Biological roots of sex differences: a longitudinal twin study

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Biological roots of sex differences:

a longitudinal twin study

Biologische wortels van sekseverschillen:
een longitudinaal tweelingenonderzoek
(met samenvatting in het Nederlands)

Proefschrift

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Chapter 1

Introduction

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1. GENERAL INTRODUCTION

There is now good evidence from studies with human and nonhuman species that events occurring during prenatal development can have life-long effects on an organism. These effects are not limited to physical characteristics, but extend to a variety of behavioral traits. Thus, for example, physical and emotional stressors experienced by rodent, monkey, and human females during pregnancy are associated with lifelong behavioral problems in their offspring (O'Connor et al., 2003; Schneider et al., 2002; Gordon, 2002).

Long-term effects of prenatal events extend beyond exposure to stress hormones. There are also marked physical and behavioral consequences of prenatal exposure to another category of hormones, those produced by the gonads ("sex hormones"). In all mammalian species studied, sexual differentiation of the reproductive system depends largely on the amount of androgens present during critical periods of prenatal life. In human beings, this critical period begins at about 7 to 8 weeks of gestation, when the testes develop and begin to secrete testosterone (Grumbach et al., 2002). The external genitalia are undifferentiated until then, and the amount of testosterone (or other androgens) determines whether they differentiate into male-typical or female-typical genitalia. With high levels of testosterone, the genitalia become the penis, scrotum, and urogenital sinus, whereas low levels of testosterone result in the development of the clitoris, labia majora, and separate vaginal and urethral canals. Intermediate levels of testosterone result in ambiguous genitalia, e.g., an enlarged clitoris with fused labia, a small penis. Sex hormones also affect the development of the internal reproductive structures.

Behavioral studies with nonhuman mammals clearly show that the same prenatal hormones responsible for the sexual differentiation of the body are also involved in the sexual differentiation of behavior (for reviews see, Becker et al., 2002; Breedlove, 1992; Goy and McEwen, 1980). In rodents, newborn females injected with high doses of androgens show behavior more typical of males than of other females, and newborn males that are castrated or given anti-

androgens show behavior more typical of females than of other males. The behaviors involved include adult sexual behavior, juvenile rough-and-tumble play, adult aggression, and maze performance. These effects are also found in rodents naturally exposed to atypical hormone levels as a result of gestating next to opposite-sex littermates.

Behavioral effects of early hormones are also found in nonhuman primates: female monkeys exposed to androgens early in development are masculinized with respect to sexual behavior, rough-and-tumble play, grooming (Goy et al., 1988), and some learning abilities (Bachevalier and Hagger, 1991; Clark and Goldman-Rakic, 1989). Studies with monkeys confirm and extend studies with rodents in two important ways. First, they illustrate the complexity of timing effects and show that there may be several distinct periods during which behavior is sensitive to androgens, even within the prenatal period, so that some behaviors are masculinized by exposure to androgens in early (but not late) gestation, whereas other behaviors are masculinized by exposure in late (but not early) gestation (Goy et al., 1988). For example, monkeys that received androgens early in development and had masculinized genitals showed increased mounting of peers and mothers and less grooming behavior, whereas those exposed to androgens later in development had normal-looking genitals but showed increased rough play and mounting of peers, but not mounting of mothers. Second, studies with monkeys show the importance of the environmental context in modifying the behavioral effects of hormones. For example, the social environment of juvenile monkeys modifies the expression of behavior that is influenced by hormones (Wallen, 1996). The behaviors that are the least variable across social contexts are the most affected by prenatal hormones.

This chapter describes the behavioral effects of sex hormones in humans and presents evidence that the findings of studies of rodents and primates also apply to humans. Most of the early studies exploring the behavioral effects of sex hormones in humans were performed with clinical samples, i.e., individuals whose hormone concentrations were unusual because of disease or accident. Such "experiments of nature" have limitations regarding alterna-

tive explanations and generalizability, so it is important to examine evidence obtained with different methods, all of which have different limitations. It is even more important to extend findings to typical populations, to examine generalizability, and this is now possible as a result of methodological advances. This chapter is organized around some of the methods used to study the behavioral effects of hormones, beginning with a study involving individuals with congenital adrenal hyperplasia (CAH), then moving to studies of typical populations involving direct measures of prenatal hormone exposure, and then to the main topic of this dissertation, studies of typical populations in which an indirect measure of hormone exposure was used.

2. EVIDENCE FROM OTHER HUMAN STUDIES

The early studies with animals showing behavioral effects of early exposure to hormones prompted studies of people with atypical hormone exposure, i.e., in which the sex hormones are higher or lower than expected for a person's sex (e.g., Money and Ehrhardt, 1972). Early studies involving such "experiments of nature" suggested that sex hormones have an important role in behavioral development, confirming the results of studies with other species, but they were criticized for methodological limitations, especially for their use of subjective measures and insufficient controls. Recent studies with improved methodology generally confirm the findings of the earlier studies, but also show the complexity of hormonal influences on behavior.

For example, the best-studied clinical condition is CAH due to 21-hydroxylase deficiency, probably because it is one of the most common problems of sexual differentiation, with an incidence of 1 in 10,000 to 1 in 15,000 live births (Grumbach et al., 2002; Therrell et al., 1998). Because of an enzymatic defect caused by a single gene, individuals with CAH produce high levels of adrenal androgens very early in gestation. Postnatal treatment with corticosteroids (and mineralocorticoids for the 75% who are salt-wasters) reduces

hormone levels, generally to normal or subnormal levels (Speiser and White, 2003). Both sexes are affected by CAH, and both have been studied behaviorally, but studies of nonhuman species have generated clearer hypotheses for females than for males. Studies with male rodents and primates show that excess androgens may masculinize or demasculinize behavior, but generally have no effect, whereas studies of female rodents and primates show that excess androgens have masculinizing and defeminizing effects. Therefore, if sex differences in human behavior are affected by the levels of androgens present during sensitive periods of development, as occurs in other species, then females with CAH should show more "male-typical" and less "female-typical" behavior than control females. And they do in many, but not all, ways, with the size of the effect depending on the type of behavior investigated. These studies have recently been reviewed (Berenbaum, 2002; Berenbaum, 2001; Collaer and Hines, 1995).

While studies with clinical populations have provided valuable information about hormonal contributors to behavior, their design is not perfect (because of the methodological limitations and concerns about generalizability, see review, Cohen-Bendahan et al., in press) and such populations are also difficult to study (because of their relatively low frequency and the sampling problems). There has thus been an increased interest in developing alternative ways to study the behavioral effects of prenatal hormones, particularly within the normal range. These methods include the *direct* measurement of hormones to which the developing fetus is exposed and *inferential* (*indirect*) measures about the fetal hormone environment, e.g. prospective studies, and the twin paradigm.

Findings for nonhuman mammals and clinical populations strongly suggest that sex hormones play an important role in the development of behavioral differences between the sexes, and probably in producing within-sex behavioral variations, but it is important to test the generality of these effects by studying typical populations. An ideal study would involve direct measurement of fetal hormones at different times during gestation, to cover possible sensitive periods, and then a follow-up behavioral study in childhood

and beyond. The ideal is obviously difficult to realize because of the risks associated with collecting serum from living fetuses. But, it is possible to obtain "snapshots" of the fetal hormone environment, from samples of peripheral hormones, which are somewhat removed from direct fetal processes.

Three types of studies have examined fetal hormones: (a) studies of perinatal hormones obtained from *umbilical cord blood* at birth; (b) studies of prenatal hormones obtained from *maternal serum* by venipuncture during routine prenatal medical care (although opinion is divided as to whether maternal blood contains fetal sex hormones); (c) studies of prenatal hormones from *amniotic fluid* obtained during routine amniocentesis for diagnosis of genetic anomalies. The results of studies investigating the possible association between testosterone in amniotic fluid and childhood behavior have been inconsistent. Although some findings support the hypothesis that testosterone has masculinizing effects, others do not or even suggest the opposite (see review, Cohen-Bendahan, in press). These inconsistencies are most parsimoniously explained by methodological limitations: most behaviors studied did not show large sex differences, and sample sizes were very small. In addition, this approach is not practical because of the long follow-up needed, with the attendant problems (Ehrhardt and Meyer-Bahlburg, 1979).

3. GENERAL CONSIDERATIONS AND CAVEATS

Before moving to the main topic, we highlight a number of theoretical and methodological issues relevant to evaluating all studies of human hormone-behavior relations.

3.1. Theoretical Issues

Timing of Effects

Organizational vs. activational effects. Two types of hormonal effects have generally been distinguished: organizational and acti-

vational (Goy and McEwen, 1980). Organizational effects produce permanent changes in the wiring and sensitivity of the brain (Phoenix et al., 1959) and are thus largely irreversible; they are most likely to occur during early development when most neural structures are established. Activational effects occur later and are associated with concurrent changes in circulating hormone levels, for example, those associated with menstrual cycle variations (Cooke et al., 1999; Kimura and Hampson, 1994); here, hormones activate neural systems that were organized early in life. The distinction between organizational and activational hormones is not as clear as once believed (Arnold and Breedlove, 1985). For example, the human brain continues to develop into adolescence, so hormones that increase at puberty may change brain structure. Organizational hormones may prime the brain by changing its responsivity to hormones that are present later in life (Clark and Galef, 1998). Nevertheless, most work is based on the distinction between organizational and activational effects, so we will continue to use this distinction here. Because circulating sex hormones are low before puberty, any hormones affecting behavioral sex differences in childhood are likely to be due to organizational effects, i.e., hormones producing changes in the structure of the brain. Behavioral sex differences in adolescents and adults may reflect effects of organizational hormones or activational effects, given the large sex differences in the levels of circulating sex hormones after puberty. The main focus of this paper is on organizational effects occurring during the prenatal period, although there is good evidence for behavioral effects of activational hormones as well (see, e.g., Gadea et al., 2003; Becker et al., 2002; O'Connor et al., 2001; Moffat and Hampson, 2000; Kimura and Hampson, 1994).

Sensitive periods for organizational effects. It is widely accepted that organizational effects are maximal during circumscribed sensitive periods when the brain is developing, but the exact sensitive periods for human behavioral effects of sex hormones are not known. Weeks 8 to 24 of gestation have long been considered the key period (e.g., Collaer and Hines, 1995; Sikich and Todd, 1988),

given data showing a testosterone surge in male fetuses then (Smail et al., 1981). Nevertheless, there is increasing recognition that there may be multiple sensitive periods, and that different brain regions (and thus different behaviors) may be affected by hormones at different times. There are only limited data on this issue in human beings (Smith and Hines, 2000; Hampson et al., 1998), but ample data from other species, including primates, illustrate this point (Goy and McEwen, 1980). For example, as mentioned above, data from rhesus macaques show that androgen exposure early in gestation masculinizes different behaviors than exposure late in gestation (Goy et al., 1988).

There may be another sensitive period shortly after birth, associated with another peak in testosterone in male infants during postnatal months 1 to 5. Its significance for human behavior is not well-studied, but some information can be gleaned from studies in infant male monkeys who exhibit the same early postnatal peak. Those studies show that neonatal testosterone is important for genital development (Chemes, 2001; Brown et al., 1999), but there is no clear evidence for its role in behavioral development: neonatal testosterone has been found to affect mother-offspring interaction in one study of juveniles (Wallen et al., 1995), but not in another study of infants (Brown and Dixson, 1999), and not to affect sex-dimorphic play or sexual behavior (Brown and Dixson, 1999; Nevison et al., 1997; Wallen et al., 1995). Given continuing postnatal brain development, it would not be surprising to find that sex hormones continue to affect the brain and behavior after birth.

Feminization as an Active or Passive Process?

Much of the evidence about both physical and behavioral sexual differentiation focuses on the masculinizing effects of androgens. For a long time, it was believed that feminization is a passive process, occurring in the absence of high levels of androgens. There is increasing recognition of the importance of other hormones for complete feminization, but much is still unknown about this process (Grumbach et al., 2002). The ovary develops at about 3 months of gestation, but does not produce estradiol until later in

embryogenesis (Wilson et al., 1981) and even then does not produce significant amounts (Bin-Abbas et al., 1999). Fetuses of both sexes are exposed to high levels of estrogens from the placenta, perhaps explaining why estrogen does not play a large role in prenatal development. Very little is known about the human behavioral effects of ovarian estrogens during early development (organizational effects) (Collaer and Hines, 1995), although much is known about their effects during adulthood (activational effects) (see, e.g., Berenbaum et al., 2003; Becker et al., 2002; Resnick and Maki, 2001; Moffat and Hampson, 2000; Maki and Resnick, 2000; Kimura and Hampson, 1994). It has been suggested that estrogen's organizational effects occur during early postnatal rather than prenatal development (Fitch et al., 1998; Fitch and Denenberg, 1998), but there is no relevant evidence. Progestins from the ovary are thought to have anti-androgenic effects, but there are also reports that they act as androgens (Collaer and Hines, 1995), and it may be important to consider that effects vary by dose (Witt et al., 1995).

Subtleties of Hormone Action

Hormone levels. In nonhuman species, hormonal effects on behavior are dose-dependent, (animals exposed to high doses change more than animals exposed to low doses), and the level of hormones necessary to masculinize or defeminize behavior varies across behaviors (a given dose changes some behaviors more than others) (Goy and McEwen, 1980; Debold and Whalen, 1975). Some behaviors may be affected only by extreme variations in hormones, whereas others may be affected by relatively minor variations. Effects may be nonlinear. For example, there may be threshold effects, such that behavior is affected only when hormone levels exceed a specific point, with no additional effect with increasing levels.

Specific hormones. Masculinizing hormones come in many forms, and each affects different aspects of physical and behavioral sexual differentiation. For example, dihydrotestosterone is the metabolite responsible for differentiation of the external genitalia (Siiteri and Wilson, 1974); dihydrotestosterone and another metabo-

lite of testosterone, testosterone propionate, have different effects on learning abilities in monkeys (Bachevalier and Hagger, 1991). Some metabolites are more potent masculinizing agents than others, e.g., dihydrotestosterone is more potent than testosterone in masculinizing the external genitalia, but this has not been well studied with respect to human behavior. In some species, it is estradiol metabolized from androgen in the brain that is responsible for the masculine-typical development of some behaviors. For example, in rodents, estradiol masculinizes sexual behavior and learning (Breedlove, 1992; Williams and Meck, 1991; Goy and McEwen, 1980), but testosterone itself or dihydrotestosterone masculinizes rough play (Meaney, 1988).

Further, the effects of specific hormones depend on other hormones present, so that the presence of one hormone may promote or prevent the effect of another hormone (Goy and McEwen, 1980). For example, as mentioned above, progesterone may provide protection against masculinizing effects of androgens (Ehrhardt et al., 1984; Hull, 1981; Hull et al., 1980; Shapiro et al., 1976).

Hormone responsivity. Individuals vary not only in the levels of hormones to which they are exposed, but also in their sensitivity to those hormones. For example, variations in androgen responsivity caused by mutations in the human androgen receptor gene result in physical variations in men ranging from complete insensitivity to androgens (and thus female differentiation) to infertility and minor undervirilization (Casella et al., 2001; McPhaul et al., 1993). There has been very little study of the behavioral effects of variations in androgen sensitivity (Comings et al., 2002), but clearly this is something worth exploring.

Cross-species comparisons

Human studies are largely motivated by studies in other species, and largely confirm those studies in the general outline, that is, in revealing the behavioral importance of sex hormones present during early development. But it is crucial to recognize that the details of hormonal influences on human behavior do not always parallel those effects in other species given species differences in physiology.

Key examples concern the sensitive periods for hormone effects and the specific hormones responsible for masculinization.

The sensitive periods differ dramatically across species, in direct relation to the timetable of brain development. In rodents, brain development occurs during both prenatal and postnatal life, so the early postnatal period continues to be a sensitive period for the effects of sex hormones. In primates, however, much of structural brain development takes place prenatally, so this represents the most important sensitive period for brain and behavioral effects of sex hormones. Nevertheless, as noted above, the postnatal period may turn out to be another sensitive period for effects of hormones on some aspects of human behavior.

The specific hormones responsible for masculinization and defeminization of the brain and behavior may also differ across species. In rodents, these processes are largely dependent on estradiol as it is converted from testosterone in the brain via the action of the aromatase enzyme ("aromatization"). Female rodents are protected from the masculinizing effects of estrogen by a protein which binds circulating estrogen and prevents it from entering the brain (for reviews, see (Fitch and Denenberg, 1998; Goy and McEwen, 1980). It is unclear whether aromatization is important for human behavior, although the limited evidence suggests that it is not, with masculinization and defeminization resulting directly from the effects of testosterone or other metabolites (see the discussions of Complete Androgen Insensitivity and exposure to diethylstilbestrol, in Cohen-Bendahan et al., in press).

3.2. Methodological Issues

Measures

Studies in other species suggest – not surprisingly – that sex hormones affect behaviors that show sex differences. Accordingly, human studies examining behavioral effects of sex hormones have been considered valid only when the measures used differentiate between typical males and females (e.g., Hines et al., 2002; Hampson et al., 1998; Berenbaum, 1998; Collaer and Hines, 1995;

Resnick et al., 1986). Further, it is generally believed that the same factors that produce between-sex differences produce within-sex variation. For example, sex differences in early androgens are hypothesized to contribute to sex differences in spatial ability, and natural variations in levels or availability of androgens among normal males and females to within-sex variability in spatial ability. But, some have advocated the use of a broad range of behavioral measures, arguing against simple extrapolation of findings in animals to humans (Finegan et al., 1992), or that hormones may produce sex differences in brain organization and behavioral processes without necessarily producing average differences in performance (Fitch et al., 1998). Studies from this perspective have been exploratory in approach (e.g., Finegan et al., 1992).

Effect Sizes

The ability to detect behavioral effects of hormones, as any effect in science, requires that the study has sufficient statistical power. Effect sizes in this field vary considerably by behavior, and the design and interpretation of all studies require attention to the size of effect expected. Behaviors that show large sex differences are the best candidates for studying effects of hormones. Table 1 lists a representative sample of sex-related behaviors, along with the direction and general size of the sex difference in standard deviation units, *d* (Cohen, 1988), estimated from the studies reviewed below and others in the literature (e.g., Halpern, 2000; Kimura, 1999; Leveroni and Berenbaum, 1998; Berenbaum and Snyder, 1995; Resnick et al., 1993; Hyde and Linn, 1988; Kramer et al., 1988; Reinisch and Sanders, 1986). The size of the sex difference should be considered in interpreting the studies described in the sections below, because a measure that does not show a large sex difference is unlikely to be strongly influenced by sex hormones. (Despite appeals to use other measures, as mentioned above, it is difficult to interpret findings when measures do not show sex differences.) It is also valuable to consider the magnitude of hormone effects in relation to the magnitude of the sex difference, because this provides an indication of the extent to which hormones are likely to be responsible for the sex difference.

Table 1. Representative sex differences in behavior.

Trait	Direction of **Sex Difference**	***d*, Size of Sex Difference** [a]
Cognitive Abilities		
Spatial ability: mental rotation	M > F	large
Spatial ability: targeting	M > F	large
Verbal ability: fluency	F > M	small to medium
Verbal ability: memory	F > M	medium
Perceptual speed and accuracy	F > M	small to medium
Personality Traits		
Sensation-seeking	M > F	medium to large
Aggression	M > F	large
Nurturance	F > M	medium
Interest in babies	F > M	medium to large
Gender-Role Behaviors		
Interest in male-typical activities	M > F	very large
Interest in female-typical activities	F > M	very large
Preference for boys as playmates	M > F	very large
Preference for girls as playmates	F > M	very large
Sexual Orientation		
Arousal to females	M > F	very large
Arousal to males	F > M	very large

[a] *d* (mean difference/standard deviation) as reported in adults.

4. CO-TWIN SEX AS AN INDIRECT INDICATOR OF PRENATAL HORMONES – THE TWIN PARADIGM

After the above-mentioned theoretical background of issues involved in the study of sex differences, we continue with an overview of the background and results of twin studies, the main topic of this thesis.

4.1. Opposite-sex Twins

4.1.1. Background

Most evidence for behavioral and physiological effects of early hormones comes from nonhuman studies in which hormones are directly manipulated. Interestingly, however, there is good evidence that behavior and physiology are influenced by naturally-occurring variations in hormones that result from an animal's position in the uterus, particularly the sex of its littermates (intrauterine position, IUP) (for reviews, see Ryan and Vandenbergh, 2002; Clark and Galef, 1998; vom Saal, 1989). Female rodents that developed between male fetuses *in utero* are less female-typical in postnatal behavior (e.g., aggression, attractiveness to males), anatomy (e.g., anogenital distance, an aspect of genital morphology), and reproductive characteristics (pubertal maturation, reproductive life) than are female animals that developed between female fetuses *in utero*. This effect extends beyond rodents. For example, female swine surrounded by male swine *in utero* are more likely to participate in and to win fights than female swine positioned between female swine *in utero* (Rohde Parfet et al., 1990). The masculinizing effect on females of gestating close to males is attributed to the transfer of testosterone from the male fetus to the adjacent female fetus (Even et al., 1992). In some species, intrauterine position affects postnatal behavior, anatomy, and physiology in male animals too, presumably by the same mechanism. For example, male gerbils that developed between two females are less masculine than those that developed between two males in reproductive characteristics and sexual behavior (Clark and Galef, 1998). The studies of intrauterine position effects are consistent with studies in which hormones are manipulated directly and shown to affect later behavior (Clark and Galef, 1998) and confirm the importance for sex-typed behavior of exposure to sex hormones early in development.

As suggested by Resnick (1993) and Miller (1994), human twins might also be affected by the sex of the co-twin, providing a parallel to the intrauterine position effect in animals, and an opportunity

to examine the human behavioral and physiological effects of prenatal exposure to higher-than-average-female (or lower-than-average male) levels of testosterone. The female member of an opposite-sex (OS) twin pair is assumed, as a result of sharing the womb with a male co-twin, to be exposed to higher levels of testosterone during prenatal development than is a female member of a (dizygotic) same-sex (SS) twin pair. Thus, OS females should be more male-typical and less female-typical than SS females. Similarly, the male member of an OS twin pair might be exposed to lower levels of testosterone than a male member of a SS twin pair, making OS males less male-typical and more female-typical than SS males. It is important to note, however, that twins also share a postnatal environment, and that children with opposite-sex siblings may be exposed to a different gender-related social environment than children with same-sex siblings (McHale et al., 2001; Stoneman et al., 1986). This complicates interpretation of differences between OS and SS twins, as considered below. In this section we briefly review human studies of opposite-sex twins designed to investigate effects of prenatal sex hormones on physical and behavioral characteristics, interpreting findings in light of the strengths and limitations of the method.

4.1.2. Findings: Physical and Behavioral Traits in Opposite-sex Female Twins

Twin studies designed to investigate the possible masculinizing effect on behavioral and physical traits of prenatal exposure to testosterone in females have yielded contradictory results.

Physical Characteristics. The majority of twin studies have focused on *physical* or *psychophysical* characteristics, possibly because they are thought to be less likely to be influenced by postnatal environmental factors (although clearly that is not always true). Most studies failed to find differences between OS and SS females on a variety of measures of reproductive characteristics and handedness. Exceptions concerned tooth size (Dempsey et al., 1999) and spontaneous otoacoustic emissions, an auditory characteristic

that shows sex differences and is related to hearing sensitivity (McFadden, 1993): in both cases OS females were masculinized relative to SS females. In contrast, click-evoked otoacoustic emissions, another auditory characteristic that shows sex differences and is related to hearing sensitivity (McFadden, 1998), did not differ significantly between OS and SS females, although OS females were considered by the investigators to show "masculine" changes and the overall results were interpreted to suggest that prenatal exposure to testosterone leads to a more masculinized pattern of otoacoustic emissions (McFadden et al., 1996). Two large-scale studies have failed to show differences between OS and SS females in reproductive characteristics (Rose et al., 2002; Loehlin and Martin, 1998), and another study failed to find effects of co-twin sex on handedness (Elkadi et al., 1999).

Cognitive Abilities. The only twin study to examine this domain found OS females to have higher spatial ability than SS females (Cole-Harding et al., 1988). Although the results are consistent with prenatal testosterone effects, they are also consistent with effects of environmental factors associated with having an opposite-sex sibling, such as the availability of toys that might facilitate spatial ability (Cole-Harding, 2002; Stoneman et al., 1986). Further, the effect has not been subject to replication.

Personality and Gender-role Behavior. Some studies have found OS females to be more masculine than SS females, on traits such as sensation-seeking (Resnick et al., 1993), rule-breaking (Loehlin and Martin, 2000), and social attitudes (Miller and Martin, 1995), although differences are not always seen (Rose et al., 2002; Loehlin and Martin, 2000). There is some suggestion of an age effect in one study (Loehlin and Martin, 2000), with differences between twin groups in younger females (mean 23.4 years) but not in older ones (mean 41.2). Although the age division does not reflect clear developmental distinctions, it is possible that testosterone effects diminish with age, as influences of other factors, such as circulating hormones and social environmental events, become salient. This suggests an important need to consider age in these studies.

Other studies showed mixed results in comparisons of OS and SS females on a variety of traits. In a large Australian study, very few traits differentiated the twin groups, with the exception of retrospectively-recalled sex-typed childhood behavior (Dawood et al., 2004). This stands in contrast to three studies in children, in which sex-typed behavior was measured and found not to differentiate OS and SS females (Rodgers et al., 1998; Henderson and Berenbaum, 1997; Elizabeth and Green, 1984), suggesting the possibility of *Type I* error with many comparisons in the Australian study. Inconsistencies across studies might reflect differences in age.

4.1.3. Findings: Physical and Behavioral Traits in Opposite-sex Male Twins

A few studies examined co-twin effects in males, hypothesizing, on the basis of rodent studies, that OS males would be demasculinized/feminized compared to SS males or non-twin males. These studies were originally focused on co-twin effects in females, but also studied males. Therefore, they examined the traits described above in studies of females. Most studies failed to find demasculinization in males with a female co-twin on physical traits, such as tooth size (Dempsey et al., 1999), psychological traits, such as social attitudes (Loehlin and Martin, 2000) and sensation-seeking (Resnick et al., 1993), spatial ability (Cole-Harding et al., 1988), sex-typed toy play (Rodgers et al., 1998), and handedness (Elkadi et al., 1999). Interestingly, however, there is some suggestion that males with a female co-twin might be demasculinized or feminized on gender-role behavior (Dawood, 2004; Elizabeth and Green, 1984), but it is not possible to know whether this reflects effects of gestating in proximity to a female or being reared with one.

4.1.4. Interpretation of Findings in Opposite-Sex Twins

The results of the studies summarized above show a mixed picture regarding effects for females of having an opposite sex co-twin and not a lot of information regarding effects for males. The positive effects in females primarily concern masculinized tooth size, spon-

taneous otoacoustic emissions, and spatial ability in female OS twins, but it is important to note that these positive findings have not been replicated. This masculinization (if replicated) is difficult to explain by gender-socialization factors. Moreover, if OS females are raised in a more male-typical environment than SS females, and this affects behavior, then there should be many and pronounced differences between OS and SS females. This is clearly not the case. Nevertheless, twin studies need to have better control for the role of the social environment. This would involve a comparison group of singleton girls with an older brother very close in age; only one twin study used such a comparison group (Henderson and Berenbaum, 1997). Although this is the best comparison currently available, it is not perfect, because same-aged siblings, as in twin pairs, might affect each other differently than siblings of different ages.

5. AIMS OF THE STUDIES PRESENTED IN THIS THESIS

The aim of the studies presented in this thesis was to investigate and elaborate the twin paradigm in a structured manner in order to develop a fairly easy method to investigate the biological basis of sex differences. We investigated different measures during important transitional phases of development, i.e. the pre-pubertal period and puberty (the longitudinal element of the study), and controlled for the possible activational effect of current circulating levels of testosterone.

Given the constraints of the limited research period, we decided to focus our research on 10- and 13-year old-children. We hypothesized that we would be able to study the organizational effects of testosterone in 10-year-old children because most children are pre-pubertal at this age. In contrast, we hypothesized that when the children were 13 years old, the surge of hormones that accompanies puberty would make it possible to measure additional activational effects of testosterone. For this reason, we established a battery of tests and questionnaires that could be used for both ages. However, the difference in mental age was rather large between the two test-

ing sessions, and we could administer only one instrument in the exact same manner in both age groups (see, *chapter 3*).

We focused on the following issues. First, because we were interested in whether the sex of the fetus is associated with maternal serum levels of steroids in human twin pregnancies (*chapter 2*). We investigated the effects of presumed prenatal exposure to testosterone on the hemispheric specialization and cognitive abilities of 10-year-old children (*chapters 3 and 4*), and then studied the behavioral effects of such prenatal exposure in the same children when they were 13 years old (*chapter 5*). At the age of 13 years, the children were retested with the task to evaluate hemispheric specialization. The results are presented at the end of *chapter 3.* In order to validate the twin paradigm, we also investigated another indirect method of evaluating the effects of prenatal exposure to sex hormones, namely the finger ratio, in our sample (*chapter 6*). In addition we tested whether the finger ratio is associated with specific characteristics in our twin sample. In *chapter 7* we finish with the results on educational achievement using the twin paradigm.

Chapter 2

Maternal serum steroid levels are unrelated to fetal sex: A study in twin pregnancies

Cohen–Bendahan CCC, van Goozen SHM, Buitelaar JK, Cohen–Kettenis PT, Twin Research and Human Genetics, in press.

ABSTRACT

Increased prenatal exposure to testosterone in girls of an opposite-sex (OS) twin pair may have an effect on the development of sex-typical cognitive and behavioral patterns. The prenatal exposure to testosterone due to hormone transfer in OS twin girls may occur in two ways, one directly via the feto-fetal transfer route within the uterus, the other indirectly through maternal-fetal transfer and based in the maternal-fetal compartment. Although some studies in singletons indeed found that women pregnant with a male fetus have higher testosterone levels during gestation than women pregnant with a female fetus, many other studies could not find any relation between the sex of the fetus and maternal serum steroid levels. Therefore at the moment it is unclear whether a pregnant women bearing a male has higher levels of testosterone than a women bearing a female. So far, no one investigated this issue in twin pregnancies. We examined the relationship between maternal serum steroid levels and sex of fetus in 17 female-female, nine male-male and 29 OS twin pregnancies.

No differences were observed between maternal serum steroid levels of women expecting single sex or mixed sex offspring's. It is concluded that the source of prenatal testosterone exposure in girls probably comes from the fetal unit, which is the direct route of fetal hormone transfer.

1. INTRODUCTION

Research on normal healthy twins may provide an opportunity to investigate the possible prenatal effects of testosterone on brain and behavior. In animal research it has been shown that exposure to steroids is influenced by the intrauterine position of the fetus. Female fetuses located between two male fetuses are exposed to higher levels of testosterone than fetuses situated between two females or one female and one male fetus (Gandelman, 1992; Vom Saal, 1989; for review, see Ryan and Vandenbergh, 2002). Fetal hormone transfer may occur in two ways: (1) more indirectly via the maternal-fetal transfer route, as Meulenberg and Hofman (1991) described; and (2) more directly via the feto-fetal transfer route. With respect to the latter route, it has been shown in rodents that testosterone can diffuse across amniotic membranes (e.g., Even et al., 1992). At present it is unclear whether similar processes operate in human opposite-sex (OS) fetal twins. Evidence in support of an increased prenatal testosterone exposure effect in female animals which were located between male fetuses comes from the observation that these female animals have anatomical characteristics and show behavioral patterns more typical of males (Ryan and Vandenbergh, 2002). The few studies that have been conducted in human opposite-sex twins suggest that female fetuses of an OS twin pair could have been masculinized in a similar way (see Miller, 1998, for review). For example, it has been found that OS twin girls have a number of spontaneous otoacoustic ear emissions (McFadden, 1993) and tooth sizes (Dempsey et al., 1999) that are more similar to males than to females. Also, OS twin girls seem to have more masculine attitudes compared to same-sex twin girls with regard to Sensation seeking behavior (Resnick et al., 1993).

Although it is presently unclear how the increased prenatal testosterone exposure – if it indeed exists – is caused, it presumably occurs through direct or indirect hormone transference between the OS fetuses.

In the seventies and early eighties of the last century several investigators tried to establish a link between fetal sex and maternal

testosterone levels (Rivarola et al., 1968; Forest et al., 1971; Dawood and Saxena, 1977; Klinga et al., 1978; Nagamani et al., 1979; Bammann et al., 1980; Glass and Klein, 1981; Rodeck et al., 1985). At the time, this information was considered to be useful in the antenatal determination of fetal sex, which in turn would be a fairly inexpensive screening tool in, for example, the diagnosis of sex-linked genetic disorders (Glass and Klein, 1981). However, this seemed not to be very productive because inconclusive results and an extensive overlap between the sexes.

Years later Meulenberg and Hofman (1991) again investigated the relationship between fetal sex and maternal testosterone serum concentrations and concluded that male fetus pregnancies were associated with higher maternal serum testosterone levels in the second half of the pregnancy. The maternal serum testosterone levels differed in this period at least 1 standard deviation among male/female fetus pregnancies. Like Bammann et al. (1980), they claimed that this could be due to "a gradient from the fetal to the maternal compartment at the level of plasma unbound testosterone" (p. 53; Meulenberg and Hofman, 1991). So far, all the previously conducted studies have focused on singleton pregnancies. Since the maternal-fetal gradient of unbound testosterone is able to cross the placenta from the male fetus towards the maternal blood circulation it would be interesting to find out whether maternal serum testosterone levels reflect the number of male fetuses the pregnant mother is carrying in her womb. If this is true, than women carrying male-male twins should have higher testosterone levels than women carrying OS twins, and this latter group of women should again have higher levels than women expecting female-female twins.

In that case, this may reflect the assumption of the (indirect) maternal-fetal route of hormone transfer between co-twins. This can be the hormonal basis for the unusual hormonal milieu wherein OS twin girls are situated and may lead to the rather sex atypical patterns that are found in females with a co-twin brother.

In summary, this present study focused on whether the sex of the fetus is associated to maternal serum steroid levels in twin preg-

nancies. Which may be an indication for the possible indirect route, i.e. via the maternal-fetal compartment, of testosterone exposure through hormone transfer during twin pregnancies.

Research in the past has shown that subfertile women may have a different endocrine profile than women without fertility problems. For example, in women with polycystic ovary syndrome, a common disorder in subfertile women, one of the three possible criteria for diagnosis is biochemical features of hyperandrogenism (Agrawal et al., 2004). Because a part of the women that participated in our study will become pregnant as a result of the treatment of their subfertility (non-spontaneous twin pregnancies) we will consider that in our analysis in order to control possible effects of different endocrine profiles.

2. MATERIALS AND METHODS

2.1. Subjects

All participants were recruited, between August 2000 and August 2002, from the Department of Obstetrics at the University Medical Center in Utrecht (The Netherlands) after ultrasonic examination had confirmed a twin pregnancy. The women were invited to participate in this study by an information letter. From the 89 women who were invited to take part, 59 agreed to participate. Eventually, the data of 55 women were used in this study because one woman had a miscarriage, in two women one of the twins died during pregnancy, and one woman withdrew from the study without giving a reason. Of these 55 women carried nine Male-Male (MM), 29 OS and 17 Female-Female (FF) twin pairs. The average age of the women was 33.9 years (SD = 3.4; range 25-41). Thirty-eight women were nulliparous. There were 29 spontaneously conceived twin pregnancies and 25 pregnancies were induced as a result of for example *in* vitro fertilization, for one woman we were unable to trace the conception form (see Table 1). A written informed consent form was obtained from all participating women.

The Medical Ethical Committee of the University Medical Center in Utrecht approved the study.

Table 1. The numbers of spontaneous and non-spontaneous pregnancies per typed twin:

	FF	DOS	MM
Spontaneous	12	12	5
Non spontaneous	4	17	4

Note: From one woman we were unfortunately unable to retrieve conception form.

2.2. Materials

Two samples of blood serum were obtained from the participants: One sample was taken at 24 weeks and one at 32 weeks of gestation. The sera was taken during a normal visiting appointment with the gynecologist. Because of logistic reasons and the longitudinal character of this study some data are missing. See Table 2 for an overview of the number of hormone samples. All samples were collected and stored at –30^{o}C until assayed.

Several steroids were measured: Testosterone (T), Progesterone (Prog), Dehydroepiandrosterone-sulfate (DHEAS), Sex hormone-binding globulin (SHBG), Estradiol (E2), and Androstenedione (A); Free Testosterone (FT) was calculated.

Testosterone was measured after diethylether extraction using an in house competitive radioimmunoassay (RIA) employing a polyclonal antitestosterone-antibody (Dr.Pratt AZG 3290). [1·,2·-^{3}H(N)]-Testosterone (NET-387, DuPont NEN Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 0.12 nmol/L and inter-assay variation was 7,6; 5,0 and 5,9% at 1,5; 4,9 and 24 nmol/L respectively (n = 45).

Androstenedione was measured after hexane-toluene extraction using an in house competitive RIA employing a polyclonal anti-androstenedione-antibody (Dr.Pratt AZG 271178). [1,2,6,7-^{3}H]-Androst-4-ene-3, 17ß-dione (TRK 454, Amersham Pharmacia biotech) was used as a tracer following chromatographic verifica-

tion of its purity. The lower limit of detection was 0.2 nmol/L and inter-assay variation was 9,0; 5,5 and 5,7% at 1,4; 5,0 and 11,5 nmol/L respectively (n = 20).

DHEAS was measured using an immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 0.1 μmol/L and inter-assay variation was 7,0; 5,9 and 4,9% at 1,0; 4,7 and 14,3 μmol/L respectively (n = 50).

SHBG was measured using an immunometric technique on an IMMULITE Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 5 nmol/L and inter-assay variation was 5,5; 4,1 and 5,3% at 14; 34 and 91 nmol/L respectively (n = 23).

Estradiol (E2) was determined using the Axsym of Abbott (Abbott Park, Ill. 60064). Sera was diluted with phosphate buffered saline (0.01 Mol at pH 7.0, containing 0.5% Bovine Serum Albumin) (second trimester serum 20x, third trimester serum 40x). Interassay variation was 5.1% at 1060 pmol/L.

Progesterone (Prog) was determined by the above-mentioned method with a dilution of 10x. Interassay variation was 5.0% at 18 nmol/L.

Free testosterone (FT) was calculated using the equations described by Dunn et al. (1981). These equations can be used to describe the relation between bound and free fraction of testosterone with other steroids and with several binding proteins. We made calculations for a system of two steroids (E2 and T) and two binding proteins (albumin and SHBG). The value of albumin in maternal serum was fixed at 35 g/L for measurements at 24 weeks gestation and 33 g/L at 32 weeks gestation.

2.3. Statistical analyses

To establish that the maternal blood serum steroid levels indeed showed sex differences we compared the mean levels of the three possible groups (female-female (FF), opposite-sex (OS), and male-male (MM)) at both time points (i.e., 24 and 32 weeks) using a

repeated measures model. Separate analyses of repeated measures for the "male" steroids (i.e. T, FT, A, DHEAS, SHBG) and for the "female" sex hormones (i.e. E2 and PROG) were conducted.

3. RESULTS

3.1. Sex

The pregnant women who participated in the study gave birth to nine MM, 29 OS, and 17 FF twins; thus 47 babies were a boy and 63 were a girl, which gives a sex ratio of 0.75. This is different from the sex ratio normally found in singletons, namely 1.045.

3.2. Group and Time differences in maternal serum steroids: T, FT, A, DHEAS, and SHBG

None of the levels of prenatal steroids displayed any statistically significant difference between the three groups (i.e., MM, OS, and FF), and only a main Time effect was found for FT ($F(1,31) = 5.29$; $p<.05$), SHBG ($F(1,31) = 10.17$; $p<.01$), and DHEAS ($F(1, 31) = 5.30$; $p<.05$) These data reflect the fact that in all groups there was a progressive increase in FT and SHBG during pregnancy, but a decrease in DHEAS (see Table 2). There were no statistically significant interactions between Group and Time, and it can therefore be concluded that the groups responded in the same manner to the effect of time for all hormones assessed.

Table 2. Mean maternal steroid sera levels in women pregnant with a twin, according to the sex of the newborn twins, (SD in parentheses).

Steroid		Maternal serum 24 weeks	$N_{24weeks}$	Maternal serum 32 weeks	$N_{32weeks}$
DHEAS (mmol/L)	FF twin	3.07 (1.60)	15	2.45 (1.30)	12
	OS twin	2.99 (1.81)	27	2.09 (1.28)	22
	MM twin	3.04 (1.35)	7	2.74 (0.83)	7
Androstenedione (nmol/L)	FF twin	8.01 (4.31)	15	6.91 (4.82)	13
	OS twin	6.20 (2.79)	27	4.99 (2.60)	21
	MM twin	6.20 (2.82)	7	7.78 (5.41)	6
SHBG (nmol/L)	FF twin	403.00 (107.50)	15	455.83 (153.52)	12
	OS twin	434.44 (75.34)	27	465.23 (81.95)	22
	MM twin	452.14 (94.95)	7	496.43 (97.03)	7
Testosterone (nmol/L)	FF twin	5.26 (3.55)	15	3.79 (2.20)	12
	OS twin	3.95 (2.21)	27	2.96 (1.82)	22
	MM twin	3.50 (1.59)	7	5.06 (3.84)	7
Progesterone (nmol/L)	FF twin	482.67 (180.32)	15	894.83 (216.60)	12
	OS twin	493.00 (138.27)	27	1046.18 (301.84)	22
	MM twin	455.86 (163.76)	7	1104.29 (297.34)	7
Estradiol (pmol/L)	FF twin	117493 (37855)	15	182200 (66079)	12
	OS twin	115559 (50799)	27	152181 (55978)	22
	MM twin	106971 (24802)	7	182771 (57781)	7
Free Testosterone (pmol/L)	FF twin	70.68 (47.36)	15	77.56 (70.14)	11
	OS twin	48.58 (29.50)	27	55.23 (38.01)	22
	MM twin	41.00 (23.68)	7	93.46 (59.16)	7

Note: male-male twin (MM); opposite-sex twin (OS); female-female twin (FF)

3.3. Group and Time differences in maternal serum sex hormones: E2 and PROG

There were no differences between the three twin groups in maternal serum levels of Estradiol and Progesterone. However, these hormone levels increased significantly over time (E2 ($F(1,34) = 39.85$; $p<.001$) and PROG ($F(1,34) = 149.80$; $p<.001$), see Table 2).

3.4. Former analysis including the conception form as a covariate

The chi-square test was not significant for the distribution of type of twin between the different conception forms, i.e. spontaneous and non-spontaneous. However, because of the possible interference of an abnormal endocrine profile in the non-spontaneous women we entered in the model in an extra analysis the way of conception. This showed that only the SHBG ($F(1,29) = 5.91$; $p<.05$) and PROG ($F(1,32) = 15.02$; $p<.001$) still increased significantly over time for all twin groups.

4. DISCUSSION

According to the hypothesis of hormone transfer between co-twins (Miller, 1998), exposure to testosterone *in utero* may occur via the maternal-fetal route apart from the feto-fetal route. This study was designed to find (indirect) support for this possibility. We predicted that if the maternal serum steroid levels depend (at least partly) on the sexes of the twin in the womb, the mothers of MM twins would show different levels of steroids in their serum as an indication of the hormone transfer via the maternal-fetal blood circulation than mothers of OS twins or FF twins. This expectation is based on the idea that the maternal serum levels are affected because of concentration-gradient, i.e. difference at the level of unbound testosterone with higher levels in the male fetus compared to the maternal concentrations (Meulenberg and Hofman, 1991). In singleton pregnancies differences in maternal serum levels of androgens of women pregnant of boys or girls have been found (e.g. Meulenberg and Hofman, 1991) but there are also studies, which have failed to find such a relationship between fetal sex and maternal serum steroid levels (e.g. Nagamani et al., 1979; Rodeck et al., 1985).

In the present study we collected serum twice (i.e., in week 24 and 32 of gestation) in nine women pregnant of MM twins, 29 women pregnant of OS twins, and 17 women pregnant of FF twins.

We were unable to establish any difference in maternal serum steroid levels between mothers carrying a MM twin pair, FF twin pair or OS twin pair.

However, the present outcome together with previous non-findings questions the reliability of the two studies in singletons that observed a difference in maternal serum steroid levels, although both did not agree on the period of the pregnancy in which the elevation of testosterone occurred (Meulenberg and Hofman, 1991; Klinga et al., 1978). In this respect our findings could be best compared to the study of Meulenberg and Hofman (1991) since their measurements were done during the same period.

Therefore, no support was found in this study for a maternal-fetal route of the fetal hormone transfer notion. It seems therefore likely that the maternal compartment of the maternal-fetal unit is a fairly different one than the fetal compartment (i.e., the amnion sac). It has been shown rather convincingly in several studies (e.g., Nagamani et al., 1979; Rodeck et al., 1985; Van de Beek et al., 2004) that amniotic fluid testosterone levels are higher when measured in the presence of a male fetus compared to a female fetus. This supports the notion that prenatal exposure to testosterone in fetuses is not so much via the more indirect maternal-fetal route, as the fetal hormone transfer notion suggests, but rather by the other route, i.e. the feto-fetal route. In animal research this mechanism has been observed more systematically and there is clear support that transport of testosterone occurs through fetal membranes (e.g., Even et al., 1992).

However, since we also observed a change in several maternal serum steroids during gestation which is assumed to be of fetal origin no matter which sex (Bammann et al., 1980), we can not completely rule out any exchange of steroids between the maternal-fetal compartment. Evidence for such an underlying assumption, that fetal and maternal compartments affect each other hormonal states, is mentioned in the literature (see, Miller, 1998).

Although the participating pregnant women were not selected on basis of the sex of their unborn children, the number of women pregnant with a MM twin pair turned out to be unexpectedly low

(i.e., nine) compared to the other types of twin pregnancies (respectively 29 OS – and 17 FF-twins). This might be because of an artifact due to hormone induced pregnancies (James, 1986; Orlebeke et al., 1993).

In sum, we must clearly be cautious in drawing any strong conclusions since it is possible that we were not able to demonstrate an effect of fetal sex on maternal serum steroid levels due to the fact that one of our sub-groups (i.e., the MM group) was small.

However, our results seem to suggest for now that any presumed increased prenatal exposure to testosterone in female fetuses sharing the womb with males, as in opposite-sex twins, occurs mainly through the direct feto-fetal route of hormone transfer in the amniotic sac and not the indirect one via the maternal-fetal unit. Since this study failed to find evidence for the maternal-fetal (indirect) route we believe that the two routes of hormone transfer are depending on different compartments. Measuring maternal serum steroid levels in the maternal unit during pregnancy does not seem to be of any use in investigating prenatal exposure to steroids in twins.

Nevertheless, final conclusions can only be drawn after our results have been replicated.

Chapter 3

Prenatal exposure to testosterone and functional cerebral lateralization: A study in same-sex and opposite-sex twin girls

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ABSTRACT

In animals it has been shown that exposure to sex hormones is influenced by intrauterine position. Thus fetuses located between two male fetuses are exposed to higher levels of testosterone than fetuses situated between two female fetuses or one female and one male fetus. In a group of opposite-sex (OS) twin girls and same-sex (SS) twin girls a potential effect of prenatal exposure to testosterone on functional cerebral lateralization was investigated. We hypothesized that prenatal exposure to testosterone would result in a more masculine, i.e. a more lateralized pattern of cerebral lateralization in OS twin girls than in SS twin girls. An auditory-verbal dichotic listening task (DLT) was used as an indirect method to study hemispheric specialization. Firstly, we established a sex difference on the DLT. Compared with SS girls, OS twin boys showed a more lateralized pattern of processing verbal stimuli. Secondly, as predicted OS girls had a more masculine pattern of cerebral lateralization, than SS girls. These findings support the notion of an influence of prenatal testosterone on early brain organization in girls.

1. INTRODUCTION

As early as the nineteenth century the superiority of each hemisphere for specific skills had been discovered (Geschwind and Galaburda, 1987). The majority of human beings are found to have a functional cerebral lateralization pattern that is characterized by the left hemisphere specializing in the processing of verbal material and the right hemisphere in nonverbal and emotional processing (Geschwind and Galaburda, 1987; Kimura, 1999). However, this patterning of functional cerebral lateralization has been demonstrated to be stronger for men than for women, i.e. males exhibit a more asymmetrical functional cerebral lateralization pattern than women do. The specific mechanism explaining this sex difference in functional cerebral lateralization is as yet unclear, but it has been suggested that the prenatal sex hormonal environment could play a role. Specifically, it has been proposed that the sex hormone testosterone (Goy and McEwen, 1980; Wisniewski, 1998; Kelso et al., 1999) during the critical period of the pregnancy, around week 16, has an important impact on the development of the fetal brain by influencing functional cerebral lateralization (Hines and Shipley, 1984; Geschwind and Galaburda, 1987; Witelson, 1991; Kelso et al., 1999). Important processes such as neuronal growth, and proliferation have been shown to be co-regulated by sex hormones during critical periods of early brain development (Goy and McEwen, 1980; Geschwind and Galaburda, 1987; Erlanger et al., 1999).

A pathway through which this might occur is the aromatization of testosterone to estradiol. From animal studies it is known that this process enables testosterone to bind to estrogen as well as to testosterone receptors, which has a critical role in the masculinization and defeminization of specific brain structures. This has less effect in females since they are protected from the masculinizing effects of estrogen via a protein called alpha-fetoprotein (AFP). AFP binds to freely circulating estrogen and prevents it from crossing the blood-brain-barrier and consequently from entering the neuron (see, Fitch and Denenberg (1998), for review). However, at present it is unclear whether similar processes operate in humans.

Since deliberately manipulating the perinatal sex hormonal environment in humans is not only impossible but also unethical, most research has focused on animal designs and on clinical human samples with a medical condition in which they have been exposed to higher levels of sex hormones either pre- or postnatally.

For example, with respect to animal studies in the Long-Evans rat a sexual dimorphism has been observed with the male rat showing a thicker right hemispheric cortex, and the female rat revealing the reversed pattern. However, when the female rat was ovariectomized at birth and the testes of the male rat were removed, the typical cerebral patterns in each sex could be altered (Goy and McEwen, 1980; Geschwind and Galaburda, 1987; Diamond, 1991). On the basis of animal research it can be concluded that the administration of testosterone (or its metabolites) to females, during pregnancy or shortly thereafter, increases male-typical patterns and decreases female-typical patterns of functional cerebral lateralization (Goy and McEwen, 1980; Geschwind and Galaburda, 1987).

Functional cerebral lateralization may be studied in humans using a dichotic listening task in which ear advantage in terms of correct responses reflects the dominance of the contralateral hemisphere for that specific material (Bouma, 1998; Bouma et al., 1998; Kimura, 1967). A right-ear advantage (REA) has been observed in normal, right-handed subjects when the stimuli consist of verbal material (Bouma, 1998), which is thought to reflect the left hemispheric dominance for language. The REA is enhanced in men since men are on average more lateralized for verbal stimuli presented to the left-hemisphere than women (Voyer, 1996; Cohen-Kettenis et al., 1998).

In clinical samples, for example women who have been exposed prenatally to diethylstilbestrol (DES), which has a masculinizing and defeminizing effect, it has been found that they have a more masculine pattern of functional lateralization. Specifically, they appear to show an enhanced right-ear superiority on the dichotic listening task compared to their unexposed control sisters (Hines and Shipley, 1984).

However, in another clinical group consisting of women with congenital androgen hyperplasia (CAH), a genetic disorder that exposes them to extremely high levels of androgens prenatally, the expected higher REA was not found (Helladay, et al., 1994; Kelso et al., 2000).

Some limitations and confounding influences of the above cited studies could hamper the extrapolation of these results to the "normal" general population. One of these is that clinical groups may have other medical problems, which might complicate the interpretation of the results. For example, it is known that a large proportion of CAH patients suffers from salt-wasting which may lead to episodes of hypotension or hyponatremia and these conditions can permanently affect the functioning of the brain (Nass and Baker, 1991).

Research on normal healthy twins may also provide an opportunity to investigate the possible prenatal effects of testosterone on brain and behavior. In animal research it has been shown that exposure to *testosterone*, or its metabolites, is influenced by the intrauterine position of the fetus. Female fetuses located between two male fetuses are exposed to higher levels of testosterone than fetuses situated between two female or one female and one male fetus (Vom Saal, 1989; Gandelman, 1992). Fetal hormone transfer may occur in different ways: (1) via the maternal-fetal transfer route as Meulenberg and Hofman (1991) have shown in their study, "As a consequence of a maternal-fetal gradient, unbound testosterone crosses the placenta from the male fetus towards the maternal circulation, whereas the opposite occurs from the maternal circulation towards the female fetus" (p.53) (see Miller 1998 for review); and (2) via the feto-fetal transfer route because it has been shown (in rodents) that testosterone can diffuse across amniotic membranes (e.g. Fels and Bosch, 1971; Even et al., 1992).

Likewise it could be predicted that prenatal exposure to testosterone of female fetuses would have effects on a variety of anatomical and behavioral aspects, in a way that differs from the typical female pattern. Such effects could be studied in female twins. Presently, however, very few human studies have investigated the

fetal hormone transfer notion in twins (e.g. Loehlin and Martin, 1998; Miller, 1998). One study reported that females from an opposite-sex (OS) twin pair had more male like spontaneous otoacoustic emissions than females from same-sex (SS) twin pairs (McFadden, 2002). In another study it was found that OS girls outperformed SS girls on spatial ability (Cole-Harding et al., 1988).

These prenatal hormonal effects on behavior and cognition, as discussed above, are also known as the organizing effects of sex hormones; such effects are seen as structural and to a large extent irreversible. Organizing effects are distinguished from the so-called activating effects of sex hormones. With these we refer to the postnatal behavioral effects that are observed as a result of concurrent changes in hormone levels, i.e. hormonal changes related to the menstrual cycle or to puberty (Cooke et al., 1999; McEwen, 1999). For example, sex differences in lateralization which can manifest themselves early in life could be due to organizing effects of testosterone, since organizing effects establish structural differences in the neural basis of the brain (Goy and McEwen, 1980). However, they can also be the consequence of circulating activating effects of testosterone, since it has been shown that circulating testosterone levels, as measured in saliva, have an effect on cerebral lateralization (e.g. Moffat and Hampson, 2000; Gadea et al., 2003).

The aim of this present study was to explore the notion of differences in prenatal testosterone exposure in SS and OS female twins by investigating whether OS twin girls as compared to SS twin girls would show a pattern of functional cerebral lateralization away from the typical female pattern. In addition, to investigate the possible circulating or activating effects of testosterone on performance, we also collected in all participants saliva and analyzed it for levels of testosterone.

2. METHOD

2.1. Subjects

The subjects in this study were recruited from the Netherlands Twin Register (NTR) of the Vrije Universteit in Amsterdam. Twins were included in this study when (1) they were born in the year 1989; (2) they had expressed an interest in the past for participating in scientific research; and (3) they were right-handed.

In total 67 opposite-sex (OS) twin girls, 67 OS twin boys, and 53 girls from same-sex (SS) dizygotic twin pairs with a mean age of 10.96 years (range: 10.43 – 11.84) agreed to participate. Written informed consent was obtained from the parents, and the twins themselves gave oral consent.

2.2. Materials and procedure

2.2.1. Dichotic listening task

A three pair digit Dichotic Listening Task (DLT; Bouma, 1998) was used to measure functional cerebral lateralization, presenting auditory-verbal stimuli. Monosyllabic numbers were presented dichotically through headphones.

To control for attentional biases which have been reported to occur in children when using free recall methods (Bryden and Allard, 1981), we used the version of the DLT in which the children were required to focus their attention on one of the ears, determined by a tone presented before presentation of the numbers. The tone indicates the order of oral report by the child. The test consisted of 18 practice trials and 20 test trials. Total presentation time was 1500 msec per trial (500 per pair). A right-ear correct-score, a right-ear missed-score, a left-ear correct-score, left-ear missed-score, and a Laterality Index (LI) were calculated.

The formula for the Laterality Index is as follows:

_ = ln [(R * _) / (ÿ* L)], where R is the number of correct right-ear responses, ÿ the number of missed right-ear responses, L the num-

ber of correct left-ear responses, and £ the number of missed left-ear responses. A negative LI lambda score indicates a left-ear superiority whereas a positive score reflects a right-ear advantage. The variation in overall accuracy among the subjects is adjusted by the use of LI because it is mathematically independent (McCormick and Witelson, 1994; Saxby and Bryden, 1984; Bryden and Sprott, 1981).

2.2.2. Hormone assay

Testosterone in saliva was measured after diethylether extraction using an in house competitive radio-immunoassay employing a polyclonal antitestosterone-antibody (Dr.Pratt AZG 3290).

[1,2,6,7-^{3}H]-Testosterone (TRK402, Amersham Nederland B.V.) was used as a tracer following chromatographic verification of its purity.

The lower limit of detection was 2.9 pg/mL and inter-assay variation was 10% at 12 pg/mL respectively (n = 180).

2.3. Statistical analyses

First, to establish that the DLT indeed showed sex differences we compared the mean LI scores of OS boys to those of the SS girls by independent-samples *t*-tests. Thereafter, we analyzed ear advantage and possible interactions with Type in a 2 X 2 (right-ear/left-ear X boys/girls) repeated measures analysis using multivariate procedures. Finally and in a similar way, we compared the two girls groups in order to establish from a statistical point of view that OS girls show a pattern of functional cerebral lateralization away from the typical female pattern. In case of directional hypotheses we used one-tailed tests.

In order to control for the effects of possible circulating free testosterone levels on performance we compared the free testosterone levels of the three groups by using a linear contrast matrix and we calculated correlations between testosterone and LI.

3. RESULTS

3.1. Sex differences: comparison between OS boys and SS girls

As expected, we found a sex difference on the mean Laterality Index (LI) score, with the OS boys showing a higher Laterality Index compared to the SS girls ($t(116) = 3.18$, $p < 0.01$, (two-tailed)). The mean LI for both groups is illustrated in Figure 1.

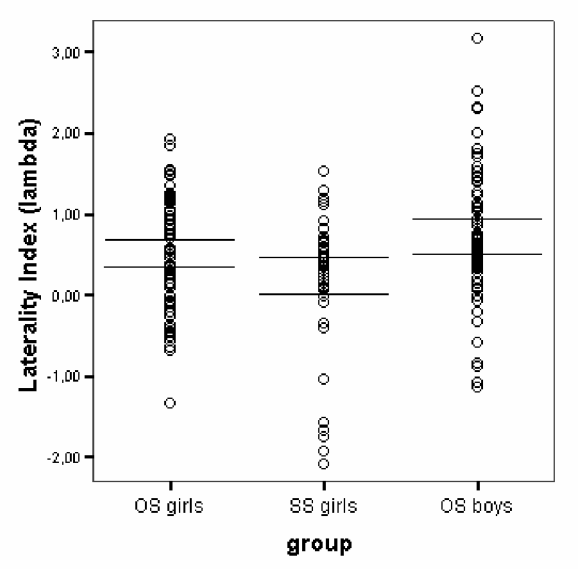


Figure 1. Scattergram of the Laterality Index (LI; Lambda) as measured with the Dichotic Listening Task (DLT) for the Opposite-sex (OS) girls, Same-sex (SS) girls, and OS boys. The error bar represents the 95% mean confidence interval LI for each group.

A main effect of ear was found indicating a clear right-ear advantage in the total (combined OS boys and SS girls) group ($F(1, 118) = 33.174$, $p < 0.001$), and we found an interaction between ear and sex with boys showing a more lateralized pattern ($F(1, 118) = 7.636$, $p < 0.01$), see Figure 2).

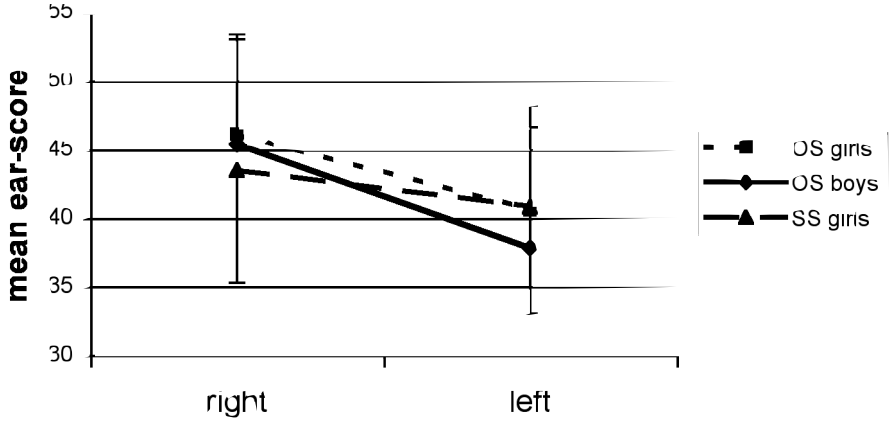


Figure 2. Separate left- and right-ear score for the Opposite-sex (OS) twin girls, Same-sex (SS) twin girls, and OS twin boys. Means and standard error scores are indicated.

3.2. Comparison between OS girls and SS girls

Likewise, a significant difference in mean LI scores ($t(117) = 2.07$, $p < 0.025$; one-tailed (see Figure 1)) was found between the OS and SS girls.

When analyzing within the separate twin girl groups, again a main effect of ear was found, indicating a right-ear advantage in girls ($F(1, 118) = 26.885$, $p < 0.001$). As predicted, we found an interaction between type (OS or SS) and ear (right or left) ($F(1, 118) = 3.267$, $p < 0.05$; see Figure 2), suggesting that the OS girl group showed a more masculine pattern of cerebral lateralization than the SS girl group.

3.3. Relation between testosterone and laterality scores

In the total sample there was no association between the LI scores and the free testosterone levels ($r = -.03$, n.s.), nor were there meaningful relations between LI and testosterone in the three separate groups, with values ranging between -.08 and .13 (and all values being not significant).

However there was a significant difference in free testosterone level, as measured at age of 10, between the three groups, with the SS girls having the highest levels (mean testosterone pg/mL = 13.33, SD = 5.38), followed by the OS girls (mean testosterone pg/mL = 12.05, SD = 4.40), and the OS boys (mean testosterone pg/mL = 10.51, SD = 3.70), ($F(2,179) = 11.739$, $p < .001$).

A paired samples test showed that the OS twin pairs differed significantly in their free testosterone levels ($t(53) = -2.76$, $p < .01$). Independent Samples testosterone test revealed a significant sex difference between the OS boys and SS girls, ($t(115) = 3.19$, $p < .01$) but the OS girls and SS girls did not differ significantly ($t(111) = 1.4$, ns).

4. DISCUSSION

The aim of the present study was to investigate whether prepubertal, 10 year old opposite-sex (OS) twin girls as compared to same-sex (SS) twin girls, would show a pattern of functional cerebral lateralization away from the typical female pattern as a result of their possible prenatal exposure to higher levels of testosterone (T) due to fetal hormone transfer. To that end we compared the performances of these two groups on a dichotic listening task (DLT), which provided us with an indirect measure of functional cerebral lateralization.

The results indicate that the OS twin girls indeed showed a less feminine and more masculine pattern of functional cerebral lateralization on the DLT as compared to the SS twin girls. Their functional cerebral asymmetry, as reflected by a right-ear superiority (i.e. left-hemispheric dominance) when processing verbal-auditory stimuli, was greater than in SS twin girls. Differences between OS and SS twin girls in the extent of prenatal exposure to androgens could at least partly be responsible for these findings, given that prenatal testosterone (T) has shown to be involved in brain organization and postnatal sex-dimorphic behavior (McGlone, 1980; Geschwind and Galaburda, 1987; Wisniewski, 1998).

A different line of research indicates that differences in performance on cerebral lateralization tasks could also be attributed to current or activating effect of sex hormones (Sanders and Wenmoth, 1998). However, we do not believe that the current findings are caused by differences between the groups in circulating testosterone levels. First, we did not find a correlation between testosterone and our functional cerebral laterality scores. The results of our testosterone analyses showed not only that girls had higher testosterone levels than boys of similar age, a finding that has been reported before (Granger et al., 1999), but also, and more importantly for the interpretation of the current results, that there were no differences in testosterone between OS and SS twin girls. In other words, the difference in lateralization performance between OS and SS twin girls, as shown by the results on the DLT, could not be attributed to differences between these two groups in concurrent testosterone levels. It seems therefore more likely that if it is true that testosterone influences lateralization performance, its role in explaining the observed difference in performance between SS and OS twin girls is more likely to have been a prenatal one.

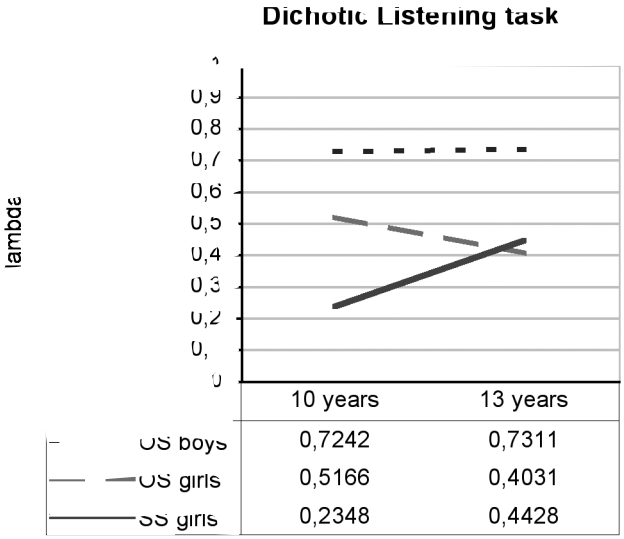
It should of course be kept in mind that hormonal and "social"-experiential influences are very difficult to separate. It remains possible that environmental factors, such as those provided by the presence of a male twin brother, have a strong influence on the general "masculinization" of the OS twin girl's behavior. However, although these effects can be expected to occur in the general domain of gender-related behavior (e.g., affecting toy or playmate preference, activity level) it is less easy to see how they could influence more specific areas of neuropsychological functioning, like hemispheric specialization.

Clearly, the possible elevated prenatal exposure to testosterone of OS twin girls, and the extent of such an effect - if it exists at all -, remains at the present unclear, and needs further investigation. Our findings are at best suggestive of such an effect on cerebral lateralization. In the future it will be interesting to find out whether similar patterns of findings are observed for other behavioral and/or

neuropsychological parameters on which males and females are known to differ. The current findings should therefore be replicated and extended in different groups of OS and SS twin girls, but the designs of these studies should also inform us whether any differences are suggestive of hormonal rather than environmental influences.

5. POSTSCRIPT

The finding of prenatal masculinization of hemispheric specialization, as measured with the DLT, in OS female twins at the age of 10 years was a primary reason to re-evaluate DLT performance when the twins were 13 years. In addition, this was the only task that we were able to use during both test occasions in the exact same manner. We expected that these differences in performance between SS and OS girls would still be present when the girls were older because we hypothesized that the differences were due to structural prenatal effects of testosterone. Unexpectedly, these differences in DLT performance were no longer present when the girls were 13 years old (see Figure).



Multivariate analyses showed the main effect of Time (10 or 13 years old) and the interaction between Group and Time not to be significant. We had not expected these interactions to be significant because we considered hemispheric lateralization to be mainly due to an organizational effect of testosterone. However, because our main interest was the possible masculinization of the OS girls in comparison to the SS females, we decided to use independent *t*-tests to analyze the data for the second test occasion, when the girls were about 13 years of age. These tests revealed a significant sex difference, with the OS boys (again as expected) showing an increased hemispheric laterality compared to the SS girls ($t(106) = -2.02$, $p < .05$). However, as noted above, we could no longer detect a more masculine hemispheric specialization in the OS girls than in the SS girls ($t(107) = .26$, ns).

The effect size for the sex difference based on the data from the first testing occasion (OS boys versus SS girls) was $d = .59$; however, the effect size for SS and OS girls was obviously lower $d = .38$. Because fewer girls were tested when they were 13 years old, the sample size may not have been large enough to detect a difference comparable to that seen when the girls were 10 years old (Cohen, 1988). Alternatively, it may also be that the effects that were found before puberty fade with age, perhaps due to postnatal experiential influences. Whether the masculinized hemispheric specialization in OS females changes again after puberty remains to be determined. In any case, free testosterone levels did not influence lateralization performance because the correlation between testosterone levels and performance was not significant in any group at 13 years.

Chapter 4

Is there an organizational or activational effect of testosterone on verbal skills and spatial ability? A twin study

Cohen-Bendahan CCC, van Goozen SHM, Cohen-Kettenis PT, Orlebeke JF, Buitelaar JK. Submitted.

ABSTRACT

There is clear evidence of a sex difference in cognitive functioning. Thus, males show enhanced spatial skills and females excel in tasks involving verbal skills. Although these sex differences are apparent in adulthood, and have been attributed to both organizing and activating effects of hormones, there is less clear evidence of similar cognitive sex differences in childhood. We compared the performance of 79 pre-adolescent boys and 59 girls on two spatial tasks and two verbal tasks. Further, because it has been suggested that opposite-sex (OS; N=79) twin girls may have been exposed to higher levels of testosterone *in utero* than same-sex (SS) twin girls, we examined a possible organizational effect of testosterone on cognitive performance in twin girls and hypothesized that prenatal exposure to testosterone would lead to a more masculine cognitive pattern in OS girls than in SS girls. However, because testosterone may also have an activating effect on cognitive abilities, we measured testosterone and related this to performance on the cognitive tasks. Although a sex difference on two of the four cognitive tasks was found, testosterone was not significantly associated with either verbal or spatial performance. Not many differences were observed between OS and SS girls, but SS girls had a better memory for verbal information than the OS girls. It is discussed that prenatal exposure to testosterone may affect the development of the certain brain structures that support verbal memory.

1. INTRODUCTION

Already thirty years ago, Maccoby and Jacklin (1974) stated that cognitive sex differences exist, i.e. men outscore women on tasks that require spatial ability, and women have better verbal skills than men. It is less clear whether these sex differences already exist in children. According to Maccoby and Jacklin, the sex difference in spatial ability does not emerge before adolescence. Differences in verbal ability are first seen before the age of three, but then they seem to disappear and to reappear again in adolescence and beyond. Many studies failed to confirm the ideas of Maccoby and Jacklin (e.g. Levine et al., 1999; Johnson and Meade, 1987). Sex differences in cognitive functioning seem to be apparent at a young age provided suitably sensitive tests are used to establish the differences (e.g. Voyer et al., 1995). For example, Johnson and Meade (1987) showed that by using appropriate tasks, sex differences in spatial performance were found in children as young as 10 years. Another possibility, which is partly related to the above, is that girls, because of their verbal precocity, use a verbal strategy to solve spatial tasks (Johnson and Meade, 1987) and that sex differences in spatial performance only become apparent in adolescence when testosterone levels rise dramatically in boys.

Various suggestions have been advanced to explain the mechanism causing sex differences. One explanation is that the differences are caused by the postnatal socialization processes (e.g. Bem, 1983 in Liben et al., 2002; Baenninger and Newcombe, 1989). Thus environmental factors that reinforce the child's biological sex, e.g. sex-differentiated play, may contribute to sex differences in cognition. This social learning theory was widely accepted as the best explanation during the early 1980s, but has since been questioned. An alternative explanation concerns the influence of sex steroids, mainly testosterone. Hormones, such as testosterone, affect some aspects of cognitive functioning through two distinct mechanisms, (1) by their organizing effects, and (2) by their activating effects. Organizing effects are based on the structural (lasting) influence of hormones on the nervous system during prenatal development as

well as very early childhood. Both maternally produced hormones and fetal hormones have been shown to exert gender-specific effects on neuro-anatomical growth. These effects may be amplified by (later) circulating hormones, especially during puberty (Goy and McEwen, 1980). However, some differences may only become manifest as an additional effect of circulating, or activational, testosterone (Goy and McEwen, 1980). Thus the elevated levels of sex hormones from puberty onward contribute to the expression of sex differences that were established prenatally by the organizing effects of testosterone.

Animal studies have shown that experimental manipulation of hormone levels in the perinatal period affects behavior and physiology later in life. Because this method cannot be used in humans, other paradigms have been used to investigate whether human cognitive sex differences are influenced by abnormal prenatal exposure of the brain to testosterone.

A different line of animal research has shown that female fetuses located between two male fetuses are exposed to higher levels of testosterone than female fetuses that are situated between two females or one female and one male fetus. This differential exposure has been shown to correlate with postnatal cognition (Galea et al., 1994), physiology, and behavior (for review see Ryan and Vandenbergh, 2002) toward the pattern of the opposite sex, i.e. away from the typical biological female pattern.

The purpose of the present study was to examine the possibility of an organizing effect of testosterone on cognitive sex differences in children by using the twin paradigm. We assumed that opposite-sex (OS) twin girls are exposed prenatally to higher levels of testosterone, and that they would as a consequence of this early exposure show a more masculine pattern of cognitive performance than same-sex (SS) twin girls. We also took account of any possible activating effects of testosterone on cognitive performance by assessing relationships between free testosterone levels and cognitive performance in 10 year old children. Age appropriate tests were selected that were comparable to tasks for which sex differences have been

demonstrated in adults. In order to verify that our tasks showed sex differences, we first compared the performance of boys and girls.

2. METHODS

2.1. Subjects

The subjects were recruited from the Netherlands Twin Register (NTR) of the Vrije Universiteit in Amsterdam. This is a population-based sample with more than 50% of all born twins registered from birth onward. Twins could be included in this study if: (1) they were born in the year 1989, and, (2) they had expressed their interest in the past to participate in research. In total 79 opposite-sex (OS) twin pairs and 59 girls from same-sex (SS) dizygotic twin pairs aged 10 years (see Table 1) agreed to participate. Written informed consent was obtained from the parents, and oral consent from the twins.

Table 1. Mean age and range for the two twin groups. Distribution of the level of education for each parent (%).

	OS twin pairs (N=79)	FF twin pairs (N=59)
Age Children (yrs) Mean (sem) Range	10.96 (.005) 10.43 – 11.75	10.94 (.005) 10.50 – 11.84
Vocabulary subtest WISC-RN (SEM)	boys: 103.22 (2.0) girls: 97.30 (2.0)	girls: 99.12 (2.2)
Educational level Mother Low Middle High	 16.2% 43.2% 40.5%	 15.7% 39.2% 45.1%
Educational level Father Low Middle High	 17.1% 44.3% 38.6%	 16.0% 38.0% 46.0%

Note. No significant differences were found between the two groups on any of these variables.

2.2. Materials

2.2.1. Card Rotation two-dimensional (RF-2D; Ekstrom et al., 1976).

By comparing five alternatives with the example figure given, the subject has to decide which alternatives are identical by rotating them in two dimensions. The subject has to identify the answers by crossing the letter above each figure. After practicing with four example items and receiving feedback about the correct answers, the subject is given 7 minutes to finish the task (30 items). A total score was calculated by allocating one point for each correct answer, including the incorrect items left uncrossed, thus giving a maximum score of 150. The effect size for sex differences in adults is between 0.30 and 0.39 (Voyer et al., 1995).

2.2.2. Line Orientation (LO; Benton et al., 1983).

Two lines shown at the top of a page have to be compared with eleven lines with different slopes at the bottom of the page. The subject has to indicate which of the eleven numbered lines correspond with the two example lines. The test contains 5 practice items followed by 30 test items. There is no time limit and a score of 1 is awarded if both answers to an item are correct. The maximum score is 30. The effect size for sex differences in adults is between 0.57 and 0.75 (Benton, 1994).

2.2.3. Dutch adaptation of Rey's (1941, 1958) Auditory-Verbal Learning Test (RAVLT; see Van den Burg and Kingma, 1999)

We would have preferred to use the California Verbal Learning Test – children's Version (CVLT-C; Delis et al., 1994), which can detect sex differences between the ages 5 and 16 years (Kramer et al., 1997), but this test is not available in Dutch. Hence we decided to use the Dutch version of the RAVLT because it detects sex differences in adults (Geffen et al., 1990).

In the RAVLT, subjects have to memorize 15 words in five consecutive trials. After each trial, the recalled words are reported verbally. The sum of these five trials represents the short-term verbal

memory. The maximum score is 75. After 30 minutes, subjects are asked to re-call the previously learned words. In the 30-minute interval verbal or memory tasks are not administered, in order to prevent any interference. The sum of the delayed recall represents long-term verbal memory. The maximum score is 15. For statistical analysis, we used adjusted scores: we corrected for age in the short-term verbal memory task (STM-RAVLT) and for STM-RAVLT score in the long-term verbal memory task (LTM-RAVLT). We calculated the effect size of the sex differences for this task using the following formula (Cohen, 1988), based on the adult data (ages 40 to 49) presented in the study of Bleecker et al. (1988):

2.2.4. Groninger Intelligence test – Word naming 1 – Verbal Ideational fluency test (VIF; Luteijn and Van der Ploeg, 1983).

This test measures verbal ideational fluency. The verbal fluency test consists of a 60-second category (animals)-naming trail. The score is the sum of correctly produced animals in 60 seconds. Separate norms are available for each sex.

2.2.5. Wechsler Intelligence Scale for Children – Revised – Subtest Vocabulary (WISC-R, Wechsler, 1974; Dutch version WISC-RN, Vandersteene et al., 1986)

To measure the verbal skill and to determine whether the girls show a verbal precocity compared to the boys we chose to administer the subtest Vocabulary of the WISC-RN (WISC-VOC).

$$d = \frac{(M_1 - M_2)}{\sqrt{[(\Sigma^2_1 + \Sigma^2_2)/2)]}} = 0.55$$

2.2.6. Hormone assay

Testosterone in saliva was measured after diethylether extraction with an in-house competitive radioimmunoassay using a polyclonal anti-testosterone-antibody (Dr.Pratt AZG 3290). [1,2,6,7-^{3}H]-Testosterone (TRK402, Amersham Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 2.9 pg/mL and inter-assay variation was 10% at 12 pg/mL (n = 180). Saliva sampling is a non-invasive, stress-free technique and saliva testosterone levels provide a very good indication of the free testosterone level in serum (Ostatníková et al., 2002).

2.2.7. Pubertal status

Pubertal status was evaluated using the Tanner staging system (Tanner, 1962). Mothers were asked which of five drawings best reflected the development of the breasts and pubic hair of their daughters or the pubic hair and scrotal development of their sons.

2.3. Statistical analyses

First, we compared the mean scores of our groups to normative data from the literature in order to assess the representativeness of our subject population, using one-sample *t*-tests. This was only possible for the tasks LO, VIF, and RAVLT. For the RF-2D no age appropriate norms were available. Second, to establish that the tasks indeed showed sex differences, we compared the mean scores of the four tasks for the OS boys with those of the SS girls by ANOVA (General Linear Model; GLM).

Thereafter, we compared the two girl groups (OS and SS) in order to test whether OS girls had a more masculine cognitive pattern than SS girls. We used one-tailed tests in case we replicated established sex differences in our sample on comparing OS boys and SS girls, and two-tailed tests whenever we failed to establish a sex difference.

In order to control for possible effects of circulating, or activational (i.e., postnatal), free testosterone on performance, we compared the free testosterone levels of the three groups by using a linear contrast matrix and calculated the Pearson correlations between testosterone levels and scores on the four cognitive tasks.

3. RESULTS

3.1. Comparison with norms found in literature

Normative data were available for the following three tasks only:

3.1.1. Line Orientation (LO)

Normative data were available for boys and girls on the LO task (N=17; Lindgren and Benton, 1980). OS boys performed much better than the boy normative group (mean difference = 2.50; $t(78) = 5.35$; $p < .001$). The twin girls also performed significantly better than the girl normative group (N=19); mean difference = 1.42; $t(137) = 3.93$; $p < .001$).

3.1.2. RAVLT

There were no separate normative data for boys and girls; we therefore compared our total sample with the available age normative group.

3.1.2.1. RAVLT - Short Term Memory

The performance of the twin group (N=216) was not significantly different from that of the normative group (N=64; Van den Burg and Kingma, 1999); the mean difference was –1.10, ($t(215) = -1.93$; ns).

3.1.2.2. RAVLT - Long Term Memory

The twin group performed significantly worse than the normative group, ($t(216) = -3.98$; $p < .001$; mean difference = -0.66).

3.1.3. Verbal Ideational Fluency

The VIF was originally designed for the ages 12 up to 76 years, and therefore we compared our groups with the youngest normative group, i.e. 12 year olds (see Luteijn and Van der Ploeg, 1983; p.43). All our subjects performed better than the normative group on the VIF. The mean difference for the boys was 1.52, ($t(78) = 3.16$; $p < .01$) and for the girls 3.47 ($t(137) = 9.14$; $p < .001$).

3.2. Sex differences

To determine a possible verbal precocity in girls relative to boys, the Dutch version of the Wechsler Intelligence Scale for Children Revised (Vandersteene et al., 1986) subtest Vocabulary, was evaluated; however, no significant difference was found ($t(136) = -1.37$, $p = .20$).

Means and standard deviations for all four cognitive tasks for each group are shown in Table 2. The GLM analyses revealed a sex difference on two tasks, namely, the Line Orientation (LO) and the

Verbal Ideational fluency test (VIF): boys performed better on the LO task and girls performed better on the VIF test. No sex differences were found on the other two tasks, see Table 2.

Table 2. Performance on each test for the three different groups

Test	OS boys M SEM	SS girls M SEM	OS girls M SEM	OS boys - SS girls	OS girls – SS girls
	N = 79	N = 59	N= 79	F/t p	F/t p
RF-2D	94.82 2.34	88.88 3.11	81.59 2.64	2.26 ns	3.21 ns
LO	23.10 0.47	20.80 0.44	20.66 0.54	12.20 .001	0.04 ns
STM-RAVLT	44.00 0.97	44.80 1.09	47.24 0.89	0.29* ns	3.09* ns
LTM-RAVLT	9.47 0.30	9.88 0.28	9.62 0.28	0.51* ns	10.87* .001
VIF	19.52 0.48	21.14 0.57	19.97 0.50	4.69 .032	2.31 ns
WISC-VOC	103.22 2.00	99.12 2.20	97.30 2.00	-1.37 ns	0.61 ns

Note. OS, Opposite-sex twin; SS, same-sex twin; M, mean score; SEM, Standard Error Mean; p, p-value; RF-2D, Card Rotation two-dimensional; LO, Line Orientation; RAVLT, Rey's Auditory-Verbal Learning Test; STM, short-term memory; LTM, Long-term memory; VIF, Verbal Ideational Fluency; WISC-VOC, WISC-RN's Vocabulary subtest; *, F-values are based upon adjusted RAVLT scores.

3.3. SS versus OS girls

We compared the results of the SS and OS girls on the two tasks showing significant sex differences, using one-tailed tests. However, no differences between the OS- and SS-girls were found on the VIF and LO (see Table 2). As past research (Voyer et al., 1995; Bleecker et al., 1988) has suggested that the other two tasks, the two-dimensional mental rotation task (RF-2D) and the verbal memory task (RAVLT; long-term and short-term), do differentiate between males and females, we examined whether performance on these tasks differed between the two groups of girls (two-tailed tests). There was no difference between the OS and SS girls on the RF-2D, ($F(1,136) = 3.21$; $p < 0.10$) but OS girls did worse on the LTM-RAVLT test (see Figure 1). Apparently, SS girls were better in memorizing information because they remembered more words than the OS girls in the long term (Table 2).

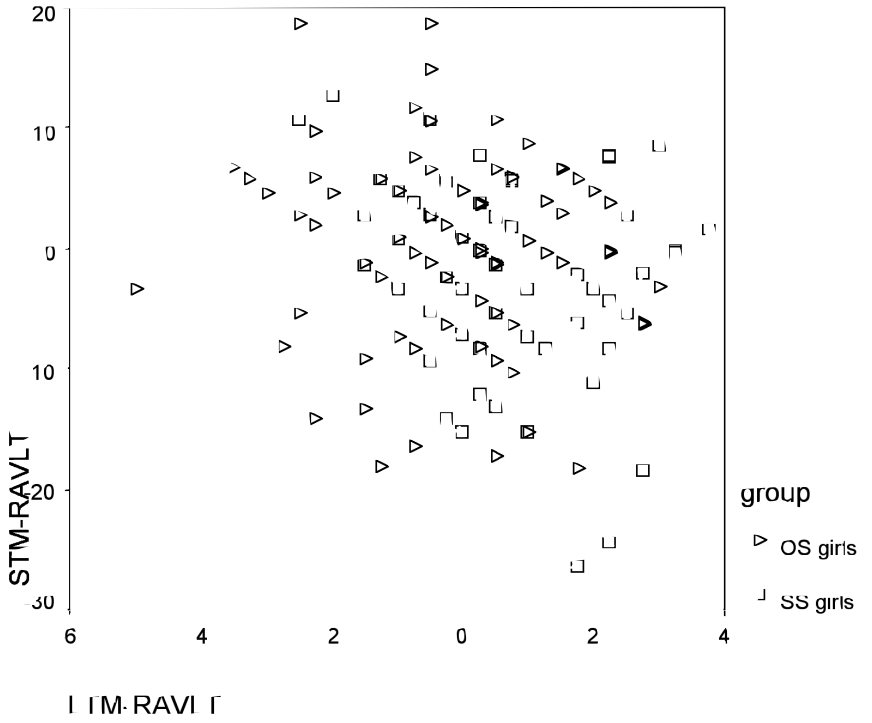


Figure 1. An overview of the relation between STM-RAVLT and LTM-RAVLT for the two girl groups

3.4. Relations between saliva free testosterone levels and the four cognitive tasks

The mean free testosterone levels of the twins are presented in Table 3. There was no significant association between performance on the four tasks and free testosterone levels in the total group (with correlation values ranging between -.04 and .09, all not significant), nor in the three separate groups, with the only exception of a significant positive correlation between LO performance and free testosterone levels in the OS girl group ($r = .24$; $p < .05$).

However, there was a significant difference in free testosterone level, between the three groups, with the SS girls having the highest levels, followed by the OS girls, and the OS boys, ($F(2,207) = 8.35$, $p<.001$). Independent Samples T-test revealed a significant sex

difference between the OS boys and the SS girls, (t(131) = 3.84, p<.001), but also SS and OS girls differed significantly (t(129) = 1.92, p<.03, one-tailed.

Table 3. Number and mean saliva testosterone level (SEM in parentheses) for the three twin groups.

	SS girls	OS boys	OS girls
N	56	77	75
pg/mL	13.43 (.70)	10.33 (.41)	11.83 (.50)

3.5. Pubertal status

The median pubertal stage for the OS girls was stage 1 for breast development and stage 1 for pubic hair, and for SS girls stage 2 for breast development and stage 2 for pubic hair. The differences were not statistically significant (chi-square = 1.25, and 3.14, for breast development and pubic hair, respectively; all ns). The OS boys had a median pubertal stage 2 for scrotal development and 2 for pubic hair.

3.6. Post-hoc estimation of power

To determine whether the non-significant findings were due to a small sample size, we performed a post-hoc power analysis, including all the variables that were non-significant in the main analysis, for the sex differences (SS girls – OS boys) and the twin girl differences (SS girls – OS girls). Both analyses indicated that the power was too low, varying between .05 and .61, respectively. Thus the relative lack of significant results was in part due to the inclusion of too few subjects.

4. DISCUSSION

The goal of the study was to look at the presumed organizing effects of testosterone on cognitive abilities. To that end we compared the performances of OS twins and female SS twins on spatial and verbal domains and related these to levels of testosterone. When we compared SS twin girls and OS twin boys, we found that pre-adolescent girls had a better verbal ability whereas pre-adolescent boys had a better spatial ability. However, this did not apply to all the investigated measures because the groups did not differ in performance on the mental rotation task (RF-2D) and the verbal memory task (RAVLT). The mental rotation findings are more-or-less in line with conclusions from an extensive meta-analysis carried out by Voyer et al. (1995) that sex differences in spatial ability before the age of 13 years are rarely observed. Moreover, the RF-2D task, which is a two-dimensional mental rotation task, provides a less clear-cut difference between males and females than the more complex, three-dimensional mental rotation task such as the Vandenberg Mental Rotation Test (RF-3D; Vandenberg and Kuse, 1978). Because of the age of our participants, we did not select the RF-3D as we expected the task would be too difficult. Fairweather (1976), and Johnson and Meade (1987) have suggested that girls may show a verbal precocity in childhood, which may overshadow an actual sex difference in spatial ability because girls use a verbal strategy to solve spatial problems. Our finding of no difference in RF-2D performance, but a better verbal fluency in girls, seems in line with this explanation. However, in that case one would also expect a sex difference on another verbal task (WISC-VOC) that was used to control for this possible precocity but here we did not find any difference. Additionally, if this were the case, then performance on the other spatial task (i.e. LO) should have been influenced in a similar way.

Our finding of an absence in sex difference on the RAVLT may be related to the test choice. Because the California Verbal Learning Test – children's Version, is not available in Dutch we had to use a "similar" test, the RAVLT. Although the RAVLT does show signifi-

cant sex differences in adults (Geffen et al., 1990) it apparently does not in children (Van den Burg and Kingma, 1999; Forrester and Geffen, 1991). Test similarity, i.e. RAVLT and California Verbal Learning Test – children's Version, does not seem to be a guarantee for finding the same results when investigating the same domain (Crossen and Wiens, 1994).

Apart from the above-mentioned difference between boys and girls (LO and VIF), our inability to detect sex differences in performance on other tasks, i.e., RF-2D and RAVLT, could have been due to the relatively small sample size as our calculations of statistical power showed.

We compared the performance of OS and SS girls on those tests (LO and VIF) which showed a sex difference in performance, to determine whether prenatal exposure to testosterone could have influenced task performance. We assumed that the OS girls would have been exposed to higher levels of testosterone *in utero* than the SS girls, and that they would show a more "masculine-type" performance on these tasks. However, this was not the case and thus our results did not support our hypothesis that prenatal testosterone exposure in OS girls would have affected their cognitive performance. Moreover, because cognitive task performance and free testosterone levels were not correlated in these 10-year-old children, we can also conclude that testosterone does not seem to have an activational effect at this age. As all children were pre-pubertal or in the very early stages of puberty, at Tanner stage 1 or 2, it remains possible that the effect of prenatal testosterone exposure will only become visible later in puberty, after the onset of menarche. As mentioned before, our sample may also have been too small to detect the (at this age perhaps still subtle) differences in cognitive performance.

It is possible that a task showing no sex differences in mean scores, still can reflect effects of sex hormones on the developing brain (Janowsky et al., 2000). For example, in men a curvilinear relationship is shown and in women a linear relationship exists between spatial ability and testosterone (Moffat and Hampson, 1996; Shute et al., 1983). We therefore also analyzed performance on

the two cognitive tasks that failed to show a sex difference in our groups, using two-tailed tests. This analysis revealed an interesting difference between OS and SS girls for the RAVLT. Although the short-term memory performance of the two groups of girls was similar, the OS girls had a significantly worse long-term verbal memory performance than the SS girls.

It is known that estrogen has a positive effect on long-term memory (e.g., Kampen and Sherwin, 1994). This finding may be explained by the fact that the SS girls had higher levels of circulating testosterone than the OS girls, and testosterone can be aromatized into estrogen. Another possibility is that the SS girls had higher levels of estrogen regardless of their testosterone levels. At this moment we cannot sort what the exact reason is of the poorer long-term verbal memory performance by the OS girls.

It is interesting that in pre-adolescent children some neuropsychological functions seem to be affected by prenatal testosterone exposure, such as the lateralization of brain functions (Cohen-Bendahan et al., 2004) whereas others are not. It is possible that the cognitive skills investigated here involve specific structures in the brain that are not influenced by the organizing effects of testosterone, in contrast to the structures involved in the task measuring verbal lateralization. Alternatively, the timing of exposure to testosterone may be important. During the critical period of early brain development some brain structures may have more testosterone receptors than others, i.e. they are more sensitive to testosterone, and react to prenatal exposure to testosterone by, for example, neuronal proliferation and growth (Goy and McEwen, 1980). The outcome of these differences in brain structure and function may become manifest as early as 10 years. Thus the tests used in these above-mentioned studies, i.e., for example, hemispheric lateralization (Cohen-Bendahan et al., 2004; chapter 3) and the cognitive findings in this paper, probably involve different areas of the brain with different dose-dependent thresholds. If the involved brain areas have a different sensitivity to testosterone, then the effect of sex hormones may become manifest from adolescence onward when much higher levels of sex hormones are present. Cole-Harding and her

colleagues (1988) have shown that adult OS females do show much more masculine spatial ability measured with the RF-3D than SS adult females. So the exhibition of traits may be dose-dependent regarding the amount of exposure during life to testosterone.

We feel that our inability to detect substantial differences between OS and SS twin girls on several cognitive functions, with the exception of long-term verbal memory function, was due to a too small sample size. Indeed, study power may be especially a problem when trying to detect subtle differences in participants of the same sex. In the future, studies with larger groups of twins, who are assessed at both pre- and postpubertal age, should be conducted to find out whether the twin paradigm can be used to investigate the possible organizational and activational effects of testosterone on cognitive functioning.

Chapter 5

Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls

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ABSTRACT

Men and women differ in temperament and personality traits, such as aggression and sensation seeking. The sex hormone testosterone could play a role in the origin of these differences, but it remains unclear how and when testosterone could have these effects. One way to investigate the prenatal exposure effect of testosterone is to compare opposite-sex (OS) and same-sex (SS) female twins. It has been suggested that OS twin girls are exposed prenatally to elevated testosterone levels and that this may result in some masculinization of their personality and behavior. We measured sexually-dimorphic traits and circulating testosterone levels in 13-year-old OS (n=74) and SS (n=55) twins.

Testosterone levels showed a clear circadian rhythm, with higher levels in the morning than in the afternoon. Testosterone was higher in boys than girls, but similar in OS and SS twin girls. Testosterone was not in any way systematically related to the different personality traits. However, a sex difference in aggression proneness was observed, and OS girls showed a more masculine pattern of aggression proneness than the SS girls. It is argued that it is unlikely that this difference is due to social factors, such as a gender-specific upbringing.

1. INTRODUCTION

While the physical differences between males and females are clear, differences in behavioral traits are less clear-cut. However, certain behaviors do show sexual dimorphism in adults. For example, men are more physically aggressive and engage in more sensation-seeking behavior than do women (e.g. Helgeson, 2002; Feij et al., 1997). Such male–female differences are thought to be even more pronounced in children (Helgeson, 2002). It is generally assumed that sex hormones, mainly testosterone, are associated with the development of sexually dimorphic adult behavior (e.g., Finkelstein et al., 1997) and exert their effect early in life, as recently emphasized by Ramirez (2003), who stated that "the origin of gender-based differences in aggression must lie in neuroendocrinological events occurring during prenatal life or early postnatal life" (p. 621). However, other authors have reported that the influence of testosterone on aggression is sometimes rather small (e.g., Mattsson et al., 1980; Olweus et al., 1980, 1988).

Sensation seeking was defined by Zuckerman (1979, p.10) as "the need for varied, novel, and complex sensations and experiences and the willingness to take physical and social risks for the sake of such experience". Men engage in more sensation seeking behavior than do women (e.g., Rosenblitt et al., 2001; Russo et al., 1991; Zuckerman et al., 1978). For example, Feij et al. (1982) showed that adult men and youths aged 12-24 years showed more overall sensation seeking behavior, as measured with the Sensation Seeking Scale (SSS) than did women and girls of the same age (Feij et al., 1997). Although scores on the Disinhibition subscale of the SSS were found to be associated with levels of sex hormones, mainly testosterone, estradiol, and estrone (Daitzman and Zuckerman, 1980), Rosenblitt et al. (2001) could not find support for an influence of testosterone on these behavioral sex differences. Temperament may be associated with personality traits such as aggression and sensation seeking. Temperamental traits, such as impatience and irritability, are supposed to be related to testosterone levels. In boys, high testosterone levels were correlated with a more impatient and

irritable temperament, which in turn increased aggressive behavior (Olweus et al., 1988).

The role of early testosterone exposure in humans can be investigated in various ways. For example, women with congenital adrenal hyperplasia (CAH) are exposed to higher levels of testosterone during gestation because of an enzyme defect. This exposure leads to masculinization of different aspects of behavior and cognition – CAH girls have been found to be more aggressive in adolescence and adulthood than their non-affected siblings or cousins (Berenbaum and Resnick, 1997). However, since these women often suffer from other medical problems, it is not easy to generalize these results to implications for the normal population.

Another approach is to look at the relation between prenatal levels of sex hormones and behavior in offspring's. For example, levels of testosterone measured in maternal serum during pregnancy may contribute to the development of gender role behavior in preschool girls but not boys (Hines et al., 2002). However, this approach is not practical because of the long follow-up needed and the attendant problems (Ehrhardt and Meyer-Bahlburg, 1979).

A third approach is to study the effects of prenatal hormone transfer in opposite-sex (OS) twins. In animals, female animals that develop *in utero* between male fetuses show a less typically female pattern of behavior and anatomy in adulthood than do female animals that developed *in utero* between female fetuses (for review see, Ryan and Vandenbergh, 2002). This may be due to exposure of the female fetus to high levels of testosterone coming from the adjacent male fetuses (Even et al., 1992). Male mice exposed to higher levels of testosterone *in utero* (because they are positioned between male fetuses) are more aggressive as adults than male mice exposed to lower levels of the hormone (because they are positioned between female fetuses) (Beatty, 1992). Similarly, female swine surrounded by male swine *in utero* are more likely to participate in fights, and to win fights, than female swine located between female swine *in utero* (Rohde Parfet et al., 1990). Again, these findings have been explained in terms of the transfer of sex hormones from the male fetuses to the adjacent female fetuses during pregnancy. This early

prenatal exposure to androgens may change the organization of the structure and the sensitivity of the brain and nervous system in adulthood (Harris, 1999). The above-mentioned examples concerned prenatal exposure to hormones via the feto-fetal route, i.e., the diffusion of testosterone across amniotic membranes, which seems to be the more direct path (e.g. Even et al., 1992; Fels and Bosch, 1971). An alternative route of exposure is the maternal-fetal transfer route (see, Meulenberg and Hofman, 1991).

Previous studies using this method have shown inconsistent results. It has been shown that prenatal exposure affected tooth size (Dempsey et al., 1999) and spontaneous otoacoustic emission in opposite-sex twin (OS) females (McFadden, 1993), which is in line with findings in males. However, click-evoked otoacoustic emissions were not significantly different between OS and same-sex (SS) female twins. McFadden et al. (1996) concluded that although the difference between the females with a male co-twin or a female co-twin did not reach statistical significance, the OS females tended to show "masculine" changes. Therefore they argued that their results added support to the underlying phenomenon that prenatal exposure to testosterone leads to a more masculinized pattern of otoacoustic emissions in OS females, and that the sex differences in otoacoustic emission are due to, and controlled by, the influence of prenatal testosterone. Cohen-Bendahan et al. (2004) recently found, in a group of pre-adolescent 10-year-old twins, that OS girls had a more masculine pattern of cerebral lateralization than did the SS girls. Resnick et al. (1993) investigated sensation-seeking behavior in female OS twins and showed that this may also be influenced by prenatal hormones. Other studies, however, could not find any indication for an effect of prenatal exposure (e.g. Rose et al., 2002; Henderson and Berenbaum, 1997).

In this study, we investigated whether sex differences in aggression, sensation seeking, and temperament are influenced by testosterone. To this end, we studied a group of 13-year-old OS and SS female twins. We assumed that the OS girl twins would have been exposed to higher levels of testosterone *in utero* than the SS girl twins, and that this would affect their behavior. Although exposure

to maternally produced hormones and hormones of the fetus itself have been shown to exert sex-specific differential neuro-anatomical growth effects, these effects may be amplified and come to expression under the influence of circulating hormones during puberty. By using this paradigm, we hoped to overcome some of the difficulties of sex dimorphic research. Since the twins were healthy, results obtained would be generalizable to the entire population. Moreover, by studying twins of the same sex, we hoped to exclude the effect of social factors such as gender-role upbringing.

2. METHOD

2.1. Participants

The participants in this study were recruited from the Netherlands Twin Register (NTR) of the Vrije University in Amsterdam, which contains information about more than 50% of all twins born in the Netherlands. Twins were included in this study if: they were born in the year 1989 and, had expressed interest in the past to participate in research. The data presented in this study are part of a longitudinal study. In total 74 opposite-sex (OS) twin pairs (boys and girls) and 55 girls from same-sex (SS) dizygotic twin pairs, aged 13 years, agreed to participate. Written informed consent was obtained from the parents, and oral consent from the twins.

2.2. Measures

Aggression, sensation seeking, and temperament were all measured with paper-and-pencil questionnaires.

2.2.1. Aggression

The Dutch translation of the Reinisch Aggression Inventory (RAI; Reinisch and Sanders, 1986) was used to investigate a child's proneness to aggressive behavior. In this test, the child is presented

with six written descriptions of situations involving an interpersonal conflict. The child can make one of four possible responses, namely, verbal aggression, physical aggression, withdrawal, or an assertive reaction. In each situation the child has to choose a response from six pairs of forced reactions corresponding to all possible combinations of the different types of responses. The effect size for sex differences has been shown to be large, between 0.7 and 1.1, for physical aggression, with boys showing more physical aggression than girls (see, Berenbaum and Resnick, 1997).

A Dutch translation of a modification of the Olweus Multifaceted Aggression Inventory (OMAI; according to Finkelstein et al., 1997) was used to investigate aggressive behavior and attitudes. The OMAI is a self-report questionnaire. Subjects have to endorse to the following six scales: Physical Aggression Against Adults and Peers; Verbal Aggression Against Adults, and Verbal Aggression against Peers; Aggressive Inhibitory Responses; and Aggressive Impulses. The OMAI has been shown to differentiate between the sexes, with boys having higher scores on all scales except of the subscale Aggressive Inhibitory Responses (Finkelstein et al., 1997).

2.2.2. Sensation Seeking

The Dutch adaptation and modification of Zuckerman's (1971) Sensation Seeking Scale V (SSS; see Feij and van Zuilen, 1984) was used. The questionnaire consists of 67 Likert-type statements divided into a total SSS and four sub-scales: Thrill and Adventure Seeking, Experience Seeking, Boredom Susceptibility, and Disinhibition. In adults, men have higher scores than women on this scale (Rosenblitt et al., 2001; Russo et al., 1991; Zuckerman et al., 1978). Feij et al. (1997) showed comparable results in a study of Dutch twins (aged 12 – 24) and adults (Feij et al., 1982).

2.2.3. Temperament

The Adolescent Temperament List (ATL; Feij and Kuiper, 1984) measures temperamental traits in adolescents, i.e. personality traits.

The ATL consists of 77 forced-choice statements of which the child has to indicate whether this is true or false for him/herself. The ATL measures the following personality traits: Extraversion, Emotionality, Impulsiveness, Thrill and Adventure Seeking, and Disinhibition/Experience Seeking. All the scales show sex differences, with girls showing higher scores than boys on all scales except for the scales, Thrill and Adventure Seeking and Disinhibition/Experience Seeking.

2.2.4. Hormone assay

The subjects were requested to collect a sample of saliva on the morning of the study immediately after awakening (i.e., before they had eaten breakfast or cleaned their teeth). Another sample was collected in the mid afternoon. Due to the fact that some children were unable to collect (enough) morning saliva by themselves, the number of samples for analysis were at the end 54 SS girls, 67 OS boys, and 70 OS girls, and regarding the afternoon saliva we analyzed 73 OS samples because one OS pair was unwilling to collect saliva. For the menarchal –premenarchal comparisons the final numbers for the morning saliva were: 66 menarchal and 58 premenarchal, and for the afternoon samples the number of samples used were: 69 menarchal and 59 premenarchal.

Testosterone in saliva was measured after diethylether extraction with an in-house competitive radio-immunoassay using a polyclonal anti-testosterone-antibody (Dr.Pratt AZG 3290). [1,2,6,7-^{3}H]-Testosterone (TRK402, Amersham Nederland B.V.) was used as a tracer The lower limit of detection was 10 pmol/L and inter-assay variation was 12.9, 8.6, and 8.3% at 55; 125, and 195 pmol/L, respectively (n = 24). Intra-assay variation was 7% (n = 150), 3.5% (n = 250), and 3.5% (n = 50) at 50, 150, and 300 pmol/L, respectively.

2.2.5. Pubertal status and menarche

Pubertal status was analyzed by questionnaire depicting five drawings of the pubertal development as described by Tanner

(1962). The girls had to identify the picture most similar to the development of their breasts and pubic hair; the boys were asked to do the same for 5 pictures of pubic hair and scrotum development.

Of the total girl group 46% were premenarchal (49% of the OS girls and 42% of the SS girls), and 54% girls menarchal (51% of the OS girls and 58% of the SS girls).

2.3. Statistical analyses

For all analyses, the data were used for one girl from each SS twin pair selected a priori at random. The circadian rhythm of testosterone and possible interactions with Type were analyzed in a 2 X 2 (morning/afternoon-sample X boys/girls) repeated measures analysis using multivariate procedures. Thereafter the two girls groups (OS/SS) were compared to determine whether OS girls showed a more female or more male circadian rhythm of testosterone.

Possible correlations between subscale scores and testosterone levels were calculated to control for possible effects of free testosterone on performance. The mean subscale scores for the OS boys were compared with those of the SS girls, using a multivariate procedure (MANOVA), to find out whether the tasks showed sex differences. Finally, the scores of the OS and SS girls were compared, using independent t-tests, to determine whether OS girls differed from SS girls. We assumed that differences between the two groups of girls could be explained by the effect of prenatal exposure to testosterone.

3. RESULTS

3.1. Circadian Rhythm of testosterone

In the group of OS boys and SS girls, testosterone levels were higher in the morning and decreased during the day ($F(1, 119) = 197.75$, $p < 0.001$) (Table 1), and the OS boys had higher levels than

the SS girls ($F(1, 119) = 197.75$, $p < 0.001$). Moreover, the decrease in testosterone levels during the day was greater in the OS boys than in the SS girls ($F(1, 118) = 7.636$, $p < 0.01$), due to the significantly higher testosterone levels in boys in the morning ($t(119) = -3.54$, $p < 0.001$).

In the girls (OS and SS), testosterone levels were also higher in the morning and decreased during the day ($F(1, 119) = 223.0$, $p < 0.001$) but there were no differences in morning ($F(1,121) = 2.64$, ns) or afternoon ($F(1, 121) = 0.08$, ns) testosterone levels between the two groups of girls. Thus, both the OS and SS girls showed the typical testosterone pattern of variation over the day.

Table 1. Mean morning and afternoon saliva testosterone levels (with SEM in parentheses) for the three twin groups, and (pre)menarchal girls.

Time sample	SS girls	OS boys	OS girls	Menarchal girls	Pre menarchal girls
Morning [pmol/L]	145.28 (7.23)	197.15 (12.75)	133.89 (5.93)	147.52 (7.14)	128.98 (5.33)
Afternoon [pmol/L]	81.96 (3.77)	89.78 (7.60)	72.96 (3.50)	81.81 (3.23)	71.93 (3.96)

Note. OS, Opposite-sex twin; SS, same-sex twin.

3.2. Menstrual cycle effects on testosterone

In order to find out whether there was an effect of menstrual cycle on free testosterone we compared circulating testosterone levels of premenarchal and menarchal girls. We hypothesized menarchal girls to have an overall higher level of free testosterone. Generally, we found in girls (both premenarchal (n=58) and menarchal (n=66)), that testosterone levels were higher in the morning and decreased during the day ($F(1, 122) = 229.7$, $p < 0.001$), however there was no interaction between menstrual cycle group and time of testosterone collection ($F(1,122) = 1.20$, ns). Still, there was a main effect of menarche, with menarchal girls having higher free testosterone levels compared to pre-menarchal girls ($F(1,122) = 5.19$, $p <$

0.03). Thus, the menarche was clearly associated with higher testosterone levels (see Table 1). There was no difference between OS and SS girls in the numbers that reached menarche, (chi-square = 0.59, ns).

Given that the menarchal status groups differed in testosterone level, we next calculated relations between our behavioral measures and morning and afternoon testosterone values respectively. There was no relation between afternoon free testosterone and any of the measures in both menarchal status groups, but only in menarchal girls significantly inverse correlations were found between morning testosterone and sensation (thrill) seeking (ATL_TAS, r = -.26; SSS_TAS, r = -.25; both $p < 0.05$) whereas we observed significantly positive correlations in premenarchal girls with sensation seeking (total SSS; r = 0.34, $p < 0.02$); SSS_DIS, r = 0.32, $p < 0.02$; SSS_ES, r = 0.35, $p < 0.01$).

3.3. Pubertal status

There was no difference in pubertal development between OS and SS girls, and this applied to both pubertal measures (breast and pubic hair; respectively chi-square = 2.76, and 1.54, all ns).

Correlations between testosterone and behavior

Since the two girl groups (OS and SS) did not show a different circadian pattern of testosterone levels, their data were combined. Overall, there were no relations between testosterone and any of the behavioral measures. In boys, a significant positive correlation was found between morning testosterone and scores on the Aggressive Impulses subscale of the OMAI and the Boredom Susceptibility subscale of the SSS, both r = -.26 ($p<.05$). In the girls, there was a positive relationship between morning testosterone and scores on the Experience Seeking subscale of the SSS (r = .21, $p<.05$) and a negative correlation with the Extraversion subscale of the ATL (r = -.21, $p<.05$).

3.4. Sex differences

All the statistically significant differences and trends were in the expected direction. On nearly all scales of the OMAI, the OS boys had significantly higher scores than the SS girls. There was only a tendency for OS boys to score higher than SS girls on the scales Aggressive Inhibitory Responses and Physical Aggression Against Adults (Table 2). On the RAI OS boys and SS girls differed significantly on all subscales, with boys showing more physical aggression. On the SSS, boys and girls differed significantly on the Disinhibition scale, with boys scoring higher on sensation seeking. Also on the ATL, boys scored significantly higher on the Disinhibition/Experience Seeking and Thrill and Adventure Seeking scales whereas girls tended to have higher scores on the Emotionality scale.

3.5. OS girls versus SS girls

As the Multivariate test was not significant, differences between the girls were tested using independent *t*-tests. The SS and OS girls differed significantly on the Withdrawal and Verbal aggression subscales of the RAI, with the OS girls showing a more masculine pattern of behavior (Table 2). On the other hand, on the SSS SS girls showed more experience-seeking behavior. No other differences between these groups were observed.

3.6. Effect size for the sex difference on the physical aggression scale of the RAI

The Effect size for the sex difference on the physical aggression scale of the RAI between the OS boys and the SS girls was 0.95, and within the OS twin-pair this was 0.86. This seems comparable to previously reported effects sizes (see Berenbaum and Resnick, 1997).

Table 2. Mean questionnaire results for the 3 twin groups, and the results of ANOVA tests for the effects of Sex (OS boys vs. SS girls) and Type (OS vs. SS girls).

Measurement scale	SS girls	OS boys	OS girls	Sex Differences		SS versus OS girls Differences	
				T	p-value	F	p-value
OMAI	*54*	*74*	*74*				
VAAA	2.42	2.93	2.43	14.21	0.001		Ns [a]
PAAP	1.71	2.20	1.77	13.18	0.001		Ns [a]
AI	1.60	1.91	1.70	5.75	0.020		Ns [a]
AIR	2.39	2.18	2.39	3.68	0.058		Ns [a]
PAAA	1.51	1.72 [b]	1.43	3.08	0.082		Ns [a]
VAAP	2.89	3.58 [b]	2.92	22.73	0.001		Ns [a]
RAI	*55*	*74*	*74*				
Physical aggression	5.29	8.54	5.70	31.64	0.001		Ns [a]
Withdrawal	10.87	7.78	9.78	25.46	0.001	1.91	0.030 [a]
Verbal aggression	9.51	11.24	10.81	11.82	0.001	2.71	0.004 [a]
Assertive reaction	10.35	8.38	9.70	13.52	0.001		Ns [a]
SSS	*55*	*73*	*73*				
TAS	40.98	42.88	40.00		Ns		Ns
ES	37.09	37.23	34.70		Ns	2.09	0.038
BS	38.69	38.70	36.53		Ns	1.75	0.082
DIS	31.31	35.21	32.63 [b]	10.20	0.002		Ns [a]
Overall SSS	11.65 [b]	12.14	11.30 [b]	3.21	0.076		Ns [a]
ATL	*55*	*73*	*73*				
DIS/ES	1.38	2.30	1.52	9.35	0.003		Ns [a]
TAS	5.64	6.75	5.37	6.17	0.014		Ns [a]
EMO	6.75	5.56	6.40	3.68	0.057		Ns [a]
EXTR	4.67	4.66	5.33 [b]		Ns		Ns
IMP	4.82	5.01	4.73		Ns		Ns

Note. OS, Opposite-sex twin; SS, same-sex twin; [a] one-tailed tests because of directional hypothesis; VAAA, Verbal aggression against adults; PAAP, Physical aggression against peers; AI, Aggressive impulses; AIR, Aggressive inhibitory responses; PAAA, Physical aggression against adults; VAAP, Verbal aggression against peers; TAS,Thrill and Adventure Seeking; ES, Experience Seeking; BS, Boredom Susceptibility; DIS, Disinhibition; DIS/ES and TAS, Sensation Seeking; EMO, Emotionality; EXTR, Extraversion; IMP, Impulsiveness; the number of participants are indicated in *Italic*s; Ns, nonsignificant; [b], N-1.

4. DISCUSSION

To get more insight into the origin of sex differences in human behavior, we compared SS and OS twin girls on various measures of sex-dimorphic behavior. We hypothesized that differences in aggression, sensation seeking, or temperament between SS and OS girls would support the notion that (prenatal) sex hormones, mainly testosterone, could have affected the development of these behavioral traits.

Research has shown a possible relation between the activational effects of current circulating testosterone and behavioral traits, besides the organizational effects of testosterone on sexually dimorphic behavior. For example, Mattsson et al. (1980) found a (rather small) link between testosterone and behavioral and personality variables and concluded that the increase in testosterone in adolescence may contribute to antisocial behavior in certain vulnerable boys. Moreover, Dabbs and de La Rue (1991) reported that the circadian rhythm of testosterone has a stronger effect on behavior than the menstrual cycle.

We found a clear circadian rhythm in testosterone levels, with higher levels in the morning, and a clear sex difference, with boys having higher overall levels than girls. This sex difference was mainly caused by the significant difference in testosterone levels in the morning; the afternoon testosterone levels were not significantly different between boys and girls. The OS and SS girls had essentially similar testosterone levels which were higher in the morning than in the afternoon.

Although some associations were found between free testosterone levels and some personality traits (e.g., Aggressive Impulses [OMAI] and Boredom Susceptibility [SSS] in boys; and Experience Seeking [SSS] and Extraversion [ATL] in girls), if there is a clear association between behavioral traits and circulating testosterone levels, we would have expected to find more and stronger correlations between testosterone and the behavioral scales. We therefore conclude that, at this age, there is no clear relationship between cir-

culating testosterone levels and the behavioral traits that we investigated.

Thus the differences between personality traits found in this study are not due to the activational effects of circulating testosterone but instead possibly due to an organizational effect of the hormone, exerted primarily in the prenatal period. The sex differences in aggression that we found were similar to the ones reported in literature, i.e. (OS) boys were more aggressive than (SS) girls. We also found a comparable effect size for the sex difference on the physical aggression scale of the RAI in our study, namely 0.95. In the literature the reported effect sizes range between the 0.7 and 1.1 (see Berenbaum and Resnick, 1997).

We hypothesized that the OS girls would show an aggression pattern more similar to that of boys than of SS girls. However, we obtained different results depending on the aggression questionnaire used. With the OMAI there were no differences between the two groups of girls, whereas with the RAI the OS girls had scores on the Withdrawal and Verbal aggression subscales that were in the expected, more masculine direction. This difference can be explained by the type of aggression measured by the two scales. The OMAI focuses more on overt aggressive behavior and only globally refers to relevant situational antecedents and the subsequent behavior (Van Goozen et al., 1995), whereas the RAI investigates the proneness to aggressive behavior. Moreover, the sensitivity to detect sex differences in aggression depends on the research method used (see Van Goozen et al., 1995). Differences are more often found when response alternatives are offered, for example a forced choice between a verbal or a physical aggression response, as in the RAI questionnaire. The differences found on the RAI are partly in line with the results of studies of women with CAH. Berenbaum and Resnick (1997) found that women with CAH had higher scores on the Physical aggression scale of the RAI than did controls.

As expected, we found a sex difference on the Sensation seeking scale (SSS), in particular Disinhibition, with OS boys having a higher score than SS girls. Again, testosterone levels did not seem to be

related to sensation seeking behavior in this age group. In older subjects androgens have been found to be associated with sensation seeking (e.g. Daitzman et al., 1978; Daitzman and Zuckerman, 1980). Interestingly, the two groups of girls differed on the subscale Experience Seeking and showed a tendency to differ on the subscale Boredom Susceptibility, with SS girls having higher scores. We cannot explain these differences because normally these scales do not show any sex differences. Our results are different from those of Resnick et al. (1993), who found that females from OS twins had higher overall scores on the SSS scale and Disinhibition and Experience Seeking subscales; however, their subjects were older and their sample was larger.

With respect to the ATL, we found two significant sex differences in the expected direction, for the subscales DIS/ES, and TAS, and one trend for Emotionality. However, no significant differences were determined for the other two subscales. This last finding is contrary to our expectations and is possibly due to the norm values being outdated (i.e. 1984; Personal communication J.A. Feij, September 2004). Finally, no indication was found for a prenatal testosterone effect as the results of both girl groups were similar.

We also looked at some aspects that could have played a role in the differences observed between the girls, such as pubertal stage and menarchal status. Both aspects did not seem to differ in such a way that it would have resulted in any alternative explanations of our findings. Pubertal development was essentially the same in both twin girl groups and there were no differences in the number of girls in each group that was either premenarchal or menarchal.

In conclusion, our data show that testosterone levels have a normal circadian rhythm in adolescent boys and girls, and that these testosterone levels do not seem to be systematically related to the personality traits that we investigated. However, the differences observed between the OS and SS twin girls were all in the predicted direction, and the OS girls showed significantly more aggressive behavior, this difference could have been caused by a greater pre-

natal exposure of the OS girls to testosterone. Although we cannot entirely exclude the possibility that environmental factors, such as gendered social norms and behavior, may have contributed to this pattern, we believe that by comparing groups of the same (female) sex we partially overcame this problem. Still, it is clear that in humans any behavioral or personality effects of prenatal testosterone exposure are hard to demonstrate, as the abundant but inconclusive literature from CAH girls or girls prenatally exposed to DES shows (Hines, 2004).

Chapter 6

Investigating the possible prenatal testosterone exposure effects on finger ratio: Is the finger pattern masculinized in female opposite-sex twins?

This chapter is based on Cohen-Bendahan CCC, van de Beek C, Berenbaum SA. Neuroscience and Biobehavioral Reviews, in press.

ABSTRACT

The development of the finger ratio, i.e., the relative length of the index finger to the ring finger, is assumed to be under the control of prenatal sex hormones. Prenatal testosterone has been suggested to stimulate the growth of the fourth digit, and estrogen the growth of the index finger. Therefore, the finger ratio is lower in men than in women. This marker has recently been extensively studied in relation to postnatal behavior. The characteristics of female members of opposite-sex (OS) and same sex (SS) twins could also indirectly reflect prenatal levels of sex hormones. In this chapter, we were primarily interested in the possible association between these two methods. We hypothesized that OS girls would have a lower (masculinized) finger ratio than SS girls, but no such a difference was found. However, we were also unable to establish a sex difference in our sample. Age and sample size may have played an important role in this outcome.

In addition, we attempted to confirm in our sample some of the reported relationships between, the finger ratio and sex-typed behavior that is supposed to be controlled by prenatal sex hormones, and finger ratio and circulating testosterone levels. We did not find the finger ratio and sex-typed behavior to be associated, but we did find the finger ratio to be negatively associated with circulating testosterone levels in boys, with high testosterone levels being associated to low finger ratios.

1. INTRODUCTION

A relatively new method for investigating the behavioral effects of prenatal exposure to sex hormones involves the use of morphological indices, specifically the finger ratio (the relative lengths of the index and ring fingers). This marker is assumed to reflect prenatal exposure to sex hormones and is studied in relation to postnatal behavior, with associations between this marker and behavior being taken to reflect the influence of prenatal sex hormones on the behaviors studied. In this chapter, we describe the rationale behind this method and briefly review the studies investigating associations between the marker and various behaviors thought to be the result of prenatal exposure to sex hormones. At the same time, we take a look at the relationship between the finger ratio method and the twin paradigm. Both methods are considered to investigate the role of prenatal sex hormones on the development of sexual dimorphisms in behavior, and thus it would be expected that findings obtained with the two methods would be associated in some way.

1.2. Finger Ratio (2D:4D)

1.2.1. Background

The ratio of the length of the second digit (index finger) to the length of the fourth digit (ring finger), the 2D:4D ratio, is assumed to be fixed by week 14 of fetal life (Garn et al., 1975). The development of the ratio has been hypothesized to be affected by testosterone, which is relatively high in male fetuses as compared with female fetuses from prenatal weeks 8 to 24 (Manning, 2002). If the 2D:4D ratio does, in fact, accurately reflect prenatal sex hormones, it would provide a simple and widely-available method for examining hormonal effects on human behavior. The evidence supporting this assumption is mostly indirect. The 2D:4D ratio is lower in men than in women, resulting from men showing longer fourth digit relative to the second digit, and women showing longer second digit relative to the fourth digit. (Other digit ratios have been

investigated and some also show sex differences (Manning et al., 2003a), but we concentrate on the 2D:4D ratio because it has been the most extensively studied.) This sex difference was noted more than 125 years ago (Ecker, 1875) and a standard method for measuring it was developed 55 years later (in Brown et al., 2002a). The finger ratio appears to be stable from age 5 (Manning et al., 1998 ; Williams et al., 2003) and shows a sex difference across races (Manning et al., 2000; Peters et al., 2002). In fact, the 2D:4D ratio shows larger ethnic than sex differences (Manning et al., 2003b). The sex difference in the 2D:4D ratio is found on both hands, although it is somewhat larger on the right than the left hand, *d*'s of .85 and .74, respectively (McFadden and Shubel, 2002).

The 2D:4D ratio is thought to be negatively related to testosterone levels in men and positively related to estrogen levels in both men and women (Manning et al., 1998). Testosterone has been suggested to stimulate the prenatal growth of the fourth digit, and estrogen to promote the growth of the index finger (Manning, 2002). Prenatal exposure to sex hormones has been considered important in the development of the ratio because the sex difference is apparent in childhood and the ratio appears not to be affected by the changing levels of sex hormones during puberty. The sex difference is suggested to arise about week 8 of gestation, when the fetal testes are formed in males. The homeobox genes, especially the HOXa and HOXd genes, are responsible for the differentiation of the urogenital system (including the gonads) (Herault et al., 1997; Peichel et al., 1997). These genes also influence the formation of human toes and fingers (Kondo et al., 1997), and a mutation in the HOXa gene leads to the hand-foot-genital syndrome, which is characterized by malformation of the digits, toes, and genitals (Mortlock and Innis, 1997). This relation between the formation of the gonads and the fingers has led to the hypothesis that finger differentiation, as measured by the 2D:4D ratio, may reflect prenatal testosterone production in humans during the first trimester of gestation (Manning et al., 1998).

There is limited direct evidence to support these assumptions. Testosterone and estradiol in amniotic fluid measured in the second

trimester were studied in relation to finger ratio in 2-year-old children (Lutchmaya et al., 2004). Results showed a negative association between the finger ratio and the ratio of fetal testosterone to estradiol, regardless of sex but more strongly for the right than the left hand. Given that finger ratio may change later in childhood, the meaning of this relation is not clear (Williams et al., 2003; Manning et al., 1998). Further, this relation was documented later in gestation than the presumed critical period for 2D:4D development (second vs. first trimester), although it may be explained if there is stability of individual differences in sex hormones across prenatal development. Studies in patients with congenital adrenal hyperplasia (CAH) are inconsistent, with some finding a more masculine finger pattern in females and males with CAH than in controls (Brown et al., 2002b; Ökten et al., 2002), but some finding no difference (Buck et al., 2003). Some of the inconsistencies may relate to differences in method: most studies in normal populations examine the finger ratio of the right hand, measured from photocopies, and this was the method used in the two studies finding differences between CAH and controls (Brown et al., 2002b; Ökten et al., 2002). The study failing to find differences used X-rays of the left hand (Buck et al., 2003).

The details of the theory and evidence regarding the 2D:4D ratio have been extensively discussed in recent reviews (Putz et al., 2004; Manning, 2002). We do not wish to provide another review, but instead we would like to consider the value of the finger ratio as a method to investigate the role of prenatal exposure to sex hormones on sex-typed behavior and its association with another "indirect" method, i.e. the twin paradigm. In the next section we elaborate on the association between groups exposed to presumed "atypical" prenatal testosterone levels and the effect on the finger ratio.

Sexual Orientation. Findings of finger ratio in relation to sexual orientation are complex. Studies in men have been motivated by two conflicting hypotheses. On the one hand, homosexual men were hypothesized to have been exposed to relatively *high* levels of testosterone *in utero*, which would be associated with a lower 2D:4D ratio than that found in heterosexual men. Two studies found this

to be the case (Rahman and Wilson, 2003; Robinson and Manning, 2000), but one did not (Williams et al., 2000). On the other hand, homosexual men also have been hypothesized to have been exposed to *lower* prenatal testosterone levels. Data from two studies are consistent with this hypothesis (Lippa, 2003; McFadden and Shubel, 2003), showing homosexual to have a higher finger ratio (on both hands) than heterosexual men; one study was large and considered effects of ethnicity (Lippa, 2003). There is no direct evidence that would favor one or the other hypothesis about prenatal testosterone exposure in gay men, but indirect evidence supports the idea that they have had a lower exposure, given their demasculinized/feminized interests and reduced spatial ability (Bailey and Zucker, 1995; McCormick and Witelson, 1991; Wegesin, 1998; but see Gladue and Bailey, 1995). Alternatively, the inconsistency may reflect population differences in 2D:4D and the fact that studies sample different populations, in this case the United Kingdom and the United States (Manning and Robinson, 2003). Mean 2D:4D appears to be constant across populations of homosexual individuals, but variable across populations of heterosexual individuals (higher in the UK than in the USA), and the data indicating hypermasculinization come from the UK and those indicating hypomasculinization from the USA (Manning and Robinson, 2003).

Studies of women have generally been motivated by the hypothesis that homosexual women have been exposed to higher levels of testosterone *in utero* than heterosexual women, consistent with their masculinized interests (Bailey and Zucker, 1995). One study failed to find a difference in finger ratio between heterosexual and homosexual women after correction for ethnicity (Lippa, 2003), but two other studies found that lesbians had a lower (masculinized) finger ratio (Rahman and Wilson, 2003; Williams et al., 2000), and another found a difference in butch-type (i.e. lesbians that consider themselves more masculine than other women), but not femme-type, lesbians (Brown et al., 2002c). A study of the finger ratio in female monozygotic twins provided an innovative way to control for genetic and environmental background factors (Hall and Love,

2003) and showed an association between finger ratio and sexual orientation. In female twins discordant for sexual orientation, the lesbian twin had a significantly lower (masculinized) finger ratio in both hands than her heterosexual co-twin, whereas in twins concordant for sexual orientation, there was no difference in the finger ratio. Thus, the data suggest that a masculinized finger ratio is associated with homosexuality in women. The evidence in men is inconsistent. Given that differences in other sex-typed traits between homosexual and heterosexual individuals are equivocal (except for interests), the inconsistency in finger ratio is not surprising.

Sex Role. The development of sex role has been related to prenatal exposure to androgens (as described above for CAH), and it is reasonable to hypothesize that a "masculinized" role would be associated with a lower finger ratio in women. One study is consistent with this: the 2D:4D ratio was lower in women who had more "masculine" scores on a sex role questionnaire (Csathó et al., 2003).

Developmental Disabilities. Given the sex difference in autism and the suggestion that it is caused by exposure to high levels of testosterone *in utero* (Baron-Cohen et al., 2004), the possible association between autism and the finger ratio has been investigated (Manning et al., 2001). The 2D:4D ratio was lower in families with autistic individuals than in the general population. Children with Asperger syndrome, which is considered a mild form of autism, had higher ratios than children with "pure" autism, with both having ratios lower than normal children.

1.2.2. Interpretation of Findings Regarding Associations with Finger Ratio

The studies discussed above assume that the 2D:4D ratio reflects the fetus's exposure to sex hormones early in gestation. It is important to bear in mind that the ratio is an indirect measure of sex hormones and is influenced by other factors. For example, the finger ratio appears to be associated with ethnicity more than with sex, and is affected by handedness. Effects are more pronounced for the right hand, and a similar asymmetry has been seen in mice (Brown et al., 2002a).

In summary, it would be interesting to investigate whether girls presumed to have been exposed to higher levels of testosterone develop a finger pattern that is more typically male, i.e. a longer 4th digit than 2nd digit, and thus a low finger ratio, than girls exposed to lower levels of testosterone. Opposite-sex (OS) twin females may serve as an ideal group to study this phenomenon. Because twins are normal and healthy, unlike individuals with CAH, information may be obtained about the effect of prenatal exposure to testosterone in the normal population.

In animal studies, intrauterine positioning during pregnancy has been related to prenatal exposure to testosterone. Female rodents that had developed between two male rodents during pregnancy showed a more masculine array of sex-typed behavior postnatally (see review, Ryan and Vandenbergh, 2002) than did females that had developed between one or no male fetuses. A human equivalent of this is the twin (or multiple) pregnancy, in which the female member of an OS twin is thought to be exposed to higher levels of testosterone prenatally than a female member of a female-female (SS) twin. Research in the past has shown that female OS girls may be masculinized because of prenatal exposure to testosterone; for example, such girls have crown sizes more typical for boys (Dempsey et al., 1999) and show more cerebral lateralization (this thesis).

1.2.3. Aim

The aim of this study was to investigate the possible effect of prenatal exposure to testosterone on the development of the finger pattern in twins. In addition, we looked at the possible association between the finger ratio, sex role behavior and identity, and current circulating levels of testosterone.

The following questions were addressed:

(1) Is there a sex difference in finger ratio between OS boys and SS girls?
(2) Do OS girls show a more masculinized finger ratio compared to SS girls?

(3) Is there a negative association between finger ratio and masculine gender role (VGV- k) in our total sample?
(4) Is there a link between current circulating free testosterone levels and finger ratio in right-handed subjects?

2.. METHOD

2.1. Participants

The digit lengths were measured in a subsample of children from a longitudinal study (see for more detail, Cohen-Bendahan et al., 2004) when the children were 13 years old. The children were asked to provide a photocopy of the ventral surface of both their hands. The photocopies were coded to conceal the twin type or sex before the fingers were measured. We received photocopies for 29 opposite-sex (OS) boys, 29 OS girls, and 26 same-sex (SS) twin girls.

2.2. Measurement of the finger ratio

The lengths of the second and fourth digits were measured to the nearest 0.5 mm on photocopies for both hands from the basal crease of the digit to the tip, using a Vernier caliper. If there were several creases at the base of the digits, the digit length was measured from the most proximal of these creases. The digit ratio (2D:4D) was calculated by dividing the length of the index finger (second digit) by the length of the ring finger (fourth digit). Two researchers independently measured the photocopies. We calculated the repeatability of our measurements in the form of Cronbach's alpha. The internal reliability of these measurements was good for both hands: Cronbach's alpha was 0.91 for the right hand and 0.90 for the left hand.

2.3. Instruments

All measurements were taken as part of a longitudinal study, with testing moments at the age of 10 and 13 years.

2.3.1. Estimation of Intellectual ability - Wechsler Intelligence Scale for Children – Revised (WISC-R, Wechsler, 1974; Dutch version WISC-RN, Vandersteene et al., 1986)

Two subscales were administered to measure Full Scale Intelligence (IQ), namely, the Vocabulary and Block Design of the WISC-RN. These two subtests show a high reliability and correlate well with the Full Scale Intelligence score and are easily converted into an estimate of Full Scale IQ (Sattler, 1988).

2.3.2. Questionnaire gender preference – Children's version (VGV-k)

A Dutch questionnaire was used to measure gender preference in children (*Vragenlijst gendervoorkeur - Kinderen* (VGV-k); Ijntema et al., unpublished). This questionnaire consists of 30 questions relating to areas concerning peer-preference, gender-role, and gender-behavior. Each question has a masculine, feminine, or neutral answer. An example of a question on behavior is: "Which sport do you prefer to play: soccer, swimming, or ballet?". The masculine answer ("soccer") receives 3 points, the neutral answer ("swimming") 2 points, and the feminine answer ("ballet") 1 point. The total score varies between 30 (very feminine) to 90 (very masculine) points. The questionnaire has been shown to clearly differentiate between boys and girls.

2.3.3. Hormone assay: current circulating T levels

Age 10 years:

Testosterone in saliva was measured after diethylether extraction with an in-house competitive radioimmunoassay, using a polyclonal anti-testosterone-antibody (Dr. Pratt AZG 3290). [1,2,6,7-^{3}H]-Testosterone (TRK402, Amersham Nederland B.V.) was used as a

tracer following chromatographic verification of its purity. The lower limit of detection was 10.1 pmol/L and inter-assay variation was 10% at 42 pmol/L (n = 180). Saliva sampling is a non-invasive, stress-free technique and saliva testosterone levels provide a very good indication of the free testosterone level in serum (Ostatníková et al., 2002).

Age 13 years:

The subjects were requested to collect a sample of saliva on the morning of the study immediately after awakening (i.e., before they had eaten breakfast or cleaned their teeth). Another sample was collected mid afternoon. Testosterone levels were measured as described above. The lower limit of detection was 10 pmol/L and inter-assay variation was 12.9, 8.6, and 8.3% at 55; 125, and 195 pmol/L, respectively (n = 24). Intra-assay variation was 7% (n = 150), 3.5% (n = 250), and 3.5% (n = 50) at 50, 150, and 300 pmol/L, respectively.

2.4. Statistical Analyses

As not all variables were normally distributed, the relationships between finger ratio and the investigated other measures were analyzed using Spearman's nonparametric rho tests. Simple linear regression analysis was performed to establish a possible link between the finger ratio and masculinization (VGV-k).

3. RESULTS

Only about 50% of the twins sent in a copy of their hands. Statistical analysis showed that there was no difference in IQ (WISC-RN) between the twins that had sent in their copy and those that had not (t (183)= 1.75, ns).

3.1. Sex differences – OS boys versus SS girls

No significant differences in 2D:4D ratio were found between the sexes for either hand, see Table 1. An analysis of statistical power based on effect sizes reported by McFadden and Shubel (2002; left

hand: .74, right hand: .85) showed a statistical power of more than .79 for the left hand and more than .90 for the right hand. Therefore, the nonsignificant result for sex differences in finger ratio in our sample was most probably not due to the small sample size.

3.2. SS- versus OS girls

The 2D:4D ratio was not different in the two girl groups, see table 1.

Table 1. Mean finger ratio, testosterone levels, and sex role in the three groups.

	OS Boys		OS girls		SS girls	
		N		N		N
Right 2D:4D.	9669(.039)	29	.9721(.035)	29	.9700(.034)	26
Left 2D:4D	.9655(.041)	29	.9681(.035)	29	.9729(.031)	26
VGV-k	72.59 (5.38)	29	55.03 (5.05)	29	53.77 (6.17)	26
Free testosterone 10yrs*	32.17 (12.26)	29	41.41 (14.63)	29	47.21 (20.87)	24
Free testosterone 13yrs [am.]*	195.46 (108.59)	28	140.07 (36.66)	28	147.48 (54.91)	25
Free testosterone 13yrs [pm.]*	83.29 (56.07)	28	68.04 (21.50)	28	84.81 (26.97)	26

Standard deviation in parenthesis; *, pmol/L.

3.3. Correlations for gender-role (masculinization) with finger ratio

Correlational and linear regression analyses between finger ratio's and masculinization scores, as measured with VGV-K, turned out to be nonsignificant, and this applied to the combined group as well as the sexes separately.

3.4. Correlations between current circulating testosterone levels and finger ratio (only right-handed subjects)

In right-handed boys, the finger ratio (both hands) was negatively correlated with the mean morning testosterone level (left hand: $r=-.61$, $p<.001$; right hand: $r=-.42$, $p<.05$; 13 years), and the finger ratio for the left hand was negatively correlated with the mean afternoon testosterone level ($r=-.56$, $p<.01$; 13 years).

3.5. Summary of the results

The present study did not replicate the finding of a sex difference in finger ratio as reported in previous studies (e.g. McFadden and Shubel, 2002; Manning, 2002). In addition, when comparing OS girls and SS girls no potential effect of prenatal exposure to testosterone on finger ratio was observed, nor was there an association between masculine gender-role behavior and finger ratio. However, in boys we found a significant negative correlation between circulating testosterone levels and finger pattern, but the pattern of results was different to the one previously reported, i.e. a nonsignificant finding (Neave et al., 2003).

4. DISCUSSION

The aim of this study was to determine whether the twin paradigm is supported by another model for (presumed) prenatal exposure to testosterone, namely the finger ratio. Researchers have proposed that the sexual dimorphism in the finger ratio is due to prenatal levels of testosterone. In addition, we also investigated the relationship between gender-role behavior (masculinity) and current circulating testosterone levels and the finger ratio in our whole sample. Some of these relations have been studied previously (Neave et al., 2003; Csathó et al., 2003; Wilson, 1983) because of the suggested association between finger ratio and prenatal exposure to testosterone.

Power analysis showed that our research groups should be large enough to detect a possible sex difference in finger ratio. However, we did not detect such a difference in our adolescent subjects. We do not think that measurement error was a likely candidate to explain our findings, because the measurements showed strong internal reliability. We also do not believe that the quality of the hand copies was a relevant issue. Sex differences in the finger ratio are reported to stabilize between 2 and 5 years of age (Williams et al., 2003; Manning, 2002), although Manning (2002) reported uncertainty regarding the stability of the finger ratio in, for example, girls before puberty. However, recently the same group published a

study in which they found sex differences in children aged between 5 and 14 years, similar to those seen in adults. There was no indication that the finger ratio changed as the children grew up (Manning et al., 2004). Nonetheless, Manning and his colleagues advocate a thorough longitudinal investigation to establish whether finger patterns change during childhood and puberty.

A different possibility that should be taken into account is that OS males could be hypomasculinized/feminized as compared to singleton males and SS males (see for review, Cohen-Bendahan et al., in press). Some studies failed to find such a hypomasculinization of sex-typical features (e.g., Elkadi et al. 1999; Dempsey et al., 1999). It has been suggested that estrogen levels are also related to the finger ratio in men (Manning, 2002). However, it is difficult to understand how prenatal exposure to estrogen is related to the development of the finger ratio (Manning et al., 1998; Lutchmaya et al., 2004), or indeed other differences between boys and girls because exposure to this hormone comes largely from the mother and is identical for all fetuses, regardless of their sex.

Prenatal exposure to testosterone is reported to be associated with the development of sex differences in the finger ratio. Studies with CAH females found a reduced finger ratio, i.e. more masculine, compared to that of control females, most probably because of prenatal exposure to high levels of adrenal androgens (Ökten et al., 2002; Brown et al., 2002). In our studies, we assumed that the OS girls would have been exposed prenatally to higher levels of testosterone than SS girls because they had shared the uterus with their co-twin brothers. However, the OS girls did not show a more masculine finger ratio. The nonsignificant finding could be explained in several ways: (1) The level of exposure to testosterone in OS females was too low to have an effect or did not take place during the appropriate period. It has been hypothesized that, to be effective, exposure to testosterone must occur before week 14 of gestation. During this period, the OS females would be most susceptible to the effects of exposure to androgens because the skin is still permeable to fluids and other dissolved solutes, thereby facilitating a direct transfer of substances (Abramovitch and Page, 1972; Brace, 1989). An indication that the level of exposure may have

been too low for any masculinizing effects to occur is that OS females do not show any obvious genital virilization, whereas CAH girls do. (2) Although statistical power analysis showed that we had enough subjects to detect a male – female sex difference, it may be that more subjects are needed when comparing girls only.

Csathó et al. (2003) and Wilson (1983) investigated a possible link between masculinity in women and digit ratio. Wilson studied this in a very unusual way, by asking women in a newspaper article to classify themselves in comparison with other females as "assertive and competitive" (read: "masculine"), "fairly average" or "gentle and feminine". The women with more masculine (lower) finger ratios classified themselves as more "assertive and competitive". We were not able to replicate this finding, because we did not find a relation between masculinization, (VGV-k score) and finger ratio.

Neave et al. (2003) found no relation between saliva testosterone and finger ratio in right-handed men (no women were included in their sample). We found that right-handed boys with higher salivary testosterone levels had a lower finger ratio. Although no comparable effect was found in the girls, however, we do not have an idea explaining why.

A recent review of the literature on the 2D:4D ratio (Putz et al., 2004) addressed some of the methodological issues of published studies (e.g., *Type I* error) and concluded that some evidence supports an association between prenatal exposure to sex hormones and the finger ratio. However, it also raised some interesting questions, such as why the 2D:4D finger ratio is not associated with other sexually dimorphic traits or similar variables which are known to be influenced by sex hormones. The reviewers suggested a role for developmental timing, that is, all traits depend on hormonal influences, but they may differ in developmental timing and therefore will not be correlated with each other.

In this present study we have tried to confirm the twin paradigm with another model for prenatal testosterone exposure, i.e. finger ratio, however, without any result. Whether this is due to the weakness of one or both methods is difficult to tell. Unfortunately, the results did not produce the desired support for our twin paradigm.

Chapter 7

Educational achievement in a national sample of Dutch female twins and their matched singleton controls: influence of social or hormonal environment?

This chapter is based on Cohen CCC, van Goozen SHM, Orlebeke JF, Buitelaar JK, Cohen–Kettenis PT. 2002. Twin Research, 5: 273-277.

ABSTRACT

The aim of this study was to investigate the role of prenatal testosterone on the development of sex differences in educational achievement. For this purpose, we compared the educational achievement of female opposite-sex twins and same-sex female twins, and their matched singleton controls with an older brother or sister. Children were selected from participants of a national test of educational achievement in the years 1993 to 1998. To ensure the representativeness of the selected groups, we also compared the achievement scores of the twins and the controls to those of the total Dutch female population tested in the same period. We analyzed the results of the following educational achievement scales: Language, Mathematics, and Information Processing. The first two scales were chosen because they show annually sex differences in the total Dutch population, whereas Information Processing does not. Results showed that there was no evidence that prenatal testosterone or environmental aspects influenced educational achievement measured with these scales.

Singleton classmates performed significantly better than the twins on all three scales, whereas the twins performed as well as the total Dutch female population. We believe that our singleton control group was not optimal and that selection bias at the level of the schools may have confounded this comparison.

1. INTRODUCTION

Researchers agree on the existence of sex differences in educational achievement in verbal and mathematical abilities. For example, girls tend to excel on verbal tests and boys perform better on math tests (e.g., Halpern, 1996; Maccoby and Jacklin, 1974). But what is the origin of these differences? Investigations have followed two main directions, namely, gender upbringing and role models. However, recently the influence of sex hormones has been emphasized. Researchers have shown that sex differences in sex-typed behavior are influenced by sex hormones that are present during prenatal development, confirming the results of studies in other mammalian species (for review see, Cohen-Bendahan et al., in press). Although most evidence comes from clinical populations in which prenatal hormone exposure is atypical for a person's sex, there is increasing evidence that prenatal exposure to sex hormones has a role in the normal population (e.g., Berenbaum, 2001; Grimshaw et al., 1995; Finegan et al., 1989).

These findings prompted us to investigate the role of testosterone in sex differences in performance of the CITO test (a national test of educational achievement for children at elementary school taken in the 6th grade). It has repeatedly been found that in the 6th grade boys outperform girls on the Mathematics and World Orientation scales of the CITO, whereas girls outperform boys on the Language scale (Uiterwijk, Personal communication, 1996, 1998; Citogroep Internal publication, 2004). No differences are found on the Information Processing scale.

Animal studies have offered us an interesting model to investigate the effects of prenatal testosterone exposure on the development of sex differences. This model is based on naturally occurring variations in hormones that result from an animal's position in the uterus and the sex of its littermates (for reviews, see Ryan and Vandenbergh, 2002; Clark and Galef, 1998; vom Saal, 1989). Female fetuses that develop between male fetuses *in utero* show less typically female behavior (e.g., aggression, attractiveness to males), anatomy (e.g., anogenital distance), and reproductive characteris-

tics (pubertal maturation, reproductive life) later in life than do female fetuses that develop between female fetuses *in utero*. This effect extends beyond rodents. For example, in swine, female fetuses surrounded by male fetuses *in utero* are more likely to participate in and to win fights later in life than female fetuses positioned between female fetuses *in utero* (Rohde Parfet et al., 1990). The masculinizing effect on females of gestation close to males is attributed to the transfer of testosterone from the male fetus to the adjacent female fetus (Even et al., 1992). The studies of the effects of intrauterine position are consistent with studies in which hormones were manipulated directly and shown to affect behavior later in life (Becker et al., 2002; Goy and McEwen, 1980). They confirm the importance of early developmental exposure to sex hormones for the development of sex-typed behavior.

The human equivalent of this model is twins (e.g., Miller, 1994; Resnick et al., 1993). Twins may also be affected by the sex of the co-twin, in parallel to the effect of intrauterine position in animals, and thus provide an opportunity to study, in humans, the effect on cognitive function of prenatal exposure to higher-than-average-female (or lower-than-average male) levels of testosterone. The female member of an opposite-sex (OS) twin pair is assumed, as a result of sharing the womb with a male co-twin, to be exposed to higher levels of testosterone during prenatal development than is a female member of a (dizygotic) same-sex (SS) twin pair. Thus, OS females would be expected to show more typically male characteristics and fewer typically female characteristics than SS females.

We conducted a nation-wide study of the educational achievement of Dutch female twins and their female classroom singleton controls. Moreover, to take the effect of the domestic environment into account, the matched control girl had to have an older sister if the twin couple consisted of two girls or she had to have an older brother if the twin couple consisted of opposite sex children. The focus of the study was on girls only because the present study is part of a larger prospective follow-up project on female twins.

The aim of the study was to examine whether the sex differences in educational achievement could be explained by prenatal brain

exposure to testosterone. Therefore we compared OS female twins to SS female twins and expected that the OS girls would show a more male-typical pattern of educational achievement than the SS girls, i.e., better score on Mathematics, and lower score on Language. In order to obtain a better understanding of environmental influences, we investigated the possible differences between the matched singleton controls. All our subjects were selected from participants of a national test of educational achievement in the years 1993 to 1998. To ensure the representativeness of the selected groups, we also compared the achievement scores of the twins and the controls to those of the total Dutch female population tested in the same period.

2. MATERIALS AND METHODS

2.1. Procedure

The study population consisted of a nation-wide sample of 6th grade subjects that participated in the "CITO" elementary test (Citogroep, *Eindtoets Basisonderwijs*, 1997; a national test of educational achievement) between the years 1993-1998. The subjects were girls with a male or female co-twin and singleton girls with an older brother or older sister. In order to protect the anonymity of the participants, the CITO organization does not register the date of birth of the participants. To find out whether schools that had taken part in the CITO test had participating twins, we searched the database for double surnames in the same school and year. Schools with at least six double surnames were contacted by letter. There turned out to be 742 schools in the Netherlands and all schools were contacted, by letter, to ask whether the names on the list belonged to a twin pair, and that if this were the case, whether they would be prepared to give the names of the twin pair and the name of a singleton female classmate - with an older brother or sister.

2.2. Sample

Using this procedure, 222 girls with a boy co-twin and 359 girls with a girl co-twin were selected as participants. We could not determine the zygosity of the twins because we did not have access to blood-samples of the twins since they were anonymous subjects to us. Data were collected via the CITO organization and schools. For comparison, 212 singleton girls with an older brother and 238 singleton girls with an older sister were selected. All participants were between 11 and 12 years old.

2.3. Measures

Educational achievement was assessed by the Dutch CITO elementary test (Citogroep, *Eindtoets Basisonderwijs*). The CITO consists of 240 multiple-choice items assessing four different intellectual skills: Language, Mathematics, Information Processing, and World Orientation. Each performance scale contains 5 or 6 subscales, with a total of 60 multiple-choice questions. Together these performance scales result in a standardized score between 501 and 550. The test is administered on 3 consecutive days.

Because we used the data for the years 1993 to 1998 and the World Orientation scale was only added to the CITO test after 1994, we analyzed the results of the performance scales Language, Mathematics, and Information Processing only.

2.4. Statistics

The Statistical Package for Social Science (SPSS) was used to analyze the data. Independent samples T-tests were used to compare the mean scores of the twin and singleton groups. In order to examine whether the results of our twin and singleton groups were representative of those of the total Dutch female population we also tested whether there were differences in educational achievement between these three groups. One sample T-Tests were used to analyze these differences.

The magnitude of the effect size was calculated according to Cohen (1988). Effect size is the difference between two means, divided by the pooled standard deviation of the two groups (twins and singletons). In general, an effect size of approximately 0.80 is considered large, 0.50 moderate, and 0.20 small (Cohen, 1988). The significance level for all analyzes was set at $p < 0.05$.

3. RESULTS

Within the twin group no significant differences were found between same-sex and opposite sex twin girls. Nor were significant differences found within the singleton group between girls with an older sister or brother.

For further analysis, we combined these subgroups into a twin group and a singleton group. Comparison of the total mean CITO test scores of singletons and twins over the years 1993-1998 revealed a significant group difference with the singleton group outperforming the twin group (mean Singletons = 537.47 (sd = 8.93), mean Twins = 535.45 (sd = 9.61), $t = 3.43$, $p < 0.001$).

We also analyzed whether a group difference was found for the three separate performance scales. Table 1 shows the mean performance of the twins and singletons on the three main scales of the CITO test. The results clearly indicate that the singletons performed significantly better than the twins on all three performance scales (see Table 1).

Finally, in order to find out whether the scores of our twin and singleton groups were representative of those of the total Dutch female population we compared the mean scores of these three groups for each performance scale. In Table 1 the mean scores for each group are presented.

Table 1. Mean CITO scale scores (1993–1998) of the total Dutch female population, the female singleton controls, and the female twins are presented together with the standard deviation (the significance of the differences is indicated)

	Population (P) N = 340877	Singletons N = 447	(S)Twins (T) N = 577	t(SXT)	t(PXT)	t(SXP)
Language	43.22(9.22)	45.29 (8.57)	43.44 (8.99)	3.32**	0.59	5.10**
Mathematics	40.57(11.46)	43.31 (10.82)	41.06 (11.35)	3.21**	1.03	5.35**
Information Processing	42.93(9.59)	44.81 (8.64)	43.19 (9.47)	2.87*	0.65	4.61**
Total		537.47 (8.93)	535.45 (9.61)	3.43**		

Note: * = $p < 0.01$, ** = $p < 0.001$

Results showed that the singleton group performed significantly better than the Dutch female population on all three achievement scales. There were no differences in performance between the twin group and the Dutch female population.

The effect sizes found between the twins and singletons for the three educational achievement scales were respectively 0.21 for Language, 0.20 for Mathematics, and 0.18 for Information Processing. Very small effect sizes were found between the twins and the total Dutch female population, namely, 0.02, 0.04, and 0.03, respectively.

4. DISCUSSION

In this chapter, we looked at the possible prenatal effects of testosterone in twins in the 6th grade. However, no differences were detected between the OS females and the SS female twins. Therefore we found no evidence for the assumption that prenatal exposure to testosterone affects the educational achievement, as measured with the annual CITO test, of OS twin girls in a masculine direction. Power analyses based on effect size between the mean scores for boys and the mean scores for girls in the population showed that

our groups should have been large enough to detect a sex difference (Personal communication H. Uiterwijk, 1996, 1998; Citogroep Internal publication, 2004). However, this may be the hitch since we were looking for a within sex variation, i.e. the comparison between female twins with a brother and female twins with a sister. In addition, we did not have access to information concerning the type of zygosity of the same-sex female-female twins. Controlling for dizygosity in SS female twins may be important and crucial in the comparison with (dizygotic) OS twins. Prenatal environmental factors that may differ between monozygotic and dizygotic twins could have affected the outcome of a comparison between the groups.

The twin paradigm has been criticized, mainly because of the possible environmental and social effects on OS girls of being bought up with a twin brother (i.e. masculine environment). Therefore we collected data from singleton girls (classmates) with an older brother or sister, in order to be able to make clear statements regarding potential environmental and experiential postnatal influences of siblings. Although this does not help to explain our lack of significant findings, it is of relevance to an overall understanding of the development of sex differences. If sex differences are influenced by environmental influences, such as role models or gender-specific cues, then we would have expected the two singleton groups to differ among each other, i.e., singletons with an older brother would score in a more male-typical manner than the female singletons with an older sister. As this was not the case, possible experiential influences are probably not important factors contributing to the development of sex differences in educational achievement. However, within sex comparisons and the sample size may have interfered with the ability to detect a significant difference between the singletons girls with an older brother or sister.

An interesting and unanticipated finding was that the female twin group (n=577) performed significantly worse on all three scales of the CITO test than did their matched singleton female controls (n=447). On the basis of these results, one could conclude that the educational achievement of twins is worse than that of their

matched singleton controls. This conclusion is in line with other studies and would imply that twins do have a disadvantage in this respect. Studies have shown that a low birth weight has a negative impact on cognitive development (e.g., Seidman et al., 1992) and it is known that twins are generally born with a lower mean birth weight than singletons.

Although we tried to compose the best possible singleton control group, the selection of the control cases by the participating schools may have had an unforeseen consequence. It is possible that our singleton control group was not as properly selected as we intended. The statistical analyses comparing the female twin data to those of female population showed that the twin mean scores were more similar to the population mean scores than to the female singleton mean scores: the scores of our singleton control group were clearly higher than those of the population mean. It is possible that this is due to a selection bias in the choice of the singleton controls by the schools participating in the study. The fact that the study involved a national comparison of female CITO test scores, and that the CITO outcomes are used to compare the performance of schools, could have influenced the selection procedure of the teachers, in that they may have chosen the cleverest girls as comparison subjects. If we examine the results of the comparisons of scores of the twins and the female national population it becomes clear that these are similar and we therefore could also conclude that there are no differences in educational achievement between female twins and singletons. The conclusion is supported by the negligible effect sizes we found.

Although we intended in this study to select the best possible control group of singletons by selecting classmates of the twins, a selection bias operative at the level of the schools may have confounded the comparison. In the future, this may be overcome by using a random selection of control cases from all possible candidates that match the selection criteria.

Chapter 8

Summary of results and general discussion

This chapter is based on Cohen-Bendahan CCC,
van de Beek C, Berenbaum SA.
Neuroscience and Biobehavioral Reviews, in press.

1. SUMMARY

1.1. Background

Evidence from animal studies seems to indicate that the prenatal hormonal milieu, as a consequence of intrauterine positioning, is associated with the expression of sexual dimorphisms in morphology and behavior. This has been explained in terms of the diffusion of androgens, mainly testosterone, among womb mates. An equivalent of this phenomenon can be found in humans, in opposite-sex twins. The females of female-male twin pairs may be exposed to higher levels of testosterone *in utero* than female same-sex twins and thus provide a natural model to investigate the role of early testosterone in the development of sex differences. Well-documented animal studies provide a good basis for this assumption (e.g., Ryan and Vandenbergh, 2002; Vom Saal, 1989).

In the present dissertation, we attempted to study the influence of testosterone on the development of sex-typed behavior by using this opposite-sex twin paradigm. We first examined whether the sex of the fetus is associated with maternal serum steroid levels (chapter 2). In chapters 3 and 4 we presented two studies on the effects of prenatal exposure to testosterone in children aged 10 years, and in chapter 5 the results of a study of the same children when they were 13 years old. In chapter 6 we evaluated another indirect method of investigating the effects of prenatal exposure to testosterone, namely, the finger ratio in our twin sample, and also tested whether the finger ratio is associated with specific characteristics. In chapter 7 we explained the results on educational achievement in terms of the twin paradigm. The results are summarized in the following sections.

1.2. Are maternal serum steroid levels related to sex of the fetuses in twin pregnancies?

Although prenatal exposure to testosterone probably occurs, there are only limited data demonstrating the transfer of testos-

terone in human multiple pregnancies. In pregnancy, hormones from one fetus may reach another fetus via transmembrane transport (throughout pregnancy) or via the maternal-fetal circulation (Meulenberg and Hofman, 1991). In this study, we were interested in whether maternal serum steroid levels are affected by the sex of the twins the mother is carrying. Meulenberg and Hofman (1991) and Klinga et al. (1978) were the only investigators to show a difference in maternal testosterone levels during gestation between mothers pregnant with a male child and those pregnant with a female child, i.e. maternal testosterone levels were higher in boy pregnancies than in girl pregnancies. However, the pregnancies were singleton pregnancies, and there have been no studies of twin pregnancies. We hypothesized that if maternal serum steroid levels depend partly on the sex of the fetus, mothers pregnant with two boys would have different levels of steroids in their serum than mothers pregnant with opposite-sex twins or female-female twins.

We did not find an effect of the sex of the fetus on the serum steroid levels of mothers pregnant with twins. Possible explanations are that hormone concentrations were not measured at an appropriate moment or that the sex of the fetus does not influence maternal serum steroid levels. The latter is in accordance with many studies in singletons (e.g. Nagamani et al., 1979; Rodeck et al., 1985). Although another possibility for the nonfinding could be because of a sample size effect – the sub-group of male-male twins was (unexpectedly) rather small. If these (non-significant) results are replicated in a larger group, it may be that an increased prenatal exposure to testosterone in OS female fetuses occurs mainly through the direct feto-fetal route of hormone transfer in the amniotic sac rather than via the indirect maternal-fetal route. It has been shown rather convincingly in several studies (e.g., Nagamani et al., 1979; Rodeck et al., 1985; Van de Beek et al., 2004) that the levels of testosterone in amniotic fluid are higher with a male fetus than with a female fetus, and animal research has clearly shown that testosterone is transported across fetal membranes (e.g., Even et al., 1992).

1.3. Prenatal testosterone and cerebral lateralization

A further goal was to investigate whether prepubertal, 10-year-old opposite-sex (OS) twin girls as compared to same-sex (SS) twin girls, show a pattern of hemispheric specialization different from the typical female pattern as a result of their possible prenatal exposure to higher levels of testosterone due to fetal hormone transfer (the twin paradigm). To this end, we compared the performance of these two groups (only right-handed subjects) on a dichotic listening task (DLT; Bouma, 1998), which provided us with an indirect measure of functional cerebral lateralization.

The results indicated that the 10-year-old OS twin girls indeed showed a less feminine and more masculine pattern of functional cerebral lateralization, i.e. hemispheric specialization, than the SS twin girls. Their functional cerebral asymmetry, as reflected by a right-ear superiority (i.e. left-hemispheric dominance) when processing verbal-auditory stimuli, was greater than in SS twin girls. Differences between OS and SS twin girls in the extent of prenatal exposure to androgens could at least partly be responsible for these findings, given that prenatal testosterone has shown to influence brain organization and postnatal sex-dimorphic behavior (McGlone, 1980; Geschwind and Galaburda, 1987; Wisniewski, 1998).

A different line of research indicates that differences in performance on cerebral lateralization tasks can be attributed to current levels of sex hormones, which exert an activating effect (Sanders and Wenmoth, 1998). However, the current findings do not seem to be caused by differences in circulating levels of testosterone between the groups because testosterone levels were not correlated with functional cerebral laterality scores in our study. Our results showed not only that girls had higher testosterone levels than boys of similar age, a finding that has been reported before (Granger et al., 1999), but also, and more importantly for the interpretation of the current results, that there were no differences in testosterone levels between OS and SS twin girls. In other words, the difference in lateralization performance between OS and SS twin girls, as

shown by the results on the DLT, could not be attributed to differences in the circulating level of testosterone between the two groups of girls. It seems therefore more likely that if testosterone influences lateralization performance, it exerts its effect prenatally.

However, when this measure was re-evaluated when the twins were 13 years old, the difference between the OS and SS girls had disappeared. There are two possible explanations for this. First, the sample may have become too small to detect such differences because fewer girls participated in this evaluation than in the evaluation when they were 10 years old. Statistical power analysis indeed confirmed our idea that the sample was too small to enable us to detect significant differences. Secondly, the prenatal effect may have actually faded with time or have been overshadowed by postnatal experiential influences.

1.4. Is there an organizational or activational effect of testosterone on verbal skills and spatial ability?

Clearly, the sexes differ on cognitive abilities in adulthood, with males excelling on spatial aspects such as mental rotation (e.g., Voyer et al., 1995) and females performing better on verbal tasks such as verbal fluency (Halpern, 2000; Hyde and Linn, 1988). The question is whether these sex differences appear in a young age as a consequence of prenatal sex hormones or whether they are dependent on the surge of sex hormones during puberty? To investigate this, we studied the performance of 10-year-old children on two spatial tasks and two verbal tasks. The results showed that on one task in each domain (i.e. spatial and verbal) the boys differed from the girls in the expected direction. We then investigated whether these differences were a result of prenatal exposure to testosterone. Although the OS females and SS females had a similar performance on these two tasks (the spatial and verbal tasks that showed sex differences), on the RAVLT long-term verbal memory the performance of the OS girls was worse than that of the SS girls. Because in these girls circulating testosterone levels did not correlate with test performance, we concluded that the difference was not caused by an

activational effect of circulating testosterone. We hypothesized that the poorer, i.e. more male-typical, long-term verbal memory of the OS girls was caused by the effect of prenatal exposure to testosterone on brain structures involved in verbal memory. This is in line with our main idea that prenatal testosterone causes fetuses to become more male typical, possibly by affecting specific structures by stimulating neuronal proliferation and growth during critical periods of brain development (Goy and McEwen, 1980).

1.5. Is there an effect of prenatal testosterone on aggression and other behavioral traits?

In this study, we investigated whether sex differences in aggression, sensation seeking, and temperament are influenced by testosterone. The influence of testosterone on these behavioral traits could be exerted prenatally, although activational effects of circulating testosterone have been shown to be of a major importance. The latter is especially relevant because the hormone levels in the 13-year-old twins have changed as a result of puberty.

Whereas 10-year-old girls had higher testosterone levels than 10-year-old boys, at 13 years boys had overall higher circulating testosterone levels than the girls, as expected at this age. In both boys and girls, testosterone levels were higher in the morning than in the afternoon.

One study using the twin paradigm investigated the behavioral trait of sensation seeking (Resnick et al., 1993). In that study, female OS twins showed more sensation-seeking behavior, having higher scores on the total SSS scale and Disinhibition and Experience Seeking subscales than female SS twins. The authors concluded that this may have been due to prenatal hormones. We were unable to replicate these results, possibly because our subjects were younger and our sample was smaller (although the other study had a smaller OS group). However, we did find that the two groups of girls differed on the subscale Experience Seeking and showed a tendency to differ on the subscale Boredom Susceptibility, with SS girls having

higher scores. We cannot explain these differences because normally these scales do not show any sex differences.

Although the other investigated traits (aggression and temperament) have been shown to be associated with circulating testosterone levels, similar studies using the twin paradigm have not yet been performed. We did not find SS and OS girls to differ with respect to temperamental behavior. However, one of the questionnaires used showed the OS girls to have more male-like aggression scores than the SS girls. This concerned an overt type of aggression, i.e. aggression proneness, whereas a measure of covert aggression did not reveal differences between the OS and SS girls. Thus the choice of behavioral trait and measurement method investigated appears to be important for detecting possible behavioral effects of prenatal exposure to testosterone. Other factors, such as circulating free testosterone levels, menarchal status, and pubertal stage, did not play a role in the outcome of our study.

1.6. Is the finger pattern masculinized in female opposite-sex twins?

Besides the use of atypical groups (e.g., girls with congenital adrenal hyperplasia (CAH)) and OS twins, and typical (prospective) studies to investigate the possible role of testosterone in the development of sex differences, there is also a fairly new line of investigation that involves biomarkers such as the finger ratio, i.e. the relative length of the second digit to the fourth digit (2D:4D). Researchers claim that the 2D:4D ratio is influenced by the effect of prenatal sex hormones up till week 14 of gestation, which is about the same period that is critical for the development of the brain. As the finger ratio and the twin paradigm are both considered to reflect the prenatal influence of hormones, we hypothesized that the two measures may be associated. Therefore we investigated whether OS girls had a more masculine finger pattern, i.e. lower finger ratio, than SS girls, but found this not to be the case. Possibly because the differences in prenatal exposure to testosterone were too low to affect finger pattern, or indeed no masculinizing effect on finger pattern exists. Still, there seems to be an association between finger

ratio and testosterone in girls with CAH, because the finger ratio was lower in girls with CAH than in girls without CAH (Brown et al., 2002b; Ökten et al., 2002), although one study failed to detect significant differences between the two groups of girls (Buck et al., 2003) but this could be attributed to external factors.

1.7. Educational achievement in a national sample of Dutch female twins and female singletons

This study was designed to look for effects of prenatal exposure to hormones on educational achievement, by comparing the educational performance of OS and SS girls, and their matched singleton female classmates with an older brother or sister.

There was no difference between OS females and SS females in terms of educational achievement. However, there were large differences between the twin group and the singleton group. Our singleton girls performed not only better than our twin girls but also than the total Dutch female population. This may be because of bias, because schoolteachers selected the singleton classmates of the twin girls and may have unconsciously (or consciously) selected the cleverer girls. Importantly, we were unable to show an effect of prenatal testosterone in OS female twins, and we also did not detect a difference between singleton girls with an older brother and singleton girls with an older sister. This suggests that the "maleness" or "femaleness" of the environment in which a girl grows up does not affect her academic performance. Overall, this may strengthen the idea that postnatal environment could be of less importance to the development of sex differences.

2. IN CONCLUSION

Much research concerns the understanding of the behavioral consequences of prenatal exposure to sex hormones in humans. There is increasing evidence, obtained with different methods, that prenatal exposure to androgens has a masculinizing effect on the

female offspring, especially at high doses of androgens and especially for sex-typed interests, spatial ability, and aspects of personality. Although it seems likely that androgens are responsible for some of the differences between the sexes in these traits, it is not clear how much they contribute to variations within males and within females. There is a need to continue to develop and validate methods for assessing these effects in typical samples, e.g., the twin paradigm.

The results of most studies using the twin paradigm have not been replicated by other investigators, and in many cases the studies have focused on only a few behavioral traits. The studies described in this thesis extend a line of research that has used OS twins to examine the role of prenatal exposure to testosterone in the development of sexual dimorphic behavior. This type of research offers a natural experimental setting to investigate the prenatal influence of hormones on sex differences. Although our research findings are mostly negative, carefully conducted studies, even when the results are negative, contribute to our understanding of the processes and causes behind sexual differentiation.

Table 1. Studies investigating prenatal exposure, using the twin paradigm

Domain	Support for twin paradigm?*	Source	Aspect, Point of interest	n	Age	Sex differences shown/ confirmed in reported study?	Female twin differences, OSF/SSF?
Cognitive	Yes	Cole-Harding et al. (1988)	Mental rotation	Max. 35 OSF** ? SSF	21-50**	Yes, males>females.	OSF>SSF, d>?, †
	Yes/No	Cohen-Bendahan (this thesis)	Verbal memory (RAVLT)	79 OSF 59 SSF	10 years	No sex difference was found in this study.	OSF=SSF for STM OSF<SSF for LTM, d's>.10
	No	Cohen-Bendahan (this thesis)	Educational achievement (CITO)	220 OSF 357 SSF	12-13 years	The study was based on annual sex difference shown with the CITO test	OSF=SSF, all d's<.1
		Cohen-Bendahan (this thesis)	Spatial ability	79 OSF	10 years 59 SSF	For one of two tasks, boys>girls	OSF=SSF, d's>.04.
		Cohen-Bendahan (this thesis)	Verbal Ideational Fluency	79 OSF10 59 SSF	years	Yes, girls>boys	OSF=SSF, d>.25.
Physical	Yes	Dempsey et al. (1999)	Tooth crown size	83 MZF 66 MZM 49 SSF 44 SSM 56 OSF 56 OSM 75 NCF 75 NCM	Twins: 7 – 62 years (90%: 10 – 25 years) Singletons: 17 – 25 years	Yes, males>females	OSF>SSF and NCF, 0<all d's<.5

Note. OS, Opposite-sex twin; OSF, females with an male co-twin; SSF, females with a female co-twin; OSM, males with female co-twin; SSM, males with male co-twin; NCF, female singleton; NCM, male singleton; MZM, monozygotic male twin; MZF, monozygotic female twin; MZ, monozygotic; DZ, dizygotic; SOAEs, Spontaneous otoacoustic emissions; CEOAs, Clicke-evoked otoacoustic emissions; RAI, Reinisch Aggression Inventory; *Twin paradigm, it is presumed that the female member of an OS twin pair is exposed to higher levels of testosterone during pregnancy, because she shares the womb with her co-twin brother, than are (dizygotic) same-sex (SS) female twins, which in turn may masculinize the OS female; **estimated data collected by personal communication with Cole-Harding (Feb. 2002); †, unable to calculate the effect size because the necessary data is not mentioned in the study; = is nonsignificant; < or > significant; CAH, congenital adrenal hyperplasia; d, effect size.

Domain	Support for twin paradigm?*	Source	Aspect, Point of interest	n	Age	Sex differences shown/ confirmed in reported study?	Female twin differences, OSF/SSF?
Physical (Continue)	Yes	McFadden (1993)	SOAEs	7 OSF 10 OSM 20 MZM 13 MZF 9 SSF 15 SSM 14 NCF 18 NCM	17 - 25 years	Yes, females>males	SSF>OSF, d>.5
		Cohen-Bendahan (this thesis)	Functional cerebral lateralization	67 OSF 53 SSF	10 years	Yes, boys>girls	OSF>SSF, d>.3
		Cohen-Bendahan (this thesis)	Saliva testosterone levels	75 OSF 56 SSF	10 years	Yes, boys<girls	OSF<SSF, d>.3
	No	Cohen-Bendahan (this thesis)	Functional cerebral lateralization	61 OSF 48 SSF	13 years	Yes, boys>girls	OSF=SSF, d<.05
		Cohen-Bendahan (this thesis)	Saliva testosterone levels	79 OSF 59 SSF	13 years	Yes, boys>girls	OSF=SSF, d<.05.
		Cohen-Bendahan (this thesis)	Finger ratio	29 OSF 26 SSF	13 years based on other studies boys	No, boys=girls, however, <girls (e.g., Manning, 2002)	OSF=SSF, d<.1
		McFadden et al. (1996)	CEOAEs	42 MZF 34 MZM 32 SSF 24 SSM 34 NCF 34 NCM 18 OSF 17 OSM	16 - 25 years	Yes, females>males	OSF=SSF, d<?, †

Domain	Support for twin paradigm?*	Source	Aspect, Point of interest	n	Age	Sex differences shown/ confirmed in reported study?	Female twin differences, OSF/SSF?
Physical (Continue)	No (Continue)	Loehlin and Martin (1998)	Reproductive functions	+/- 650 OSF +/- 1450 SSF	OSF 39.66 SSF 42.63	The study was related to findings in animal literature (e.g. Vom Saal et al., 1990)	OSF=SSF, all d's<.1
		Elkadi et al. (1999)	Left-handedness	59 OS21 – 25 years 40 SSF 21 SSM		No, however, males> females (e.g. Oldsfield, 1971).	OS=SS, d<.1
		Rose et al. (2002)	Reproductive functions	762 SSF 783 OSF 466 SSF 486 OSF 7528 SSF 4767 OSF	11 years 11 years 14 years 14 years 28 years 28 years	The study was related to findings in literature (e.g. Koch, 1966 in Rose et al., 2002)	OSF=SSF, d<?, †
Behavioral/ psychological	Yes	Resnick et al. (1993)	Sensation Seeking (SSS)	51 OS 112 SSF 26 SSM 174 MZF 59 MZM	16 – 70 years	Yes, males>females except of experience seeking.	Disinhibition: OSF>SSF, d>.3; Experience seeking: OSF>SSF, d>.3; Overall SSS: †
		Miller and Martin (1995)	Attitudes	>6000 twins	Adult	No, however, OSF had shown in another study to have more masculine attitudes (Miller, 1994).	OSF>SSF, d>? †
	Yes/No	Loehlin and Martin (2000)	Dimensions of masculinity-femininity	+/- 3000 adult twins +/- 3200 young twins	24 – 87 years (mean 41.2) 17 – 28 years (mean 23.2)	Yes, the effect sizes are reported, varying from medium to large. Females> males on dimension Worried, the others dimensions males>females.	Adult: all measures: OSF=SSF, all d's<.1. Young: rule-breaking: OSF>SSF, d>.1; all other measures: OSF=SSF, all d's<.2.

Domain	Support for twin paradigm?*	Source	Aspect, Point of interest	n	Age	Sex differences shown/ confirmed in reported study?	Female twin differences, OSF/SSF?
Behavioral/ psychological (Continue)	Yes/No (Continue)	Dawood et al. (2004)	Sexually dimorphic traits, e.g. childhood sex-typical behavior.	980 MZ pairs 928 DZ pairs 1085 singletons (3077 females, and 1824 males)	19 – 52 years (mean 30.9)	Yes, all showed sex <differences, males>females.	Sex-typed childhood behavior: OSF>SSF, d>.1; all other measures OSF=SSF, all d's<.1.
		Cohen-Bendahan (this thesis)	Sensation seeking, aggression, temperament	74 OSF 55 SSF	13 years	Yes, all found differences were in the expected direction	SSS experience seeking: OSF < SSF, d<-.3; RAI withdrawal: OSF < SSF, d<-.3; RAI verbal aggression: OSF > SSF, d > .4 All other scales: OSF = SSF, d < .2
	No	Elizabeth and Green (1984)	Sex-typed play and sex-role	702 twin pairs	4 – 12 years (mean 6.77)	Yes, total behavioral score was different, males > females. Feminine-masculine rating scale also discriminated, males > females.	Total sex-role behavioral score: OSF=SSF, d<?, †
		Henderson and Berenbaum (1997)	Sex-typed play	35 OSF 36 SSF 20 NCF with older brother	3 – 8 years	No, however, the study was based upon effects seen in CAH girls (e.g. Berenbaum and Snyder, 1995). CAH girls exposed to higher levels of testosterone play more time with boys' toys than controls.	OSF=SSF or NCF, d<.1.

Domain	Support for twin paradigm?*	Source	Aspect, Point of interest	n	Age	Sex differences shown/ confirmed in reported study?	Female twin differences, OSF/SSF?
	No (Continue)	Rodgers et al. (1998)	Sex-typed toy play	32 OS 27 SSF 24 SSM	7 – 12 years	Yes, boys>girls with masculine toys; girls>boys with feminine toys.	OSF=SSF, d's>.25.

2.1. Interpretation of Findings in Opposite-Sex Twins

The results of the studies presented in this thesis together with the studies mentioned in the literature show a mixed picture regarding the effects of having an opposite sex co-twin. Therefore the validity of the twin paradigm for investigating the role of testosterone in the development of sex differences is uncertain (see Table 1 for an overview of all these studies per domain). Some studies show female OS twins to have a more masculine pattern than female SS twins regarding tooth size (Dempsey et al., 1999), spontaneous otoacoustic emissions (McFadden, 1993), hemispheric lateralization (Cohen-Bendahan et al., 2004; Chapter 3), free testosterone levels (this thesis; Chapter 3), long-term verbal memory (this thesis; Chapter 4), aggression proneness (this thesis; Chapter 5), and spatial ability (Cole-Harding et al., 1988), but it is important to note that most of these findings have not been replicated, at least not in the same age group. If replicated this masculinization effect would be difficult to explain solely in terms of gender socialization factors. Moreover, if being raised in a more male-typical environment would affect behavior, then there should be many, and more pronounced differences between OS and SS females. This is clearly not the case. Our study on educational achievement (Chapter 7) also showed that having an older brother or sister did not affect the educational achievement of singleton girls, and therefore the social environment is perhaps not as important to the development of sex differences. Nevertheless, when conducting studies using the twin paradigm one needs to control for the role of the social environment. Such a control could be a singleton girl with an older brother who is very close in age. So far, only one twin study used such a comparison group although there were no significant findings (Henderson and Berenbaum, 1997). We used a similar control group in the studies described in this thesis, but unfortunately this control group probably suffered from a selection bias, at the level of the parents of the twins (the singletons selected by the twin parents were extremely intelligent compared to the twins, there was a difference of at least 1 standard deviation). This made the envisioned control

group, i.e. singleton female schoolmates with an older brother, unsuitable as controls. Time constraints meant that it was not possible to reselect and test a new control group. Although female singletons with an older brother form the best comparison currently available, this is not a perfect comparison group because same-aged siblings, as in twin pairs, might affect each other differently than siblings of different ages.

The lack of some of the expected differences between OS and SS females is open to several interpretations. First, of course, it is possible that prenatal sex hormones do not affect behavior within the normal range, suggesting that effects in clinical populations (e.g., girls with CAH) cannot be generalized to normal populations. But the consistency of results across clinical conditions and obtained with other methods as for example prospective studies in the typical population (Cohen-Bendahan et al., in press) would appear to make this unlikely.

Secondly, twins may not provide a good model for assessing the behavioral effects of prenatal sex hormones. There are only limited data demonstrating the transfer of testosterone in human multiple pregnancies. Androgens may be transferred from one twin to the other early in pregnancy, because steroids readily cross the fetal membranes (and placenta) and the fetal skin is permeable to hormones dissolved in amniotic fluid (Abramovitch and Page, 1972; Brace, 1989). Later in pregnancy, changes in the fetal skin prevent the simple diffusion of amniotic fluid constituents (Abramovitch and Page, 1972), but hormones from one twin may reach the other twin via transmembrane transport and the maternal-fetal circulation (Meulenberg and Hofman, 1991). If these mechanisms of transfer differ in their effectiveness, and if the brain is more sensitive to androgens during certain periods, then the effect of androgens from a male co-twin may vary throughout pregnancy. Moreover, the separate placenta protects female twins to a certain extent from possible exposure to testosterone from their male womb mate. The genitalia of female OS twins are clearly not masculinized, but the exposure to testosterone from a male co-twin may not be high enough to achieve this. Even if female OS twins are exposed to

testosterone from a male co-twin, the levels might not be very high, and the effect may be counteracted by other hormones, or the exposure may not occur during key sensitive periods. Female fetuses in other species are probably exposed to relatively higher levels of testosterone than are human female twins, because the former have multiple littermates. Intrauterine positioning effects in rodents primarily reflect differences between females that gestate between two males and those that gestate between two females; effects are weaker for females that gestate next to only one male. In addition, androgen exposure in twins may be affected by other factors associated with twin pregnancies, such as left-right position in the uterus (which influences how much blood is received from the mother and from the co-twin), placentation (whether the placentas are separated or fused), number of chorions between the fetal compartments, and twin differences in growth. Some of these factors may differ in monozygotic (MZ) versus dizygotic (DZ) twin pairs and, unfortunately, not all studies restricted their samples of same-sex twins to the latter.

Thirdly, the behavioral effects of intrauterine position may be small, and samples may not have been large enough to detect subtle effects due to prenatal testosterone exposure. In other species, effects are moderate to large, but behavior is also affected by other environmental factors, such as housing conditions and maternal stress (Huizink et al., 2004; Kaiser and Sachser, in press; Van den Bergh et al., in press; Loehlin and Martin, 1998). Many of the twin studies described above used measures that show small to moderate sex differences, and most studies did not have sufficient power to detect even smaller within-sex twin differences.

This introduces the main problem of our studies, the sample size. We believe that the sexes vary in gender-related behavior, which is expressed along a continuum with on one end the most-masculine typed behavior and on the other the most-feminine typed behavior. On average, boys lean toward the masculine side and girls toward the feminine side. Thus while OS girls are female, compared with SS girls they may show fewer feminine characteristics and more mas-

culine characteristics — see the schematic overview in the next figure regarding the skill mental rotation:

While samples need not be large to detect inter-sex differences, larger samples or more sensitive measures are needed to detect intra-sex differences. This was shown to be the case in our study of DLT. While a significant difference was found between OS and SS girls at 10 years of age, this was no longer the case when the girls were 13 years old, when fewer girls returned for the follow-up assessment.

Fourthly, the effect of prenatal testosterone on brain structures may not become visible until after puberty. Puberty is the stage in which the rush of sex hormones finalizes prenatal effects by making brain structures become more "masculine" or "feminine". However, some aspects, such as hemispheric lateralization and verbal memory, were already masculinized in the OS girls before the pubertal hormone spurt. Differences in other behavioral traits, for example, aggression, mental rotation (Cole-Harding et al., 1988), or sensation seeking (Resnick et al., 1993), may develop well after puberty. It still remains to be determined whether prenatal exposure to hormones "primes" the brain to the later effect of hormones at puberty. Moreover, if there is an effect in childhood but not after puberty, does this mean that postnatal socialization overrules the early effect of hormones?

We found that when 10 years old, the OS girls had a more male-typical long-term verbal memory and a hemispheric specialization, and that when they were 13 years old, the OS girls showed more aggression proneness, whereas the hemispheric lateralization was no longer detected (possibly because of the small sample size). A weakness of this study is that the same test battery should have been used for the same girls at both assessments. Unfortunately, the relatively large developmental gap between the two ages made it

very difficult to use the instruments on both testing sessions, because, at the second testing occasion they were no longer appropriate.

2.2. Final conclusion and recommendations for studies of OS twins as reflections of prenatal hormones

The studies that have been conducted as part of this thesis and the existing studies are not particularly encouraging in terms of the value of the twin design for investigating the behavioral effects of prenatal hormones. It is therefore not clear whether this method should continue to be used. Existing studies have limitations (e.g., relatively low statistical power, some combined MZ and DZ twins) that make it difficult to draw strong inferences about the lack of effects. The method also has several advantages compared to other methods for studying hormone effects, such as the relatively high frequency of fraternal twinning (about 1 in 150 births worldwide), the availability of twin registries, and the fact that there are no ethical problems inherently associated with the method. Nevertheless, personal communications with other scientists involved in twin studies suggest that many have failed to find differences between OS and SS twins, but the bias against the reporting of negative results in peer-reviewed journals means that such results are not always available to investigators. This makes it difficult to fully evaluate the utility of the twin method for studying the behavioral effects of prenatal hormones and may result in misdirected research efforts.

Our main intention was to clarify the role of prenatal testosterone in the development of sex differences. To this end, we compared OS females, who are presumed to be exposed to higher levels of testosterone during gestation and therefore may have developed a more masculine pattern of performance and behavior, with SS females. This approach reduces, but does not exclude, the influence of postnatal experiences. Differences in tooth size (Dempsey et al., 1999), otoacoustic emissions (McFadden, 1993), and our own findings regarding hemispheric specialization, behavior, and cognition support the hypothesis of testosterone having a prenatal effect on sex

differences in behavior. When taking all the reported findings into account it is clear that within each domain positive effects have been observed, but one has to ask oneself whether these are not *Type I* errors? Possibly, since we are dealing with subtle differences (i.e. within the female sex), the use of quite crude measures (i.e., paper-and-pencil questionnaires), investigate different types of variables, and use a relatively small sample, we may erroneously have drawn the wrong conclusions. In future investigations these "problems" should be kept in mind and non findings should also be published, in order to create more scientific understanding of the development of sex differences. The existence of various international twin registries should not make that too difficult to accomplish.

REFERENCES

Abramovitch DR, Page KR. 1972. Pathways of water exchange in the fetoplacental unit at midpregnancy. The Journal of Obstetrics and Gynaecology for the British Commonwealth, 79:1099-1102.

Agrawal R, Sharma S, Bekir J, Conway G, Bailey J, Balen AH, Prelevic G. 2004. Prevalence of polycystic ovaries and polycystic ovary syndrome in lesbian women compared with heterosexual women. Fertility and sterility, 82:1352-1357.

Arnold AP, Breedlove SM. 1985. Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. Hormones and behavior, 19:469-498.

Bachevalier J, Hagger C. 1991. Sex differences in the development of learning abilities in primates. Psychoneuroendocrinology, 16:177-188.

Baenninger M, Newcombe N. 1989. The role of experience in spatial test performance: A meta-analysis. Sex Roles, 20:327-344.

Bailey JM, Zucker KJ. 1995. Childhood sex-typed behavior and sexual orientation: A conceptual and quantitative review. Developmental psychology, 31:43-55.

Bammann BL, Coulam CB, Jiang N-S. 1980. Total and free testosterone during pregnancy. American journal of obstetrics and gynecology, 137:293-298.

Baron-Cohen S, Lutchmaya S, Knickmeyer R. 2004. Prenatal testosterone in mind. Cambridge, MA: MIT Press.

Beatty WW. 1992. Gonadal hormones and sex differences in nonreproductive behaviors, in: A.A. Gerall, H. Moltz, and I.L. Ward (Eds.), Handbook of neurobiology. Plenum Press, New York, pp. 85-128.

Becker JB, Breedlove SM, Crews D, McCarthy MM (ed.). 2002. Behavioral endocrinology, 2nd ed. Cambridge, MA: MIT Press.

Benton AL, Hamsher Kd.S, Varney NR, Spreen O. 1983. Contributions to neuropsychological assessment; A clinical manual. Oxford University Press, NY.

Benton AL. 1994. Neuropsychological assessment. Annual Review of Psychology, 45:1-23.

Berenbaum SA. 1998. How hormones affect behavioral and neural development. Developmental neuropsychology, 14:175-196.
Berenbaum SA. 2001. Cognitive function in congenital adrenal hyperplasia. Endocrinology and metabolism clinics of North America, 30:173-192.
Berenbaum SA, Moffat S, Wisniewski AB, Resnick SM. 2003. Neuroendocrinology: Cognitive effects of sex hormones. In de Haan M, Johnson MH (ed.) The cognitive neuroscience of development. New York: Psychology Press; p. 207-235.
Berenbaum SA, Resnick SM. 1997. Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. Psychoneuroendocrinology, 22:505-515.
Berenbaum SA, Snyder E. 1995. Early hormonal influences on childhood sex-typed activity and playmate preferences: Implications for the development of sexual orientation. Developmental psychology, 31:31-42.
Bin-Abbas B, Conte FA, Grumbach MM, Kaplan SL. 1999. Congenital hypogonadotropic hypogonadism and micropenis: effect of testosterone treatment on adult penile size. Why sex reversal is not indicated. The journal of pediatrics, 134:579-583.
Bleecker ML, Bolla-Wilson K, Agnew J, Meyers DA. 1988. Age-related sex differences in verbal memory. Journal of clinical psychology, 44(3):403-11.
Bouma JM. 1998. Dichotic listening and hemispheric specialization: Theoretical considerations and clinical implications. In: R. Licht, A. Bouma, W. Slot and W. Koops, Child Neuropsychology. Reading disabilities and more …. Eburon, Delft.
Bouma JM, Mulder J, Lindeboom J. 1998. Neuropsychologische diagnostiek. Een handleiding uit de praktijk. Lisse: Swets and Zeitlinger. Deel II, 1–28.
Brace RA. 1989. Amniotic fluid dynamics. In Creasy RK, Resnik R (ed.) Maternal-fetal medicine: Principles and practice. Philadelphia: Harcourt Brace Jovanovich, p. 128-135.
Breedlove SM. 1992. Sexual dimorphism in the vertebrate nervous system. The journal of neuroscience, 12:4133-4142.

Brown GR, Dixson AF. 1999. Investigation of the role of postnatal testosterone in the expression of sex differences in behavior in infant rhesus macaques (Macaca mulatta). Hormones and behavior, 35:186-194.

Brown WM, Finn CJ, Breedlove SM. 2002a. Sexual dimorphism in digit-length ratios of laboratory mice. The anatomical record, 267:231-234.

Brown WM, Hines M, Fane BA, Breedlove SM. 2002b. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. Hormones and behavior, 42:380-386.

Brown WM, Finn CJ, Cooke BM, Breedlove SM. 2002c. Differences in finger length between self-identified «butch» and «femme» lesbians. Archives of sexual behavior, 31:123-128.

Brown GR, Nevison CM, Fraser HM, Dixson AF. 1999. Manipulation of postnatal testosterone levels affects phallic and clitoral development in infant rhesus monkeys. International journal of andrology, 22:119-128.

Bryden MP, Allard F. 1981. Do auditory perceptual asymmetries develop? Cortex, 17:313-318.

Bryden MP, Sprott DA. 1981. Statistical determination of degree of laterality. Neuropsychologia, 19:571-582.

Buck JJ, Williams RM, Hughes IA, Acerini CL. 2003. In utero exposure and 2nd to 4th digit length ratio — comparisons between healthy controls and females with classical congenital adrenal hyperplasia. Human Reproduction, 18:976-979.

Csathó A, Osvath A, Bicsak E, Karadi K, Manning J, Kallai J. 2003. Sex role identity related to the ratio of second to fourth digit length in women. Biological psychology, 62:147-156.

Casella R, Maduro MR, Lipshultz LI, Lamb DJ. 2001. Significance of the polyglutamine tract polymorphism in the androgen receptor. Urology, 58:651-656.

Chemes HE. 2001. Infancy is not a quiescent period of testicular development. International journal of andrology, 24:2-7.

Citogroep. 1997. Eindtoets Basisonderwijs 1997, Arnhem.

Citogroep. 2004. Intern rapport, Arnhem.

Clark MM, Galef BG. 1998. Effects of intrauterine position on the behavior and genital morphology of litter-bearing rodents. Developmental neuropsychology, 14:197-211.

Clark AS, Goldman-Rakic PS. 1989. Gonadal hormones influence the emergence of cortical function in nonhuman primates. Behavioral neuroscience, 103:1287-1295.

Cohen J. 1988. Statistical power analysis for the behavioral sciences, 2nd ed. New York: Academic Press.

Cohen-Bendahan CCC, Buitelaar JK, van Goozen SHM, Cohen-Kettenis PT. 2004. Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. Psychoneuroendocrinology, 29:911-916.

Cohen-Bendahan CCC, van de Beek C, Berenbaum SA. (in press). Prenatal sex hormone effects on child and adult sex-typed behavior: Methods and findings. Neuroscience and Biobehavioral Reviews.

Cohen-Kettenis PT, van Goozen SHM, Doorn CS, Gooren LJG. 1998. Cognitive ability and cerebral lateralization in transsexuals. Psychoneuroendocrinology, 6:631-641.

Cole-Harding S. 2002. Personal communication.

Cole-Harding S, Morstad AL, Wilson JR. 1988. Spatial ability in members of opposite-sex twin pairs (Abstract). Behavior Genetics, 18:710.

Collaer ML, Hines M. 1995. Human behavioral sex differences: a role for gonadal hormones during early development? Psychological bulletin, 11:55-107.

Comings DE, Muhleman D, Johnson JP, MacMurray JP. 2002. Parent-daughter transmission of the androgen receptor gene as an explanation of the effect of father absence on age of menarche. Child Development, 73:1046-1051.

Cooke BM, Tabibnia G, Breedlove SM. 1999. A brain sexual dimorphism controlled by adult circulating androgens. Proceedings of the National Academy of Sciences of the United States of America, 96:7538-7540.

Crossen JR, Wiens AN. 1994. Comparison of the Auditory-Verbal Learning Test (AVLT) and California Verbal Learning Test

(CVLT) in a sample of normal subjects. Journal of clinical and experimental neuropsychology, 16(2):190-4.
Dabbs JM jr, de La Rue D. 1991. Salivary testosterone measurements among women: relative magnitude of circadian and menstrual cycles. Hormone research, 35:182-184.
Daitzman R, Zuckerman M. 1980. Disinhibitory sensation seeking, personality and gonadal hormones. Personality and individual differences, 1:103-110.
Daitzman R, Zuckerman M, Sammelwitz P, Ganjam V. 1978. Sensation seeking and gonadal hormones. Journal of biosocial science, 10:401-408.
Dawood K, Bailey JM, Loehlin JC, Martin NG. 2004. Are opposite-sex twins sex-atypical? Paper presented at the Gender Development Research Conference, San Francisco.
Dawood MY, Saxena BB. 1977. Testosterone and dihydrotestosterone in maternal and cord blood and amniotic fluid. American journal of obstetrics and gynecology, 129:37-42.
Debold JF, Whalen RE. 1975. Differential sensitivity of mounting and lordosis control systems to early androgen treatment in male and female hamsters. Hormones and behavior, 6:196-209.
Delis DC, Kramer JH, Kaplan E, Ober BA. 1994. The California verbal learning test – children's version. San Antonio, TX: Psychological Corporation.
Dempsey PJ, Townsend GC, Richards LC. 1999. Increased tooth crown size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. American journal of human biology,11:577-586.
Diamond MC. 1991. Hormonal effects on the development of cerebral lateralization. Psychoneuroendocrinology, 16:121-129.
Dunn JF, Nisula BC, Rodbard D. 1981. Transport of steroid hormones: Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. The journal of clinical endocrinology and metabolism, 53:58-68.
Ecker A. 1875. Some remarks about a varying character in the hands of humans. Arch Anthropol, 8:68-74.

Ehrhardt AA, Meyer-Bahlburg HF. 1979. Prenatal sex hormones and the developing brain: effects on psychosexual differentiation and cognitive function. Annual review of medicine, 30:417-430.
Ehrhardt AA, Meyer-Bahlburg HFL, Feldman JF, Ince SE. 1984. Sex-dimorphic behavior in childhood subsequent to prenatal exposure to exogenous progestogens and estrogens. Archives of sexual behavior, 13:457-477.
Ekstrom RB, French JW, Harman HH. 1976. Kit of factor-referenced cognitive tests. Educational testing service, Princeton, NJ.
Elizabeth PH, Green R. 1984. Childhood sex-role behaviors: Similarities and differences in twins. Acta Geneticae Medicae et Gemellologiae, 33:173-179.
Elkadi S, Nicholls MER, Clode D.1999. Handedness in opposite and same-sex dizygotic twins: testing the testosterone hypothesis. Neuroreport, 10:333-336.
Erlanger DM, Kutner KC, Jacobs AR. 1999. Hormones and cognition: Current concepts and issues in neuropsychology. Neuropsychology review, 9:175-207.
Even MD, Dhar MG, vom Saal FS. 1992. Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position phenomenon in rat. Journal of reproduction and fertility, 96:709-716.
Fairweather H. 1976. Sex differences in cognition. Cognition, 4:231-280.
Feij JA. 2004. Personal communication.
Feij JA, Dekker PH, Koopmans JR, Boomsma DI., 1997. Nieuwe normen en stabiliteitsgegevens voor de spanningsbehoeftelijst (SBL). Nederlands tijdschrift voor de psychologie en haar grensgebieden, 52:131-134.
Feij JA, Kuiper CM. 1984. Adolescenten Temperament Lijst. Swets and Zeitlinger, Lisse.
Feij JA, van Zuilen RW. 1984. Spanningsbehoeftelijst. Swets and Zeitlinger, Lisse.
Feij JA, van Zuilen RW, Gazendam A. 1982. De ontwikkeling van een Nederlandse vragenlijst voor sensation seeking: De spanningsbehoeftelijst (SBL) (The development of a dutch sensation

seeking questionnaire: the Spanningsbehoeftelijst). Gedrag tijdschrift voor de psychologie, 10:364-383.
Fels E, Bosch LR. 1971. Effects of prenatal administration of testosterone on ovarian function in rats. American journal of obstetrics and gynecology, 111:964-969.
Finegan JA, Bartleman B, Wong PY. 1989. A window for the study of prenatal sex hormone influences on postnatal development. The journal of genetic psychology, 150:101-112.
Finegan JK, Niccols GA, Sitarenois G. 1992. Relations between testosterone levels and cognitive abilities at 4 years. Developmental psychology, 28:1075-1089.
Finkelstein JW, Susman EJ, Chinchilli VM, Kunselman SJ, D'arcangelo MR, Schwab J, Demers LM, Liben LS, Lookingbill G, Kulin HE. 1997. Estrogen or testosterone increases self-reported aggressive behaviors in hypogonadal adolescents. The journal of endocrinology and metabolism, 82:2423-2438.
Fitch RH, Cowell PE, Denenberg VH. 1998. The female phenotype: Nature's default? Developmental neuropsychology, 14:213-231.
Fitch RH, Denenberg VH. 1998. A role for ovarian hormones in sexual differentiation of the brain. The behavioral and brain sciences, 21:311-352.
Forest MG, Ances IG, Tapper AJ, Migeon, CJ. 1971. Percentage binding of testosterone, androstenedione and dehydroisoandrosterone in plasma at the time of delivery. The Journal of clinical endocrinology and metabolism, 32:417-425.
Forrester G, Geffen G. 1991. Performance measures of 7- to 15-year-old children on the auditory verbal learning test. The clinical neuropsychologist, 5(4):345-359.
Gadea M, Gómez C, González-Bono E, Salvador A, Espert R. 2003. Salivary testosterone is related to both handedness and degree of linguistic lateralization in normal women. Psychoneuroendrocrinology, 28:274-287.
Galea LA, Ossenkopp KP, Kavaliers M. 1994. Performance (reacquisition) of a water-maze task by adult meadow voles: effects of age of initial task acquisition and in utero environment (litter sex-ratio). Behavioural brain research, 63(2):177-85.

Gandelman R. 1992. Psychobiology of behavioral development. Oxford: University Press.

Garn SM, Burdi AR, Babler WJ, Stinson S. 1975. Early prenatal attainment of adult metacarpal-phalangeal ranking and proportions. American journal of physical anthropology, 43:327-332.

Geffen GM, Moar KJ, O'Hanlon AP, Clark CR, Geffen LB. 1990. The auditory verbal learning test (Rey): Performance of 16-86 year olds of average intelligence. The clinical neuropsychologist, 4:45-63.

Geschwind N, Galaburda AM. 1987. Cerebral lateralization: Biological mechanisms, associations, and pathology. MIT Press, Cambridge, MA.

Gladue BA, Bailey JM. 1995. Spatial ability, handedness, and human sexual orientation. Psychoneuroendocrinology, 20:487-497.

Glass AR, Klein T. 1981. Changes in maternal serum total and free steroid levels in early pregnancy: Lack of correlation with fetal sex. American journal of obstetrics and gynecology, 140:656-660.

Gordon HW. 2002. Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology, 27:115-126.

Goy RW, Bercovitch FB, McBrair MC. 1988. Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. Hormones and behavior, 22:552-571.

Goy RW, McEwen BS. 1980. Sexual differentiation of the brain. Cambridge: MIT Press.

Granger DA, Schwartz EB, Booth A, Arentz M. 1999. Salivary testosterone determination in studies of child health and development. Hormones and behavior, 35:18-27.

Grimshaw GM, Sitarenios G, Finegan JK. 1995. Mental rotation at 7 years: Relations with prenatal testosterone levels and spatial play experience. Brain and cognition, 29:85-100.

Grumbach MM, Hughes IA, Conte FA. 2002. Disorders of sex differentiation. In Larsen PR, Kronenberg HM, Melmed S, Polonsky KS (ed.) Williams textbook of endocrinology, 10th ed. Philadelphia: W.B. Saunders.

Hall LS, Love CT. 2003. Finger-length ratios in female monozygotic twins discordant for sexual orientation. Archives of sexual behavior, 32:23-28.
Halpern DF. 2000. Sex differences in cognitive abilities, 3rd ed. Mahwah, NJ: Erlbaum.
Hampson E, Rovet JF, Altmann D. 1998. Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Developmental neuropsychology, 14:299-320.
Harris JA. 1999. Review and methodological considerations in research on testosterone and aggression. Aggression and violent behavior, 4:273-291.
Helgeson VS. 2002. The psychology of gender. Prentice-Hall, New Jersey.
Helladay J, Siwers B, Ritzén EM, Hugdahl K. 1994. Normal lateralization for handedness and ear advantage in a verbal dichotic listening task in women with congenital adrenal hyperplasia (CAH). Neuropsychologia, 32:875-880.
Henderson BA, Berenbaum SA. 1997. Sex-typed play in opposite-sex twins. Developmental Psychobiology, 31:115-123.
Herault Y, Fraudea N, Zákány J, Duboule D. 1997. Ulnaless (Ul), a regulatory mutation inducing both loss-of function and gain-of-function of posterior HoxD genes. Development, 124:3493-3500.
Hines M. 2004. Brain Gender. Oxford University Press, New York.
Hines M, Golombok S, Rust J, Johnston KJ, Golding J. 2002. Testosterone during pregnancy and gender role behavior of preschool children: A longitudinal, population study. Child Development, 73:1678-1687.
Hines M, Shipley C. 1984. Prenatal exposure to diethylstilbestrol (DES) and the development of sexual dimorphic cognitive abilities and cerebral lateralization. Developmental psychology, 20:81-94.
Huizink AC, Mulder EJC, Buitelaar JK. 2004. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychological bulletin, 130:115-142.
Hull EM. 1981. Effects of neonatal exposure to progesterone on sexual behavior of male and female rats. Physiology and behavior, 26:401-405.

Hull EM, Franz JR, Snyder AM, Nishita JK. 1980. Perinatal progesterone and learning, social and reproductive behavior in rats. Physiology and behavior, 24:251-256.

Hyde JS, Linn MC. 1988. Gender differences in verbal ability: a meta-analysis. Psychological bulletin, 104:53-69.

James WH. 1986. Hormonal control of sex ratio. Journal of theoretical biology, 118:427-441.

Janowsky JS, Chavez B, Orwoll E. 2000. Sex steroids modify working memory. Journal of cognitive neuroscience, 12(3):407-414.

Johnson ES, Meade AC. 1987. Developmental patterns of spatial ability: an early sex difference. Child Development, 58:725-740.

Kaiser S, Sachser N. (in press). The Effects of Prenatal Social Stress on Behaviour: Mechanisms and Function. Neuroscience and Biobehavioral Reviews.

Kampen D, Sherwin BB. 1994. Estrogen use and verbal memory in healthy postmenopausal women. Obstetrics and gynecology, 83:979-983.

Kelso WM, Nicholls MER, Warne GL. 1999. Effects of prenatal androgen exposure on cerebral lateralization in patients with congenital adrenal hyperplasia (CAH). Brain and cognition, 40:153-156.

Kelso WM, Nicholls MER, Warne GL, Zacharin M. 2000. Cerebral lateralization and cognitive functioning in patients with congenital adrenal hyperplasia. Neuropsychology, 14:370-378.

Kimura D. 1967. Functional asymmetry of the brain in dichotic listening. Cortex, 3:163-168.

Kimura D. 1999. Sex and Cognition. MIT Press, Cambridge, MA.

Kimura D, Hampson E. 1994. Cognitive pattern in men and women is influenced by fluctuations in sex hormones. Current directions in psychological science, 3:57-61.

Klinga K, Bek E, Runnebaum B. 1978. Maternal peripheral testosterone levels during the first half of pregnancy. American journal of obstetrics and gynecology, 131:60-62.

Kondo T, Zakany J, Innis JW, Duboule D. 1997. Of fingers, toes, and penises. Nature, 390:29.

Kramer JH, Delis DC, Daniel M. 1988. Sex differences in verbal learning. Journal of clinical psychology, 44:907-915.

Kramer JH, Delis DC, Kaplan E, O'Donnell L, Prifitera A. 1997. Developmental sex differences in verbal learning. Neuropsychology, 11(4):577-584.

Leveroni CL, Berenbaum SA. 1998. Early androgen effects on interest in infants: Evidence from children with congenital adrenal hyperplasia. Developmental neuropsychology, 14:321-340.

Levine SC, Huttenlocher J, Taylor A, Langrock A. 1999. Early sex differences in spatial skill. Developmental Psychology, 35(4):940-949.

Liben LS, Susman EJ, Finkelstein JW, Chinchilli VM, Kunselman S, Schwab J, Dubas JS, Demers LM, Lookingbill G, Darcangelo MR, Krogh HR, Kulin HE. 2002. The effects of sex steroids on spatial performance: a review and an experimental clinical investigation. Developmental Psychology, 38(2):236-253.

Lindgren SD, Benton AL. 1980. Developmental patterns of visuospatial judgement. Journal of pediatric psychology, 5:217-225.

Loehlin JC, Martin NG. 2000. Dimensions of psychological masculinity-femininity in adult twins from opposite-sex and same-sex pairs. Behavior Genetics, 30:19-28.

Loehlin JC, Martin NG. 1998. A comparison of adult female twins from opposite-sex and same-sex pairs on variables related to reproduction. Behavior Genetics, 28:21-27.

Lippa RA. 2003. Are 2D:4D finger-length ratios related to sexual orientation? Yes for men, no for women. Journal of personality and social psychology, 85:179-188.

Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT. 2004. 2nd to 4th digit ratios, fetal testosterone and estradiol. Early human development, 77:23-28.

Luteijn F, Van der Ploeg FAE. 1983. Groninger Intelligence test. Lisse: Swets and Zeitlinger.

Maccoby EE, Jacklin CN. 1974. The psychology of sex differences. Stanford, CA: Stanford University Press.

Maki PM, Resnick SM. 2000. Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition. Neurobiology of aging, 21:373-383.

Manning JT. 2002. Digit ratio: a pointer to fertility, behavior and health. New Brunswick, NJ: Rutgers University Press.

Manning JT, Barley L, Walton J, Lewis-Jones DI, Trivers RL, Singh D, Thornhill R, Rohde P, Bereczkei T, Henzi P, et al. 2000. The 2nd:4th digit ratio, sexual dimorphism, population differences, and reproductive success. evidence for sexually antagonistic genes? Evolution and human behavior, 21:163-183.

Manning JT, Baron-Cohen S, Wheelwright S, Sanders G. 2001. The 2nd to 4th digit ratio and autism. Developmental medicine and child neurology, 43:160-164.

Manning JT, Callow M, Bundred PE. 2003a. Finger and toe ratios in humans and mice: implications for the aetiology of diseases influenced by HOX genes. Medical hypotheses, 60:340-343.

Manning JT, Henzi P, Venkatramana P, Martin S, Singh D. 2003b. Second to fourth digit ratio: ethnic differences and family size in English, Indian, and South African populations. Annals of human biology, 30:579-588.

Manning JT, Robinson SJ. 2003. 2nd to 4th digit ratio and a universal mean for prenatal testosterone in homosexual men. Medical hypotheses, 61:303-306.

Manning JT, Scutt D, Wilson J, Lewis-Jones DI. 1998. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Human reproduction, 13:3000-3004.

Manning JT, Stewart A, Bundred PE, Trivers RL. 2004. Sex and ethnic differences in 2nd to 4th digit ratio of children. Early human development, 80(2):161-168.

Mattsson A, Schalling D, Olweus D, Löw H, Svensson J. 1980. Plasma testosterone, aggressive behavior, and personality dimensions in young male delinquents. Journal of the American Academy of Child Psychiatry, 19: 476-490.

McCormick CM, Witelson SF. 1991. A cognitive profile of homosexual men compared to heterosexual men and women. Psychoneuroendocrinology, 16:69-76.
McCormick CM, Witelson SF. 1994. Functional cerebral asymmetry and sexual orientation in men and women. Behav. Neurosci. 108, 525-531.
McEwen BS. 1999. Permanence of brain sex differences and structural plasticity of the adult brain: Commentary. Proceedings of the National Academy of Sciences of the United States of America, 96, 7128-7130.
McFadden D. 1993. A masculinizing effect on the auditory systems of human females having male co-twins. Proceedings of the National Academy of Sciences of the United States of America, 90:11900-11904.
McFadden D. 1998. Sex differences in the auditory system. Developmental Neuropsychology, 14:261-298.
McFadden D. 2002. Masculinization effects in the auditory system. Archives of sexual behavior, 31:99-111.
McFadden D, Loehlin JC, Pasanen EG. 1996. Additional findings on heritability and prenatal masculinization of cochlear mechanisms: click-evoked otoacoustic emissions. Hearing Research, 97:102-119.
McFadden D, Shubel E. 2003. The relationships between otoacoustic emissions and relative lengths of fingers and toes in humans. Hormones and behavior, 43:421-429.
McFadden D, Shubel E. 2002. Relative lengths of fingers and toes in human males and females. Hormones and behavior, 42:492-500.
McGlone J. 1980. Sex differences in human brain asymmetry: A critical survey. The behavioral brain sciences, 3:215-263.
McHale SM, Updegraff KA, Helms-Erikson H, Crouter AC. 2001. Sibling influences on gender development in middle childhood and early adolescence: a longitudinal study. Developmental Psychology, 37:115-125.
McPhaul MJ, Marcelli M, Zoppi S, Griffin JE, Wilson JD. 1993. Genetic basis of endocrine disease 4. The spectrum of mutations

in the androgen receptor gene that causes androgen resistance. The journal of clinical endocrinology and metabolism, 76:17-23.
Meaney MJ. 1988. The sexual differentiation of social play. Trends Neurosciences, 11:54-58.
Meulenberg PM, Hofman JA. 1991. Maternal testosterone and fetal sex. Journal of Steroid Biochemistry and Molecular Biology, 39:51-54.
Miller EM. 1994. Prenatal sex hormone transfer: A reason to study opposite-sex twins. Personality and individual differences, 17(4):511-529.
Miller EM. 1998. Evidence from opposite-sex twins for the effects of prenatal sex hormones. In L. Ellis and L. Ebertz (Eds.), Males , Females, and Behavior. Towards biological understanding, p. 27-57.
Miller EM, Martin N. 1995. Analysis of the effect of hormones on opposite-sex twin attitudes. Acta Geneticae Medicae et Gemellologiae, 44:41-52.
Moffat SD, Hampson E. 1996. A curvilinear relationship between testosterone and spatial cognition in humans: possible influence of hand preference. Psychoneuroendocrinology, 21(3):323-337.

Moffat SD, Hampson E. 2000. Salivary testosterone concentrations in left-handers: an association with cerebral language lateralization? Neuropsych; 14:71-81.
Money J, Ehrhardt AA. 1972. Man and woman, boy and girl. Baltimore: Johns Hopkins University Press.
Mortlock DP, Innis JW. 1997. Mutation of HOXA 13 in hand-foot-genital syndrome. Nature genetics, 15:179-180.
Nagamani M, McDonough PG, Ellegood JO, Mahesh VB. 1979. Maternal and amniotic fluid steroids throughout human pregnancy. American journal of obstetrics and gynecology, 134:674-680.
Nass R, Baker S. 1991. Androgen effects on cognition: congenital adrenal hyperplasia. Psychoneuroendrocrinology, 16:189-201.
Neave N, Laing S, Fink B, Manning JT. 2003. Second to fourth digit ratio, testosterone, and perceived male dominance. Proceedings of the Royal society of London. Series B, 270:2167-2172.

Nevison CM, Brown GR, Dixson AF. 1997. Effects of altering testosterone in early infancy on social behavior in captive yearling rhesus monkeys. Physiology and behavior, 62:1397-1403.

O'Connor DB, Archer J, Hair WM, Wu FCW. 2001. Activational effects of testosterone on cognitive function in men. Neuropsychologia, 39:1385-1394.

O'Connor TG, Heron J, Golding J, Glover V. 2003. Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. Journal of child psychology and psychiatry, 44:1025-1036.

Ökten A, Kalyoncu M, Yaris N. 2002. The ratio of second- and fourth-digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Early human development, 70:47-54.

Olweus D, Mattsson A, Schalling D, Low H. 1980. Testosterone, aggression, physical, and personality dimensions in normal adolescent males. Psychosomatic medicine, 42:253-269.

Olweus D, Mattsson A, Schalling D, Low H. 1988. Circulating testosterone levels and aggression in adolescent males: a causal analysis. Psychosomatic medicine, 50:261-272.

Orlebeke JF, Boomsma DI, Eriksson AW. 1993. Epidemiological and birth weight characteristics of triplets: a study from the Dutch twin register. European journal of obstetrics, gynecology, and reproductive biology, 50:87-93.

Ostatníková D, Pastor K, Putz Z, Dohnányiová M, Mat'a_eje A, Hampl R. 2002. Salivary testosterone levels in preadolescent children. BMC Pediatrics, 2:5.

Peichel CL, Prabhakaran B, Vogt TF. 1997. The mouse Ulnaless mutation deregulates posterior HoxD gene expression and alters appendicular patterning. Development, 124:3481-3492.

Peters M, Tan U, Kang Y, Teixeira L, Mandal M. 2002. Sex-specific finger-length patterns linked to behavioral variables: consistency across various human populations. Perceptual Motor Skills, 94(1):171-81.

Phoenix CH, Goy RW, Gerall AA, Young WC. 1959. Organizing action of prenatally administered testosterone propionate on the

tissues mediating mating behavior in the female guinea pig. Endocrinology, 65:369-382.
Putz DA, Gaulin SJC, Sporter RJ, McBurney DH. 2004. Sex hormones and finger length. What does 2D:4D indicate? Evolution and human behavior, 25:182-199.
Rahman Q, Wilson GD. 2003. Sexual orientation and the 2nd to 4th finger length ratio: evidence for organising effects of sex hormones or developmental instability? Psychoneuroendocrinology, 28:288-303.
Ramirez JM. 2003. Hormones and aggression in childhood and adolescence. Aggression and violent behavior, 8:621-644.
Reinisch JM, Sanders SA. 1986. A test of sex differences in aggressive response to hypothetical conflict situations. Journal of personality and social psychology, 50:1045-1049.
Resnick SM, Berenbaum SA, Gottesman II, Bouchard TJ. 1986. Early hormonal influences on cognitive functioning in congenital adrenal hyperplasia. Developmental psychology, 22:191-198.
Resnick SM, Gottesman II, McGue M. 1993. Sensation seeking in opposite-sex twins: An effect of prenatal hormones? Behavior Genetics, 23:323-329.

Resnick SM, Maki PM. 2001. Effects of hormone replacement therapy on cognitive and brain aging. Annals of the New York academy of sciences, 949:203-214.
Rivarolo MA, Forest MG, Migeon CJ. 1968. Testosterone, androstenedione and dehydroepiandrosterone in plasma during pregnancy and at delivery: Concentration and protein binding. The Journal of clinical endocrinology and metabolism, 28:34-40.
Robinson SJ, Manning JT. 2000. The ratio of 2nd to 4th digit length and male homosexuality. Evolution and human behavior, 21:333-345.
Rodeck CH, Gill D, Rosenberg DA. 1985. Testosterone levels in midtrimester maternal and fetal plasma and amniotic fluid. Prenatal diagnosis, 5:175-181.
Rodgers CS, Fagot BI, Winebarger A. 1998. Gender-typed toy play in dizygotic twins: A test of hormone transfer. Sex Roles, 39:173-184.
Rohde Parfet KA, Ganjam VK, Lamberson WR, Rieke AR, Vom Saal FS, Day BN. 1990. Intrauterine position effects in female swine:

Subsequent reproductive performance, and social and sexual behavior. Applied animal behaviour science, 26, 349-362.
Rose RJ. (in press). Prenatal programming of behavior: A twin-study perspective. Neuroscience and Biobehavioral Reviews.
Rose RJ, Kaprio J, Winter T, Dick DM, Viken RJ, Pulkkinen L, Koskenvuo M. 2002. Femininity and fertility in sisters with twin brothers: Prenatal androgenization? Cross-sex socialization? Psychological science, 13:263-267.
Rosenblitt JC, Soler H, Johnson SE, Quadagno DM. 2001. Sensation seeking and hormones in men and women: exploring the link. Hormones and behavior, 40:396-402.
Russo MF, Lahey BB, Christ MAG, Frick PJ, McBurnett K, Walker JL, Loeber R, Stouthamer-Loeber M, Green S. 1991. Preliminary development of sensation seeking scale for children. Personality and individual differences, 12:399-405.
Ryan BC, Vandenbergh JG. 2002. Intrauterine position effects. Neuroscience and Biobehavioral Reviews, 26:665-678.
Sanders G, Wenmoth D. 1998. Verbal and music dichotic listening tasks reveal variations in functional cerebral asymmetry across the menstrual cycle that are phase and task dependent. Neuropsychologia, 36:869-874.
Sattler JM. 1988. Assessment of children. 3rd Ed. San Diego State University.
Saxby L, Bryden MP. 1984. Left-ear superiority in children for processing auditory emotional material. Developmental psychology, 20, 1, 72-80.
Schneider ML, Moore CF, Kraemer GW, Roberts AD, DeJesus OT. 2002. The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. Psychoneuroendocrinology, 27:285-298.
Seidman DS, Laor A, Gale R, Stevenson DK, Mashiach S, Danon YL. 1992. Birth weight and intellectual performance in late adolescence. Obstetrics and gynecology, 79:543-546.
Shapiro BH, Goldman AS, Bongiovanni AM, Marino JM. 1976. Neonatal progesterone and feminine sexual development. Nature, 264:795-796.

Shute VJ, Pellegrino JW, Hubert L, Reynolds RW. 1983. The relationship between androgen levels and human spatial abilities. Bulletin of the psychonomic society, 21:465-468.

Siiteri PK, Wilson JD. 1974. Testosterone formation and metabolism during male sexual differentiation in the human embryo. The journal of clinical endocrinology and metabolism, 38:113-125.

Sikich L, Todd RD.1988. Are the neurodevelopmental effects of gonadal hormones related to sex differences in psychiatric illnesses? Psychiatric developments, 6:277-309.

Smail PJ, Reyes FI, Winter JSD, Faiman C. 1981. The fetal hormone environment and its effect on the morphogenesis of the genital system. In Kogan SJ, Hafez ESE (ed.) Pediatric andrology. The Hague, Netherlands: Martinus Nijhoff, p. 9-19.

Smith LL, Hines M. 2000. Language lateralization and handedness in women prenatal exposed to diethylstilbestrol (DES). Psychoneuroendocrinology, 25:497-512.

Speiser PW, White PC. 2003. Congenital adrenal hyperplasia. The new English journal of medicine, 349:776-788.

Stoneman Z, Brody GH, MacKinnon CE. 1986. Same-sex and cross-sex siblings: Activity choices, roles, behavior, and gender stereotypes. Sex Roles, 15:495-511.

Tanner JM. 1962. Growth at adolescence: with a general consideration of the effects of hereditary and environmental factors upon growth and maturity form birth to maturity. 2nd.Ed. Blackwell, Oxford.

Therrell BL, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, Gonzalez J, Gunn S. 1998. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. Pediatrics, 101:583-590.

Uiterwijk H. 1996. Personal communication.

Uiterwijk H. 1998. Personal communication.

Van de Beek C, Thijssen JHH, Cohen-Kettenis PT, van Goozen SHM, Buitelaar JK. 2004. Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: What is the best source of information to investigate the effects of fetal hormonal exposure? Hormones and Behavior, 46:663-69.

Vandenberg SG, Kuse AR. 1978. Mental rotation: A group test of three-dimensional spatial vizualization. Perceptual and motor skills, 47:599-604.

Van den Bergh BRH, Mulder EJH, Mennes M, Glover V. (in press). Antenatal maternal anxiety and stress and the behavioral development of the fetus and child: links and possible mechanisms. Neuroscience and Biobehavioral Reviews.

Van den Burg W, Kingma A. 1999. Performance of 225 Dutch school children on Rey's Auditory Verbal Learning Test (RAVLT): Parallel test-retest reliabilities with an interval of 3 months and normative data. Archives of Clinical Neuropsychology, 14(6):545-559.

Vandersteene G, van Haasen PP, de Bruyn EEJ, Coetsier P, Pijl YL, Poortinga YH, Lutje Spelberg HC, Spoelders-Claes R, Stinissen J. 1986. WISC-R, Wechsler Intelligence Scale for Children-revised. Nederlandstallige uitgave [Dutch version]. Lisse: Swets and Zeitlinger B.V.

Van Goozen SHM, Cohen-Kettenis PT, Gooren LJG, Frijda NH, Van de Poll NE. 1995. Gender differences in behaviour: Activating effects of cross-sex hormones. Psychoneuroendocrinology, 20:343-363.

Vom Saal F. 1989. Sexual differentiation in litter-bearing mammals: Influence of sex of adjacent fetuses in utero. Journal of animal science, 67:1824-1840.

Voyer D. 1996. On the magnitude of laterality effects and sex differences in functional lateralities. Laterality, 1:51-83.

Voyer D, Voyer S, Bryden MP. 1995. Magnitude of sex differences in spatial abilities: A meta-analysis and consideration of critical variables. Psychological Bulletin, 117(2):250-270.

Wallen K. 1996. Nature needs nurture: The interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. Hormones and behavior, 30:364-378.

Wallen K, Maestripieri D, Mann DR. 1995. Effects of neonatal testicular suppression with a GnRH antagonist on social behavior in group-living juvenile rhesus monkeys. Hormones and behavior, 29:322-337.

Wechsler D. 1974. Manual for the Wechsler intelligence scale for children-revised (WISC-R). New York: Psychological corporation.

Wegesin DJ. 1998. A neuropsychologic profile of homosexual and heterosexual men and women. Archives of sexual behavior, 27:91-108.

Williams CL, Meck WH. 1991. The organizational effects of gonadal steroids on sexually dimorphic spatial ability. Psychoneuroendocrinology, 16:155-176.

Williams JH, Greenhalgh KD, Manning JT. 2003. Second to fourth finger ratio and possible precursors of developmental psychopathology in preschool children. Early human development, 72:57-65.

Williams TJ, Pepitone ME, Christensen SE, Cooke BM, Huberman AD, Breedlove NJ, Breedlove TJ, Jordan CL, Breedlove SM. 2000. Finger-length ratios and sexual orientation. Nature, 404:455-456.

Wilson GD. 1983. Finger length as an index of assertiveness in women. Personality and individual differences, 4:111-112.

Wisniewski AB. 1998. Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. Psychoneuroendocrinology, 23:519-547.

Witelson SF. 1991. Neural sexual mosaicism: Sexual differentiation of the human temporo-parietal region for functional asymmetry. Special Issue: Neuroendocrine effects on brain development and cognition. Psychoneuroendocrinology, 16:131-153.

Witt DM, Young LJ, Crews D. 1995. Progesterone modulation of androgen-dependent sexual behavior in male rats. Physiology and behavior, 57:307-313.

Zuckerman M. 1979. Sensation seeking: Beyond the optimal level of arousal. Lawrence Erlbaum, Hillsdale, New Jersey.

Zuckerman M. 1971. Dimensions of sensation seeking. Journal of consulting and clinical psychology, 36:45-52.

Zuckerman M, Eysenck SBG, Eysenck HJ. 1978. Sensation seeking in England and America: Cross-cultural, age, and sex comparisons. Journal of consulting and clinical psychology, 46:139-149.

NEDERLANDSE SAMENVATTING

Inleiding. Onderzoek wijst uit dat de seksen[1] zowel lichamelijke verschillen als ook verschillen in cognitie en gedrag vertonen. Zo blijken mannen gemiddeld over een beter ruimtelijk inzicht te beschikken, meer spanningsbehoefte te hebben, maar ook vaker fysieke agressie te vertonen. Vrouwen daarentegen hebben gemiddeld betere taalvaardigheden, perceptiesnelheid en verbaal geheugen. Deze verschillen worden bij dezelfde gemiddelde IQ's waargenomen en sommige hiervan zijn al voor de puberteit aanwezig. Voor 1990 werd aangenomen dat deze tweedeling voornamelijk veroorzaakt werd doordat de omgeving de genderontwikkeling beïnvloedde en dat dus de ouders als rolmodel fungeerden voor hun kinderen. De jongens zouden hun vaders imiteren en de meisjes hun moeder. In de loop der daaropvolgende jaren werd het duidelijk dat er meer is dan alleen deze omgevingsinvloeden.

De mens is eigenlijk vrouw als "default" (standaardinstelling d.w.z. een situatie die wordt toegewezen, zich voordoet, en alleen door middel van actieve interventie kan deze aangepast worden). Dit blijkt uit het feit dat pas als het hormoon testosteron wordt aangemaakt (rond week 7 van de zwangerschap) bij de mannelijke XY foetus de externe geslachtskenmerken ontstaan die typerend zijn voor de man. Er wordt bij de andere sekse-typische kenmerken ook gespeculeerd dat vroege prenatale testosteron een belangrijke rol zou hebben in het ontstaan van de sekseverschillen. Onderzoek verricht bij groepen met atypische hormoonspiegels toont aan dat het prenataal geslachtshormoon testosteron een grote rol heeft in het ontstaan van deze sekseverschillen.

Het doel van het onderzoek beschreven in dit proefschrift is om te onderzoeken of er meer duidelijkheid verkregen kan worden met

[1] Met de term *sekse* wordt meer nadruk gelegd op het biologische geslacht; bij *gender* benadrukt men meer het sociale facet van mannelijkheid en vrouwelijkheid.

betrekking tot de rol van prenataal testosteron op de ontwikkeling van sekseverschillen.

De aanpak. Uit dieronderzoek blijkt dat als een vrouwelijke foetus zich tijdens de zwangerschap in de baarmoeder tussen twee mannelijke foetussen in ontwikkelt, zij na de geboorte meer testosteron in haar bloed heeft. Verder vertonen dit soort meisjes meer mannelijk gedrag (bijvoorbeeld meer agressie), gedragen ze zich minder actief en hebben meer mannelijke lichaamskenmerken in vergelijking tot meisjes die zich naast een of geen jongetje in de baarmoeder hebben ontwikkeld. Concluderend ziet het er naar uit dat blootstelling aan testosteron tot vermannelijking leidt bij dieren. Bovenstaand model is in principe ook toepasbaar op de mens. Indien het delen van en zich in elkaars nabijheid bevinden in de baarmoeder door een jongen en een meisje tot mogelijke verhoging van testosteron spiegels kan leiden bij het meisje en op haar beurt weer tot vermannelijking van andere aspecten bij dit meisje, dan kunnen we veronderstellen dat de jongen-meisje (OS) tweeling hiervoor als geschikt equivalent bij de mens zou kunnen dienen. Hier wordt aldus verondersteld dat het meisje wordt blootgesteld aan hogere testosteron waarden dan meisjes die niet de baarmoeder hebben gedeeld met een broer. Daarom hebben wij de meisjes van een OS tweeling vergeleken met die van een meisje uit een meisje-meisje (SS) tweeling. Een verder plus-punt van dit model is dat het een groep proefpersonen betreft die in principe normale hormoonniveaus hebben. Hierdoor kunnen we de uitslagen beter generaliseren naar de algehele populatie. Ethisch gezien zijn er ook geen beperkingen van toepassing die het toetsen van de hypothese bemoeilijken.

Het onderzoek. We hebben op twee momenten onderzoek gedaan bij een groep OS tweelingen en een groep SS tweelingen, namelijk toen ze 10 en 13 jaar oud waren. Tevens hebben we een groep eenlingmeisjes als controle benaderd via de tweelingen. Achteraf bleek echter dat deze eenlingmeisjes op een zeer belangrijk kenmerk (namelijk IQ) zodanig verschilden van de tweelingen dat

ze slecht bruikbaar waren als controles. De onderzoeksvraag in dit proefschrift is of het meisje uit de OS tweeling een mannelijker patroon vertoond in vergelijking met een meisje uit de SS tweeling. Deze hypothese wordt verondersteld omdat het OS meisje tijdens de zwangerschap blootgesteld is geweest aan hogere niveaus van testosteron aangezien zij de baarmoeder heeft gedeeld met haar broertje.

In de komende paragrafen zal ik de uitkomsten (per hoofdstuk) bespreken.

Hoofdstuk 2. We hebben afzonderlijk van het hoofdonderzoek een bloedonderzoek verricht bij een groep vrouwen die zwanger was van een tweeling. In de literatuur wordt gesproken over twee routes waarop de mogelijke blootstelling aan verhoogd testosteron bij vrouwelijk foetussen zou kunnen plaatsvinden: (1) binnen de baarmoeder (deze directe route is alleen aangetoond bij dieren en is lastig om bij de mens aan te tonen met de huidige beschikbare technieken) en (2) de indirecte route via de moederlijke bloedcirculatie. Deze route verloop als volgt: vanuit de jongen stroomt testosteron over naar zijn moeder en vervolgens via de moeder naar het meisje. Op deze laatste route hebben we onderzoek gericht. Wij hebben gemeten of er een verschil is in de hoeveelheid testosteron in het moederlijke bloed op twee verschillende meetmomenten (rond week 24 en week 32). Onze verwachting ten aanzien van het hormoon testosteron was dat vrouwen die een jongen-jongen tweeling droegen de hoogste niveaus van testosteron zouden hebben, gevolgd door vrouwen die een OS tweeling droegen en dat de laagste niveaus van testosteron hoorden bij de vrouwen zwanger van een SS (meisje-meisje) tweeling. Dit bleek echter niet zo te zijn. Het enige dat we hebben kunnen aantonen was dat er gedurende de zwangerschap een aantal hormoonspiegels veranderden. Aangezien we bij de werving geen kennis hadden van het geslacht van de toekomstige tweeling bleek naderhand in onze groep van 55 vrouwen een zeer geringe hoeveelheid van jongen-jongen tweelingen aanwezig te zijn. Deze scheve verdeling in groepsgrootte kan

de uitslag hebben vertekend aangezien de hormoongemiddelden voor de kleine groep van jongen-jongen tweeling niet representatief zou kunnen zijn. Bovendien blijkt er uit de literatuur dat ook bij eenling zwangerschappen er dikwijls geen verband aangetoond kan worden tussen de sekse van het kind en de hormoonniveaus die bij moeder gemeten worden. De mogelijke blootstelling van een meisje binnen een OS tweeling aan testosteron gebeurt naar alle waarschijnlijkheid eerder rechtstreeks door middel van uitwisseling van hormonen binnen de baarmoeder tussen de leden van een tweelingpaar, dan via de door ons onderzochte route, namelijk de maternale circulatie.

Hoofdstuk 3. In een groep van 67 OS en 53 SS tweelingen van 10 jaar hebben we de invloed van mogelijke blootstelling aan prenataal testosteron onderzocht op verbale hersenspecialisatie (lateralisatie van het brein). Mannen hebben een sterke hersenlateralisatie, dat wil zeggen dat de functies specifieker door een bepaalde hersenhelft worden vertegenwoordigd dan bij de vrouwen waar de functies vaker over beide hersenhelften verdeeld zijn. Uiteraard nog steeds de belangrijkste centra voor taal in de linker hersenhelft en de ruimtelijke centra in de rechter hersenhelft. Mannen tonen daardoor een sterker rechteroor voorkeur voor verbale stimuli (in verband met de sterke verbindingen met de linker hersenhelft, de "taal"-helft). Uit het onderzoek kwam naar voren dat de OS meisjes een meer vermannelijkte breinspecialisatie hebben in vergelijking met de meisjes van een SS tweeling. Er was geen relatie met de circulerende testosteronspiegels zoals die op het moment van testafname gemeten zijn in het speeksel.

Alhoewel we deze taak hebben herhaald op 13 jarige leeftijd, bleek er toen geen mannelijker patroon meer te bestaan in de OS meisjes. De verklaring hiervoor zou kunnen zijn dat de omgevingsinvloeden dit verschil hebben laten verdwijnen. Dat zou kunnen betekenen dat het blootstellingseffect prenataal slechts voor een bepaalde duur is. Het zou ook zo kunnen zijn dat de onderzoeksgroepsgrootte een cruciale rol heeft gespeeld aangezien er bij de tweede meting een iets kleinere groep meewerkte en dat deze groep

niet meer groot genoeg was om het verschil nogmaals aan te tonen. Daarbij is van belang om op te merken dat de vergelijkingen die in dit proefschrift worden gedaan vergelijkingen zijn die binnen de vrouwelijke sekse gebeuren en niet tussen de seksen. Deze vergelijkingen binnen de vrouwelijke sekse vergt naar alle waarschijnlijkheid meer proefpersonen.

Hoofdstuk 4. We hebben bij een groep 10-jarigen tweelingen, 79 OS en 59 SS, mogelijke prenatale testosteron invloeden onderzocht op cognitief functioneren. Hiervoor hebben we twee spatiele taken en twee verbale taken gebruikt. In elk domein werd in een van de twee gekozen taken een sekseverschil gevonden. Dit toont aan de mogelijke sekseverschillen al in het vroege puberteitsstadium kunnen worden aangetoond. Om mogelijke activerende (circulerende) testosteron invloeden uit te sluiten op de taken hebben we speekselmonsters afgenomen bij de tweelingen aan de hand waarvan we hun huidige testosteron spiegels konden bepalen. Interessant (maar volgens verwachting) was dat de testosteronspiegels op deze leeftijd hoger waren bij de meisjes dan bij de jongens, waarbij de SS meisjes de hoogste waarden hadden gevolgd door de OS meisjes en de laagste spiegels werden gemeten bij de jongens. Om de mogelijke rol van testosteron aan te tonen hebben we de taken vergeleken tussen de OS meisjes (die mogelijkerwijs zijn blootgesteld tijdens de zwangerschap aan hogere niveaus van testosteron en daardoor een meer mannelijk cognitief patroon hebben ontwikkeld) en SS meisjes (waarbij deze blootstelling aan testosteron tijdens de zwangerschap niet heeft plaatsgevonden). De twee taken die (eerder) een sekseverschil hadden aangetoond lieten geen verschil tussen deze meisjes zien maar een verbale geheugentaak (die eerder geen sekseverschil had opgeleverd) liet wel op het lange termijn geheugen een verminderde prestatie in de OS meisjes zien. Dit wil zeggen dat de meisjes een minder goed geheugen hadden voor verbaal materiaal op de lange termijn dan de SS meisjes. Het korte termijn geheugen verschilde niet tussen beide groepen. Bovendien bleek dat deze prestaties niet beïnvloed werden door de huidige testosteron spiegels.

Hoofdstuk 5. In dit hoofdstuk hebben we onderzocht in hoeverre sekseverschillen in agressie, spanningsbehoefte en temperament worden veroorzaakt door de invloed van testosteron. In de literatuur is zowel de prenatale als postnatale invloed van testosteron aangetoond. Aangezien de kinderen zich op het moment van testafname al in een begin tot gevorderd stadium van de puberteit bevonden (ze waren 13 jaar ten tijde van het onderzoek), hebben we het verband met de actuele hormoonspiegels ook meer diepgaand onderzocht. Tijdens dit meetmoment waren de spiegels in de jongens hoger dan in de meisjes (dit in tegenstelling tot de meting op 10 jaar), verder vertoonden alle kinderen een duidelijk circadiaan bioritme, hoog in de ochtend en een afname in de middag. Er was echter geen verschil meer in hormoonspiegels tussen de twee meisjes groepen (de SS en OS tweeling meisjes). Spanningsbehoefte was al eens eerder onderzocht door een groep onderzoekers (Resnick et al., 1993). Zij hadden destijds aangetoond dat de OS vrouwen meer spanningsbehoefte (dus een mannelijker patroon) lieten zien dan SS vrouwen. De onderzoekers zagen een mogelijke rol van testosteron in de uitslag; de OS vrouwen waren waarschijnlijk tijdens de zwangerschap blootgesteld aan hogere niveaus van testosteron en dit had hun spanningsbehoefte vermannelijkt. Wij hebben deze uitkomst niet kunnen repliceren. Mogelijk komt dat doordat wij te maken hadden met een jongere onderzoekspopulatie. Tevens was onze gehele onderzoeksgroep kleiner (ofschoon zij in principe een kleinere OS groep hadden).

Op het gebied van temperament hebben wij geen verschil kunnen aantonen tussen de twee groepen meisjes. Wel was er een verschil tussen de OS en SS tweeling meisjes in de mate van agressie; de OS meisjes toonden meer agressie geneigdheid dan de SS meisjes. Een andere agressie vragenlijst leverde geen verschil op. Dit komt waarschijnlijk door het gebruik van een andere meetmethode voor het meten van agressie. Dit benadrukt het feit dat de methode waarop een variabele wordt onderzocht van groot belang kan zijn in het vinden van verschil tussen groepen.

Hoofdstuk 6. Naast de tweeling methode (zoals besproken in dit proefschrift) voor het verkrijgen van kennis met betrekking tot de rol van testosteron in het ontstaan van sekseverschillen, hebben we ook andere methodes zoals atypische groepen (kinderen met genetische afwijking bijvoorbeeld) en prospectieve studies. Tegenwoordig wordt er ook gebruik gemaakt van een indirecte methode, namelijk de vingerratio: de relatieve lengte van de wijsvinger ten opzichte van de ringvinger. Onderzoekers suggereren dat de vingerratio wordt aangelegd tot aan week 14 van de zwangerschap en in dezelfde periode ontwikkelen zich ook de hersenen. Zowel het tweeling model als de vingerratio zouden onder invloed staan van prenatale geslachtshormoon niveaus in de baarmoeder. Wij verwachtten daarom ook een bepaalde associatie te vinden tussen deze beide methodes. We hebben onderzocht of de OS meisjes mogelijk een mannelijker vingerratio patroon hebben, dat wil zeggen een lagere vingerratio. Wij hebben dit niet kunnen aantonen in onze onderzoekspopulatie. Dit komt mogelijk door een lagere blootstelling aan testosteron in de OS meisjes groep (relatief gezien ten opzichte van jongens en meisjes met een genetische afwijking). De mogelijkheid dat er helemaal geen masculiniserend effect van prenataal testosteron op de vingerratio bestaat lijkt onwaarschijnlijk aangezien onderzoek in de atypische (medische) populaties hebben aangetoond dat dit wel degelijk het geval lijkt te zijn.

Hoofdstuk 7. Deze studie is opgezet om de mogelijke rol van prenataal testosteron te onderzoeken in het ontstaan van sekseverschillen op een aantal onderdelen van de CITO toets (een toets die aan het eind van het basisonderwijs wordt gehouden om advies uit te kunnen brengen ten aanzien van het te volgen vervolgonderwijs). Jaarlijks worden dezelfde sekseverschillen geconstateerd op de verschillende onderdelen van de CITO-eindtoets basisonderwijs: meisjes scoren (in vergelijking tot jongens) hoger op het onderdeel Taal en de jongens scoren (in vergelijking tot de meisjes) hoger op de onderdelen Rekenen en Wereldorientatie. Er wordt geen verschil tussen beiden geslachten gevonden op Informatieverwerking. In dit verband is een grote groep 11-12 jarige meisjes opgespoord uit de

afname jaren 1993 – 1998 van de CITO. 222 OS meisjes, 359 SS meisjes, 212 eenling meisjes met een maximaal 3 jaar oudere broer en 238 eenling meisjes met een maximaal 3 jaar oudere zus. (De laatste twee groepen zijn geselecteerd door de aangeschreven scholen zelf.) Er zijn geen verschillen gevonden tussen de twee verschillende tweelinggroepen. Wel was er een opvallend en significant verschil tussen de prestaties van de tweelingen en de eenlingen, ten nadele van de tweelingmeisjes. De eenlingen scoorden op alle onderdelen beter dan de tweelingen. Hoewel de prestatie van de eenlinggroepen ook sterk afweek van de algehele meisjes populatie in dezelfde afname jaren. Namelijk ook daar weer scoorden zij beter op alle onderdelen. Er was geen verschil tussen de algehele meisjes populatie en de door ons gevonden tweelingmeisjes. Dit verschil in prestatie zou kunnen komen doordat de leerkrachten niet bewust waren van het doel van dit onderzoek (en zij wel bewust zijn van het belang van een goede prestatie van hun leerlingen op de CITO voor hun school) en daarom onbewust (of bewust) een slim eenlingmeisje voor ons hebben geselecteerd.

Desalniettemin is er geen aanwijzing gevonden voor enige prenatale invloed van testosteron op de schoolprestatie zoals gemeten met de CITO. Verder hebben we ook niet kunnen aantonen dat de eenlingmeisjes met een oudere broer verschilden van de eenlingmeisjes met een oudere zus. Dit zou erop kunnen wijzen dat de "mannelijkheid" of "vrouwelijkheid" van de omgeving van het kind minder invloed heeft op de academische prestatie van een kind.

Conclusie. Uit de studies die zijn beschreven in dit proefschrift en de eerder vermelde bevindingen uit de literatuur kan worden geconcludeerd dat de waarde van tweelingen om de mogelijke rol van testosteron te onderzoeken minder bemoedigend is dan we hadden gehoopt. Het is ons daarom ook niet duidelijk of deze methode in de toekomst verder moet worden gebruikt. De bestaande studies maken het vrijwel onmogelijk om duidelijke gevolgtrekkingen te doen door het uitblijven van enige aantoonbare invloed. De methode op zich heeft zijn voordelen in vergelijking met de

andere methodes voor de studie van de rol van testosteron; de relatieve incidentie van twee-eiige tweelingen (ongeveer 1 op de 150 geboortes wereldwijd), het bestaan van tweelingenregisters en het vermijden van ethische problemen bij dit soort onderzoek. Desalniettemin hebben verschillende gedragsgenetici aangegeven dat zij regelmatig geen effect in OS meisjes hebben kunnen constateren. Jammer genoeg speelt het voorselectie beleid van wetenschappelijke bladen vaak een cruciale rol in onze onwetendheid van dit soort bevindingen doordat zij geneigd zijn om alleen significante resultaten te rapporteren en niet de niet-significante bevindingen. Dit maakt het daarom ook moeilijk voor ons om goed en volledig de bruikbaarheid van deze tweelingmethode tot het uiterste te evalueren en dit zou kunnen leidden tot ongewenste verspilling van tijd en inspanning, naast het verspillen van onderzoeksgeld.

Het hoofddoel van dit proefschrift was om meer duidelijkheid te verschaffen omtrent de rol van prenataal testosteron in de ontwikkeling van sekseverschillen. Hiervoor hebben we een groep meisjes uit een OS tweeling vergeleken met een groep meisjes uit een (dizygote) SS tweeling. Elk meisje van een OS tweeling wordt namelijk verondersteld te zijn blootgesteld aan hogere niveaus van testosteron tijdens de zwangerschap (dan een meisje uit de SS tweeling) doordat zij de baarmoeder deelde met haar tweelingbroertje. Door deze blootstelling aan hogere niveaus van testosteron wordt zij verondersteld een meer masculien patroon van cognities, gedrag en zelfs fysiologie te hebben ontwikkeld, in vergelijking met een meisje uit de SS tweeling. Deze benadering verkleint de impact van de postnatale omgevingsinvloeden, maar sluit ze niet uit. Verschillen op het gebied van tandgrootte, otoakoestische emissies (bepaalde maat gemeten in het oor), en onze eigen bevindingen met betrekking tot de hersenspecialisatie, gedrag en cognitie lijken de hypothese ten aanzien van de mogelijke rol van vroege testosteron te ondersteunen. Bovendien, als we al deze positieve bevindingen op een rij zetten vinden we dat in alle domeinen (namelijk het gedrags-, fysieke-, en het cognitieve-domein) een effect is aangetoond. Maar als we alle in de literatuur vermelde uitkomsten in overweging nemen dan

moeten we ons wel afvragen of de relatief kleine hoeveelheid positieve uitslagen die we hebben gevonden geen vorm van *Type I* fout zijn. Dat wil zeggen, dat we de nulhypothese (= geen verschil) ten onrechte verwerpen en aannemen dat er een verschil is dat er eigenlijk niet is. Dit is heel goed mogelijk vanwege een aantal redenen. Op de eerste plaats hebben we te maken met geringe verschillen (omdat we meten binnen de vrouwelijke sekse). Op de tweede plaats hebben we dikwijls gebruik gemaakt van grove meetinstrumenten (bijvoorbeeld. papier-en-potlood vragenlijsten). Op de derde plaats hebben we verschillende soorten variabelen onderzocht en tot slot hebben we gebruik gemaakt van een relatief kleine onderzoeksgroep. Hierdoor is het mogelijk dat we de verkeerde conclusies hebben getrokken. In de toekomst zouden we dit soort "problemen" moeten zien te vermijden. Daarbij komt dat we ook niet-significante verschillen moeten publiceren om zo een beter wetenschappelijk gefundeerd beeld te kunnen creëren van het onderzoeksterrein (zoals in ons geval de theorie achter de sekseverschillen). Vanwege het bestaan van de verschillende (internationale) tweelingenregisters lijkt dit niet al te moeilijk om te verwerkelijken.

DANKWOORD

Lieve mensen,

Nu is het dan eindelijk zover: het moment is aangebroken om iedereen te bedanken die ik in de loop der jaren ben tegengekomen en die een klein of groot beetje hebben bijgedragen in dit proces.

Als eerste zijn er natuurlijk **mijn ouders** die mij altijd onvoorwaardelijk hebben gesteund in alles wat ik heb gedaan en iedere keer weer klaar staan als ik ze nodig heb. Daar ben ik jullie heel erg dankbaar voor. Mijn **broer Sam** die alles wel prima vindt, maar mij nog regelmatig vraagt "Wanneer ga je nu het echte geld verdienen?!" (Ja, dat vraag ik mezelf ook wel eens af). And **Lina** (mijn bijna (?) schoonzus not to forget), you are great, please keep on sending hilarious e-mails, good spirit, and positive vibes, but remember I AM CE Lina! ;-)

Jan, Peggy en Stephanie, jullie hebben mij de mogelijkheid geboden om dit project uit te voeren en af te ronden. Langzaamaan zijn jullie alledrie weggegaan uit Utrecht, maar vanuit al jullie verblijfplaatsen (gelukkig bestaat e-mail) hebben jullie mij laten weten wat jullie van mijn gedachtespinsels vonden en dat heb ik zeer gewaardeerd. Bedankt voor jullie bijdrage aan het gehele proces!

Ko, jij hebt mij altijd gemotiveerd en je bent er altijd als ik je nodig heb. Jij bent mijn grote voorbeeld, ook al heb ik niet meer de ambitie om net als jij prof te worden. Ik hoop altijd je oogappel te blijven en je vertrouwen niet te schenden. Nu wacht ik jouw reactie af of het boekje voldoet aan je verwachtingen.

Judith, lachen, klagen, alles doen we al meer dan 10 jaar samen. Ik ben zo trots op het feit dat jij mijn beste vriendin bent. En laten we eerlijk zijn we weten allebei dat jij deze prestatie ook neer had kunnen zetten. Ik weet in ieder geval zeker dat het mij zonder jou niet was gelukt. Ik verheug me erop om weer eens als vanouds te borrelen, shoppen, of gewoon op de bank te hangen en het samen

fijn te hebben. Zo nu en dan schoot dat er een beetje bij in maar nu heb ik weer zeeën van tijd. Hele dikke kus. Thanx lieve Juutje.

Mijn Personal Coach, lieve **Nicolle**, jij bent een geschenk "from heaven" geweest, de gesprekken, de gevoelens: alles! Als ik ooit een advies moet geven aan een hoge pief met een positieve suggestie ten opzichte van het OIO-schap (ja ja ik behoor tot een uitstervend soort) dan is het wel het toekennen van een PC. Dat is toch echt een top prioriteit. Iedereen moet iemand hebben die je zonder gene kan wijzen op steken die je laat vallen. Dat heb jij voor mij gedaan, bovendien heb je mij zo nu en dan ook de goede richting opgeduwd, met name in het laatste jaar. Ik zou het niet hebben gered zonder jou, maar bovenal ben jij mijn maatje. Van al die jaren AZU/UMC hebben we samen toch een prachtige tijd gemaakt met veel lachen maar ook huilen, echte vriendschap!

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Claudine en Deborah, van de Winterberg-Clan; we hebben alweer een aanzet gedaan voor een volgende reis (Jahaaa, ik ben niet de enige die het wel eens zoekt buiten de grenzen van dit land). Leuk he, mijn boekje is af, maar voor jullie zal het ook snel (z)over zijn. Ik kan me nog herinneren die eerste periode van de O(A)IO-tijd, bizar met z'n tienen op een kamer (het was écht een soort tuin met ons als plantjes, die wetenschappelijke groei lieten zien), maar die tijd heeft mij wel deze vriendschappen opgeleverd. Deborah en Claudine, beiden hebben jullie een prachtig karakter (om trots op te

zijn) en staan jullie altijd klaar om te helpen en nooit is iets teveel moeite voor jullie.

Heddeke, jonge psychiater in spé, de laatste tijd is het contact wat verminderd aangezien we beiden toch wat veranderingen in ons leventje hebben meegemaakt. Toch ben ik je niet vergeten, tot binnenkort schat.

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Therése, jou wil ik niet vergeten te noemen want zonder jouw hulp bij de selectie van de tweelingen was het natuurlijk nooit gelukt. Bedankt nogmaals.

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En de **normale wereld vriendjes en vriendinnetjes**, er is echt een wereld buiten de wetenschap en jullie hebben allemaal laten zien dat die ook oh zo mooi is,door de leut- en kletsavonden (middagen), film, uitjes, skiën, feestjes, muziek, samengevat de andere leuke dingen des levens. Zonder al deze zaken had ik het nooit

gered. Ik ben en blijf nog steeds van de partij alleen nu weer met volle kracht. Bedankt en ik hoop dat het zo zal blijven!

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Y tu **Marcelo**, mi marido siempre con palabras buenas y con mucha simpatia. Sin ti este momento no seria possible. Me has motivado y dado mucha confianza durante todo este tiempo aunque no entiendes nada de lo que yo hago. Ah Ah Ah (Ha Ha Ha).

En natuurlijk het licht in ons leven, de kleine knul met zijn lichtbruine krulletjes, oh wat ben ik trots op jou, zelfs meer dan op dit boekje. Al met al hoop ik dat dit een voorbeeld voor jou zal zijn, dat met volharding en wil heel veel lukt. Laten we samen lekker van ons leven genieten want jij bent het meer dan waard.

Dit is het, afgerond en wel, en dít ga ik vieren met jullie allen samen. Here we go…

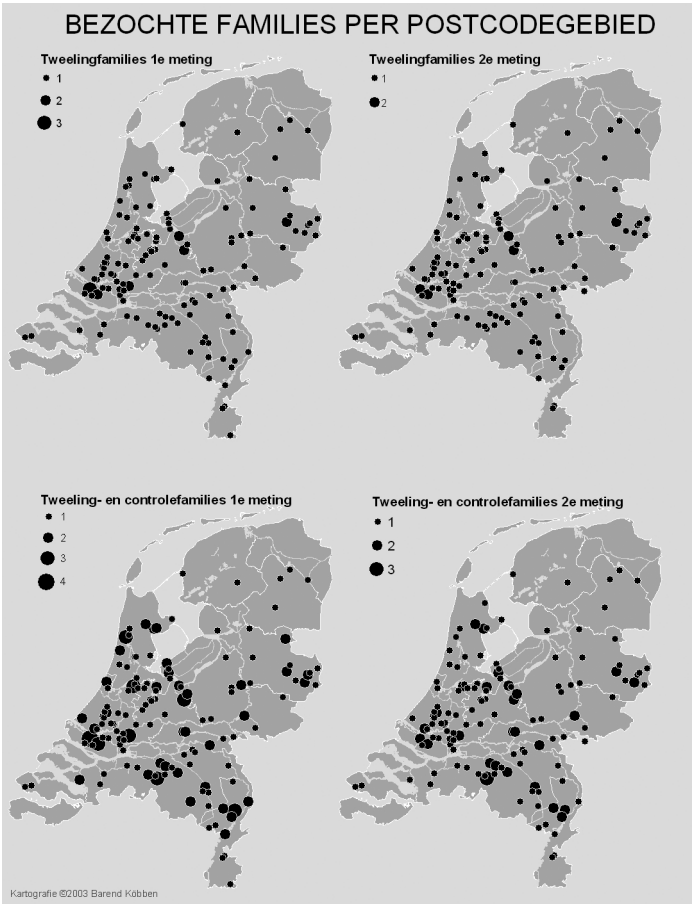
Liefs,
Celina

Ps. Als laatste wil ik nog even lieve Tsnefje (alias: Chef, Snuf) noemen. Alle jaren was hij erbij, jammer genoeg heeft hij het einde net niet gered. Ik mis hem heel erg.

CURRICULUM VITAE

Celina Cohen-Bendahan werd geboren op 31 januari 1970 te Leeuwarden. Op haar twaalfde is zij samen met haar broer en ouders naar Israel geëmigreerd. In 1988 behaalde zij haar "VWO"-diploma aan de middelbare school Kfar Batya (Raanana). Hierna heeft zij twee jaar het Israëlische leger gediend in het plaatsje Ofakiem (Negev-woestijn) waar zij werkte met tieners en les gaf aan jonge criminelen.
In 1990 is zij teruggekomen naar Nederland om te studeren aan de Vrije Universiteit te Amsterdam. Daar is zij in 1996 afgestudeerd in de Fysiologische Psychologie. Tijdens haar stage waarbij zij werkte met transseksuelen is haar enthousiasme gerezen voor het onderzoek naar sekseverschillen en de invloed van geslachtshormonen (op cognitie en gedrag). Gelukkig kon ze direct na deze stage aan de slag als onderzoeksassistent in het Universitair Medisch Centrum Utrecht bij de afdeling Kinder- en Jeugdpsychiatrie. Het onderzoek was wederom gericht op de invloed van prenatale geslachtshormonen op cognitie en gedrag maar nu bij jonge kinderen. In 1998 is ze begonnen met het onderzoek dat tot deze dissertatie heeft geleid.
Sinds 4 augustus 2002 is zij gelukkig getrouwd met haar Spaanse Marcelo. Op 20 september 2003 is hun gezin uitgebreid met een zoon, Immanuel.

BIJLAGE


BEZOCHTE FAMILIES PER POSTCODEGEBIED
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