotestosterone (DHT) (Figure 2).

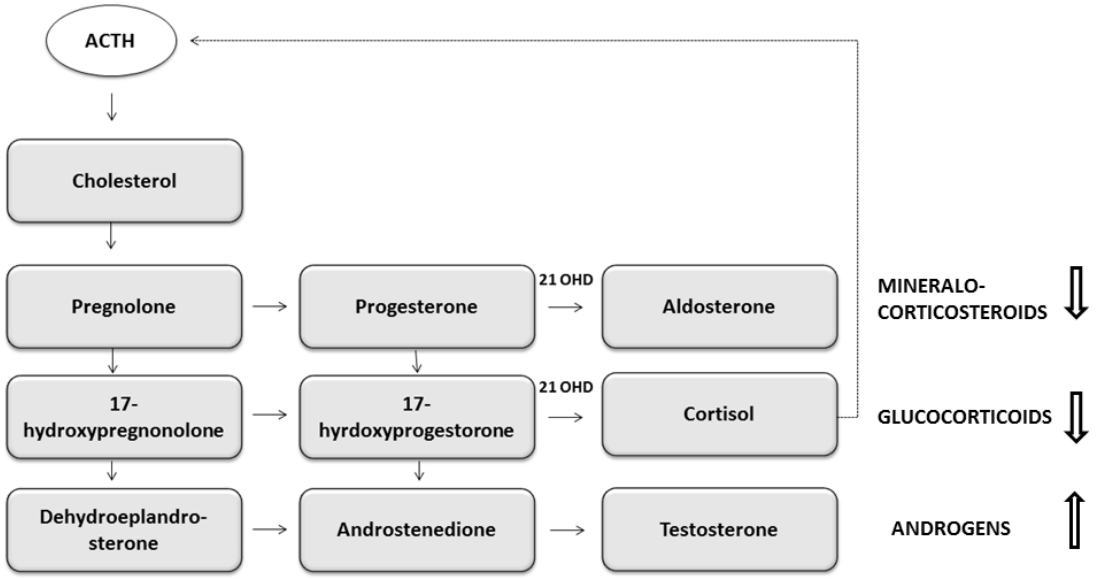


Figure 2. A simplified adrenal pathway and major steroid hormones in CAH. In most of the cases CAH is caused by a 21-hydroxylase deficiency (21OHD) due to mutations in the 21-hydroxylase gene (*CYP21A2).* 21-hydroxylase is one of the enzymes necessary in the conversion of cholesterol to cortisol. Low levels of cortisol are a signal for the brain to increase cortisol synthesis (increase the activity of the HPA axis), which, in the case of 21-hydroxylase deficiency, leads to excessive synthesis of androgens of adrenal origin.

Females with CAH are fertile with a potential for pregnancy when treated with life-long glucocorticoid replacement and surgical correction of the external genitalia. A common treatment policy recommends an establishment of the definitive endocrine diagnosis and treatment as early as possible, assignment of the infant to female gender and feminizing surgery of the virilized external genitalia (LWPES/ESPE, 2002).

To have a child with genital ambiguity is a traumatizing experience for the family. In addition, the reconstructive surgery can cause great emotional stress for both the child and her family. Moreover, the surgical outcome and sexual function are not always optimal, especially in females who are severely virilized (Nordenstrom et al., 2010). The fertility rate is still low in women with CAH, although it has improved significantly during the past years owing to earlier treatment of CAH, as well as surgical advances in genital reconstruction (Lo & Grumbach, 2001). Women with CAH report relatively low levels of sexual activity (although in an uncontrolled study) (Morgan, Murphy, Lacey, & Conway, 2005). The sexual debut occurs two years later among women with CAH as compared to controls (Frisen et al., 2009). In

the same study, 13% of women with CAH had not debuted sexually, as compared to less than 2% in the age-matched control group (ibid.).

The genital malformations in CAH can nowadays be reduced by prenatal treatment with DEX and, consequently, reconstructive surgery can be avoided (Lajic, et al., 2004).

1.2 PRENATAL DEXAMETHASONE TREATMENT OF CHILDREN AT RISK FOR CAH

Prenatal treatment with dexamethasone (DEX) has been administered since the mid-1980s to reduce the genital malformations and thereby avoid reconstructive surgery (Chrousos et al., 1985; David & Forest, 1984). The rationale for prenatal DEX treatment of foetuses at risk for CAH is to suppress the foetal adrenal cortex in order to reduce the levels of adrenal androgens and thus normalize sex differentiation in female foetuses with SV or SW CAH.

Virilization of external genitalia by androgens occurs from 6 to 8 weeks of gestation. To be fully effective, DEX treatment has to be started in the 6^{th}–7^{th} postmenstrual week and continued until the results of the prenatal diagnosis are available (generally by *CYP21A2* genotyping of foetal DNA obtained by chorionic villus sampling, CVS, in gestational weeks 11–12). This means that 7 out of 8 foetuses (boys and unaffected girls) are treated unnecessarily during early gestation. Girls with CAH are treated until term. Treatment safety has been reported to be acceptable, at least in the short-term perspective, based on findings of normal pre- and postnatal growth, and the reported side-effects do not appear to follow a particular pattern (Lajic, et al., 2004). Nevertheless, long-term effects are just starting to be addressed due to the fact that the oldest children treated are now reaching early adulthood. Results from experimental animal models have also raised concerns regarding possible future effects on metabolism, cognition and emotional development in adult life (de Vries et al., 2007; Hauser et al., 2008; Matthews, 2001; Seckl, 2004).

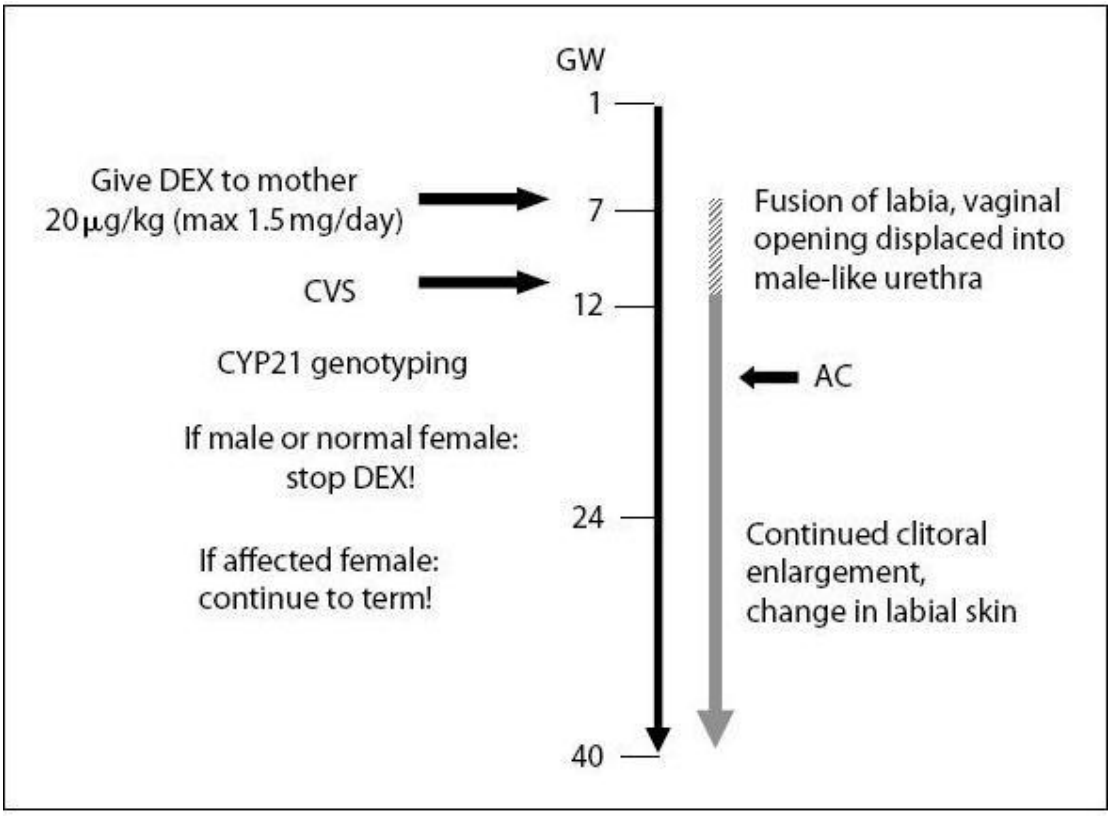


Figure 3. The treatment protocol for the prenatal dexamethasone treatment of children at risk for congenital adrenal hyperplasia.

1.2.1 Maternal side-effects of prenatal DEX treatment

The focus of this thesis is on prenatally DEX-treated children, while follow-ups of mothers have been reported in detail elsewhere (Ritzén, 2001; "Technical report: congenital adrenal hyperplasia. Section on Endocrinology and Committee on Genetics," 2000). Reported maternal adverse effects have been marked weight gain, mood swings, nervousness, irritability, hypertension, glucose intolerance and severe striae with permanent scarring, chronic epigastric pain, gastroenteritis, increased facial hair growth and cushingoid facial features (ibid). Increased weight gain during the first trimester and more cutaneous striae were the only statistically significant differences between the dexamethasone-treated mothers and controls in the only controlled (retrospective) study (Lajic, Wedell, Bui, Ritzen, & Holst, 1998). However, the discrepancy in weight between treated women and their controls had disappeared by the end of the pregnancy. There was a tendency for mothers treated full-term (7 cases) to report more side-effects than those treated only during the first trimester of pregnancy. One third of the Swedish mothers who received

dexamethasone treatment in the study by Lajic et al. (1998) would not choose treatment in a future pregnancy. In other studies (Forest, David, & Morel, 1993; Mercado, Wilson, Cheng, Wei, & New, 1995), almost all the mothers said that they would choose to undergo the same treatment during a next pregnancy.

1.3 FOETAL CNS DEVELOPMENT DURING EARLY GESTATION

The central nervous system (CNS) is particularly sensitive during foetal development. A major negative impact on the foetal CNS can be associated with gross alterations in CNS structure and functioning, while subclinical effects can result in developmental delay and lower scores on measures of CNS functioning such as IQ. Subtle effects on CNS functioning may not even be captured by routine neuropsychological assessment, despite an impact on the child's everyday life when the challenge level is increased, such as multitasking, distraction and/or new tasks (Dennis, 2000).

Generally, drugs affect the foetal brain at lower doses than in the case of adults. Different aspects of the CNS are affected depending on the developmental phase of the foetus. In early embryonic development cell proliferation occurs within the neural tube and by week 5 of gestation, the basic features of the CNS can be identified. After 6 weeks of gestation, the neuroblasts begin to migrate to their permanent locations, where the differentiation begins. Neuronal differentiation includes processes such as development of cell bodies, selective cell death, dendritic and axonal growth, and synaptogenesis (Anderson, Northam, Hendy, & Wrennall, 2001; Jessell & Sanes, 2000). Between the 6^{th} and 12^{th} gestational week many regions of the brain develop, such as the dopaminergic nuclei in the midbrain, the hypothalamic areas and certain areas of the hippocampus, as well as areas of the striatum, amygdala and neocortex (Bayer, Altman, Russo, & Zhang, 1993).

In the foetal CNS, glucocorticoids are essential for normal brain development affecting such processes as cell proliferation and neuronal growth and differentiation (Matthews, 2001), modulating neurotransmitter systems and regulating the plasticity and circuitry, as well as affecting (supressing) myelin content in the brain (Belanoff, Gross, Yager, & Schatzberg, 2001). Excessive GC levels are known to be detrimental to the foetal CNS. Foetal exposure to excess GC can be of different origins, such as maternal stress or maternal treatment with synthetic GC.

1.4 GLUCOCORTICOIDS

The endogenous glucocorticoid (cortisol, hydrocortisone) is essential for life in regulating or supporting several important metabolic, immunological, cardiovascular and homeostatic functions. In addition to DEX, several synthetic GCs have been developed for therapeutic use, and the synthetic GCs differ in pharmacokinetics (such as half-life) and pharmacodynamics (such as mineralocorticoid potency). GCs act via two types of receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR).

Cortisol has a 10 times higher affinity for the MR than for the GR (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998), which is why the MRs are occupied in a non-stress baseline state while the GR become occupied when the levels of free cortisol rise, i.e. during a stress response. However, DEX binds predominantly to the GR regardless of the state of the organism.

The MR is expressed primarily in the limbic system, while the GR is present in both subcortical and cortical structures with the highest density being found in the hippocampus, parahippocampal gyrus, paraventricular nucleus and other hypothalamic nuclei, as well as the cortex (McEwen et al., 1987). Thus, cortisol is normally regulated by the hypothalamic-pituitary-adrenal axis (HPA axis) (Figure 4) while further control of HPA axis activity occurs at extrahypothalamic sites such as the hippocampus and the amygdala. In primates the prefrontal cortex is particularly dense in GRs (Patel et al., 2000; Sanchez, Young, Plotsky, & Insel, 2000).

The hippocampus is not only involved in regulation of stress and the HPA axis, but also in learning and long-term memory (Kandel, Kupfermann, & Iversen, 2000). The amygdala is associated with processing of emotional information, for example fear, as well as social information, such as facial expressions (Iversen, Kupfermann, & Kandel, 2000). The prefrontal cortex is involved in the regulation of behaviour (executive control), with regard to motor as well as cognitive and affective processes (Gazzaniga, Ivry, & Mangun, 1998) (chapter 11, p. 423–464). Thus, a wide range of cognitive and affective processes can be influenced by GCs (see also the section Brain development in CAH).

Table 1. Timetables of neurogenesis in selected brain regions in the rat and human foetus (adapted from Bayer et al., 1993).

CNS region	*Rat embryonic (E) or postnatal (P) age in days*	*Human foetal age in weeks*
Pontine and Medullary Nuclei		
Locus coeruleus	E11-E13	3.5-5.7
Raphe nuclei	E11-E15	3.5-7.0
Reticular formation	E11-E15	4.1-7.0
Midbrain Dopaminergic Nuclei		
Substantia nigra (compacta and reticulata)	E13-E15	5.3-7.0
Ventral tegmental areas (lateral and medial)	E13-E16	5.3-7.4
Amygdala (most areas)	E13-E17	5.3-7.9
Thalamus	E13-E18	5.3-9.9
Hypothalamic Region		
Hypothalamus proper (most areas)	E13-E18	5.3-9.9
Lateral and medial preoptic areas	E12-E17	4.1-7.9
Periventricular	E15-E19	6.7-14.9
Sexually dimorphic nuclei	E15-E19	6.7-14.9
Hippocampal Region		
Entorhinal cortex and subiculum	E14-E19	5.8-11.9
Hippocampus (CA1, CA3ab, CA3c)	E16-E20	7.1-14.9
Dentate granule cells	P0-P15	19-35.9
Striatum and Pallidum		
Pallidum	E12-E16	4.1-7.4
Striatum (Caudoputamen complex and n. accumbens)	E16-E22	7.1-18.9
Neocortex and limbic cortex		
Most neocortical and limbic cortical neurons	E 14-E20	5.8-14.9

Thus, the activity of the HPA axis mediates physical "allostasis", the adaptive responses of the body that maintains homeostasis in response to stress, but it is also essential for modulation of behavioural adaptation during acute stress, and regulates arousal, alertness, and cognition (Charmandari, Kino, Souvatzoglou, & Chrousos, 2003; Lupien et al., 2002). The "allostasis" is not only produced by mediators generated by the HPA axis but also by the immune system and the autonomic nervous system (ANS). The brain is both a controller and a target of the three systems, and also effectuates allostatic processes by activation of nerve cells and release of neurotransmitters (McEwen, 2002).

1.4.1 The connection of the HPA axis to the SAM

The HPA axis activity is bidirectionally connected to the sympathetic-adrenomedullar system (SAM), which mediates a variety of rapid, visceral responses to stress, such as heart rate and vasoconstriction. Cortisol increases the rate and strength of heart contractions, sensitizes blood vessels to the action of noradrenaline and affects many metabolic functions. These actions prepare the body (and mind) to meet a stressful situation. The emergency reaction**,** or fight-or-flight reaction**,** is mediated by catecholamines. In the CNS, the SAM is regulated by the hypothalamus, which also regulates the HPA axis, and thus the secretion of cortisol in the endocrine stress response. There are a number of other physiological mediators that are also activated during a stress response and are adaptive in the short run. When the allostatic systems remain turned on during a longer period of time, e.g. during prolonged stress, the mediators can produce a wear and tear on the body and the brain, an "allostatic load", which can be damaging for the organism (McEwen, 2002).

Like the activity of the HPA axis, SAM also regulates the cognitive state and specific cognitive functions in order to facilitate the individual's ability to cope with acute stress. The self-perceived coping ability, in turn, strongly affects the perceptions of stress and, in addition, the activity in both the HPA axis and SAM. Thus, the stress reactivity may affect both cognitive performance and affective reactions to a stressor (Kapoor, Petropoulos, & Matthews, 2008).

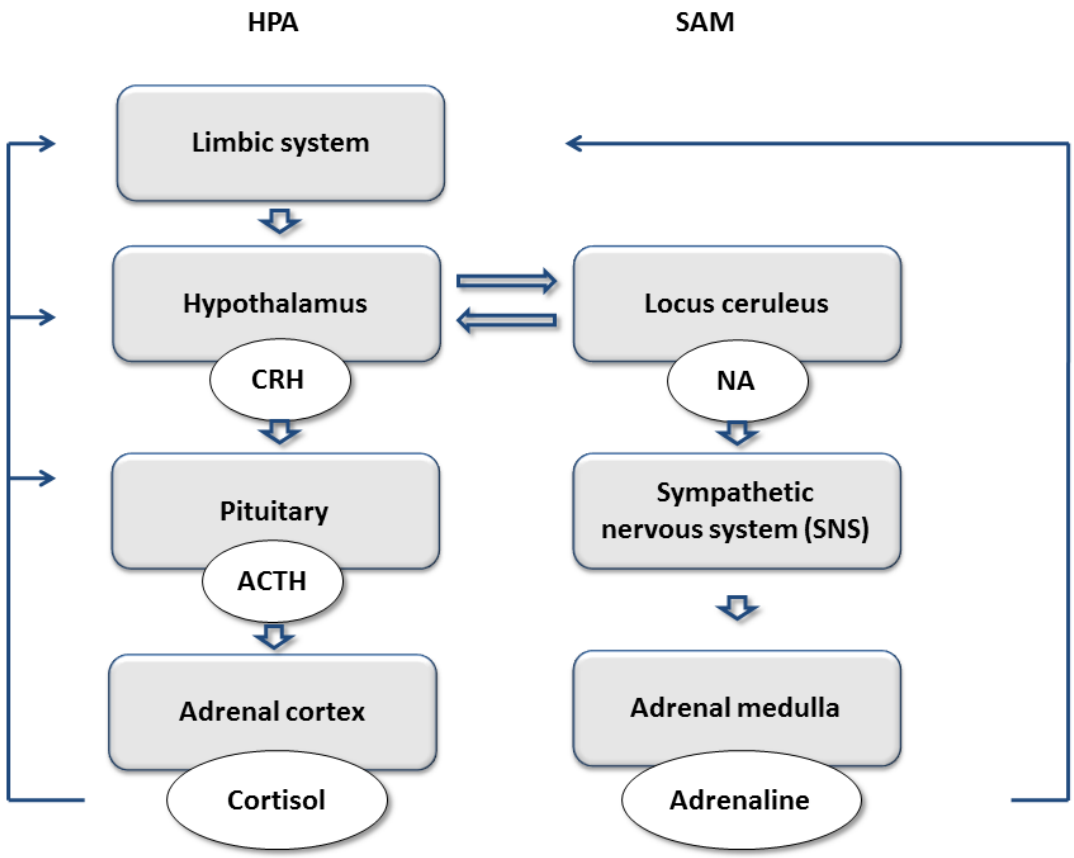


Figure 4. The hypothalamic-pituitary-adrenal axis activity is bidirectionally connected to the sympathetic-adrenomedullar system.

Note: CRH = corticotropin-releasing hormone; ACTH = adrenocorticotropin hormone; NA = noradrenaline.

1.4.2 Behavioural effects of excessive glucocorticoids

GC signalling may have different effects in a mature versus a developing brain, and prenatal exposure to excess GC has yet other consequences (McEwen, et al., 1987). The effects on the adult CNS are often reversible, at least to some degree, while effects on a foetus can be organizational, i.e. have an imprinting effect and "programming" of the behaviour and certain physiological functions for long periods of time and even for the entire life span of the individual (Charmandari, et al., 2003). It should be noted that behavioural effects including cognition are not necessarily mediated by structural changes in the brain, since the activity of the HPA axis is essential for modulation of behavioural adaptation during acute stress and regulates arousal, alertness and cognition (Charmandari, et al., 2003; Lupien, et al., 2002). Thus, permanent changes in the reactivity of the HPA axis program both cognitive

performance and emotional reactions in the affected individual (Charmandari, et al., 2003; Kapoor, et al., 2008).

1.4.2.1 Effects of glucocorticoids in adults

In a normal daily fluctuation of cortisol, an increase is seen in the morning after awakening (awakening cortisol response, ACR), after which levels successively decrease during the day (Clow, Thorn, Evans, & Hucklebridge, 2004). Both low (Heim, Ehlert, & Hellhammer, 2000) and high cortisol concentrations (Bauer et al., 2000; Kirschbaum et al., 1995), as well as aberrant ACR (Clow, et al., 2004), have been associated with chronic stress. Although seemingly inconsistent, these results may be logical in view of the fact that HPA axis reactivity is affected by several factors that may differ in different study populations, such as season of the measurement (J. A. King et al., 2000); awakening time (Clow, et al., 2004); age and gender (S. L. King & Hegadoren, 2002); the individual's IQ (Tennes & Kreye, 1985); nicotine abuse (Kirschbaum & Hellhammer, 1994); psychiatric illness, such as depression (Burke, Davis, Otte, & Mohr, 2005; Jansen et al., 1999), anxiety (McBurnett et al., 1991; van Goozen et al., 1998), post-traumatic stress disorder (Heim, et al., 2000); as well as psychoactive drugs (Joyce, Donald, Nicholls, Livesey, & Abbott, 1986; Kariyawasam, Zaw, & Handley, 2002).

In adults, excessive GC has been studied in diseases such as Cushing's syndrome (an endocrine disorder characterized by sustained hypercortisolism, either due to an endogenous overproduction of cortisol or medical treatment with synthetic GC), as well as in healthy subjects in an experimental design using synthetic GC [for a review, see (Belanoff, et al., 2001)]. Most studies on negative effects of GC have focused on high rather than low GC levels, although too low levels of cortisol are also thought to be negative for the CNS (ibid.).

The structural effects of excessive endogenous or exogenous GC have consisted in a higher ventricle-brain ratio, enlargement of ventricles and hippocampal atrophy, as well as cerebral and cerebellar cortical atrophy (ibid.). Among psychiatric symptoms, excessive GC may increase the risk for depression, anxiety and agitation (Belanoff, et al., 2001). A wide range of cognitive effects have also been demonstrated such as negative effects on general intellectual ability, memory, visual and spatial reasoning, concentration, attention and distractibility, as well as impulse inhibition, working memory and other executive functions (Belanoff, et al., 2001). Thus, exposure to prolonged stress or high levels of GC impair prefrontal cognitive

functions (Arnsten, 1999). However, short-term stress can enhance cognitive functions, such as attention and memory (Lupien, Gillin, & Hauger, 1999), as well as executive functions (Hirvikoski et al., 2011), thus facilitating the individual's ability to cope with stress. In theory, both poor recovery (repeatedly sustained high cortisol levels after a stressor) and abnormally low stress reactivity (low cortisol) could worsen the individual's ability to cope with stressors in everyday life.

It should be noted, however, that the association between stress and cognition may also be related to the SAM, and not only GC (Figure 4). Different kinds of stressors elicit stress (Cinciripini, 1986; Kirschbaum, Pirke, & Hellhammer, 1993; McEwen, 2006), but physiological stress responses may not be identical to different types of stressors (stimulus-response specificity) (Lundberg & Frankenhaeuser, 1980). For example, cognitive stressors drive predominantly stress responses from the autonomic nervous system (ANS) (Lundberg & Frankenhaeuser, 1980).

1.4.2.2 Effects of glucocorticoids in children

There are few studies on the effects of exogenous administration of GCs on cognitive performance in children, for obvious ethical reasons. Administration of high-dose prednisone to children with chronic asthma caused more severe impairment of verbal memory, as well as more symptoms of anxiety and depression than low doses (Bender, Lerner, & Poland, 1991). Synthetic GCs used in the (postnatal) treatment of CAH have been considered to be the most reasonable explanation for the white-matter abnormalities and/or temporal lobe atrophy observed in the treated children with CAH (see sections Biological factors influencing behaviour in CAH and Brain development in CAH).

1.4.2.3 Glucocorticoid effects during foetal life

In the foetal CNS, GCs are essential for normal brain development affecting such processes as cell proliferation and neuronal growth and differentiation, but excessive levels are known to be detrimental to the CNS (Matthews, 2001). Foetal exposure to excess GC can be of different origin, such as maternal stress or maternal treatment with synthetic GC.

In humans, gestational stress and elevated maternal endogenous GC during pregnancy have been shown to be associated with developmental delays, behavioural abnormalities and emotional problems in children (Huizink, Mulder, & Buitelaar, 2004; Rice, Jones, & Thapar, 2007; Weinstock, 2001). However, the outcome may

also be moderated by genetic risk factors, as well as the postnatal environment (Graham, Heim, Goodman, Miller, & Nemeroff, 1999). All trimesters have been regarded as vulnerable periods depending on the outcome factor(s) in focus (Huizink, et al., 2004; Rice, et al., 2007; Weinstock, 2001). In a prospective study (S. King & Laplante, 2005), it was found that the children whose mothers were exposed to moderate-high stress during the 1st or 2nd trimester showed significant impairment of both general intellectual ability and play behaviour at two years of age, as compared to a low-stress group.

It should be observed that the foetus is normally protected more against maternal cortisol than against synthetic GCs because placental enzyme 11βHSD2 rapidly inactivates most [50–90% (Benediktsson, Calder, Edwards, & Seckl, 1997)] of the maternal cortisol to inert cortisone, thus minimizing foetal exposure (Seckl & Meaney, 2004), while synthetic GCs, such as betamethasone and DEX, easily cross the placenta. It should also be noted that maternal stress also induces other processes that could affect the foetus. First, in pregnant primates, the placenta becomes an important transient endocrine unit that is activated by maternal stress and acts as a source of ACTH, CRH and other hormones (Petraglia, Florio, Nappi, & Genazzani, 1996). Second, maternal stress also strongly activates the sympathetic-adrenomedullar system (SAM, Figure 4), which may reduce uteroplacental blood flow because catecholamines control vasomotor activity, i.e. the activity which controls the size of the blood vessels. Thus, high cortisol levels due to maternal stress and medical treatment with synthetic GC may not be completely comparable.

The effects of synthetic GCs have been studied in children at risk of preterm delivery. In this group synthetic GCs are widely used to enhance the maturation of the foetal lung in order to avoid respiratory distress. A long-term follow-up study of individuals exposed to a single prenatal course of betamethasone showed no effect on neurological, cardiovascular, psychiatric or cognitive functions at the age of 30 (Dalziel, Lim, et al., 2005; Dalziel, Walker, et al., 2005). However, more hyperactivity, attention disorders and externalizing problems have been recognized in preschool children who received repeated antenatal betamethasone therapy compared to children who received a single dose (French, Hagan, Evans, Mullan, & Newnham, 2004), while general intellectual ability was not affected (French, et al., 2004; Wapner et al., 2007). Preterm children with respiratory distress syndrome who received a one-week course of postnatal DEX therapy were compared to a placebo control group at school age. The DEX group showed significantly poorer motor

development, a lower Full-Scale IQ, as well as lower scores on three out of four WISC-III Indexes, i.e. Perceptual Organization, Freedom of Distractibility and Processing Speed. The Verbal Comprehension Index did not differ between the two groups (Yeh et al., 2004). These differences were not detected in an earlier follow-up of the same cohort at two years of age (Yeh et al., 1998), thus illustrating the need for long-term follow-up studies.

The pre- and perinatal treatment of preterm children is different from the prenatal treatment of children at risk for CAH, and the effects of GCs may also vary depending on which synthetic GC is chosen (Baud & Sola, 2007; Heine & Rowitch, 2009). Nonetheless, studies on the treatment of preterm children may provide important information on mechanisms by which synthetic glucocorticoids exert their effects. These mechanisms have been studied in more detail in animal models.

1.4.3 Animal models of foetal glucocorticoid exposure

Experimental data from animals exposed to prenatal corticosteroids have demonstrated adverse effects on somatic development, as well as on cognition and other aspects of behaviour. In rats, a range of additional side-effects such as low birth weight and hypertension (Celsi et al., 1998); decreased size of the hippocampus and affected short-term memory (Seckl & Miller, 1997); impaired learning and memory functions (Emgard et al., 2007); alterations in forebrain development as well as noradrenergic and cholinergic neurotransmitters (Kreider et al., 2005); persistent effects on serotonergic and dopaminergic systems (Slotkin, Kreider, Tate, & Seidler, 2006); alterations in size and organization of midbrain dopaminergic populations, including a feminization or demasculinization of the three-dimensional cytoarchitecture in males (McArthur, McHale, & Gillies, 2007); aberrant sexual behaviour (Holson, Gough, Sullivan, Badger, & Sheehan, 1995); heightened vulnerability to oxidative stress in cerebellar granule cells (Ahlbom, Gogvadze, Chen, Celsi, & Ceccatelli, 2000); as well as alteration of two transcription factors known to be involved in brain cell differentiation (Slotkin, Zhang, McCook, & Seidler, 1998). Reduced exploratory behaviour and behavioural inhibition have also been observed in the same species exposed to prenatal DEX treatment either during the entire gestational period or during late gestation (Welberg, Seckl, & Holmes, 2001). However, the developmental timetable of the foetus differs significantly in rats and humans (Bayer, et al., 1993) (Table 1). Many of the brain areas that develop in rat from mid- to late gestation are formed in humans during the first trimester of

pregnancy, such as certain hypothalamic and hippocampal areas (Bayer, et al., 1993). There are also differences in GR distribution in the brain. Primates have more GR in the frontal lobes than the levels described in rodents, in which the well-known glucocorticoid-hippocampus link was originally established (Patel, et al., 2000; Sanchez, et al., 2000). Moreover, rats are considered to be corticosensitive while primates are corticoresistant species (Seckl, 2004).

In rhesus monkeys, a single high dose of DEX in late gestation resulted in altered hippocampal architecture. Moreover, multiple injections of DEX over a 24-hour period caused more severe damage on hippocampal neurons than a single injection with the same total dose (Uno et al., 1990). In a study of long-term postnatal sequelae, juvenile prenatally DEX-treated (doses of 5 mg/kg) rhesus monkeys had significantly higher plasma cortisol at baseline and post-stress than controls, as well as a reduction in hippocampal volume (Uno et al., 1994). Prenatal administration of DEX to African vervet monkeys was associated with metabolic effects after doses of 120 and 200 µg/kg/day as well as an exaggerated cortisol response to mild stress after doses of 200 µg/kg/day (de Vries, et al., 2007). Findings in common marmosets exposed to DEX (5 mg/kg/day) during early gestation (days 42–48 of a gestational period of 144 days) indicate that foetal glucocorticoid overexposure can lead to abnormal development of motor and social behaviours (Hauser, et al., 2008).

As mentioned before, most of the animal models have been designed to imitate the perinatal treatment of premature children or have used high doses of DEX. Little is therefore known about the effects of prenatal DEX treatment as used in CAH.

1.5 BEHAVIOURAL ASPECTS OF CAH

Although the prenatal DEX treatment of CAH is administered in order to reduce or avoid virilization of external genitalia in girls with CAH, there are also possible effects on the CNS and behaviour. These effects may not only be due to direct influence of excess GC but may also be due to a GC effect on sex hormones. Sex hormones have a major effect on the development of brain and behaviour. Effects of abnormally high levels of prenatal androgens on cognition and different dimensions of gender have been studied in individuals with CAH who have not been treated prenatally. Consequently, these behaviours may also be affected by the prenatal DEX treatment that brings the level of androgens to normal.

1.5.1 Cognition and lateralization

1.5.1.1 Intelligence

A possibility of selective sampling in clinic-based studies, together with small sample sizes, may explain the earlier finding of an elevated IQ in patients with CAH (Sinforiani et al., 1994). These results were not confirmed in other studies (Helleday, Bartfai, Ritzen, & Forsman, 1994; Kelso, Nicholls, Warne, & Zacharin, 2000; Merke et al., 2003). A few studies have found that patients with salt-wasting CAH have lower IQs than patients with simple-virilizing CAH (Helleday, Bartfai, et al., 1994; Nass & Baker, 1991) or lower than controls (Johannsen et al., 2006). However, no association between disease characteristics and intelligence was found in a recent study showing no evidence of intellectual deficit in either females or male patients with CAH (Berenbaum, Bryk, & Duck, 2010).

1.5.1.2 Lateralization: handedness

A higher incidence of left-handedness (Kelso, et al., 2000; Nass & Baker, 1991; Tirosh, Rod, Cohen, & Hochberg, 1993) or less consistent right-handedness (Mathews et al., 2004) among CAH patients has been found, thus supporting the hypothesis that prenatal androgen exposure causes a shift in cerebral lateralization toward right-hemisphere dominance (Geschwind & Behan, 1982; Geschwind & Galaburda, 1985). However, not all studies confirm the finding of a higher incidence of left-handedness among CAH patients (Helleday, Siwers, Ritzen, & Hugdahl, 1994; Malouf, Migeon, Carson, Petrucci, & Wisniewski, 2006).

1.5.1.3 Sex-specific cognition

Sex differences have been observed in certain cognitive tasks in children as young as 3–5 months old (Moore & Johnson, 2008; Quinn & Liben, 2008). Boys perform better in some spatial tasks such as mental rotation, while girls perform better in certain verbal tasks such as verbal memory and fluency (Halpern, 2000; Kimura, 2000). These sex differences observed in children, adolescents and adults (ibid.) are thought to be influenced by sex hormones (Hines, 2010). Thus, prenatal androgens are not only thought to shift lateralization in handedness, but they are also thought to exert other organizing effects and influence cognition.

In females with CAH, it is hypothesized that a shift towards a male-typical cognitive pattern could occur. This hypothesis has been supported by studies showing better performance in some, although not all, spatial tasks in girls, adolescents and women with CAH as compared to unaffected relatives (Hampson, Rovet, & Altmann,

1998; Hines et al., 2003; Resnick, Berenbaum, Gottesman, & Bouchard, 1986), but they were not confirmed by all studies (Malouf, et al., 2006). One study of spatial navigation ability reported that females with salt-wasting CAH (and an expected highest level of *in utero* exposure to androgens) performed similarly to both control males and CAH males, whereas evident sex differences were observed in milder forms of the disorder and in controls (Mueller et al., 2008). However, advanced bone age (an indicator of long-term childhood exposure to testosterone) was correlated with improved spatial navigation, i.e. not only prenatal but also postnatal androgens may have influenced spatial ability in these females.

In males with CAH the picture is different: poorer spatial ability has been observed in boys, adolescents and men with CAH as compared to unaffected controls (Hampson, et al., 1998; Hines, et al., 2003; Puts, McDaniel, Jordan, & Breedlove, 2008). The observations of better spatial ability in females with CAH and poorer spatial ability in males with CAH, as compared to unaffected sex-matched controls, may support the hypothesis of a curvilinear relationship ("inverted U shape") between androgens and spatial performance with intermediate levels of testosterone being associated with better spatial functioning (Moffat & Hampson, 1996).

Apart from spatial abilities, there are few studies on other sex-specific cognitive functions in CAH, such as verbal fluency, a cognitive function favouring females. However, a few studies showed inferior results in verbal tests in children with CAH (Plante, Boliek, Binkiewicz, & Erly, 1996) and women with CAH (Helleday, Bartfai, et al., 1994) as compared to controls, and a small study showed poorer verbal fluency in girls with CAH than in controls (Inozemtseva, Matute, & Juarez, 2008).

The reason why tests of spatial abilities are the most frequently used measures of sex-specific cognition in the CAH literature is probably that the focus has been on masculinization rather than defeminization. Moreover, spatial abilities (especially mental rotation and targeting) show the largest effect sizes among different measures of sex-specific cognition (Hines, 2010). Sex differences are small to medium-sized in most functions in which sex differences have been observed and studies of clinical groups are often relatively small in size, thus easily resulting in type II problems (failing to detect a difference due to low statistical power). However, what can be inferred from the existing studies on the effect of prenatal androgens is that boys and girls should be considered separately when analysing results in neuropsychological tests and other aspects of behaviour.

1.5.1.4 Learning disabilities

A risk for learning disabilities in CAH has been hypothesized for two reasons. First, there are possible risk factors due to disease complications in the neonatal period but also later, such as hypoglycaemia and salt crisis (see the section Biological factors influencing behaviour in CAH). Second, there is a possible negative effect of excess prenatal androgens on learning abilities, which has been hypothesized to result in the male predominance among children with learning disabilities, as suggested by the Geschwind-Belan-Galaburda (GBG) theory (Geschwind & Behan, 1982; Geschwind & Galaburda, 1985).

A specific deficit in computation ability has been reported (Nass & Baker, 1991), but not confirmed by other studies. Likewise, a small study showed elevated rates of reading disabilities in girls with CAH as compared to controls (n = 11 in both groups) (Inozemtseva, et al., 2008). Although there is not much evidence that patients with CAH are more likely to have learning disabilities (of clinical significance i.e. possible to diagnose) compared with their CAH-unaffected relatives, it should be noted that this issue has not been well studied with appropriate assessments. It is also possible that learning disabilities occur in children with severe and poorly treated CAH (due to disease complications), but not generally in the CAH population. However, a study in 11 children with CAH, their 5 CAH-unaffected siblings and 16 controls suggested a high rate of language/learning disabilities in both children with CAH and their CAH-unaffected siblings as compared to controls (Plante, et al., 1996). The authors suggested that the language/learning disability seemed to be a familial one and may be related to elevated androgen levels since also in CAH-unaffected heterozygote siblings androgens may be elevated, although to a lesser degree than in CAH-affected individuals (New et al., 1983). Moreover, a potential hormonal contribution of a heterozygote mother to the foetus may affect the developing CNS, and there might also be a possible linkage between the CAH genes and one or more genes in the adjacent region on chromosome 6 that may affect brain development and subsequent language/learning skills (Plante, et al., 1996).

1.5.1.5 Social cognition

In an fMRI study (discussed in more detail below in the section Brain development in CAH) (Ernst et al., 2007), the CAH group showed poorer differentiation of adverse emotions (anger or fear) from neutral facial expression as compared to healthy controls. Impairments in social cognition may affect social interactions negatively. In

a study on the association between high levels of prenatal testosterone and later autistic traits measured with the Autism Spectrum Quotient (AQ) rating scale, females with CAH scored significantly higher than their CAH-unaffected female relatives (Knickmeyer et al., 2006). Females with CAH reported especially high scores (more problems) in subscales measuring social skills and imagination.

It should be observed that these results could indicate just another aspect of masculinization of the brain and behaviour since there are also sex differences in the normal population. With regard to social perception, females have been shown to be more adept than males (Wood, Heitmiller, Andreasen, & Nopoulos, 2008; Wood, Murko, & Nopoulos, 2008). Thus, the differences between females with and without CAH do not necessarily indicate autistic traits. This is, however, an interesting area of research and not much is known about the effects of the prenatal androgens on social cognition. A recent study has shown an association between prenatal androgens (measured in the umbilical cord) and development of social communication (or "pragmatic language") in healthy girls (Whitehouse et al., 2010), but this has not been studied in girls with CAH.

1.5.2 Dimensions of gender

In contrast to earlier unidimensional theories of gender typing, recent theories emphasize the importance of integrating disparate perspectives and multiple dimensions of gender (Didonato & Berenbaum, 2011). *Gender role behaviour*, sometimes called sex-typical behaviour (the behaviour typical of one gender versus the other in a given historical time and place), is not synonymous with *gender role identification/sex role identification,* which refers to the child's self-perception of gender-related personality disposition (Boldizar, 1991), i.e. identification with a masculine or feminine sex role or "psychological masculinity or femininity". Moreover, gender role behaviour is differentiated from *sexual orientation*, i.e. the overall responsiveness to male versus female sex partners, not necessarily identical with the gender of the actual partner(s) or with the *sexual identity* as a heterosexual, homosexual or bisexual. *Gender identity* is yet another dimension of gender and refers to the basic sense of belonging to the male or female gender or something else, for instance, an intersex identity (Meyer-Bahlburg, 2001). Additional dimensions or components of gender and gender development are *sex-specific cognition* (mentioned in the section Cognition and lateralization in CAH), as well as *sex differences in psychopathology and personality* (Blakemore, Berenbaum, & Liben, 2009).

1.5.2.1 Gender-role behaviour

There is great variability in gender-role behaviour in the general population. This variability is probably accounted for by a number of factors such as gender-related genes, hormonal mechanisms (pre- and postnatal) and psychosocial factors or socialization. When compared with their sisters or other same-sex controls, females who have CAH engage in more male-typical childhood play, have more male-typical interests in adolescence, are more likely to report the use of physical aggression in conflict situations, are less interested in infants, marriage, motherhood and feminine appearance, score lower on measures related to empathy, intimacy, the need for social relations, maternal/nurturant behaviour and succorance, and are more likely to choose male-typical vocations (Berenbaum, 2001; Berenbaum, Duck, & Bryk, 2000; Berenbaum & Resnick, 1997; Dittmann, Kappes, Kappes, Borger, Meyer-Bahlburg, et al., 1990; Dittmann, Kappes, Kappes, Borger, Stegner, et al., 1990; Hines, 2006, 2010; Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2004b; Nordenstrom, et al., 2010; Nordenstrom, Servin, Bohlin, Larsson, & Wedell, 2002). These behaviour shifts have been observed also in girls and women who are treated with GC from infancy on (Meyer-Bahlburg, 2001). There is also a dose-response relationship between disease severity and degree of masculinization of behaviour in girls with CAH (playing with gender-role atypical toys) (Berenbaum, et al., 2000; Nordenstrom, et al., 2002). These results are considered to support the view that prenatal androgen exposure has a direct organizational effect on the human brain so as to determine certain aspects of sex-typed behaviour.

Nonetheless, there are also critics who argue that the physical differences in girls with CAH may lead their parents into treating them differently from CAH-unaffected girls, i.e. (not necessary consciously) influencing their behaviour in the male direction. Opposing these assumptions are results from a study using structured toy-play observation as an assessment method; when parents played with their daughters with CAH, they did not influence them to play in a more masculine way, but rather the opposite (Nordenstrom, et al., 2002).

1.5.2.2 Sexual orientation

Most women with CAH are heterosexual, but the rates of bisexual and homosexual orientation are increased compared to CAH-unaffected female relatives (Dittmann, Kappes, & Kappes, 1992; Meyer-Bahlburg, Dolezal, Baker, & New, 2008). The concept of bisexual/homosexual orientation refers to behaviours such as sexual

imagery, sexual attraction, and, to a lesser degree, overt homosexual involvement (Meyer-Bahlburg, 2001). Bisexual/homosexual orientation was not only predicted by the degree of prenatal androgenization (Frisen, et al., 2009; Meyer-Bahlburg, et al., 2008), but also by masculinization of childhood behaviour in women with CAH (Meyer-Bahlburg, et al., 2008).

1.5.2.3 Gender identity

While changes in gender-role behaviour and sexual orientation can be related to the severity of the disease, and therefore to prenatal androgenization (Cohen-Bendahan, van de Beek, & Berenbaum, 2005; Hines, 2006; Nordenstrom, et al., 2002; Servin, Nordenstrom, Larsson, & Bohlin, 2003), much less is known about what determines a person's gender identity (Meyer-Bahlburg, et al., 2004b). In girls and women with CAH, gender identity does not seem to be affected in most cases, although the observed percentage of serious problems with gender identity (5.2%) is higher than the prevalence of female-to-male transsexuals in the general population of chromosomal females (Dessens, Slijper, & Drop, 2005).

1.5.2.4 Sex differences in personality

Personality has been studied in CAH with regard to traits that usually show a sex difference, such as aggression. Males are more likely than females to show aggressive behaviour across species, ages and situations, and these differences have been hypothesized to be influenced partly by early hormones (Berenbaum & Resnick, 1997). Using questionnaires completed by mothers as the method of assessment, it has been observed that 3 to 11-year-old girls with CAH are more aggressive and active than their CAH-unaffected sisters, while there were no differences between boys with CAH and their CAH-unaffected brothers (Pasterski et al., 2007). As expected, unaffected boys were more aggressive and active than unaffected girls (ibid.). Corresponding results were observed in another study showing higher aggression in adolescent and adult females with CAH than in CAH-unaffected relatives (Berenbaum & Resnick, 1997). In this study, the difference in children (CAH-affected compared to non-CAH relatives) was not statistically significant and the sample sizes were quite small.

In a Swedish study (Helleday, Edman, Ritzen, & Siwers, 1993), 22 women with CAH were compared to 22 controls on the Karolinska Scales of Personality (KSP) (Schalling & Edman, 1993). It has been observed earlier that 8 out of 15 KSP subscales show significant sex differences (ibid.), and the CAH group differed

significantly on two of these eight scales, both in a masculine direction (Helleday, et al., 1993). Thus, the CAH group showed a high, male-level score for Detachment (distance in social relations as reflected by behaviours such as avoiding involvement in other people's personal life; not getting close to people) and a lower score for Indirect Aggression (reflected by behaviours such as slamming of doors, spreading gossip about people one dislikes) as compared to female controls.

A recent study included both males and females with CAH (age ranging from 12 to 45 years), as well as their CAH-unaffected relatives as controls, and focused on four aspects of personality: physical aggression, dominance, tender-mindedness and interest in infants (Mathews, Fane, Conway, Brook, & Hines, 2009). Females with CAH were less tender-minded and reported more physical aggression and less interest in infants compared to female controls. Males with CAH were less dominant, more tender-minded and reported less physical aggression than control males (ibid.).

1.5.3 Psychological well-being and perception of health

Children and young adult females with CAH did not differ from CAH- unaffected female relatives on parental ratings of psychopathology (the Child Behaviour Check List, CBCL), self-rating of positive versus negative emotionality, or self-image (comprising aspects of psychopathology and adjustment) (Berenbaum, Korman Bryk, Duck, & Resnick, 2004). In the same study, males with CAH were not different from unaffected males, with the exception of more reported negative affect at older ages (ibid.). Correspondingly, women with CAH have been reported to be psychologically well adjusted and they did not show increased psychiatric illness or deficits in social adjustment compared to population data (Morgan, et al., 2005). However, in a controlled study, women with CAH reported a poorer quality of life as well as more affective distress than controls (Johannsen, Ripa, Mortensen, & Main, 2006).

Yet, in another controlled study, general psychological well-being did not differ between women with CAH and controls (Frisen, et al., 2009). However, with regard to specific disease-related questions, one third of the women with CAH reported that the disease affected their relationship with their partner since they reported feeling "inhibited" or "ashamed of the appearance of my genitals". In a population-based follow-up of all Norwegian adult patients with CAH (Nermoen, Husebye, Svartberg, & Lovas, 2010), it was observed that women with CAH had only 21% of the expected number of children compared with the general population.

Moreover, 40% of the women with CAH (age range 19–80 years) reported that they had never had a gynaecological investigation as an adult (ibid.).

In the aforementioned study on personality (Helleday, et al., 1993), a non-significant trend ($.05 < p < .10$) was observed in the subscale Psychasthenia consisting of items such as "in order to get something done, I have to spend more energy than most others" and "I think I must economize my energy". In the more recent population-based study (Nermoen, et al., 2010), perception of general health and vitality were especially deteriorated in the CAH group (consisting of 72 of 101 identified patients), although scores on all eight subscales of the instrument used (SF-36 Health Survey) indicated significant impairment as compared to normative data from the Norwegian general population. However, among the quality of life scores, only one of the 16 items (item no.2: health, being physically fit and vigorous) was significantly lower than the norm (ibid.).

The literature on psychological well-being, psychosocial adjustment and self-perceived health may seem to be contradictory. However, the focus of the studies and assessment methods used vary between the studies and may at least partly explain the differences. Many of the standard instruments used are not devised to capture body-image or sexuality-related problems. Also, studying younger age groups may not show possible problems with sexuality, especially not problems related to sexual function that become apparent at older ages. The use of collateral information and rating scales, such as the CBCL, may not capture difficulties observed in clinical interviews (Wassenberg, Max, Koele, & Firme, 2004). Based on a clinically structured diagnostic interview, the Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (KSADS-PL), a high prevalence of attention-deficit hyperactivity disorder (ADHD) has been reported among boys with CAH (18.2%) (Mueller et al., 2010), which can be compared to the prevalence of approximately 5% in population-based studies (an approximately 3 boys : 1 girl ratio), although choice of informant, criteria for symptom count, definitions of subtypes and gender differences influence the prevalence estimates of ADHD (Ullebo, Posserud, Heiervang, Obel, & Gillberg, 2011). Mueller et al (2010) also observed an increased rate of disruptive behaviour disorders in boys with CAH. In girls with CAH, ADHD was observed in 4.8%, i.e. not more often than in the population. However, both girls and boys with CAH showed increased rates of anxiety disorders relative to the population norm (ibid.). The study consisted of relatively small samples, and additional studies, also including a non-psychiatric

control group, are needed. Since rating scales such as the CBCL are known to be less sensitive than clinical interviews (Wassenberg, et al., 2004), epidemiological studies based on symptom ratings may also underestimate the prevalence of psychiatric problems in the population, thus impeding comparisons between clinical studies and population-based epidemiological studies.

1.5.4 Biological factors influencing behaviour in CAH

It is important to note that the prenatal androgens and prenatal DEX treatment are not the only biological factors influencing behaviour in CAH-affected children. Brain development and behaviour may be affected by changes in hormones such as excess prenatal exposure to androgens and adrenocorticotropic hormone (ACTH) and reduced cortisol. Before appropriate (postnatal) treatment is established, children may be at risk of salt-wasting and hypoglycaemia, which can adversely affect the brain. Previously, before all neonates were screened for CAH, complications were likely to be more severe in males than in females due to later diagnosis and treatment. In Sweden, all neonates have been screened for CAH since 1986.

When treated, individuals with CAH may be exposed to excess glucocorticoids because of the difficulties mimicking the diurnal rhythm of cortisol production (LWPES/ESPE, 2002). Moreover, GC replacement therapy not only influences levels of cortisol but also levels of other hormones such as ACTH, which in turn may have an impact on cognition (Veith, Sandman, George, & Kendall, 1985). Hypotension, dehydration and hyponatraemia can result in cognitive impairment in patients with salt-wasting syndrome (Nass & Baker, 1991). Therefore, all behavioural data on prenatally DEX-treated children at risk for CAH should also be analyzed separately for children who are CAH-affected and treated postnatally.

1.5.5 Brain development in CAH

White matter abnormalities, which did not correlate with clinical or cognitive characteristics, were observed in 4 out of 15 patients (27%) who underwent magnetic resonance imaging (Sinforiani, et al., 1994). Similarly, the white matter abnormalities and/or temporal lobe atrophy observed in 18 out of 39 patients (46%) were not associated with age, type of CAH (salt-wasting versus simple virilizing) or treatment status (under- or oversuppression; compliance) as analysed using a series of chi-square tests (Nass et al., 1997). In addition, 8 of 39 patients with CAH were noted to have Chiari malformations and 9 of 39 patients had an abnormality of the pituitary region (ibid.). Likewise, 4 out of 7 patients had abnormalities of the pituitary on MRI

despite standard GC replacement therapy (Speiser, Heier, Serrat, New, & Nass, 1995). Moreover, patients with CAH showed high basal ACTH levels as well as ACTH hyperresponsiveness to oCRH stimulation test, and the authors suggested that the inevitable periods of under- and overexposure to GC in CAH patients may, over time, cause abnormalities of the HPA axis (ibid.).

Not all studies report white matter abnormalities in CAH: in a small study in which scans of 7 patients with CAH, their 3 CAH-unaffected siblings and 10 controls were read by a neuroradiologist blinded to the subjects' identity, no white matter abnormalities in CAH cases were observed (Plante, et al., 1996). However, an atypical pattern of perisylvian asymmetries was observed in the majority of CAH patients and in all of their siblings, and the authors suggest an elevated familial rate for language-based learning disabilities (see also the section Learning disabilities).

In a more recent study (Bergamaschi et al., 2006), half of the patients were subjected to a long-term follow-up with repeated scanning (they were included in the previous cohort, Sinforiani et al., 1994) while the other half were included and scanned for the first time. Ten (45%) out of 22 patients with CAH had white matter abnormalities on MRI and the MRI findings did not change over time in the subjects who were scanned a second time. The authors suggest that hormone imbalance during brain maturation may be involved in the white matter abnormalities; it has been observed that GCs influence the proliferation of oligodendrocyte precursors (oligodendrocytes' main function is the insulation of the axons in CNS with myelin, i.e. the white matter) (Alonso, 2000). Moreover, corticosteroid replacement treatments could also contribute to white matter abnormalities. Supraphysiological doses of GC are sometimes required to suppress adrenal androgen production and these high doses could interfere with normal myelination (Huang, Harper, Evans, Newnham, & Dunlop, 2001). Bergamaschi et al. (2006) also suggested that aldosterone deficiency in CAH could lead to subclinical cerebral ischaemia (microangiopathic/microvascular damage) since aldosterone has a direct local regulatory function on the cerebral arterial structure.

Areas of the brain known to be affected by hormones of the HPA axis and androgens are of special interest in brain imaging studies of CAH. Merke et al. (2003) studied the cerebrum, ventricles, temporal lobe, hippocampus and amygdala in a structural MRI study in 27 children with CAH and 47 controls. A significant decrease in amygdala volume was observed in both males and females with CAH as compared to sex- and age- matched controls; however, the effect size in girls (Cohen's $d = 1.56$)

was almost twice as large as in boys (d = .91). The organizational effects of androgens on the development of the CNS were suggested to be the most likely explanation of these findings. The amygdala is dense in androgen receptors (DonCarlos, Garcia-Ovejero, Sarkey, Garcia-Segura, & Azcoitia, 2003) and androgens modulate amygdala size in animals and humans (Goldstein et al., 2001). The authors also suggest that prenatal GC deficiency with resulting alterations in HPA axis regulation or some combination of sex steroid excess and GC deficiency may contribute to the growth and development of the amygdala (Merke, et al., 2003). In the same study, adverse effects on the hippocampus due to GC therapy were not observed in children with CAH.

In another study from the same research group (Ernst, et al., 2007), a virilized amygdala function was observed in girls with CAH using functional MRI, while no differences were observed between boys with CAH and controls. More specifically, in response to negative facial emotions, girls with CAH activated the amygdala bilaterally significantly more than healthy female controls, and the pattern in activation was similar to that in control boys (ibid.). However, when the same study group was analysed in an extended fMRI study (Mazzone et al., 2011), boys with CAH also showed a different activation pattern compared to controls. When fMRI data were analysed with regard to successfully encoded fearful faces, boys with CAH showed significant activations in the amygdala, hippocampus, and anterior cingulate relative to unaffected males, while girls with CAH demonstrated deactivations relative to unaffected females in the same regions. The CAH group showed poorer memory performance than the controls. In an another extended study, the same research group found memory deficits for negatively arousing pictures in both males and females with CAH compared to healthy controls (Maheu et al., 2008), while there were no group differences in memory recall for positive or neutral pictures. These finding suggest that an early steroid imbalance may affect memory for negative material in children with CAH. The hormonal dysfunctions during critical periods of development may lead to abnormal brain organization and function. The sample sizes were also small in the extended studies.

In the above-mentioned structural MRI study, girls with CAH did not have male-specific CNS characteristics (Merke, et al., 2003). In contrast, a male-typical pattern of activation was observed in adolescent girls with CAH in the inferior parietal lobule (LPI) in a functional MRI study from the same research group (Ernst, et al., 2007). The LPI has been associated with sex differences in visual-spatial

abilities (Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000). These results supported the hypothesis of an early organizational effect of testosterone on brain function in girls with CAH. In the same study, a general effect of CAH diagnosis (i.e. an effect across sex) was observed on the fusiform gyrus, a structure that processes visual social stimuli such as faces and body movements. The authors suggested that these results reflect impaired corticosteroid function rather than an effect of sex steroids since the effect is disease-specific (main effect of CAH) rather than sex-specific (interaction effect).

Taken together, several studies have shown white-matter abnormalities in both females and males with CAH. A hypothesized mechanism behind these abnormalities is impaired myelination due to endogenous hormonal imbalance or supraphysiological GC replacement therapy, or both, together with possible additional mechanisms such as microangiographic damage related to an aldosterone deficiency. However, a clear association between the white-matter abnormalities and disease-related factors (such as type of CAH, SW versus SV) has not been shown. Moreover, the functional implications of the white-matter anomalies are not known. In addition to white-matter abnormalities, recent series of studies have shown abnormalities in structure and function of the amygdala, together with behavioural correlates (impaired social cognition).

1.6 LONG-TERM FOLLOW-UP STUDIES OF PRENATAL DEX TREATMENT OF CAH

Long-term developmental effects of prenatal DEX treatment of children at risk for CAH have been assessed in two previous studies, both designed as parental questionnaire studies. A pilot study suggested that prenatally DEX-exposed pre-school children showed more shyness, greater emotionality, less sociability and more internalizing problems (Trautman, Meyer-Bahlburg, Postelnek, & New, 1995), while a study focusing on parent-rated cognitive and motor development could not find any significant negative effects (Meyer-Bahlburg et al., 2004a). These studies are described in more detail in the section General Discussion. Studies with direct assessments of the DEX-treated children are lacking.

2 AIMS

The aims of the thesis were to examine whether prenatal exposure to DEX affects the following aspects of development:

1. Cognition and school performance (Study I)

2. General adaptation, temperament, or long-term affective development, especially social anxiety (Study II)

3. Gender role behaviour (Study III)
   – as part of the study on gender role behaviour, to develop and evaluate a new assessment instrument (Studies III and IV)

3 METHODS

3.1 STUDY POPULATIONS AND PROCEDURES

In Sweden, between the years 1985 and 1995, 40 foetuses at risk of being affected with severe CAH were treated with dexamethasone from postmenstrual week 6 to 7 until the prenatal diagnosis was available to prevent prenatal virilization of affected females (Lajic, et al., 1998). Treatment was offered to women who have previously given birth to a child with severe CAH. The maximum dose was 20 µg/kg per day, based on pre-pregnancy maternal weight, divided into three doses. Based on the results of a mutation analysis in chorionic villus biopsies at weeks 10 to 12, the treatment was discontinued if the foetus was male or an unaffected female, whereas CAH-affected female foetuses were treated until term. All families were first contacted by means of an invitation letter containing two information letters, one for the parents and one for the child. The final cohort comprised 26 children (12 boys, 14 girls; refusal rate 35%) between 7 and 17 years old (M = 10.95, SD = 2.33 years).

The studies included in this thesis were retrospective. Thus, none of the families were initially treated within the framework of a clinical study, and this fact is most probably the major reason for the relatively high refusal rate (35%). To analyse whether there were any differences between the families/children that took part in the study, as compared to those who refused participation, we performed an analysis of the two groups using data from a previous questionnaire study on the same population (Lajic, et al., 1998), as well as data collected from the managing paediatrician. The composition of the cohort that participated in the study did not differ from that of the children/families that refused participation with regard to (1) behavioural problems of the child; (2) psychosocial problems in the family; (3) maternal side-effects induced by DEX; and (4) the maternal attitude towards future treatment.

The DEX-exposed children – both CAH-unaffected and those with CAH – were compared to sex and age-matched healthy controls. The controls were recruited from two sources. First, siblings of children treated with growth hormone (with presumed similar psychosocial prerequisites to those of the study group, i.e. having an older sibling on long-term medical treatment) were included: 10 (four boys and six girls) out of 28 children (refusal rate 64%) between 7 and 12.5 years of age (9.94 ± 2.02). Second, in order to create a large enough control group, the Swedish National Registry was used to randomly recruit 25 children matched for age and sex (11 boys

and 14 girls, refusal rate 69%) between 7 and 17 years old (10.55 ± 2.46). Data from these control groups were used in Studies I, II and III. Since there were no differences between the two control groups (siblings to the growth hormone-treated children versus children recruited through the Swedish National Registry) on any of the measures, the controls were combined to form a single control group.

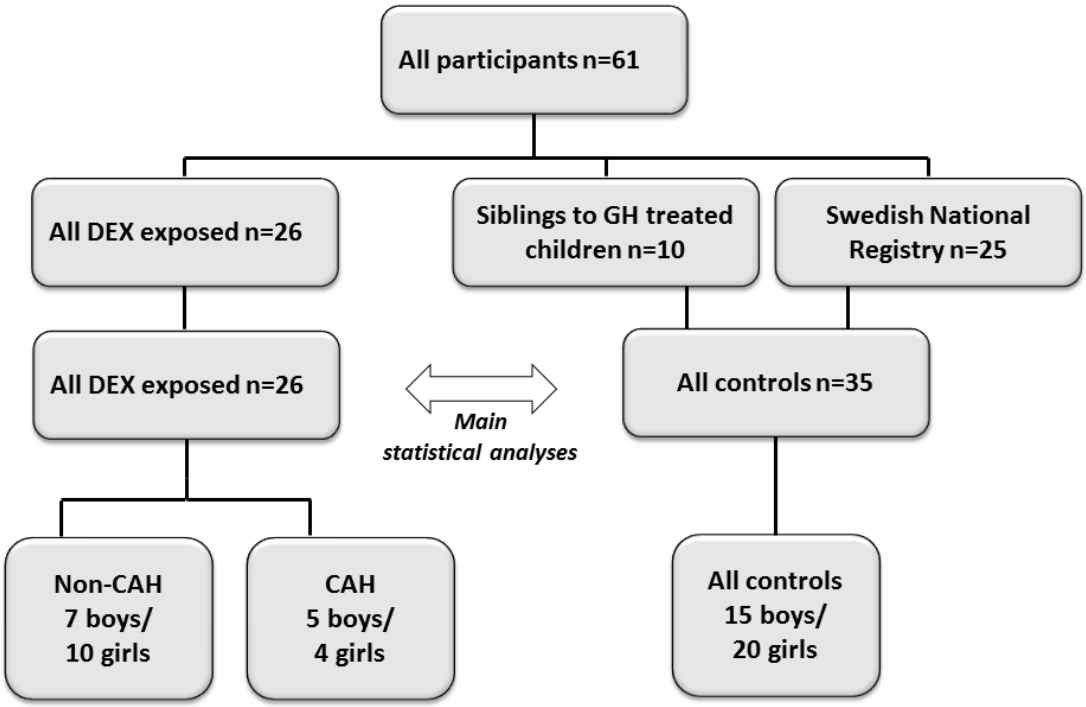


Figure 5. Participants in studies I-III comprised of 26 DEX exposed children and 35 age- and sex-matched controls. In studies III-IV, data from an additional control group of 180 children was used to evaluate a new assessment instrument.

No somatic examination was performed in the control group, while in the CAH group, somatic diseases were known from their case files: one CAH-affected boy had had diabetes mellitus since 2 years of age; one unaffected girl had rheumatoid arthritis; and another unaffected, short-term treated girl was under investigation for proteinuria. One CAH-affected girl was not able to complete the neuropsychological tests due to low intellectual performance (Study I), but parental questionnaires were obtained for this child (Study II).

Among the DEX-treated children, 54% lived in cities in comparison with 97% of the controls (χ^2 test, $p < 0.001$). There were no significant differences in parental socioeconomic status as estimated by parental educational level (χ^2 test, $p = 0.162$)

between the two groups. Moreover, the treated *versus* non-treated children were comparable regarding birth length, birth weight and gestational length (all *p*s < 0.10).

In Studies III and IV, data from an additional ‘group of 180 school-age children was used. This group was recruited to evaluate the Karolinska Inventory of Gender Role Behaviour (KI-GRB), developed for the current project. The children were recruited from two schools: one school in Stockholm, with 4309 habitants/km^2 (Statistics Sweden: www.scb.se), and one in Enköping, a small town with 33 habitants/km^2 (ibid). In total, 180 children from 13 different classes took part in the study. In Study III, data from children from grade 2 to 6 (i.e. the same age range as for most children in the other study groups) were analysed, while in Study IV, all 180 children took part.

In Studies I, II and III, the same clinical psychologist performed the standardized assessments for all families. The neuropsychological tests were administered in a fixed order and the questionnaires were completed by the children and their parents when visiting the clinical psychologist. Thus, both parents and children had the opportunity to ask for clarification of items (Achenbach, 1991). Parents and children completed the questionnaires independently of each other. In Studies III and IV, all data from the 180 children were collected by the same person, who had a MSc degree in psychology. The questionnaires and the cognitive tests were completed in a fixed order. Children who were 8 years old (grade 2) completed the tasks individually with the test leader and could thus get help with the reading and comprehension of the items. Children from grade 4 to 8 were tested in their class-rooms, so that all participating pupils from the same class completed the tasks at the same time.

3.2 ASSESSMENT OF BEHAVIOUR

3.2.1 Neuropsychological tests

The neuropsychological tests used in the projects are well-standardized and frequently used assessment instruments with good psychometric properties. General intellectual ability, or IQ, was assessed in Study I using a short version (Donders, 1997) of the Wechsler Intelligence Scales for Children – IV (WISC-III) (Wechsler, 2003). Learning and memory were assessed using the Developmental Neuropsychological Assessment (NEPSY) (Korkman, Kirk, & Kemp, 1998). Moreover, a test of spatial memory was administered (Anderson, Lajoie, & Bell,

1997). Manual preference, a test of handedness, is from a previous version of the NEPSY test battery (Korkman, 1988). Executive functions were assessed using subtests of Wechsler scales (Kaplan, Fein, Morris, & Delis, 1991; Wechsler, 2003) and the Stroop test (Golden & Freshwater, 1998).

The Wechsler Intelligence Scale for Children – IV (Wechsler, 2003) has been normed in Sweden and the index scores and scaled scores were used in order to facilitate comparison with the population mean. However, the Span Board test is not included in the WISC – III, and these raw scores were transformed into scaled scores using the norm tables for the corresponding verbal test, Digit Span, from WISC – III. The NEPSY test has been normed in Sweden, but the manual does not include the scaled scores (the comparison with the population norm is done using percentile intervals), and to enable comparison between the different tests, we transformed the NEPSY raw scores into scaled scores according to the American manual (Korkman, et al., 1998). Also, although not normed in Sweden, the results in the Stroop Color and Word Test (Golden & Freshwater, 1998) were transformed into T-scores for the sake of simplicity. The results of the statistical analyses were analogous using the raw data or the T-scores.

3.2.2 Questionnaires

Children's self-rating scales were used in Studies I, II and IV. The children estimated their scholastic ability by completing a subscale from the Self-Perception Profile for Children (Harter, 1985). The children also completed the Social Anxiety Scale for Children–Revised (SASC-R) (la Greca, Dandes, Wick, Shaw, & et al., 1988). Children's Sex Role Inventory, CSRI (Boldizar, 1991), was used in Studies III and IV to measure sex role identification.

Parental rating scales were used in Studies I and II. The Child Behavior Checklist for Ages 4–18 (Achenbach, 1991) was used for parental evaluation of the children's scholastic performance, adaptive functioning and behavioural problems. Parents also completed the Social Phobia and Anxiety Inventory for Children – Parental Rating (SPAI-C-P) (Beidel, Turner, & Morris, 1995; Higa, Fernandez, Nakamura, Chorpita, & Daleiden, 2006), while children's temperament was measured indirectly via parental ratings of the Emotionality-Activity-Sociability-Shyness Temperament Survey for Children (EAS) (Buss & Plomin, 1984).

3.2.3 Other behavioural assessment instruments

The Karolinska Inventory of Gender Role Behaviour (KI-GRBI) was developed for the current project. The KI-GRB surveys children's favourite toys, activities, same-sex versus other-sex friends and preferred future occupation. Thus, behavioural dimensions of gender are assessed with open-ended questions posed directly to the child.

3.3 STATISTICAL METHODS

In study I, a series of analyses of variance (ANOVA) was used to compare the prenatally treated children with healthy controls. In case of significant differences, the analyses were re-run for three groups, i.e. the healthy controls, prenatally DEX-treated CAH-unaffected children and prenatally DEX-treated CAH-affected children also treated postnatally with synthetic GC. This allowed us to compare healthy controls with otherwise healthy DEX-treated children (Figure 5).

In Study II, most of the variables were strongly positively skewed (i.e. many low ratings) and therefore non-parametric statistics were applied. DEX-exposed children were compared to unexposed children using the Mann-Whitney U-test, while categorical data were analysed using χ^2 tests. Statistically significant findings were re-evaluated using the Kruskall-Wallis test for comparisons of the three groups: DEX-treated CAH-unaffected children, DEX-treated CAH-affected children and controls.

The first part of Study III was focused on evaluation of the KI-GRB using data from 160 school-age children. Correlation analyses were used for inter-rated agreement (Pearson's correlation and Interclass Correlations, ICC). Underlying dimensions of the KI-GRB were studied using a principal component analysis (PCA) with varimax rotation. The KI-GRB subscales (Masculine, Feminine and Neutral behaviours) were correlated with corresponding categories in Children's Sex Role Inventory, CSRI (Boldizar, 1991).

In Study IV, developmental trajectories (effect of age/grade), sex differences as well as sex by grade interaction effects were analysed using two-way ANOVAs. Moreover, Pearson's correlations as well as multiple regression analyses were used to study the association between the different dimensions of gender.

4 ETHICAL CONSIDERATIONS

4.1 ETHICAL DILEMMA WITH THE TREATMENT

The prenatal DEX treatment of children at risk for CAH harbours an ethical dilemma since 7 out of 8 foetuses (and their mothers) are treated in early pregnancy without any obvious benefit. This is why the prenatal DEX treatment is considered controversial and has been debated in the scientific literature (Brook, 2000; Miller, 1999; New, 2001).

Based on rather few (mostly short-term) follow-up studies (Lajic, Nordenstrom, & Hirvikoski, 2008), the treatment has been considered almost totally free of side-effects (Forest, Morel, & David, 1998; New, 2001), while based on the same literature, other authors have called attention to possible long-term sequelae (Miller, 1998) and encouraged large prospective controlled studies which include careful long-term follow-ups (Ritzen, 1998). In Sweden, this kind of study has been taking place since 2000 as part of a European prospective multicentre study (Lajic, et al., 2004). However, since it takes many years before the results of this kind of extensive long-term prospective study will be available, the retrospective study presented in the current thesis was undertaken.

4.2 ETHICAL DILEMMA WITH THE RETROSPECTIVE STUDY DESIGN

To perform a retrospective study constituted further ethical dilemmas; many of the mothers commented that they had not even thought about the prenatal treatment since it had been administered, and it was possible that the participation in the study caused some worry among the families. Moreover, it is possible that not all of the treated children knew that they had been treated prenatally, and this kind of information after several years may have been both disturbing and confusing for them (and possibly one reason for the relatively high refusal rate since some parents also may have chosen not to tell their children about the treatment).

Not performing a follow-up would, however, leave the families (and us) with even greater uncertainty and the benefits of conducting the study were considered to weigh more heavily than the drawbacks; or, in other words, it was considered a greater ethical dilemma not to offer a long-term follow-up for the treated children and their families.

4.3 FORMAL ETHICAL APPROVAL

All children and their parents were given oral and written information about the aims and procedures of the studies before the examinations. They were also informed that the participation was voluntary. The names of the participants were replaced with registration numbers and/or codes to ensure anonymity. Approval from the Research Ethics Committee of Karolinska Institutet and the Stockholm Research Ethics Committee was obtained before the studies were begun.

5 RESULTS

5.1 COGNITION AND SCHOLASTIC PERFORMANCE (STUDY I)

Parental ratings of overall school performance (Child Behaviour Check List School Scale) showed good scholastic competence in the DEX-exposed group as compared to controls. Thus, there were no differences between the two groups with regard to need for special teaching or occurrence or problems in school. These results were in accord with the normal performance in all measures of learning and long-term memory from the NEPSY observed in the direct neuropsychological assessment of DEX-treated children and controls (Hirvikoski et al., 2007) (Figure 6). In addition to the NEPSY subtests, there were no differences between the DEX-exposed children and the controls in the test of spatial memory (memory for locations) (Anderson, et al., 1997), although, in this test, ceiling effects were observed.

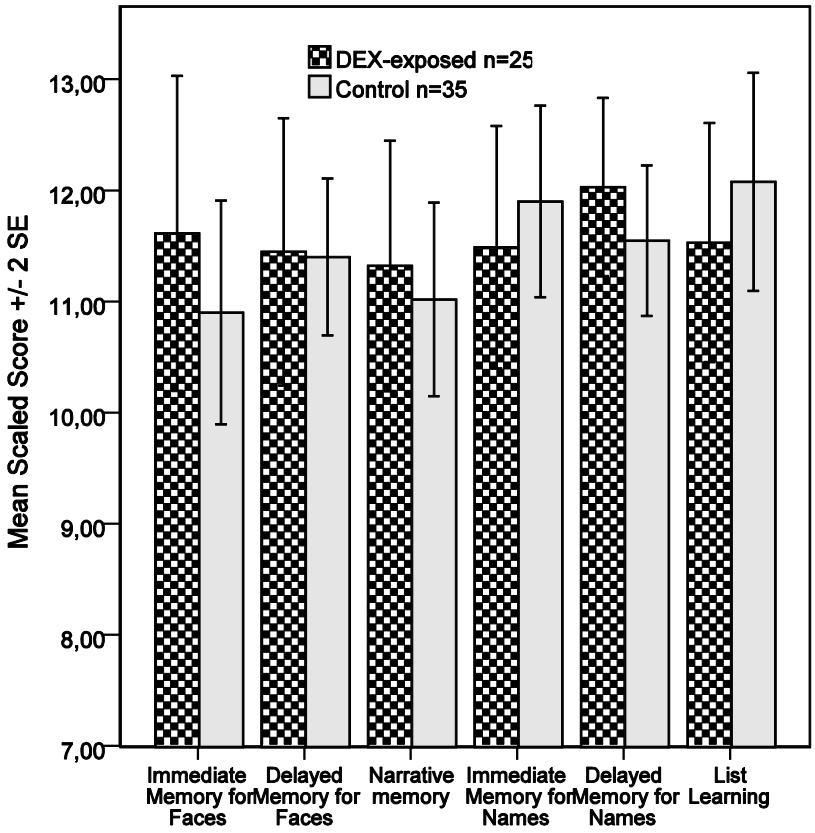


Figure 6. There were no differences between the DEX-treated children and controls in the NEPSY tests of learning and memory.

Moreover, DEX-exposed children had comparable results to those of controls in measures of psychometric intelligence, i.e. full-scale IQ, as well as the Verbal Comprehension Index, Perceptual Organization Index and Speed of Processing Index. However, in the Freedom of Distractibility Index, a significant difference was observed between the DEX-treated children and the control group, which was indicative of a negative effect on verbal working memory (Figure 7).

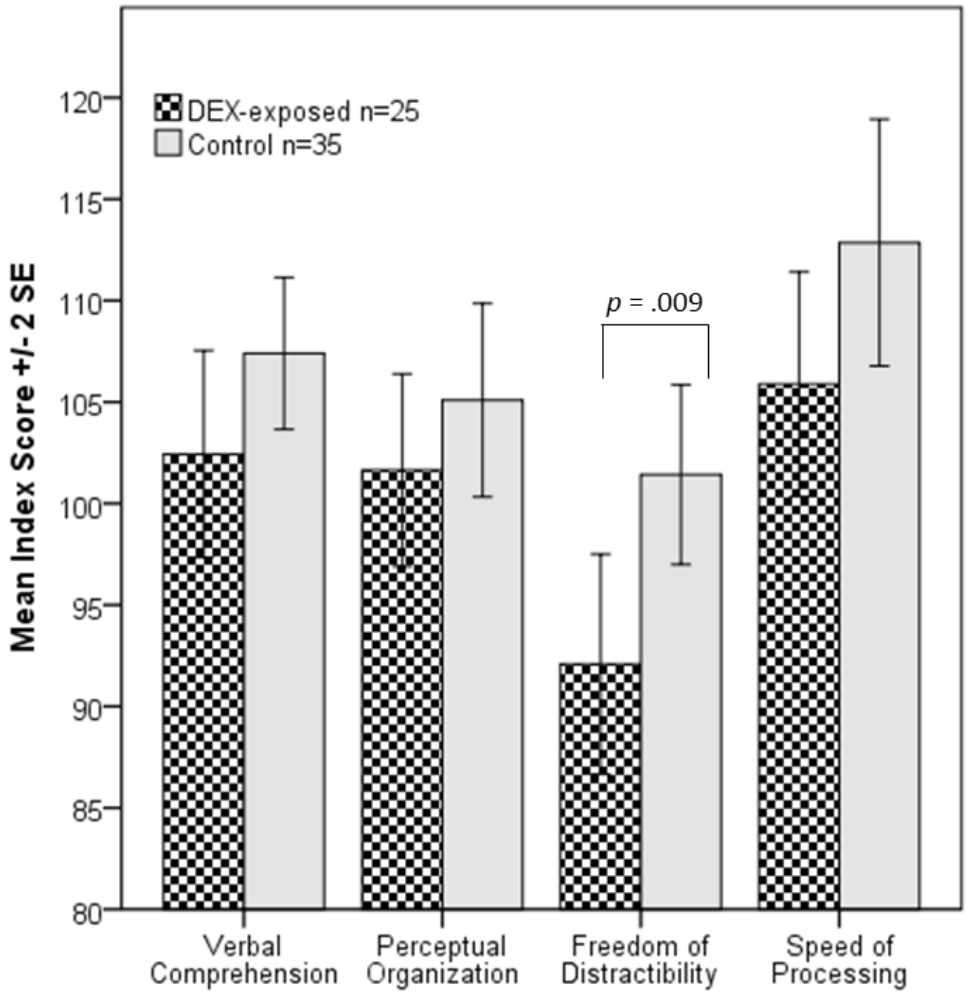


Figure 7. The DEX exposed group performed significantly more poorly than the control group in Freedom of Distractibility Index while the other indices did not show any statistically significant differences between the two groups.

A more detailed analysis of the test included in the Freedom of Distractibility Index showed that the between-group difference reached significance in the Digit Span Test, a test of verbal working memory. This difference remained significant also on controlling for FSIQ and social anxiety in an analysis of covariance. Pair-wise *post hoc* comparisons showed that both CAH-affected and CAH-unaffected DEX-treated children had lower results in the Digit Span tests compared to controls (Figure

8). After the Bonferroni correction for multiple comparisons, the difference remained significant only for the CAH-unaffected, short-term treated children. However, as can be observed in Figure 8, this may have been simply due to a small sample size in the CAH-affected group (treated with DEX prenatally and hydrocortisone postnatally); the mean values were basically the same for both DEX-treated groups. In addition, a non-significant statistical trend in the Span Board Test, a measure of visual-spatial working memory, was observed. However, since this group difference did not reach significance, no further analysis of this test was performed.

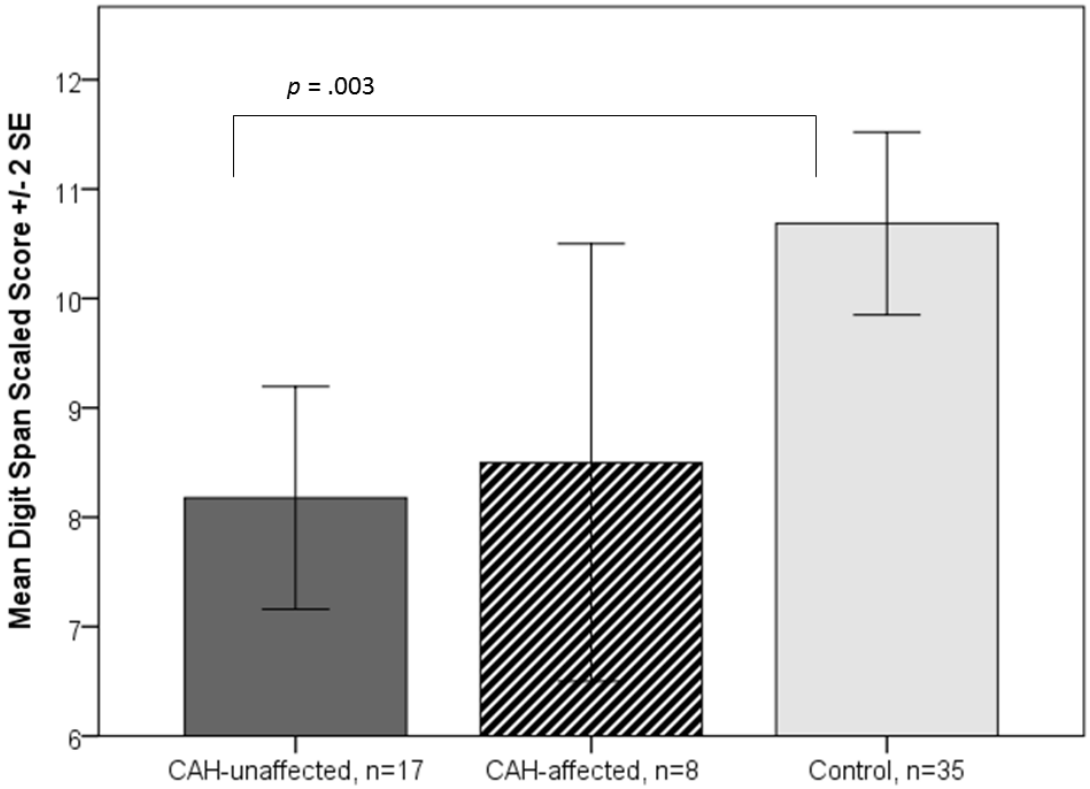


Figure 8. The CAH unaffected DEX exposed children performed poorer than the controls in the Digit Span test, while the difference between the CAH affected children and the control group no longer reached significance in the post hoc tests.

As already mentioned, the DEX-treated children did not differ from the controls on the parent-rated CBCL School Scale. However, scholastic ability was also measured as self-rating using the Scholastic Competence subscale from The Self-Perception Profile for Children (Harter, 1985), and in these self-ratings DEX-exposed children reported significantly lower results compared to controls. Moreover, children's self-perceived scholastic ability correlated positively with verbal working

memory capacity measured by the Digit Span Test (r = .41, p = .004). Thus, the children who reported low self-perceived scholastic ability were those who performed less well on the Digit Span Test (Figure 9).

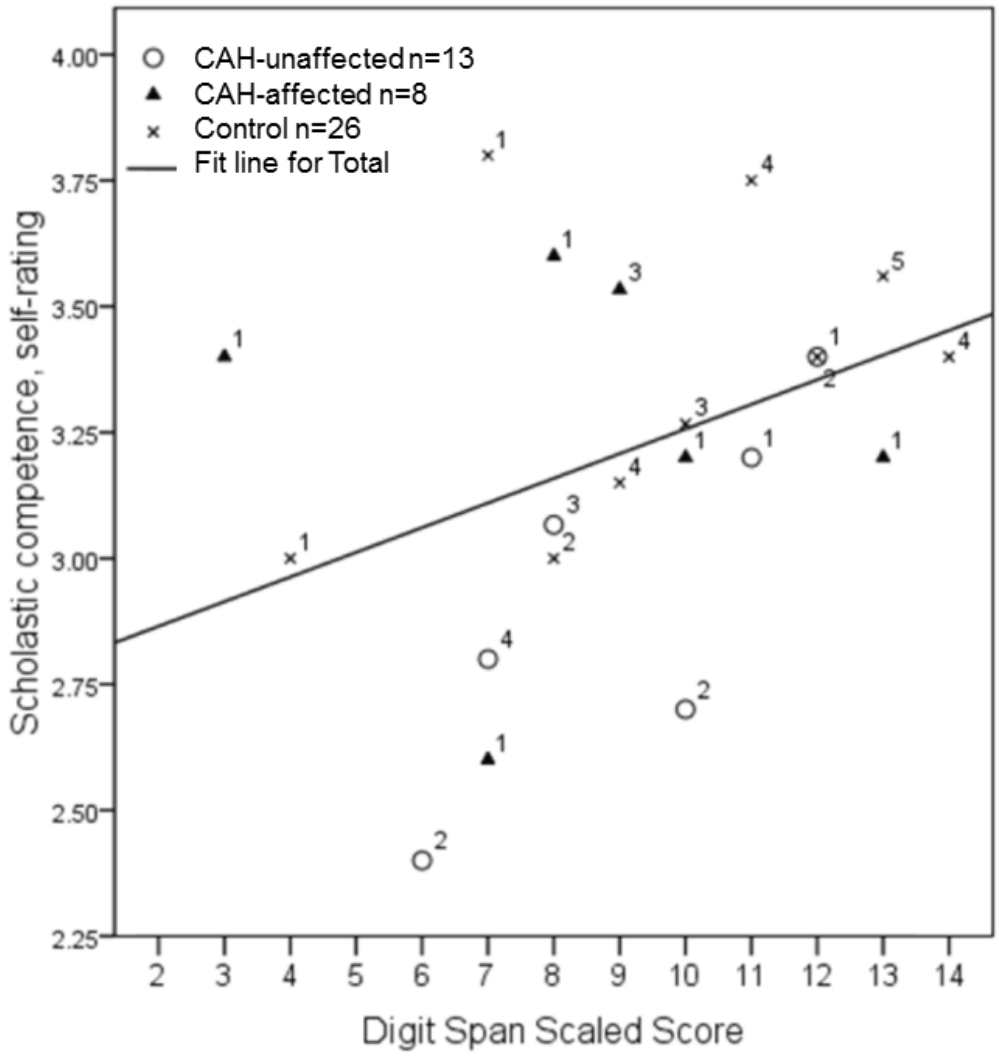


Figure 9. Verbal working memory capacity measured with the Digit Span test correlated positively with children's self-perceived scholastic ability (r = .41, p = .004).

The Stroop Colour Word Test consists of three subtests; speeded reading, speeded naming of colours and colour word/interference, a measure of impulse inhibition. The DEX-treated group had significantly lower results in both speeded reading and speeded naming of colours; however, when adjusted for FSIQ these differences no longer reached significance. Likewise, a non-significant tendency to a lower speed of processing in the DEX-treated group seemed to be an effect of slight (non-significant) differences in IQ and, consequently, dropped to below $p > .20$ when IQ was entered as a covariate in an ANCOVA.

In the NEPSY Manual Preference, a trend (p = .067, Figure 10) showed more consistent right-handedness in the DEX-treated group (n = 25), including CAH-affected children; in this group all children (n = 8) performed all tasks included in the test with the right hand, the standard deviation thus being zero (0). Categorized as left- or right-handedness, none of the DEX-treated children and three children in the control group were left-handed (2 x 2 χ^2 test, p = .13). However, in behaviours that seldom occur – 10% of the normal population are left-handed (Gilbert & Wysocki, 1992) – a larger study group may be needed in order to attain statistical power and reach significance.

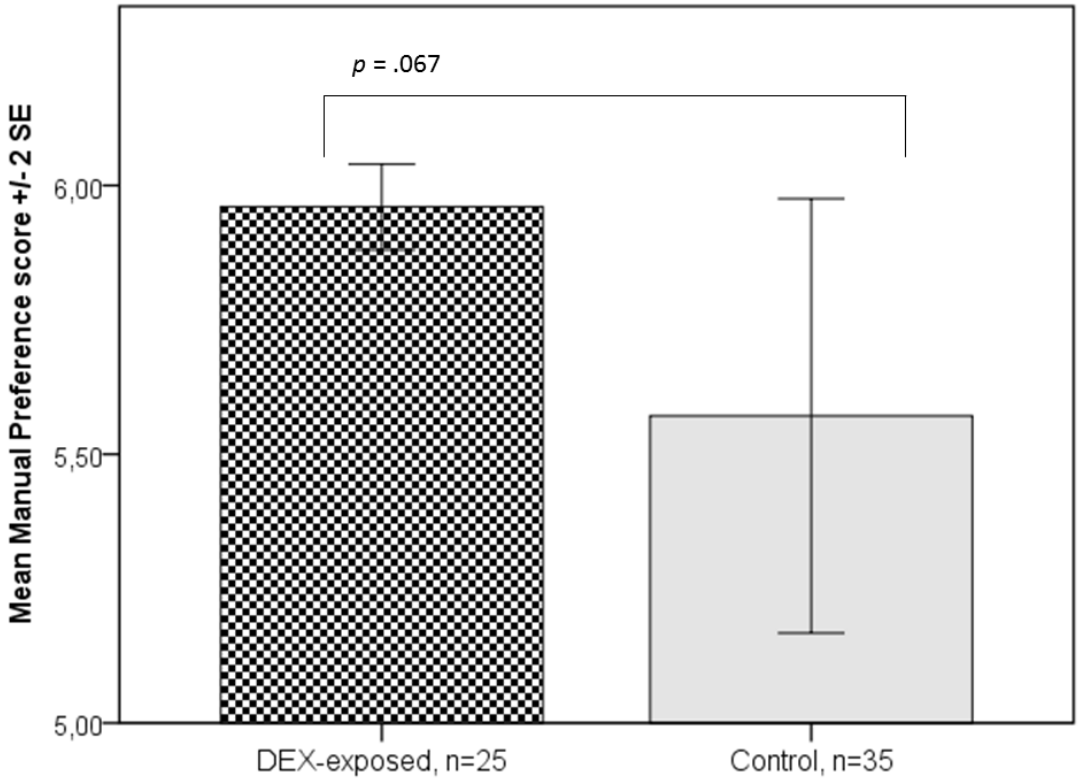


Figure 10. The DEX-exposed group showed a tendency to more consistent right-handedness. However, this difference did not reach significance (p = .067), probably due to low power.

5.2 ADJUSTMENT, WELL-BEING AND TEMPERAMENT (STUDIES I - II)

Parental ratings showed good overall adjustment in DEX-exposed children, as compared to controls, regarding both general competence and psychological well-being. Thus neither the CBCL total competence score nor any of the problem

subscales differed between the DEX-treated group and the control group (both *p*s < .10) (Figure 11).

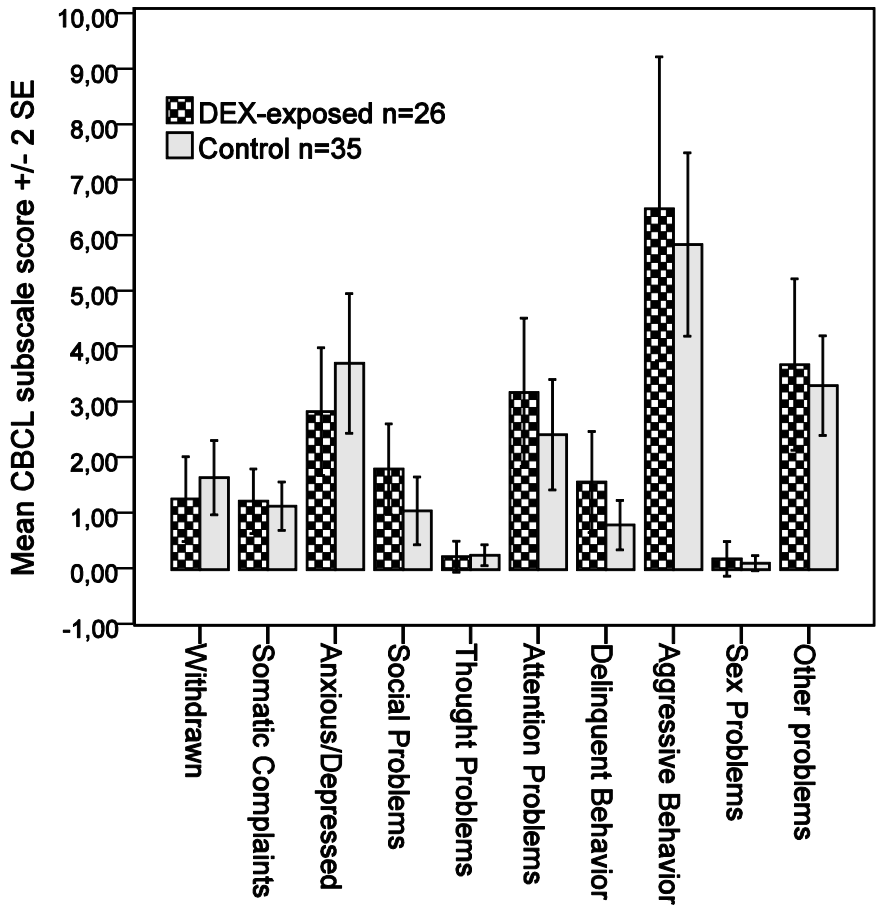


Figure 11. There were no statistically significant differences between the DEX-exposed group and the control group in the CBCL problem subscales.

No between-group differences were observed with the exception of results from the Sociability scale in the Emotionality Activity Shyness Temperament Survey for Children (Buss & Plomin, 1984). In this measure, CAH-unaffected children who were treated short-term were rated as slightly more sociable than the controls by their parents. CAH-affected girls and boys were rated similarly to controls.

Owing to the results in the pilot study by Trautman et al. (1995) suggesting that DEX-exposed children showed more shyness, greater emotionality, less sociability and more internalizing problems, we had a special focus on social anxiety among other behavioural problems. In the Swedish cohort, increased social anxiety was observed in the self-ratings (Social Anxiety Scale for Children – Revised, SASC-R) (Figure 12). However, when analysed for three groups (CAH-unaffected, CAH-

affected and controls), only the CAH-unaffected, short-term-treated group differed significantly from the controls.

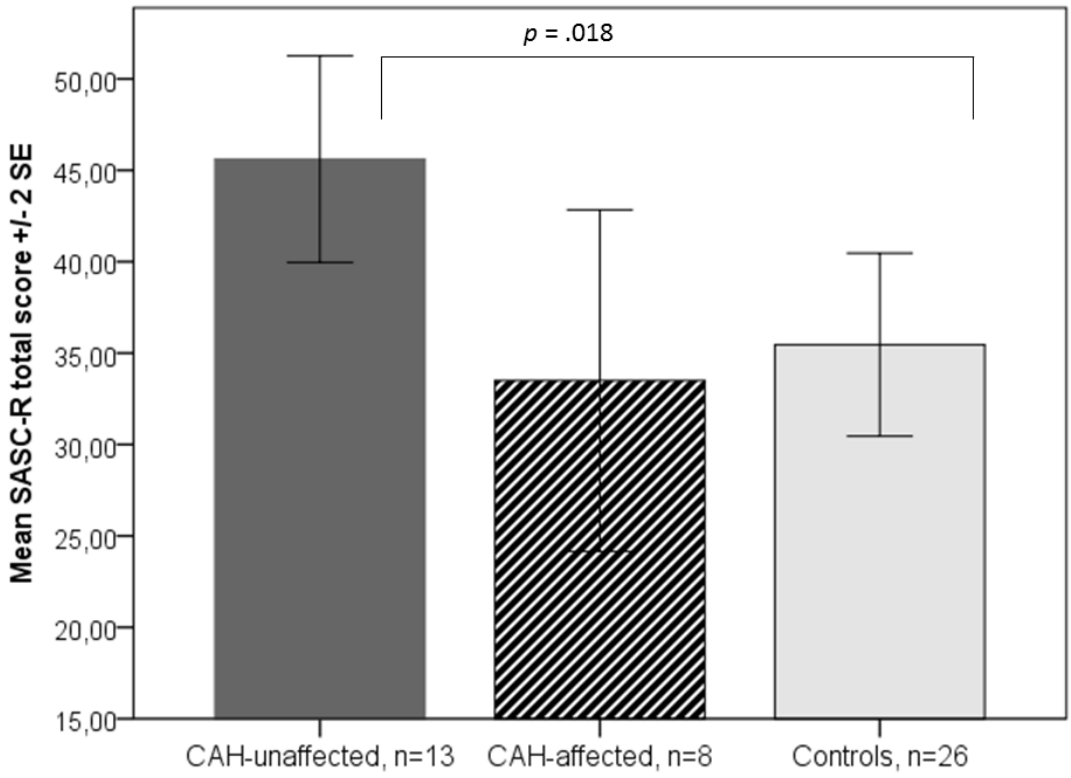


Figure 12. The CAH-unaffected DEX-exposed group reported more social anxiety than controls.

Note: Only children ≥ 8 years old completed the self-rating questionnaires.

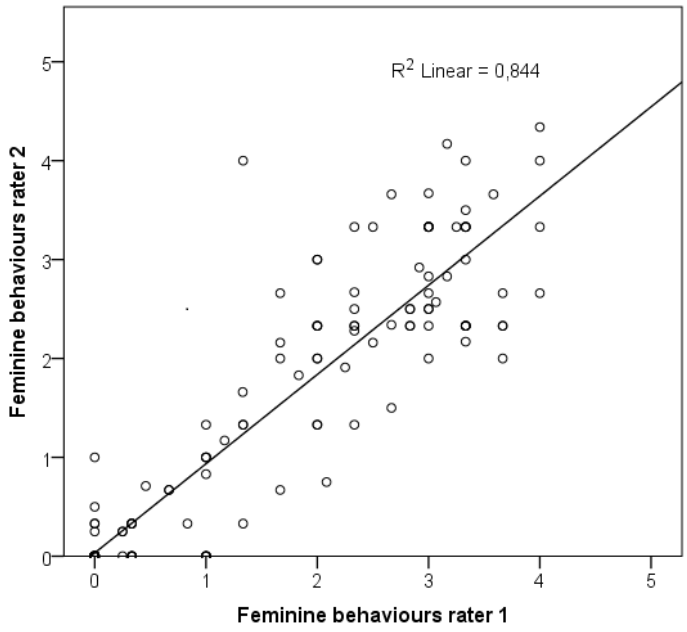
The observed differences in the children's self-ratings of social anxiety were not confirmed in parental ratings of social anxiety. No differences between the DEX-treated and the control group were observed in the Social Phobia and Anxiety Inventory for Children – Parent Report (SPAI-C-P) or on the EAS Shyness subscale. These parental ratings showed a strong correlation with each other (r = .57) while the correlation between parental ratings of social anxiety (SPAI-C-P) and the children's self-ratings of social anxiety (SASC-R) was weaker (r = .31). The modest parent-child agreement on social anxiety is consistent with previous studies on cross-informant agreement (Achenbach, McConaughy, & Howell, 1987).

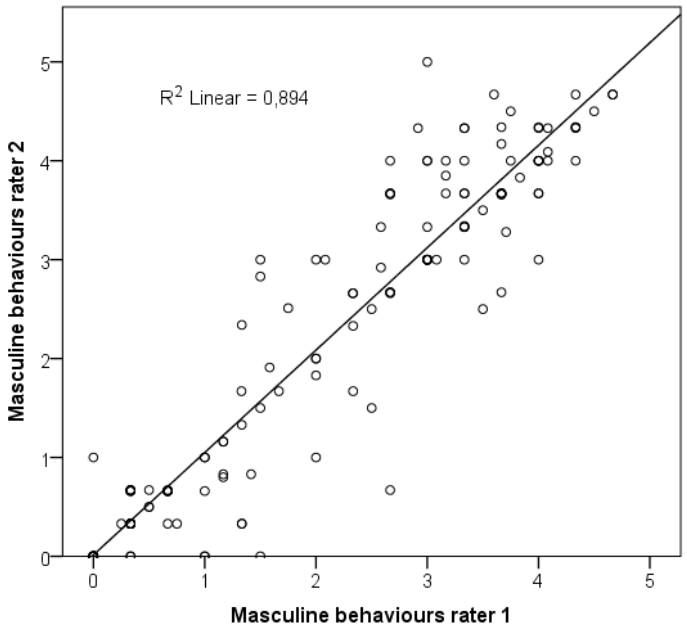
5.3 GENDER ROLE BEHAVIOUR (STUDIES III-IV)

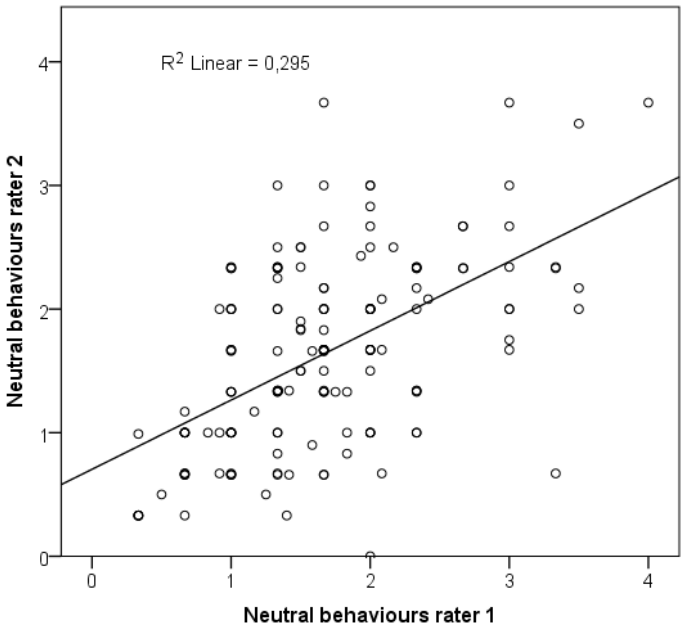
Gender role behaviour was investigated in the last part of the project. In order to measure gender role behaviour, a new inventory was designed (the Karolinska Inventory of Gender Role Behaviour, KI-GRB), and therefore evaluation of this new inventory was performed partly in Study III and in Study IV. Here, the results on the KI-GRB as such are described first, followed by the results in DEX-treated children and the sex- and age-matched controls.

In Study III, the KI-GRB was compared with a widely used instrument developed to measure another aspect of gender: the Children's Sex Role Inventory, CSRI (Boldizar, 1991). A principal component analysis with a varimax rotation showed that the underlying dimensionality of the KI-GRB was clearly described by the three subscales (Feminine, Masculine and Neutral behaviours); the first factor included the Feminine (positive loading) and Masculine (negative loading) subscales and the second the Neutral behaviours subscale. Contrary to the results in the KI-GRB, the PCA of the CSRI revealed a one-factor solution, indicating that all subscales measure the same underlying dimension. Moreover, children's sex explained a very large proportion of variance in the KI-GRB Feminine and Masculine subscales, while the effect sizes were smaller in the CSRI (the Ki-GRB Feminine subscale, $\eta_p^2 = .44$; the CSRI Feminine subscale, $\eta_p^2 = .17$; the KI-GRB Masculine subscale, $\eta_p^2 = .42$; the CSRI Masculine subscale, $\eta_p^2 = .07$). Finally, the KI-GRB scores showed a significant interaction between the child's sex and residential area (urban/rural living), indicative of a socialization effect on gender role behaviour. Girls in small towns/rural areas reported more feminine behaviours than girls in urban areas, while boys in rural areas showed less feminine behaviours than boys in urban areas ($\eta_p^2 = .11$, a medium-sized effect). Correspondingly, boys in small towns/rural areas reported more masculine behaviours than boys in urban areas, while girls in rural areas showed fewer masculine behaviours than girls in urban areas ($\eta_p^2 = .08$, a medium-sized effect).

In Study IV we investigated sex differences and the developmental trajectories in, as well as associations between, three dimensions of gender: gender role behaviour (the KI-GRB), sex-specific cognition (the Verbal fluency test, VFT; the Mental rotation test, MRT) and sex role identification (the CSRI) in 180 school-age children. We observed sex differences in all dimensions of gender. The effect of sex was largest in the KI-GRB (gender role behaviour).


R2 Linear = 0,844
Feminine behaviours rater 2
Feminine behaviours rater 1
0
1
2
3
4
5


R2 Linear = 0,894
Masculine behaviours rater 2
Masculine behaviours rater 1
0
1
2
3
4
5


R2 Linear = 0,295
Neutral behaviours rater 2
Neutral behaviours rater 1
0
1
2
3
4

Sex differences in gender role behaviour were not stable across time: children in grades 5 and 6 (11–13 years old) reported the least stereotypical patterns in GRB – although also in these ages, the effects of sex were large on both Feminine and Masculine subscales, both *d*s > 1. Both older (grade 8: 14–15 years old) and younger (grades 2 and 4: 8–11 years old) children showed very large sex differences in masculine and feminine behaviours. The largest sex differences were observed in grade 4 (on the KI-GRB Feminine subscale, d = 5.77; on the KI-GRB Masculine subscale, d = 7.31). Moreover, GRB correlated with both cognitive tests (VFT and MRT) in girls and with the MRT in boys, while sex role identification was not associated with the other dimensions of gender. Thus, the dimensions of gender did not develop in isolation, but the developmental trajectories were related to each other. This could imply that anything that influences one dimension of gender, such as gender role behaviour, may also have direct or indirect effects on other dimensions of gender, such as sex-specific cognition, or *vice versa*.

The main focus of the studies on gender role behaviour was, however, not on the new instrument (KI-GRB), but on the possible effects of prenatal DEX treatment on gender role behaviour as measured with the new instrument. The focus of this study was on the CAH-unaffected short-term treated children, although all analyses were also recalculated including CAH-affected children. The DEX-exposed CAH-unaffected children showed a non-significant tendency to less masculine behaviours (p = .13). Moreover, there was a larger variation in the behaviour of the DEX-treated CAH-unaffected boys on all three KI-GRB subscales. The DEX-exposed CAH-unaffected boys also showed more neutral behaviours than the control boys, while the DEX-treated CAH-unaffected girls did not differ from the control girls after adjusting for the residential area. This interaction effect was medium-sized (η_p^2 = .10). An analogous result was obtained when all DEX-exposed children (also CAH-affected) were included.

Figure 13. Correlation between rater 1 and rater 2 on the KI-GRB three subscales. Inter-rater reliability was good for feminine and masculine behaviours, and moderate for neutral behaviours. (Previous page).

6 GENERAL DISCUSSION

The fact that the prenatal dexamethasone treatment of children at risk for CAH has to be started early in pregnancy, i.e. several weeks before the diagnostic status of the foetus is known, leads to unnecessary treatment in 7 out of 8 treated cases (CAH-unaffected children and boys with CAH). This is the basis of the great ethical dilemma of the treatment: can we tolerate any negative effects in children that do not need the treatment? For girls with CAH, possible side effects could be weighed against the advantages of the treatment, such as reduction or elimination of virilization in the external genitalia, and thus possibility avoiding surgical corrections and possible problems with body-image and sexual function. However, in CAH-unaffected girls and all boys, there are no gains with the treatment and, consequently, tolerance for negative effects should be low.

Considering the ethical dilemma connected with the prenatal DEX treatment of CAH together with the disturbing results from animal studies, it is surprising that so few long-term follow-up studies have been conducted. The ethical dilemma and concerns with the treatment have been recognized in recent reviews (Merce Fernandez-Balsells et al., 2010; Vos & Bruinse, 2010); however, only two research groups have conducted original follow-up studies. Specifically, prior to our studies, only two studies have been published and none of these was based on direct examination of the treated children but on collateral information (parent-completed questionnaires). A pilot study designed as a postal maternal questionnaire survey studied psychological well-being in the DEX-treated children (Trautman, et al., 1995), while an extended questionnaire study by the same researchers (Meyer-Bahlburg, et al., 2004a) focused on parent-reported cognitive and motor outcomes. In the latter study, data on psychological well-being were not reported. A comparison of our studies with these previously published studies is complicated because different assessment methods have been used in different age groups (Table 2). In total, five studies have now been published: two reports focus mainly on behavioural problems and temperament (Hirvikoski et al., 2008; Trautman, et al., 1995), two focus on cognitive and motor development as well as school performance (Hirvikoski, et al., 2007; Meyer-Bahlburg, et al., 2004a) and one pilot study on gender role behaviour (Hirvikoski, Lindholm, Lajic, & Nordenstrom, 2011) (Table 2).

Table 2. Summary of the long-term follow-up studies on prenatal dexamethasone treatment of children at risk for congenital adrenal hyperplasia.

	Participants		***Results from different assessment methods***	
	DEX-exposed children	***Control group***	***Parental questionnaires***	***Direct assessment of the children***
Trautman & al. (1995)	n = 26 (3 CAH-affected)	n = 14 Untreated children at risk for CAH (3 CAH-affected)	No differences in general development (*R-DPDQ/MCDI*) or temperament (*ITQ/TTQ/ BSQ*). DEX-treated children had higher scores on Shyness and Emotionality, lower on Sociability (*EAS*), and more Internalizing and Total Problems (*CBCL*).	N/A
	Age reported for the total study group (n = 40): 6 months – 5.5 years old Mean age = 2.5 (±1.3) years			
Meyer-Bahlburg et al. (2004)	n = 36 0–15 months old	n = 15 0–15 months old	No differences on general development (*KIDS/ R-DPDQ*)	N/A
	n = 89 15 months to 6 y old	n = 126 15 months to 6 y old	No differences on general development (*CDI/R-DPDQ*)	N/A
	n = 44 6–12 y old	n = 162 6–12 y old	No differences on school performance (*CBCL School scale)*	N/A
	Total n = 174 (48 CAH-affected: 31 girls, 17 boys)	Total n = 313 (195 CAH-affected: 100 girls, 95 boys)		

	Participants		*Results from different assessment methods*	
	DEX-exposed children	***Control group***	***Parental questionnaires***	***Direct assessment of the children***
Hirvikoski et al. (2007)	n = 26 7-17 y old M = 10.95 (± 2.33) (9 CAH-affected: 4 girls, 5 boys)	n = 35 7-17 y old M = 10.38 (± 2.33) Healthy controls	No differences on school performance (*CBCL School scale)*	CAH-unaffected children reported poorer scholastic competence (*SPPC*) and increased social anxiety (*SASC-R*). No differences in IQ (*WISC-III*), handedness, learning or memory (*NEPSY*). CAH-unaffected children had poorer working memory (*WISC-III*).
Hirvikoski et al. (2008)	Same cohort as Hirvikoski et al. (2007)		No differences on behavioural problems or adjustment (*CBCL*). No differences on shyness (*SPAI-C-P*). DEX-treated children had higher scores on Sociability (*EAS*).	Correlation between parental (*SPAI-C-P*) and children's self-ratings (*SASC-R*) of social anxiety shyness was modest.
Hirvikoski et al. (2011)	n = 26 7–17 y old M = 10.95 (± 2.33) (9 CAH-affected: 4 girls, 5 boys)	n = 35 7–17 y old M = 10.38 (± 2.33) Healthy controls		CAH-unaffected DEX-treated boys showed more neutral behaviours than the controls (KI-GRB) while CAH-unaffected DEX-treated girls did not differ from the controls after adjusting for residential area. A non-significant tendency to less masculine behaviours in the DEX-treated CAH-unaffected children was observed.

Note: R-DPDQ = Revised Denver Prescreening Developmental Questionnaire; MCDI = Minnesota Child Development Inventory; CBCL = Child Behaviour Checklist; ITQ = Infant Temperament Questionnaire; TTQ = Toddler Temperament Questionnaire; BSQ = Behavioural Style Questionnaire; EAS = EAS Temperament Survey for Children; KIDS = Kent Infant Development Scale; CDI = Child Development Inventory; WISC-III = Wechsler Intelligence Scales for Children; NEPSY = Develop- mental Neuropsychological Assessment; SPPC = Self-Perception Profile for Children; SASC-R = Social Anxiety Scale for Children–Revised; SPAI-C-P = Social Phobia and Anxiety Inventory for Children – Parent Report; KI-GRB = Karolinska Inventory of Gender Role Behaviour.

Thus, a pilot study designed as a postal maternal questionnaire survey suggested that prenatally DEX-exposed preschool children showed more shyness, greater emotionality and less sociability, as well as more internalizing problems (Trautman, et al., 1995). Data on the children's temperament or behavioural problems were not presented in the extended questionnaire study by the same researchers (Meyer-Bahlburg, et al., 2004a). Our cohort comprised school-age children (Hirvikoski, et al., 2008) and used the same questionnaires when applicable. No between-group differences were observed in the parental ratings with the exception of results from the Sociability scale in the Emotionality Activity Shyness Temperament Survey for Children. Regarding this measure, children who were treated with DEX were rated as slightly more sociable than the controls by their parents; however, on comparing three groups (CAH affected, CAH unaffected and controls), this difference did not reach significance, probably due to low power. On examining the mean values, it was observed that the CAH-unaffected, short-term-treated group showed higher scores on Sociability than the other two groups.

In our results (Study II), no differences were observed in parent-rated shyness or social anxiety, and thus our results did not confirm the results of Trautman et al. (1995). However, in the Swedish cohort, increased social anxiety was observed in the CAH-unaffected, short-term-treated group when self-rated. Consequently, the correlation between parental ratings and children's self-ratings was weak. The modest parent-child agreement on social anxiety is consistent with previous studies on cross-informant agreement (Achenbach, et al., 1987). Given that parents and children focus on different aspects of child psychopathology, multi-source assessment procedures are recommended (Achenbach, et al., 1987). The sensitivity of different assessment methods is another important consideration when comparing different studies. Questionnaires are often less sensitive than structured clinical interviews in capturing clinical problems (Wassenberg, et al., 2004), let alone in identifying subclinical effects that are even more difficult to detect, but may still result in impairment in everyday life when the child encounters new or challenging tasks or during a stressful period of life.

In the previous study focusing on cognition and motor development (Meyer-Bahlburg, et al., 2004a), no differences were observed in the parental ratings between the DEX-treated children and controls. In addition, there were no differences in parental ratings of the school performance of DEX-exposed, school-age children (Child Behaviour Check List School Scale) as compared to controls in either of the

two published studies (Hirvikoski, et al., 2007; Meyer-Bahlburg, et al., 2004a). These results were in accord with the normal performance in major cognitive measures such as IQ, learning and long-term memory observed in our study using direct neuropsychological assessments of DEX-treated children and controls (Hirvikoski, et al., 2007). Nevertheless, specific negative effects were noted regarding verbal working memory and on the children's perceptions of their scholastic competence (Hirvikoski, et al., 2007) and these effects remained significant also on controlling for full-scale IQ and social anxiety. In addition, a non-significant statistical trend in a measure of visuo-spatial working memory was observed.

Working memory is one of the key executive functions. It is the ability to maintain information for a mental operation during a short period of time (Baddeley, 1986). At school, WM is important for such abilities as mental arithmetic and reading comprehension. Moreover, WM is crucial for the executive components of the learning situation, for example, organizing and planning, and the ability to remember instructions while using them to guide one's own behaviour. Not unexpectedly, in our material, verbal WM capacity was significantly correlated with children's self-perception of their scholastic ability. However, parental scoring of school performance was not associated with WM capacity.

Thus, regardless of the observed large effect sizes for the differences in verbal working memory (Cohen's d = .95) and children's self-perceived scholastic competence (Cohen's d = .82) in our study (Hirvikoski, et al., 2007), there were no differences in the number of DEX-treated children (*versus* controls) who attended special classes or special schools, had repeated a class or had "any academic or other problems in school", as reported by their parents. Likewise, we could not detect an elevated rate of learning disability diagnoses, such as dyslexia, among the DEX-exposed children. These results indicate subclinical effects on WM and scholastic performance, regardless of the "large" effect sizes. In this context it should also be noted that, according to Cohen (Cohen, 1988) (p.25), "The terms 'small', 'medium', and 'large' are relative, not only to each other, but to the area of behavioural science or even more particularly to the specific content and research method being employed in any given investigation."

It is possible that children with a somewhat lower WM capacity (i.e. not in a clinical range but slightly lower than that of their age group), as in the current study, may reach the same results as same-age peers although through an extra effort. Therefore, subjectively, they may perceive a lower scholastic capacity as a result of

the increased strain while their parents and teachers may note normal performance in school work.

Working memory functions are bilateral prefrontal functions; however, verbal WM is predominantly associated with effective connectivity in the left prefrontal cortex, whereas visuo-spatial WM functions are primarily related to the right prefrontal cortex (Arnsten, 2006; Schlosser, Wagner, & Sauer, 2006). Moreover, the catecholaminergic and dopaminergic prefrontal subcortical networks and the connectivity of the prefrontal regions with certain temporal (in the verbal WM) and parietal (in the visual-spatial WM) regions, as well as cerebellar regions, are implicated in working memory (ibid.). Hence, like most complex cognitive functions and other behaviours, working memory emerges from a neural network and not from a single brain structure.

The last part of the project focused on gender role behaviour in prenatally DEX-treated children at risk for CAH. Parental rating scales are often used to assess gender role behaviour. Using parental questionnaires may, however, lead to partly biased responses: for example, it is known that parents perceive their daughters and sons differently also when there are no differences in behaviour (Mondschein, Adolph, & Tamis-LeMonda, 2000). Alternatives to parental rating scales were inventories that provide the child with lists of activities and toys that are rated as desirable or non-desirable, a procedure that may lead to demand characteristics. Demand characteristics refer to an unconscious systematic change in behaviour due to the study participants' interpretation of the experiment's purpose.

Because of the lack of a suitable assessment instrument, we also developed a short, structured inventory to survey gender role behaviour in school-age children. This inventory, the Karolinska Inventory of Gender Role Behaviour (KI-GRB), was evaluated in an additional sample of 180 school-age children. We concluded that the inter-rater agreement was good for masculine and feminine behaviours and moderate for neutral behaviours. The results of the principal component analysis supported the underlying dimensions of the inventory, i.e. clearly described the three subscales of the KI-GRB. Sex differences were very large in both KI-GRB Feminine and KI-GRB Masculine subscales, although there was also a significant effect of age. Children who were 11 to 13 years old reported the least stereotyped pattern in GRB (although the ES for masculine and feminine behaviours were still > 1.0, i.e. a large effect), while both younger (8–10 years old) and older (14–15 years old) children showed

very large sex differences in both feminine (d = 3.33–5.77) and masculine (d = 2.53–7.31) behaviours.

Moreover, we observed a medium-size effect of living area (urban/rural) on gender role behaviour, indicative of a socialization effect. Children's results on the KI-GRB subscales correlated with both cognitive tests in girls (Verbal fluency test, VFT, and Mental rotation test, MRT) and with the MRT in boys. Thus, the dimensions of gender did not develop in isolation, but the developmental trajectories were related to each other. Consequently, anything – biological or sociocultural factors or both - that has an impact on one dimension of gender may not only have an isolated effect on that dimension but may also affect other aspects of gender development. These effects may be direct (the factor influencing both dimensions simultaneously) or indirect (an influence on one dimension may lead to later effects on another dimension, for example, through practicing effects).

The main focus of Study III was on the effects of the prenatal DEX treatment on GRB. We chose to focus on the CAH-unaffected, short-term-treated children (i.e. the children who do not benefit from the prenatal treatment *per se*), but we also performed all analyses including the CAH-affected children treated both pre- and postnatally with GC. We observed a larger variability in the DEX-treated boys on all three KI-GRB subscales, as compared to controls. DEX-exposed, CAH-unaffected boys also showed more neutral behaviours than the control boys, while the DEX-treated, CAH-unaffected girls did not differ from control girls after adjusting for the site of residence. There was also a non-significant tendency (p = 0.13) to less masculine behaviours in the CAH-unaffected, DEX-treated children. Recalculation of the analyses including the CAH-unaffected children showed analogous results.

The possible effects could be due to a direct effect of excess GC or to a GC effect on sex hormones, or a combination of both. A reduction in testosterone has been observed in rats after prenatal DEX treatment (Lalau, Aubert, Carmignac, Gregoire, & Dupouy, 1990), while there are no human studies on this subject. In the current study, androgens were not measured during the treatment and therefore we do not know how the DEX treatment affected androgens in the short-term-treated, CAH-unaffected children. Certain indirect markers of prenatal androgens have been used in research, such as the 2D:4D digit ratio (Puts, et al., 2008), although the validity of this marker has been questioned (Hickey et al., 2010). Another marker of the prenatal androgen level is handedness. According to the Geschwind-Behan-Galaburda theory (GBG) (Geschwind & Behan, 1982; Geschwind & Galaburda, 1985), high prenatal

androgen exposure inhibits development of the left hemisphere and causes a shift in cerebral lateralization towards right-hemisphere dominance, thus increasing the incidence of left-handedness or weaker dextrality. In support of this theory, a higher incidence of left-handedness has been observed among CAH patients in most studies (see Introduction). However, in addition to the CAH studies, the evidence for the association between high prenatal androgens and left-handedness is scarce (Vuoksimaa, Eriksson, Pulkkinen, Rose, & Kaprio, 2010). On the contrary, there are also studies showing an association between higher levels of prenatal testosterone, as measured in amniotic fluid, and increased right-handedness in girls (but not in boys) (Grimshaw, Bryden, & Finegan, 1995), thus supporting the callosal theory, meaning that left-handedness is caused by decreasing levels of testosterone that affect structural and functional development of temporoparietal regions of the brain, such as structure of the isthmus of the corpus callosum and the functional asymmetry in this region (Witelson & Nowakowski, 1991). Also, there are theories (focusing on other behaviours than handedness) which postulate that the relationship between androgens and sex-specific cognition, such as mental rotation ability, is curvilinear ("inverted U shape"), indicating that intermediate levels are associated with optimal performance. Thus, both prenatal (Grimshaw, Sitarenios, & Finegan, 1995; Puts, et al., 2008; Vuoksimaa, et al., 2010) and postnatal (Gouchie & Kimura, 1991; Moffat & Hampson, 1996) androgens have been suggested to correlate positively with spatial ability in females, but negatively with spatial ability in males. Consequently, also with regard to handedness, any effect on prenatal androgen levels could have different consequences for girls and boys. To sum up, there are different theories about the association between prenatal androgens and the development of handedness (or cognition) and the use of indirect measures of prenatal androgens has not been validated. Thus we do not know much about the prenatal androgen levels of prenatally DEX-treated children at risk for CAH.

7 CONCLUSIONS

On performing a long-term follow-up of prenatal dexamethasone treatment of children at risk for CAH, it was concluded that the general adjustment of the treated children was good. Major cognitive measures, such as IQ, learning and memory, showed normal results for the DEX-exposed children as compared to sex- and age-matched controls, as well as population norms. However, a possible negative effect on verbal working memory was observed – especially among CAH-unaffected short-term-treated children, i.e. children who do not benefit from the treatment – and the verbal WM capacity correlated positively with the children's self-perceived difficulties in scholastic capacity. In measures of temperament, psychopathology and psychological well-being, no differences between DEX-treated children and controls were observed in parental ratings, with the exception of DEX-exposed children being rated as being more sociable by their parents as compared to controls. Regarding shyness, no differences between the DEX-exposed group and controls were observed in parental ratings, while in the children's self-ratings, the CAH-unaffected, short-term-treated children reported more shyness than children in the control group. To measure gender role behaviour, a new instrument was developed and evaluated in an additional group of school-age children. In DEX-exposed CAH-unaffected short-term treated boys, more neutral behaviours were observed as compared to control boys, while the DEX-treated CAH-unaffected girls did not differ from the control girls after adjusting for the site of residence.

Taken together, these studies indicate that prenatal DEX treatment of CAH probably does not cause major behavioural problems, while there may be some effects on executive functions, social anxiety (shyness) and possibly on gender role behaviour (in boys). However, a small sample size, a relatively high refusal rate and the retrospective design of the studies limited the conclusiveness of the results. Studies comprising larger cohorts are needed.

The possible negative effects in our studies were observed either in the DEX-treated group as a whole or in the short-term-treated, CAH-unaffected children. The latter group does not benefit from the treatment *per se* and therefore no side-effects should be tolerated. In Sweden, we have decided to put the prenatal DEX treatment of CAH on hold until our findings have been either replicated or contradicted in other cohorts and larger study groups.

We hope that other centres will also conduct long-term retrospective follow-up studies on all treated children, as well as prospective studies on ongoing treatments. In this way our results could be replicated or challenged, as well as meaningful meta-analyses may be performed to obtain more conclusive results. These results would guide clinicians and investigators in their decisions on the future of the prenatal DEX treatment of children at risk for CAH. Until then, families should be offered thorough information on the benefits as well as possible side effects of the treatment.

8 FUTURE PERSPECTIVES

The results of the studies in this thesis have contributed to the decision to put the prenatal DEX treatment of CAH in Sweden on hold. In other countries, the controversial treatment is still administered while follow-up studies are scarce.

It should be noted that prenatal effects on the foetus do not have to affect birth weight in order to have imprinting effects on the CNS (de Vries, et al., 2007). Moreover, symptoms may not become obvious until later in life. Thus, additional long-term follow-up studies of individuals treated with DEX are essential. Moreover, future studies should not only focus on major cognitive abilities and gross developmental milestones, but also on more subtle effects. General intellectual development, learning and long-term memory are of course essential for everyday functioning, but other aspects, such as executive functions (for example, response inhibition, working memory and set shifting) should be investigated in greater detail over time because between 5 and 13 years of age there is a rapid development of many key executive functions, such as working memory (Korkman, et al., 1998; Nichelli, Bulgheroni, & Riva, 2001; Westerberg, Hirvikoski, Forssberg, & Klingberg, 2004), although it is also known from recent brain imaging studies that the human CNS continues to mature at least up to the age of 25 (Lenroot & Giedd, 2006; Shaw et al., 2008). Development of temperament, psychological well-being and dimensions of gender (such as gender role behaviour, sex-specific cognition) should also be further investigated.

Another important area for future studies is vulnerability to stress. The GC programming of the HPA axis does indeed occur in primates (de Vries, et al., 2007). Synthetic GCs given during pregnancy affect the HPA axis at the level of the brain, the pituitary and the adrenal gland, possibly via modulation of the GC and the mineralocorticoid receptors (Kapoor, et al., 2008). The effects may be exerted via facilitated CRF signalling in the amygdala (Meaney, Szyf, & Seckl, 2007). A direct impact on specific brain structures is not the only factor that may affect cognitive performance, since HPA-axis activity is also implicated in the regulation of arousal and cognition (which in turn are associated with each other). In animal studies, indications of sex differences with respect to prenatal DEX treatment and HPA function and feedback regulation have been observed (Banjanin, Kapoor, & Matthews, 2004; Liu, Li, & Matthews, 2001).

Sex differences may also occur in possible effects on behaviour, including cognition, while not much is known about prenatal treatment in CAH. Sex differences as well as individual differences in vulnerability – together with the likely subclinical nature of the effects - may complicate the interpretation of data and require large study groups. In addition to HPA-axis regulation of the CNS, an impact on the neurotransmitters or white matter may also affect signalling and connectivity and hence complex cognitive functions and behaviours that emerge from neural networks.

The potential GC programming of the HPA axis may not only alter stress reactivity but may also be associated with the metabolic syndrome. The risk factors for metabolic and cardiovascular disease (such as lipid profile, glucose metabolism, kidney function and blood pressure) should be investigated. Finally, epigenetics has attracted increasing interest with regard to GC regulation and long-term imprinting effects. Selective methylation and demethylation can permanently alter gene expression (Meaney, et al., 2007), and the changes can be inherited to the next generation. Transgenerational effects have been demonstrated in the rat (Drake, Walker, & Seckl, 2005). Whether or not these effects take place in connection with prenatal DEX treatment in humans remains to be investigated.

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10 REFERENCES

Achenbach, T. M. (1991). *Manual for the child behavior checklist /4-18 and 1991 profile*: Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M., McConaughy, S. H., & Howell, C. T. (1987). Child/adolescent behavioral and emotional problems: implications of cross-informant correlations for situational specificity. *Psychol Bull, 101*(2), 213-232.

Ahlbom, E., Gogvadze, V., Chen, M., Celsi, G., & Ceccatelli, S. (2000). Prenatal exposure to high levels of glucocorticoids increases the susceptibility of cerebellar granule cells to oxidative stress-induced cell death. *Proc Natl Acad Sci U S A, 97*(26), 14726-14730.

Alonso, G. (2000). Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. *Glia, 31*(3), 219-231.

Anderson, V., Lajoie, G., & Bell, R. (1997). *Neuropsychological assessment of the school-aged child.*

Anderson, V., Northam, E., Hendy, J., & Wrennall, J. (2001). *Developmental Neuropsychology: A Clinical Approach.* East Sussex: Psychology Press.

Arnsten, A. F. (1999). Development of the cerebral cortex: XIV. Stress impairs prefrontal cortical function. *J Am Acad Child Adolesc Psychiatry, 38*(2), 220-222.

Arnsten, A. F. (2006). Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *J Clin Psychiatry, 67 Suppl 8*, 7-12.

Baddeley, A. (1986). *Working memory*. Oxford: Clarendon Press.

Banjanin, S., Kapoor, A., & Matthews, S. G. (2004). Prenatal glucocorticoid exposure alters hypothalamic-pituitary-adrenal function and blood pressure in mature male guinea pigs. *J Physiol, 558*(Pt 1), 305-318.

Baud, O., & Sola, A. (2007). Corticosteroids in perinatal medicine: how to improve outcomes without affecting the developing brain? *Semin Fetal Neonatal Med, 12*(4), 273-279.

Bauer, M. E., Vedhara, K., Perks, P., Wilcock, G. K., Lightman, S. L., & Shanks, N. (2000). Chronic stress in caregivers of dementia patients is associated with reduced lymphocyte sensitivity to glucocorticoids. *J Neuroimmunol, 103*(1), 84-92.

Bayer, S. A., Altman, J., Russo, R. J., & Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology, 14*(1), 83-144.

Beidel, D. C., Turner, S. M., & Morris, T. L. (1995). A new inventory to assess social phobia in children: The Social Phobia and Anxiety Inventory for Children. *Psychological Assessment, 7*, 73-79.

Belanoff, J. K., Gross, K., Yager, A., & Schatzberg, A. F. (2001). Corticosteroids and cognition. *J Psychiatr Res, 35*(3), 127-145.

Bender, B. G., Lerner, J. A., & Poland, J. E. (1991). Association between corticosteroids and psychologic change in hospitalized asthmatic children. *Ann Allergy, 66*(5), 414-419.

Benediktsson, R., Calder, A. A., Edwards, C. R., & Seckl, J. R. (1997). Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. *Clin Endocrinol (Oxf), 46*(2), 161-166.

Berenbaum, S. A. (2001). Cognitive function in congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am, 30*(1), 173-192.

Berenbaum, S. A., Bryk, K. K., & Duck, S. C. (2010). Normal intelligence in female and male patients with congenital adrenal hyperplasia. *Int J Pediatr Endocrinol, 2010*, 853103.

Berenbaum, S. A., Duck, S. C., & Bryk, K. (2000). Behavioral effects of prenatal versus postnatal androgen excess in children with 21-hydroxylase-deficient congenital adrenal hyperplasia. *J Clin Endocrinol Metab, 85*(2), 727-733.

Berenbaum, S. A., Korman Bryk, K., Duck, S. C., & Resnick, S. M. (2004). Psychological adjustment in children and adults with congenital adrenal hyperplasia. *J Pediatr, 144*(6), 741-746.

Berenbaum, S. A., & Resnick, S. M. (1997). Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 22*(7), 505-515.

Bergamaschi, R., Livieri, C., Uggetti, C., Candeloro, E., Egitto, M. G., Pichiecchio, A., et al. (2006). Brain white matter impairment in congenital adrenal hyperplasia. *Arch Neurol, 63*(3), 413-416.

Blakemore, J. E. O., Berenbaum, S. A., & Liben, L. S. (2009). *Gender Development*. New York: Psychology Press.

Boldizar, J. P. (1991). Assessing sex typing and androgyny in children: The Children's Sex Role Inventory. *Developmental Psychology, 27*(3), 505-515.

Brook, C. G. (2000). Antenatal treatment of a mother bearing a fetus with congenital adrenal hyperplasia. *Arch Dis Child Fetal Neonatal Ed, 82*(3), F176-181.

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology, 30*(9), 846-856.

Buss, A. H., & Plomin, R. (1984). *Temperament: Early developing personality traits*: Hillsdale, N. J.: Lawrence Erlbaum.

Celsi, G., Kistner, A., Aizman, R., Eklof, A. C., Ceccatelli, S., de Santiago, A., et al. (1998). Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. *Pediatr Res, 44*(3), 317-322.

Charmandari, E., Kino, T., Souvatzoglou, E., & Chrousos, G. P. (2003). Pediatric stress: hormonal mediators and human development. *Horm Res, 59*(4), 161-179.

Chrousos, G. P., Evans, M. I., Loriaux, D. L., McCluskey, J., Fletcher, J. C., & Schulman, J. D. (1985). Prenatal therapy in congenital adrenal hyperplasia. Attempted prevention of abnormal external genital masculinization by pharmacologic suppression of the fetal adrenal gland in utero. *Ann N Y Acad Sci, 458*, 156-164.

Cinciripini, P. M. (1986). Cognitive stress and cardiovascular reactivity. II. Relationship to atherosclerosis, arrhythmias, and cognitive control. *Am Heart J, 112*(5), 1051-1065.

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress, 7*(1), 29-37.

Cohen-Bendahan, C. C., van de Beek, C., & Berenbaum, S. A. (2005). Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neurosci Biobehav Rev, 29*(2), 353-384.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). New York: Psychology Press.

Dalziel, S. R., Lim, V. K., Lambert, A., McCarthy, D., Parag, V., Rodgers, A., et al. (2005). Antenatal exposure to betamethasone: psychological functioning and health related quality of life 31 years after inclusion in randomised controlled trial. *Bmj, 331*(7518), 665.

Dalziel, S. R., Walker, N. K., Parag, V., Mantell, C., Rea, H. H., Rodgers, A., et al. (2005). Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. *Lancet, 365*(9474), 1856-1862.

David, M., & Forest, M. G. (1984). Prenatal treatment of congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. *J Pediatr, 105*(5), 799-803.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joels, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocr Rev, 19*(3), 269-301.

de Vries, A., Holmes, M. C., Heijnis, A., Seier, J. V., Heerden, J., Louw, J., et al. (2007). Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. *J Clin Invest, 117*(4), 1058-1067.

Dennis, M. (2000). Childhood Medical Disorders and Cognitive Impairment: Biological Risk, Time, Development, and Reserve. . In K. O. Yeates, M. D. Ris & H. G. Taylor (Eds.), *Pediatric Neuropsychology: Research, Theory, and Practice* New York: The Guilford Press.

Dessens, A. B., Slijper, F. M., & Drop, S. L. (2005). Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. *Arch Sex Behav, 34*(4), 389-397.

Didonato, M. D., & Berenbaum, S. A. (2011). The Benefits and Drawbacks of Gender Typing: How Different Dimensions are Related to Psychological Adjustment. *Arch Sex Behav, 40*(2), 457-463.

Dittmann, R. W., Kappes, M. E., & Kappes, M. H. (1992). Sexual behavior in adolescent and adult females with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 17*(2-3), 153-170.

Dittmann, R. W., Kappes, M. H., Kappes, M. E., Borger, D., Meyer-Bahlburg, H. F., Stegner, H., et al. (1990). Congenital adrenal hyperplasia. II: Gender-related behavior and attitudes in female salt-wasting and simple-virilizing patients. *Psychoneuroendocrinology, 15*(5-6), 421-434.

Dittmann, R. W., Kappes, M. H., Kappes, M. E., Borger, D., Stegner, H., Willig, R. H., et al. (1990). Congenital adrenal hyperplasia. I: Gender-related behavior and attitudes in female patients and sisters. *Psychoneuroendocrinology, 15*(5-6), 401-420.

DonCarlos, L. L., Garcia-Ovejero, D., Sarkey, S., Garcia-Segura, L. M., & Azcoitia, I. (2003). Androgen receptor immunoreactivity in forebrain axons and dendrites in the rat. *Endocrinology, 144*(8), 3632-3638.

Donders, J. (1997). A short form of WISC-III for clinical use. *Psychological assessment, 9*(15-20).

Drake, A. J., Walker, B. R., & Seckl, J. R. (2005). Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am J Physiol Regul Integr Comp Physiol, 288*(1), R34-38.

Emgard, M., Paradisi, M., Pirondi, S., Fernandez, M., Giardino, L., & Calza, L. (2007). Prenatal glucocorticoid exposure affects learning and vulnerability of cholinergic neurons. *Neurobiol Aging, 28*(1), 112-121.

Ernst, M., Maheu, F. S., Schroth, E., Hardin, J., Golan, L. G., Cameron, J., et al. (2007). Amygdala function in adolescents with congenital adrenal hyperplasia: a model for the study of early steroid abnormalities. *Neuropsychologia, 45*(9), 2104-2113.

Forest, M. G., David, M., & Morel, Y. (1993). Prenatal diagnosis and treatment of 21-hydroxylase deficiency. *J Steroid Biochem Mol Biol, 45*(1-3), 75-82.

Forest, M. G., Morel, Y., & David, M. (1998). Prenatal treatment of congenital adrenal hyperplasia. *Trends Endocrinol Metab, 9*(7), 284-289.

French, N. P., Hagan, R., Evans, S. F., Mullan, A., & Newnham, J. P. (2004). Repeated antenatal corticosteroids: effects on cerebral palsy and childhood behavior. *Am J Obstet Gynecol, 190*(3), 588-595.

Frisen, L., Nordenstrom, A., Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P. O., et al. (2009). Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab, 94*(9), 3432-3439.

Gazzaniga, M. S., Ivry, R. B., & Mangun, G. R. (1998). *Cogntive Neuroscience: The Biology of the Mind*. New York: W.W.Norton & Company.

Geschwind, N., & Behan, P. (1982). Left-handedness: association with immune disease, migraine, and developmental learning disorder. *Proc Natl Acad Sci U S A, 79*(16), 5097-5100.

Geschwind, N., & Galaburda, A. M. (1985). Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Arch Neurol, 42*(5), 428-459.

Gilbert, A. N., & Wysocki, C. J. (1992). Hand preference and age in the United States. *Neuropsychologia, 30*(7), 601-608.

Golden, C. J., & Freshwater, S. M. (1998). *The Stroop Color and Word Test - A Manual for Clinical and Experimental Uses*. Chicago: Stoelting Co.

Goldstein, J. M., Seidman, L. J., Horton, N. J., Makris, N., Kennedy, D. N., Caviness, V. S., Jr., et al. (2001). Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cereb Cortex, 11*(6), 490-497.

Gouchie, C., & Kimura, D. (1991). The relationship between testosterone levels and cognitive ability patterns. *Psychoneuroendocrinology, 16*(4), 323-334.

Graham, Y. P., Heim, C., Goodman, S. H., Miller, A. H., & Nemeroff, C. B. (1999). The effects of neonatal stress on brain development: implications for psychopathology. *Dev Psychopathol, 11*(3), 545-565.

Grimshaw, G. M., Bryden, M. P., & Finegan, J. A. (1995). Relations Between Prenatal Testosterone and Cerebral Lateralization in Children. *Neuropsychology, 9*(1), 68-79.

Grimshaw, G. M., Sitarenios, G., & Finegan, J. A. (1995). Mental rotation at 7 years: relations with prenatal testosterone levels and spatial play experiences. *Brain Cogn, 29*(1), 85-100.

Gron, G., Wunderlich, A. P., Spitzer, M., Tomczak, R., & Riepe, M. W. (2000). Brain activation during human navigation: gender-different neural networks as substrate of performance. *Nat Neurosci, 3*(4), 404-408.

Halpern, D. F. (2000). *Sex Differences In Cognitive Abilities*. Mahwah: Lawrence Erlbaum Associates.

Hampson, E., Rovet, J. F., & Altmann, D. (1998). Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Developmental Neuropsychology, 14*(2-3), 299-320.

Harter, S. (1985). *The self-perception profile for children: Revision of the perceived competence scale for children*: University of Denver.

Hauser, J., Knapman, A., Zurcher, N. R., Pilloud, S., Maier, C., Diaz-Heijtz, R., et al. (2008). Effects of prenatal dexamethasone treatment on physical growth, pituitary-adrenal hormones, and performance of motor, motivational, and cognitive tasks in juvenile and adolescent common marmoset monkeys. *Endocrinology, 149*(12), 6343-6355.

Heim, C., Ehlert, U., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology, 25*(1), 1-35.

Heine, V. M., & Rowitch, D. H. (2009). Hedgehog signaling has a protective effect in glucocorticoid-induced mouse neonatal brain injury through an 11betaHSD2-dependent mechanism. *J Clin Invest, 119*(2), 267-277.

Helleday, J., Bartfai, A., Ritzen, E. M., & Forsman, M. (1994). General intelligence and cognitive profile in women with congenital adrenal hyperplasia (CAH). *Psychoneuroendocrinology, 19*(4), 343-356.

Helleday, J., Edman, G., Ritzen, E. M., & Siwers, B. (1993). Personality characteristics and platelet MAO activity in women with congenital adrenal hyperplasia (CAH). *Psychoneuroendocrinology, 18*(5-6), 343-354.

Helleday, J., Siwers, B., Ritzen, E. M., & Hugdahl, K. (1994). Normal lateralization for handedness and ear advantage in a verbal dichotic listening task in women with congenital adrenal hyperplasia (CAH). *Neuropsychologia, 32*(7), 875-880.

Hickey, M., Doherty, D. A., Hart, R., Norman, R. J., Mattes, E., Atkinson, H. C., et al. (2010). Maternal and umbilical cord androgen concentrations do not predict digit ratio (2D:4D) in girls: a prospective cohort study. *Psychoneuroendocrinology, 35*(8), 1235-1244.

Higa, C. K., Fernandez, S. N., Nakamura, B. J., Chorpita, B. F., & Daleiden, E. L. (2006). Parental assessment of childhood social phobia: psychometric properties of the social phobia and anxiety inventory for children-parent report. *J Clin Child Adolesc Psychol, 35*(4), 590-597.

Hines, M. (2006). Prenatal testosterone and gender-related behaviour. *Eur J Endocrinol, 155 Suppl 1*, S115-121.

Hines, M. (2010). Sex-related variation in human behavior and the brain. *Trends Cogn Sci, 14*(10), 448-456.

Hines, M., Fane, B. A., Pasterski, V. L., Mathews, G. A., Conway, G. S., & Brook, C. (2003). Spatial abilities following prenatal androgen abnormality: targeting and

mental rotations performance in individuals with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 28*(8), 1010-1026.

Hirvikoski, T., Lindholm, T., Lajic, S., & Nordenstrom, A. (2011). Gender role behaviour in prenatally dexamethasone-treated children at risk for congenital adrenal hyperplasia - a pilot study. *Acta Paediarica*, 2011 Mar 9. doi: 10.1111/j.1651-2227.2011.02260.x. [Epub ahead of print].

Hirvikoski, T., Nordenstrom, A., Lindholm, T., Lindblad, F., Ritzen, E. M., & Lajic, S. (2008). Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *Eur J Endocrinol, 159*(3), 309-316.

Hirvikoski, T., Nordenstrom, A., Lindholm, T., Lindblad, F., Ritzen, E. M., Wedell, A., et al. (2007). Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *J Clin Endocrinol Metab, 92*(2), 542-548.

Hirvikoski, T., Olsson, E. M., Nordenstrom, A., Lindholm, T., Nordstrom, A. L., & Lajic, S. (2011). Deficient cardiovascular stress reactivity predicts poor executive functions in adults with attention-deficit/hyperactivity disorder. *J Clin Exp Neuropsychol, 33*(1), 63-73.

Holson, R. R., Gough, B., Sullivan, P., Badger, T., & Sheehan, D. M. (1995). Prenatal dexamethasone or stress but not ACTH or corticosterone alter sexual behavior in male rats. *Neurotoxicol Teratol, 17*(4), 393-401.

Huang, W. L., Harper, C. G., Evans, S. F., Newnham, J. P., & Dunlop, S. A. (2001). Repeated prenatal corticosteroid administration delays myelination of the corpus callosum in fetal sheep. *Int J Dev Neurosci, 19*(4), 415-425.

Huizink, A. C., Mulder, E. J., & Buitelaar, J. K. (2004). Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? *Psychol Bull, 130*(1), 115-142.

Inozemtseva, O., Matute, E., & Juarez, J. (2008). Learning disabilities spectrum and sexual dimorphic abilities in girls with congenital adrenal hyperplasia. *J Child Neurol, 23*(8), 862-869.

Iversen, S., Kupfermann, I., & Kandel, E. R. (2000). Emotional States and Feelings. In E. R. Kandel, J. H. Schwartz & T. M. Jessell (Eds.), *Principles of Neural Science*. New York: McGraw-Hill.

Jansen, L. M., Gispen-de Wied, C. C., Jansen, M. A., van der Gaag, R. J., Matthys, W., & van Engeland, H. (1999). Pituitary-adrenal reactivity in a child psychiatric population: salivary cortisol response to stressors. *Eur Neuropsychopharmacol, 9*(1-2), 67-75.

Jessell, T. M., & Sanes, J. R. (2000). The Development of the Nervous System. In K. ER, S. JH & J. TM (Eds.), *Principles of Neural Science*. New York: McGraw-Hill.

Johannsen, T. H., Ripa, C. P., Mortensen, E. L., & Main, K. M. (2006). Quality of life in 70 women with disorders of sex development. *Eur J Endocrinol, 155*(6), 877-885.

Johannsen, T. H., Ripa, C. P., Reinisch, J. M., Schwartz, M., Mortensen, E. L., & Main, K. M. (2006). Impaired cognitive function in women with congenital adrenal hyperplasia. *J Clin Endocrinol Metab, 91*(4), 1376-1381.

Joyce, P. R., Donald, R. A., Nicholls, M. G., Livesey, J. H., & Abbott, R. M. (1986). Endocrine and behavioral responses to methylphenidate in normal subjects. *Biol Psychiatry, 21*(11), 1015-1023.

Kandel, E. R., Kupfermann, I., & Iversen, S. (2000). Learning and Memory. In E. R. Kandel, J. H. Schwartz & T. M. Jessell (Eds.), *Principles of Neural Science*. New York: McGraw-Hill.

Kaplan, E., Fein, D., Morris, R., & Delis, D. (1991). *WAIS-R as a neuropsychological instrument*. New York: The Psychological Corporation.

Kapoor, A., Petropoulos, S., & Matthews, S. G. (2008). Fetal programming of hypothalamic-pituitary-adrenal (HPA) axis function and behavior by synthetic glucocorticoids. *Brain Res Rev, 57*(2), 586-595.

Kariyawasam, S. H., Zaw, F., & Handley, S. L. (2002). Reduced salivary cortisol in children with comorbid Attention deficit hyperactivity disorder and oppositional defiant disorder. *Neuro Endocrinol Lett, 23*(1), 45-48.

Kelso, W. M., Nicholls, M. E., Warne, G. L., & Zacharin, M. (2000). Cerebral lateralization and cognitive functioning in patients with congenital adrenal hyperplasia. *Neuropsychology, 14*(3), 370-378.

Kimura, D. (2000). *Sex and Cognition*. Massachusetts: The MIT Press.

King, J. A., Rosal, M. C., Ma, Y., Reed, G., Kelly, T. A., Stanek, E. J., 3rd, et al. (2000). Sequence and seasonal effects of salivary cortisol. *Behav Med, 26*(2), 67-73.

King, S., & Laplante, D. P. (2005). The effects of prenatal maternal stress on children's cognitive development: Project Ice Storm. *Stress, 8*(1), 35-45.

King, S. L., & Hegadoren, K. M. (2002). Stress hormones: how do they measure up? *Biol Res Nurs, 4*(2), 92-103.

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology, 19*(4), 313-333.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81.

Kirschbaum, C., Prussner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., et al. (1995). Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosom Med, 57*(5), 468-474.

Knickmeyer, R., Baron-Cohen, S., Fane, B. A., Wheelwright, S., Mathews, G. A., Conway, G. S., et al. (2006). Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia. *Horm Behav, 50*(1), 148-153.

Korkman, M. (1988). NEPSY: An adaptation of Luria's investigation for young children. *Clinical Neuropsychologist, 2*(4), 375-392.

Korkman, M., Kirk, U., & Kemp, S. (1998). *NEPSY: A developmental neuropsychological assessment*. San Antonio: San Antonio, TX: The Psychological Corporation.

Kreider, M. L., Aldridge, J. E., Cousins, M. M., Oliver, C. A., Seidler, F. J., & Slotkin, T. A. (2005). Disruption of rat forebrain development by glucocorticoids: critical perinatal periods for effects on neural cell acquisition and on cell signaling cascades mediating noradrenergic and cholinergic neurotransmitter/neurotrophic responses. *Neuropsychopharmacology, 30*(10), 1841-1855.

la Greca, A. M., Dandes, S. K., Wick, P., Shaw, K., & et al. (1988). Development of the Social Anxiety Scale for Children: Reliability and concurrent validity. *Journal of Clinical Child Psychology, 17*(1), 84-91.

Lajic, S., Nordenstrom, A., & Hirvikoski, T. (2008). Long-term outcome of prenatal treatment of congenital adrenal hyperplasia. *Endocr Dev, 13*, 82-98.

Lajic, S., Nordenstrom, A., Ritzen, E. M., & Wedell, A. (2004). Prenatal treatment of congenital adrenal hyperplasia. *Eur J Endocrinol, 151 Suppl 3*, U63-69.

Lajic, S., Wedell, A., Bui, T. H., Ritzen, E. M., & Holst, M. (1998). Long-term somatic follow-up of prenatally treated children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab, 83*(11), 3872-3880.

Lalau, J. D., Aubert, M. L., Carmignac, D. F., Gregoire, I., & Dupouy, J. P. (1990). Reduction in testicular function in rats. I. Reduction by a specific gonadotropin-releasing hormone antagonist in fetal rats. *Neuroendocrinology, 51*(3), 284-288.

Lenroot, R. K., & Giedd, J. N. (2006). Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev, 30*(6), 718-729.

Liu, L., Li, A., & Matthews, S. G. (2001). Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. *Am J Physiol Endocrinol Metab, 280*(5), E729-739.

Lo, J. C., & Grumbach, M. M. (2001). Pregnancy outcomes in women with congenital virilizing adrenal hyperplasia. *Endocrinol Metab Clin North Am, 30*(1), 207-229.

Lundberg, U., & Frankenhaeuser, M. (1980). Pituitary-adrenal and sympathetic-adrenal correlates of distress and effort. *Journal of Psychosomatic Research, 24*(3-4), 125-130.

Lupien, S. J., Gillin, C. J., & Hauger, R. L. (1999). Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: a dose-response study in humans. *Behav Neurosci, 113*(3), 420-430.

Lupien, S. J., Wilkinson, C. W., Briere, S., Menard, C., Ng Ying Kin, N. M., & Nair, N. P. (2002). The modulatory effects of corticosteroids on cognition: studies in young human populations. *Psychoneuroendocrinology, 27*(3), 401-416.

LWPES/ESPE. (2002). Consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. *J Clin Endocrinol Metab, 87*(9), 4048-4053.

Maheu, F. S., Merke, D. P., Schroth, E. A., Keil, M. F., Hardin, J., Poeth, K., et al. (2008). Steroid abnormalities and the developing brain: declarative memory for

emotionally arousing and neutral material in children with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 33*(2), 238-245.

Malouf, M. A., Migeon, C. J., Carson, K. A., Petrucci, L., & Wisniewski, A. B. (2006). Cognitive outcome in adult women affected by congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res, 65*(3), 142-150.

Mathews, G. A., Fane, B. A., Conway, G. S., Brook, C. G., & Hines, M. (2009). Personality and congenital adrenal hyperplasia: possible effects of prenatal androgen exposure. *Horm Behav, 55*(2), 285-291.

Mathews, G. A., Fane, B. A., Pasterski, V. L., Conway, G. S., Brook, C., & Hines, M. (2004). Androgenic influences on neural asymmetry: Handedness and language lateralization in individuals with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 29*(6), 810-822.

Matthews, S. G. (2001). Antenatal glucocorticoids and the developing brain: mechanisms of action. *Semin Neonatol, 6*(4), 309-317.

Mazzone, L., Mueller, S. C., Maheu, F., Vanryzin, C., Merke, D. P., & Ernst, M. (2011). Emotional memory in early steroid abnormalities: an FMRI study of adolescents with congenital adrenal hyperplasia. *Dev Neuropsychol, 36*(4), 473-492.

McArthur, S., McHale, E., & Gillies, G. E. (2007). The size and distribution of midbrain dopaminergic populations are permanently altered by perinatal glucocorticoid exposure in a sex- region- and time-specific manner. *Neuropsychopharmacology, 32*(7), 1462-1476.

McBurnett, K., Lahey, B. B., Frick, P. J., Risch, C., Loeber, R., Hart, E. L., et al. (1991). Anxiety, inhibition, and conduct disorder in children: II. Relation to salivary cortisol. *J Am Acad Child Adolesc Psychiatry, 30*(2), 192-196.

McEwen, B. S. (2002). Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. *Neurobiol Aging, 23*(5), 921-939.

McEwen, B. S. (2006). Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism, 55*(10 Suppl 2), S20-23.

McEwen, B. S., Chao, H., Spencer, R., Brinton, R., Macisaac, L., & Harrelson, A. (1987). Corticosteroid receptors in brain: relationship of receptors to effects in stress and aging. *Ann N Y Acad Sci, 512*, 394-401.

Meaney, M. J., Szyf, M., & Seckl, J. R. (2007). Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med, 13*(7), 269-277.

Mercado, A. B., Wilson, R. C., Cheng, K. C., Wei, J. Q., & New, M. I. (1995). Prenatal treatment and diagnosis of congenital adrenal hyperplasia owing to steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab, 80*(7), 2014-2020.

Merce Fernandez-Balsells, M., Muthusamy, K., Smushkin, G., Lampropulos, J. F., Elamin, M. B., Abu Elnour, N. O., et al. (2010). Prenatal dexamethasone use for the prevention of virilization in pregnancies at risk for classical congenital adrenal hyperplasia because of 21-hydroxylase (CYP21A2) deficiency: a systematic review and meta-analyses. *Clin Endocrinol (Oxf), 73*(4), 436-444.

Merke, D. P., Fields, J. D., Keil, M. F., Vaituzis, A. C., Chrousos, G. P., & Giedd, J. N. (2003). Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. *J Clin Endocrinol Metab, 88*(4), 1760-1765.

Meyer-Bahlburg, H. F. (2001). Gender and sexuality in classic congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am, 30*(1), 155-171, viii.

Meyer-Bahlburg, H. F., Dolezal, C., Baker, S. W., Carlson, A. D., Obeid, J. S., & New, M. I. (2004a). Cognitive and motor development of children with and without congenital adrenal hyperplasia after early-prenatal dexamethasone. *J Clin Endocrinol Metab, 89*(2), 610-614.

Meyer-Bahlburg, H. F., Dolezal, C., Baker, S. W., Carlson, A. D., Obeid, J. S., & New, M. I. (2004b). Prenatal androgenization affects gender-related behavior but not gender identity in 5-12-year-old girls with congenital adrenal hyperplasia. *Arch Sex Behav, 33*(2), 97-104.

Meyer-Bahlburg, H. F., Dolezal, C., Baker, S. W., & New, M. I. (2008). Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. *Arch Sex Behav, 37*(1), 85-99.

Miller, W. L. (1998). Prenatal treatment of congenital adrenal hyperplasia: a promising experimental therapy of unproven safety. *Trends Endocrinol Metab, 9*(7), 290-293.

Miller, W. L. (1999). Dexamethasone treatment of congenital adrenal hyperplasia in utero: an experimental therapy of unproven safety. *J Urol, 162*(2), 537-540.

Moffat, S. D., & Hampson, E. (1996). A curvilinear relationship between testosterone and spatial cognition in humans: possible influence of hand preference. *Psychoneuroendocrinology, 21*(3), 323-337.

Mondschein, E. R., Adolph, K. E., & Tamis-LeMonda, C. S. (2000). Gender bias in mothers' expectations about infant crawling. *J Exp Child Psychol, 77*(4), 304-316.

Moore, D. S., & Johnson, S. P. (2008). Mental rotation in human infants: a sex difference. *Psychol Sci, 19*(11), 1063-1066.

Morgan, J. F., Murphy, H., Lacey, J. H., & Conway, G. (2005). Long term psychological outcome for women with congenital adrenal hyperplasia: cross sectional survey. *BMJ, 330*(7487), 340-341; discussion 341.

Mueller, S. C., Ng, P., Sinaii, N., Leschek, E. W., Green-Golan, L., VanRyzin, C., et al. (2010). Psychiatric characterization of children with genetic causes of hyperandrogenism. *Eur J Endocrinol, 163*(5), 801-810.

Mueller, S. C., Temple, V., Oh, E., VanRyzin, C., Williams, A., Cornwell, B., et al. (2008). Early androgen exposure modulates spatial cognition in congenital adrenal hyperplasia (CAH). *Psychoneuroendocrinology, 33*(7), 973-980.

Nass, R., & Baker, S. (1991). Androgen effects on cognition: congenital adrenal hyperplasia. *Psychoneuroendocrinology, 16*(1-3), 189-201.

Nass, R., Heier, L., Moshang, T., Oberfield, S., George, A., New, M. I., et al. (1997). Magnetic resonance imaging in the congenital adrenal hyperplasia population: increased frequency of white-matter abnormalities and temporal lobe atrophy. *J Child Neurol, 12*(3), 181-186.

Nermoen, I., Husebye, E. S., Svartberg, J., & Lovas, K. (2010). Subjective health status in men and women with congenital adrenal hyperplasia: a population-based survey in Norway. *Eur J Endocrinol, 163*(3), 453-459.

New, M. I. (2001). Prenatal treatment of congenital adrenal hyperplasia: author differs with technical report. *Pediatrics, 107*(4), 804.

New, M. I., Lorenzen, F., Lerner, A. J., Kohn, B., Oberfield, S. E., Pollack, M. S., et al. (1983). Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. *J Clin Endocrinol Metab, 57*(2), 320-326.

Nichelli, F., Bulgheroni, S., & Riva, D. (2001). Developmental patterns of verbal and visuospatial spans. *Neurol Sci, 22*(5), 377-384.

Nordenstrom, A., Frisen, L., Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P. O., et al. (2010). Sexual function and surgical outcome in women with congenital adrenal hyperplasia due to CYP21A2 deficiency: clinical perspective and the patients' perception. *J Clin Endocrinol Metab, 95*(8), 3633-3640.

Nordenstrom, A., Servin, A., Bohlin, G., Larsson, A., & Wedell, A. (2002). Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure

assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. *J Clin Endocrinol Metab, 87*(11), 5119-5124.

Pasterski, V., Hindmarsh, P., Geffner, M., Brook, C., Brain, C., & Hines, M. (2007). Increased aggression and activity level in 3- to 11-year-old girls with congenital adrenal hyperplasia (CAH). *Horm Behav, 52*(3), 368-374.

Patel, P. D., Lopez, J. F., Lyons, D. M., Burke, S., Wallace, M., & Schatzberg, A. F. (2000). Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *J Psychiatr Res, 34*(6), 383-392.

Petraglia, F., Florio, P., Nappi, C., & Genazzani, A. R. (1996). Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. *Endocr Rev, 17*(2), 156-186.

Plante, E., Boliek, C., Binkiewicz, A., & Erly, W. K. (1996). Elevated androgen, brain development and language/learning disabilities in children with congenital adrenal hyperplasia. *Dev Med Child Neurol, 38*(5), 423-437.

Puts, D. A., McDaniel, M. A., Jordan, C. L., & Breedlove, S. M. (2008). Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies. *Arch Sex Behav, 37*(1), 100-111.

Quinn, P. C., & Liben, L. S. (2008). A sex difference in mental rotation in young infants. *Psychol Sci, 19*(11), 1067-1070.

Resnick, S. M., Berenbaum, S. A., Gottesman, I. I., & Bouchard, T. J. (1986). Early Hormonal Influences on Cognitive Funtioning in Congenital Adrenal Hyperplasia. *Developmental Psychology, 22*(2), 191-198.

Rice, F., Jones, I., & Thapar, A. (2007). The impact of gestational stress and prenatal growth on emotional problems in offspring: a review. *Acta Psychiatr Scand, 115*(3), 171-183.

Ritzen, E. M. (1998). Prenatal treatment of congenital adrenal hyperplasia: a commentary. *Trends Endocrinol Metab, 9*(7), 293-295.

Ritzén, E. M. (2001). Prenatal dexamethasone treatment of fetuses at risk for congenital adrenal hyperplasia: benefits and concerns. *Semin Neonatol, 6*, 357-362.

Sanchez, M. M., Young, L. J., Plotsky, P. M., & Insel, T. R. (2000). Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. *J Neurosci, 20*(12), 4657-4668.

Schalling, D., & Edman, G. (1993). *The Karolinska Scales of Personality (KSP). An Inventory for Assessing Temperament Dimensions Associated with Vulnerability for Psychosocial Deviance* Stockholm: The Department of Psychiatry, The Karolinska Institute.

Schlosser, R. G., Wagner, G., & Sauer, H. (2006). Assessing the working memory network: studies with functional magnetic resonance imaging and structural equation modeling. *Neuroscience, 139*(1), 91-103.

Seckl, J. R. (2004). Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol, 151 Suppl 3*, U49-62.

Seckl, J. R., & Meaney, M. J. (2004). Glucocorticoid programming. *Ann N Y Acad Sci, 1032*, 63-84.

Seckl, J. R., & Miller, W. L. (1997). How safe is long-term prenatal glucocorticoid treatment? *JAMA, 277*(13), 1077-1079.

Servin, A., Nordenstrom, A., Larsson, A., & Bohlin, G. (2003). Prenatal androgens and gender-typed behavior: a study of girls with mild and severe forms of congenital adrenal hyperplasia. *Dev Psychol, 39*(3), 440-450.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., et al. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci, 28*(14), 3586-3594.

Sinforiani, E., Livieri, C., Mauri, M., Bisio, P., Sibilla, L., Chiesa, L., et al. (1994). Cognitive and neuroradiological findings in congenital adrenal hyperplasia. *Psychoneuroendocrinology, 19*(1), 55-64.

Slotkin, T. A., Kreider, M. L., Tate, C. A., & Seidler, F. J. (2006). Critical prenatal and postnatal periods for persistent effects of dexamethasone on serotonergic and dopaminergic systems. *Neuropsychopharmacology, 31*(5), 904-911.

Slotkin, T. A., Zhang, J., McCook, E. C., & Seidler, F. J. (1998). Glucocorticoid administration alters nuclear transcription factors in fetal rat brain: implications for the use of antenatal steroids. *Brain Res Dev Brain Res, 111*(1), 11-24.

Speiser, P. W., Heier, L., Serrat, J., New, M. I., & Nass, R. (1995). Failure of steroid replacement to consistently normalize pituitary function in congenital adrenal hyperplasia: hormonal and MRI data. *Horm Res, 44*(6), 241-246.

Technical report: congenital adrenal hyperplasia. Section on Endocrinology and Committee on Genetics. (2000). *Pediatrics, 106*(6), 1511-1518.

Tennes, K., & Kreye, M. (1985). Children's adrenocortical responses to classroom activities and tests in elementary school. *Psychosom Med, 47*(5), 451-460.

Tirosh, E., Rod, R., Cohen, A., & Hochberg, Z. (1993). Congenital adrenal hyperplasia and cerebral lateralizations. *Pediatr Neurol, 9*(3), 198-201.

Trautman, P. D., Meyer-Bahlburg, H. F., Postelnek, J., & New, M. I. (1995). Effects of early prenatal dexamethasone on the cognitive and behavioral development of young children: results of a pilot study. *Psychoneuroendocrinology, 20*(4), 439-449.

Ullebo, A. K., Posserud, M. B., Heiervang, E., Obel, C., & Gillberg, C. (2011). Prevalence of the ADHD phenotype in 7- to 9-year-old children: effects of informant, gender and non-participation. *Soc Psychiatry Psychiatr Epidemiol*.[Epubl ahead of print].

Uno, H., Eisele, S., Sakai, A., Shelton, S., Baker, E., DeJesus, O., et al. (1994). Neurotoxicity of glucocorticoids in the primate brain. *Horm Behav, 28*(4), 336-348.

Uno, H., Lohmiller, L., Thieme, C., Kemnitz, J. W., Engle, M. J., Roecker, E. B., et al. (1990). Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. *Brain Res Dev Brain Res, 53*(2), 157-167.

van Goozen, S. H., Matthys, W., Cohen-Kettenis, P. T., Gispen-de Wied, C., Wiegant, V. M., & van Engeland, H. (1998). Salivary cortisol and cardiovascular activity during stress in oppositional-defiant disorder boys and normal controls. *Biol Psychiatry, 43*(7), 531-539.

Wapner, R. J., Sorokin, Y., Mele, L., Johnson, F., Dudley, D. J., Spong, C. Y., et al. (2007). Long-term outcomes after repeat doses of antenatal corticosteroids. *N Engl J Med, 357*(12), 1190-1198.

Wassenberg, R., Max, J. E., Koele, S. L., & Firme, K. (2004). Classifying psychiatric disorders after traumatic brain injury and orthopaedic injury in children: adequacy of K-SADS versus CBCL. *Brain Inj, 18*(4), 377-390.

Wechsler, D. (2003). *Wechsler Intelligence Scale for Children-Fourth Edition: Technical and interpretive manual*. San Antonio, TX: Psychological Corporation.

Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol, 65*(5), 427-451.

Veith, J. L., Sandman, C. A., George, J. M., & Kendall, J. W. (1985). The relationship of endogenous ACTH levels to visual-attentional functioning in patients with congenital adrenal hyperplasia. *Psychoneuroendocrinology, 10*(1), 33-48.

Welberg, L. A., Seckl, J. R., & Holmes, M. C. (2001). Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience, 104*(1), 71-79.

Westerberg, H., Hirvikoski, T., Forssberg, H., & Klingberg, T. (2004). Visuo-spatial working memory span: a sensitive measure of cognitive deficits in children with ADHD. *Child Neuropsychol, 10*(3), 155-161.

Whitehouse, A. J., Maybery, M. T., Hart, R., Mattes, E., Newnham, J. P., Sloboda, D. M., et al. (2010). Fetal androgen exposure and pragmatic language ability of girls in middle childhood: implications for the extreme male-brain theory of autism. *Psychoneuroendocrinology, 35*(8), 1259-1264.

Witelson, S. F., & Nowakowski, R. S. (1991). Left out axons make men right: a hypothesis for the origin of handedness and functional asymmetry. *Neuropsychologia, 29*(4), 327-333.

Wood, J. L., Heitmiller, D., Andreasen, N. C., & Nopoulos, P. (2008). Morphology of the ventral frontal cortex: relationship to femininity and social cognition. *Cereb Cortex, 18*(3), 534-540.

Wood, J. L., Murko, V., & Nopoulos, P. (2008). Ventral frontal cortex in children: morphology, social cognition and femininity/masculinity. *Soc Cogn Affect Neurosci, 3*(2), 168-176.

Vos, A. A., & Bruinse, H. W. (2010). Congenital adrenal hyperplasia: do the benefits of prenatal treatment defeat the risks? *Obstet Gynecol Surv, 65*(3), 196-205.

Vuoksimaa, E., Eriksson, C. J., Pulkkinen, L., Rose, R. J., & Kaprio, J. (2010). Decreased prevalence of left-handedness among females with male co-twins: evidence suggesting prenatal testosterone transfer in humans? *Psychoneuroendocrinology, 35*(10), 1462-1472.

Yeh, T. F., Lin, Y. J., Huang, C. C., Chen, Y. J., Lin, C. H., Lin, H. C., et al. (1998). Early dexamethasone therapy in preterm infants: a follow-up study. *Pediatrics, 101*(5), E7.

Yeh, T. F., Lin, Y. J., Lin, H. C., Huang, C. C., Hsieh, W. S., Lin, C. H., et al. (2004). Outcomes at school age after postnatal dexamethasone therapy for lung disease of prematurity. *N Engl J Med, 350*(13), 1304-1313.