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INFLUENCE OF TESTOSTERONE AND ANDROGEN RECEPTOR POLYMORPHISM ON VISUAL-SPATIAL COGNITION IN ADULT MEN

(Spine title: Testosterone, androgen receptor, and spatial cognition)

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by

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/

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ABSTRACT

The nature of the relationship, if any, between visual-spatial cognition and androgens in humans remains controversial. Possible associations between spatial ability, testosterone (T), and the length of the CAG polymorphism in the androgen receptor gene were investigated in 106 healthy undergraduate males. Using multiple linear regression, T concentrations and CAG repeat length significantly contributed to the prediction of performance on the Paper Folding Test. Some differences between left- and right-handed participants were observed. A secondary objective was to study the association between salivary T, CAG repeat length, and depressive affect across the entire mood spectrum in the same sample of subjects. CAG repeat length and T levels significantly contributed to the prediction of somatic symptoms of depression, but not to overall depression scores. These findings suggest that taking CAG repeat status into consideration may be informative in studies looking at the associations between androgens and either spatial ability or mood.

Keywords: Testosterone; Androgens; CAG repeat length; Spatial cognition; Depressive affect; Human

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

Page

CHAPTER 1

INTRODUCTION

Androgens are steroid hormones secreted by the gonads in response to stimulation from the hypothalamus and pituitary. In humans, the most well-known androgen is the hormone testosterone (T). Although it is a misnomer, T and other androgens are generally labelled "male sex hormones" because of their masculinizing effects in a variety of mammalian species. Research has also found that androgens may play a regulatory role in the central nervous system of humans and other animals. This effect occurs through the binding of androgens to androgen receptors (ARs) in the brain. Binding causes a series of structural changes to occur in the AR, which in turn result in the regulation of the actions of a wide range of neurotransmitters and neuropeptides (Rubinow & Schmidt, 1996; Genazzani et al., 1992). The effects of such binding can occur both during fetal and neonatal development and later during adult life (Rubinow & Schmidt, 1996; Swerdloff, Wang, Hines, & Gorski, 1992). During the periods of pre- and perinatal development, AR binding has been found to impact the structure of the brain, including the size of brain nuclei, synaptic formation, and axonal and dendritic branching (Gorski, Gordon, Shryne, & Southam, 1978; Williams & Meck, 1991; Breedlove, 1992). In adulthood, AR binding has been found to have neuroprotective effects against oxidative stress (Ahlbom, Prins, & Ceccatelli, 2001), apoptosis (Hammond et al., 2001), and excitotoxicity in hippocampal neurons (Pouliot, Handa, & Beck, 1996), while also having growth-promoting properties (Tirassa et al., 1997; Morse, DeKosky, & Scheff, 1992).

Based on the number and sites of androgen-related changes in the brain, it seems plausible to infer that androgens might also have effects on behaviour. Studies in humans and in other species have found that androgens modulate a number of behaviours including sexual function, aggression, cognitive abilities, and mood (Mullins & Levine, 1968; Kwan, Greenleaf, Mann, Crapo, & Davidson, 1983; Bouissou, 1983; Dabbs, Ruback, Frady, Hopper, & Sgoutas, 1988; Moffat & Hampson, 1996; Wang et al., 1996). Focused investigation of the impact of T and other androgens on cognitive abilities and mood in humans reveals that these sex steroids are more specifically associated with visual-spatial cognition and depression, respectively (Seidman & Roose, 2000; Rubinow & Schmidt, 1996; Swerdloff et al., 1992).

Androgens and Visual-Spatial Cognition

Visual-spatial cognition refers to the set of abilities needed to perceive or reason about the position of objects or their movements in space (Hampson, 1995). These abilities have been classified into a number of different categories, including, among others, spatial orientation (SO) and spatial visualization (SV) (French, 1951; Borich & Bauman, 1972; Ekstrom, French, Harman, & Derman, 1976). SO is defined as the ability to imagine an object after accounting for a change in its orientation (e.g. mental rotation), while SV is defined as the ability to imagine how a depicted object will appear after it has been manipulated in some way (e.g. being folded) (French, 1951). Together, SO and SV represent the two major skills needed to carry out spatial reasoning tasks (Ekstrom et al., 1976; Kozhevnikov & Hegarty, 2001).

Developmental Effects of Androgens on Visual-Spatial Cognition

Various studies have investigated the association between androgens and spatial reasoning in humans. Some have focused on whether exposure to androgens early in life has a developmental effect on spatial abilities, while others have studied the effects of these hormones in adulthood. Testing of the developmental effect has been attempted through studies of people diagnosed with congenital adrenal hyperplasia (e.g. Resnick, Berenbaum, Gottesman, & Bouchard, 1986; Hampson, Rovet, & Altmann, 1998; Helleday, Bartfai, Ritzen, & Forsman, 1994; Baker & Ehrhardt, 1974; McGuire, Ryan, & Omenn, 1975), or individuals exposed to diethylstilbestrol in utero (e.g. Reinisch & Sanders, 1992; Hines & Shipley, 1984; Hines & Sandberg, 1996), through studies of androgen levels in umbilical cord blood (Jacklin, Wilcox, & Maccoby, 1988) or in amniotic fluid (Grimshaw, Sitarenios, & Finegan, 1995) which are then correlated with cognitive measures obtained from the same individuals later in life, and through opposite-sex twin studies (Cole-Harding, Morstad, & Wilson, 1988). Overall, these studies have produced inconclusive results; nonetheless they do provide some preliminary evidence that spatial abilities later in life may be influenced by prenatal exposure of the brain to androgens.

Congenital adrenal hyperplasia is an inherited condition in which an enzyme deficiency in the adrenal cortex causes a significant over-production of androgens that begins prenatally and lasts until diagnosis, usually in early infancy. In females, this results in their exposure to male-like (i.e. higher) levels of androgens in utero. If spatial abilities are influenced by early androgen levels, then girls with congenital adrenal hyperplasia ought to have better spatial skills than non-affected females. Studies by

Resnick et al. (1986), Hampson et al. (1998), and Hines et al. (2003) have corroborated this hypothesis, finding that women with congenital adrenal hyperplasia performed significantly better than their unaffected female siblings on tests of spatial abilities. Similarly, a recent investigation found that young women with the most severe form of congenital adrenal hyperplasia, who are likely to be exposed to higher levels of androgens in utero than other forms of congenital adrenal hyperplasia, performed significantly better on a test of spatial navigation than unaffected control females, and performed equally as well as unaffected males and males with congenital adrenal hyperplasia (Mueller et al., 2008). Some studies have not, however, found the same pattern of results. Baker and Ehrhardt (1974), McGuire et al. (1975), Helleday et al. (1994), and Malouf, Migeon, Carson, Petrucci, and Wisniewski (2006) all failed to find improved spatial abilities in females with congenital adrenal hyperplasia. This may be partly due to poor choice of spatial tests in some of the studies. Nevertheless, a recent meta-analysis concluded that women with congenital adrenal hyperplasia perform better than control women on tasks of spatial ability that generally show a male advantage (Puts, McDaniel, Jordan, & Breedlove, 2008).

Diethylstilbestrol is a synthetic estrogen that appears to have a masculinizing effect on the brain in rodents. Studies of children of mothers who were treated with diethylstilbestrol during pregnancy have found that women exposed to diethylstilbestrol in utero do not exhibit improved visual-spatial skills compared to their non-exposed sisters (Hines & Shipley, 1984; Hines & Sandberg, 1996). This implies that if androgens do have a developmental effect on spatial abilities, it is unlikely to be mediated by the aromatization of testosterone into estrogen.

Investigation of androgen levels in umbilical cord blood of normal newborns followed by cognitive testing at six years of age found that higher levels of androgens were correlated with poorer spatial performance in childhood (Jacklin et al., 1988). These results are opposite to the directionality of prenatal androgen exposure and future spatial ability suggested by the female congenital adrenal hyperplasia data. This could be explained by the fact that androgen levels in cord blood are not representative of the androgen levels in the fetus when the brain is most sensitive to the effects of androgens, which is considered to be in early or midgestation (Hines, 1982). In contrast, Grimshaw et al. (1995) found a positive correlation between T levels in amniotic fluid obtained at 14 to 20 weeks gestation and future performance on a mental rotation test in a sample of seven-year-old girls.

The lone opposite-sex twin paradigm study, which is based on the principle that small amounts of T diffuse from the male to the female fetus in utero, found that female twins in opposite-sex pairs performed better on a mental rotation test than females with a female twin (Cole-Harding et al., 1988). Again, this highlights a potential organizational effect of prenatal androgens on future visual-spatial skills.

In addition to these investigations of the developmental effects of androgens on visual-spatial cognition, studies of the correlation between putative somatic markers of prenatal androgens and current spatial skills have been employed. One such marker is the second to fourth finger (digit) length ratio (2D:4D). The 2D:4D ratio is significantly lower in men than in women, and this is hypothesized to be due to the differential exposure of each sex to androgens prenatally (Manning, Scutt, Wilson, & Lewis-Jones, 1998). Investigation of the link between individual differences in the 2D:4D ratio and the

level of spatial abilities in men has produced a variety of results. Some studies have found that lower, more masculinized 2D:4D ratios are associated with better spatial performance (e.g. Manning & Taylor, 2001; McFadden & Schubel, 2003; Sanders, Bereckzei, Csatho, & Manning, 2005; Kempel et al., 2005; Peter, Manning, & Reimers, 2007). In contrast, one study found a significant curvilinear relationship between 2D:4D and a spatial targeting task (Falter, Arroyo, & Davis, 2006). Unfortunately, many other studies have failed to find any detectable association between spatial scores and digit length (e.g. Austin, Manning, McInroy, & Mathews, 2002; Coolican & Peters, 2003; Rahman, Wilson, & Abrahams, 2004; Putz, Gaulin, Sporter, & McBurney, 2004; Falter et al., 2006; Hampson, Ellis, & Tenk, 2008; Loehlin, Medland, & Martin, 2009). Thus, whether the finger length ratio is a reliable index of variance in spatial abilities in men still remains elusive.

Adulthood Effects of Androgens on Visual-Spatial Cognition

A larger body of work has investigated the link between spatial reasoning and androgen levels in human adults. This has been accomplished using several methods. Some studies have measured individual differences in androgen levels and their link with spatial performance (e.g. Martin, Wittert, Burns, & McPherson, 2008; Yang, Hooven, Boynes, Gray, & Pope, 2007; Moffat & Hampson, 1996). Other studies have investigated the effects of natural fluctuations in androgen levels (e.g. Celec, Ostatnikova, Putz, & Kudela, 2002; Sanders, Sjodin, & de Chastelaine, 2002; Kimura & Toussaint, 1991), or have exogenously manipulated androgen levels and examined the resulting effects on spatial tasks (e.g. Cherrier et al., 2007; Van Goozen, Cohen-Kettenis, Gooren, Frijda, & Van de Poll, 1994; Janowsky, Oviatt, & Orwoll, 1994).

Christiansen and Knussmann (1987) studied 117 men between the ages of 20 and 30 and found that all significant correlations between participants' current T and dihydrotestosterone (DHT) (a metabolite of T) levels and their spatial performance were in the positive direction.

A study by Hooven, Chabris, Ellison, and Kosslyn (2004) found that higher levels of endogenous T were associated with lower error rates and faster response times on a computerized mental rotation task (MRT) in a small sample of university-aged American males. A similar study, using an identical methodology, conducted in a British sample, failed to find any association (Falter et al., 2006), as did a cross-cultural replication in a Chinese sample. The latter study, however, did find that men with higher levels of T had significantly faster response times on the MRT, as the task increased in difficulty, compared to men with lower levels of T (Yang et al., 2007). A recent investigation by Martin et al. (2008) found that free T levels in healthy middle-to-older aged men were not directly linked with MRT performance, but were indirectly associated with MRT through the mediation of two other abilities: processing speed and working memory. More specifically, higher T levels resulted in slower processing speed, which in turn accounted for poorer working memory and MRT performance. Another study conducted in middle-to-older aged men also provided evidence for a negative association between endogenous T levels and spatial cognition, albeit through a direct path. In this case, low free T levels led to better performance on tasks of SV than high free T levels (Yonker, Eriksson, Nilsson, & Herlitz, 2006). Using a younger sample, Moffat and Hampson (1996) tested 40 male and 40 female undergraduate students on visual-spatial and verbal cognitive tests and found that male participants' T levels were significantly and negatively correlated

with spatial performance, whereas females had a significant positive correlation between T levels and one of the two spatial tests given, which tapped into SV. Interestingly, this pattern of results was only observed in right-handed subjects. When spatial performance was analysed across the entire range of T levels by combining both right-handed males and females an inverted U-shaped relationship was discovered. Nonlinear associations between T levels and spatial reasoning have also been reported by several other studies (Shute, Pellegrino, Hubert, & Reynolds, 1983; Gouchie & Kimura, 1991).

Circulating T levels in humans vary over the course of hours, days, weeks, months, and years (Dabbs, 1990; Place & Nichols, 1991). As a result, some studies have been conducted to see if spatial performance changes as a function of the natural fluctuations of T. Mackenberg, Broverman, Vogel, and Klaiber (1974) tested healthy male volunteers between the ages of 12 and 50 on a battery of cognitive tests in both the morning (9:30 a.m.) and in the afternoon (3:30 p.m.) and found that participants performed better on tasks of spatial ability in the afternoon than in the morning. T, by nature, declines in concentration across the day, with the highest levels between 6:00 to 8:00 a.m., followed by a sharp decrease in late morning that becomes more stable over the mid-day, and reaches its lowest levels between 6:00 to 8:00 p.m. (Dabbs, 1990; Leymarie, Roger, Castanier, & Scholler, 1974; Ahokoski et al., 1998). Thus the results of the Mackenberg et al. (1974) study suggest that better spatial performance occurs during a time of day when T levels are generally low. Moffat and Hampson's (1996) study included an analysis of spatial performance differences in early vs. late morning testing in right-handed subjects and found that spatial ability was significantly stronger for males tested at 10:15 a.m., when T levels are lower, than for men tested at 8: 15 a.m.,

while females showed the opposite pattern of results. Similarly, Sanders et al. (2002) found that men performed significantly better on a MRT in the afternoon/evening than in the early morning, while women showed a decline in MRT scores later in the day compared to early in the morning, albeit non-significantly. These findings are in line with the Mackenberg et al. (1974) study and fit with the inverted curvilinear relationship between T and spatial abilities hypothesized by Moffat and Hampson (1996), which suggests that men with T levels that fall in the lower part of the normal male range and women with T levels that fall in the higher part of the normal female range have the optimal T concentrations for spatial performance. Celec et al. (2002) studied changes in T levels across the menstrual cycle in women and a similar 30 day period in men, and found a similar curvilinear relationship between T levels and spatial skills. Women performed best on tasks of SO and SV at midcycle, when T levels were at their highest, while men performed best on these same tasks during the beginning and the end of the 30 day period, when their T levels were lowest. A peak in T levels was found on the 18th day of the cycle in men, which corresponded to worse spatial ability. Kimura and Toussaint (1991) assessed whether spatial cognition abilities in men varied across the seasons, since T levels in men are known to be higher in fall than in spring (Valero & Fuentes, 1998). Again, they found that men performed better on spatial tasks when T levels were lower, which in this case corresponded to the spring season.

A number of studies have investigated the effect of exogenous sources of androgens on visual-spatial cognition. These studies are of importance because they can help determine if there is a true causal relationship between androgens and spatial ability. Using a placebo-controlled design, Janowsky et al. (1994) had healthy older men between

the ages of 60 and 75 wear scrotal patches containing 15 mg of T every day over the course of three months. After 12 weeks of T treatment, spatial abilities had improved in the treated men compared to their performance prior to hormone treatment. Cherrier et al. (2007) randomly assigned 57 healthy older men to receive weekly injections of either 50, 100 or 300 mg of T enanthate or saline injections for six weeks. They were tested on a neuropsychological battery at baseline, week three and week six of treatment, and after a six week washout period. T supplementation improved spatial abilities in men who demonstrated *moderate* increases in T levels during the T administration compared to baseline, but not in men who experienced large or no to low increases in T levels. In contrast, O'Connor, Archer, Hair, and Wu (2001) found that young eugonadal men who received 200 mg of T enanthate weekly for eight weeks performed significantly worse on a test of spatial ability after four weeks of treatment compared to eugonadal men in the placebo group tested at the same point in time. Wolf et al. (2000) provided a single injection of 250 mg of T enanthate to 30 elderly men in their mid 60's and found that the T injection had no significant effect on any cognitive tasks including MRT. In one of the only studies looking at the effect of exogenous T administration on cognitive abilities in women, Aleman, Bronk, Kessels, Koppeschaar, and Honk (2004) found that a single administration of 0.5 mg of sublingual T significantly improved visual-spatial ability in healthy young women when tested on an MRT four to five hours after T administration. Similarly, Van Goozen et al. (1994) found that female-to-male transsexuals undergoing cross-sex hormone treatment through T therapy, which caused their T levels to increase from low to physiologically normal male concentrations, performed better on a spatial

rotated figures task after three months of T treatment compared to their performance prior to the onset of T supplementation.

Based on the evidence outlined above, it is clear that the direction of the relationship, if any, between androgens and visual-spatial abilities in human adults still remains elusive. Some studies have found a positive relationship (Christiansen & Knussmann, 1987), while others have found a negative relationship (Yonker et al., 2006), a curvilinear relationship (Shute et al., 1983), and even no relationship at all (Halari et al., 2005). These inconsistencies in the spatial cognition literature could be explained by a number of factors. One possibility is that biological sex plays a role in the type of association seen between androgens and spatial ability. Among the studies that have looked at androgens and spatial abilities in females, several have failed to find effects, but where an association has been found, it is invariably positive. The findings for males, however, are inconsistent. Thus conclusions about the direction of the relationship between androgens and spatial skills might be better served by being made for each sex individually, rather than for human adults as a whole. Age is another factor that needs to be considered. Most studies have found a positive correlation between T and spatial abilities in elderly groups (for a review see Beauchet, 2006), who typically have lower endogenous T production than young people, while the pattern for younger-aged samples is again inconsistent. Handedness might be another relevant variable. Based on the only study of its kind, Moffat and Hampson (1996) found that hand preference moderated the relationship between spatial cognition and T, suggesting that it could be an important individual difference variable in understanding the direction of the association between androgens and spatial ability.

Another potential explanation for the lack of clear relationship is the fact that different studies used different tests to evaluate spatial abilities, which may differ in their sensitivity to assessing spatial performance. Also, the spatial tests administered do not all tap into the same subcategories of visual-spatial cognition (e.g. SO, SV) and it is possible that androgens influence each of these subcategories in different ways. The methods by which androgens are sampled or administered to participants are other possible reasons for the conflicting findings. Blood contains both sex hormone binding globulin-bound, albumin-bound, and non-bound (free) T. Only T that is free or bound to albumin and not sex hormone binding globulin is considered to be biologically active because it can readily pass through cell membranes and thus can exert an effect on the body and brain. As a result, studies that employ blood sampling of T may less accurately represent the true androgen status of participants and may produce only weak correlations with spatial performance. Saliva, however, contains only bioavailable (i.e. free and albumin-bound) T. Thus salivary sampling of T has the potential to produce clearer correlations with spatial abilities than serum sampling. With regard to exogenous T administration, different synthetic hormones, dosages of T, length of treatment, and routes of administration all play a role in explaining the variable results seen in the literature. Recall that androgens exert their influence on the brain by binding to ARs in various brain structures including the hypothalamus, amygdala, hippocampus, and the temporal cortex (Pelletier, 2000). Thus, another explanation for the inconsistencies in the spatial cognition data is that spatial functioning may not only be dependent on androgen levels but may also be influenced by the number and affinities of ARs in the brain. Interestingly, the functionality of the AR is mediated in part by genetic factors including

the length of a polymorphic polyglutamine stretch, made up of a variable number of trinucleotide (CAG) repeats, in exon 1 of the AR gene located on the X chromosome (Zitzmann, 2009) (Figure 1.1). Normal CAG stretches range between 6 to 39 repeats, with an average of 20 to 22 (Giovannucci et al., 1997). Evidence suggests that longer CAG repeat stretches are associated with lower levels of AR activity, while shorter CAG repeat lengths confer better receptor functionality upon androgen binding (Chamberlain, Driver, & Miesfeld, 1994; Kazemi-Esfarjani, Trifiro, & Pinsky, 1995; Krithivas et al., 1999).

The length of the CAG polymorphism in the AR gene has been associated empirically with conditions that are androgen-related, including prostate cancer (Clark, Irvine, & Coetzee, 2003), infertility (Yong, Loy, & Sim, 2003), male-pattern baldness (Sawaya & Shalita, 1998), cardiovascular risk factors (Zitzmann et al., 2001a), and bone density loss (Zitzmann et al., 2001b). In the case of prostate cancer, for example, where higher androgen levels influence the development of the disease, reports suggest that short CAG repeats increase the risk for the cancer (for reviews see Westberg & Eriksson, 2008). Consequently, it seems plausible that genetic variation in the AR could influence visual-spatial abilities, which are also hypothesized to be modulated in part by androgens. To our knowledge, only one other study has investigated the link between CAG repeat status and cognitive functioning. In a sample of 301 elderly men enrolled in the Study of Osteoporotic Risk in Men, Yaffe et al. (2003) measured participants' CAG repeat length and their performance on three cognitive tests: the Mini-Mental Status Examination, which is a global measure of cognitive decline, the Trails B test, which is a measure of visual scanning, visual sequencing, and executive functioning, and the Digit Symbol test,

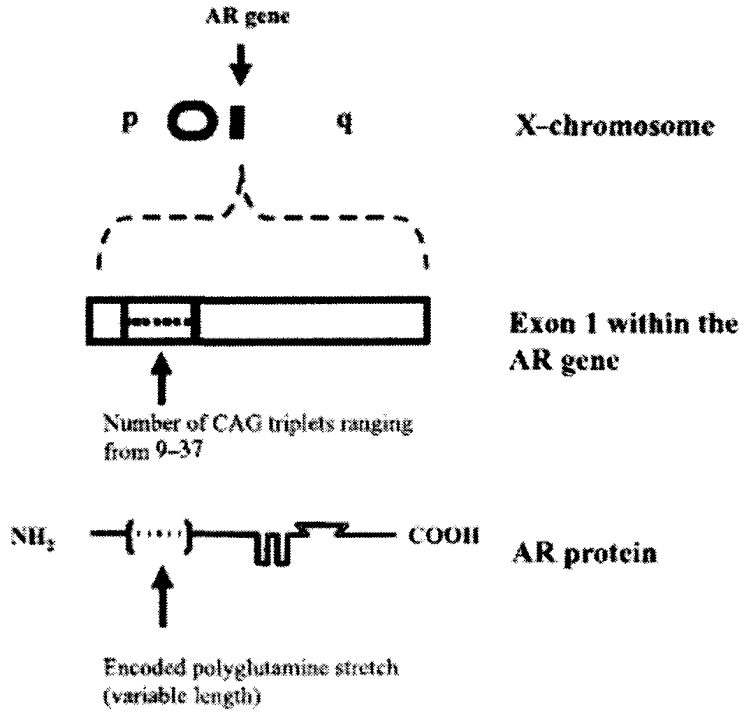


Figure 1.1. An image depicting the AR gene in the X chromosome. Located within exon 1 of the AR gene is the CAG polymorphism. A variable number of CAG repeats in the AR gene results in the encoding of a polyglutamine stretch of variable length within the AR protein. The number of CAG repeats, and by extension, the length of the polyglutamine stretch influences the activity of the AR (Source: Zitzmann & Nieschlag, 2003).

which is a measure of attention and psychomotor performance. They found that longer CAG repeat lengths were associated with significantly poorer performance on all three cognitive tests and that a greater number of men classified in the two groups containing the longest repeat lengths met criteria for cognitive impairment than those classified in the group with short repeats.

Based on these findings, the CAG polymorphism could potentially play a role in cognitive functioning and would be of value to measure in studies that attempt to assess the link between current androgen levels and spatial abilities. Such measurement could, at present, help clear up inconsistencies in the literature on spatial cognition and T in males. Because the AR gene is X-linked, males carry only a single AR gene, while women carry two, potentially different, AR genes, one inherited from each parent. Unfortunately, women experience a phenomenon known as X-inactivation, whereby only one of their X chromosomes in each of their somatic cells is active. The determination of which X chromosome will be active in each cell occurs in the embryo and is a random process, making women mosaic, with patches of tissue characterized by one active X over the other. The distribution of active Xs in the female body can only be known by analysing tissue from the specific bodily region of interest. As a result, without access to brain regions critical for spatial cognition, it would be impossible to determine which AR gene is influencing androgenic effects on spatial abilities in women. Men, instead, only have one X chromosome and by extension only one AR gene which can modulate androgen action and possibly visual-spatial skills (Migeon, 2007).

Androgens and Depressive Affect

Depressed mood is another behaviour that has been associated with androgens in humans. Evidence of a potential relationship between these two variables has come from reports that hypogonadal men often complain of loss of libido, dysphoria, fatigue, and irritability, which are also symptoms commonly associated with major depression (for reviews see Amiaz & Seidman, 2008). While more controversial, another source of evidence stems from the fact that the natural slowing of hypothalamic-pituitary-gonadal (HPG) axis function in men over the age of 50 and the resulting reduction in T levels has been associated with increased incidence of depressive illness (Shores et al., 2004). As a result, a number of studies have been conducted to determine if low androgen levels in adulthood are concurrently linked with depressive affect (e.g. Wang et al., 1996; Pope, Kouri, & Hudson, 2000; Feldman et al., 2002; Seidman et al., 2009).

Some researchers have attempted to answer this question by conducting studies of T replacement in non-depressed hypogonadal men. The rationale behind these investigations is that by exogenously increasing T to normal, physiological levels in men with low gonadal function, changes in mood will become apparent if mood and androgen levels are truly correlated with one another. Wang et al. (1996) employed such a paradigm to study 51 hypogonadal men who received T replacement for 60 days. Participants were randomly assigned to receive either 200 mg of T enanthate once every 20 days, 2.5 mg of sublingual T cyclodextrin three times a day for 60 days, or 5.0 mg of the same sublingual T three times a day for 60 days, and were withdrawn from previous T replacement for at least 6 weeks before commencing the study. Results found that compared to their baseline self-report ratings, T replacement was associated with

significant increases in positive mood parameters such as energy level, friendliness, and sense of well-being, and significant decreases in negative mood parameters such as anger, irritability, sadness, tiredness and nervousness. Furthermore, these improvements in mood persisted in a group of 30 participants assigned to the 5.0 mg sublingual T category who continued to receive treatment for six months. In another investigation by Wang et al. (2000), transdermal administration of T through a gel in hypogonadal men aged 19-68 years for 180 days was similarly associated with significant improvements in positive moods and significant decrements in negative moods, as assessed by the same self-report mood questionnaire used in the Wang et al. (1996) study, compared to pre-treatment ratings. Again, follow-up of 123 participants who continued to receive transdermal T replacement for up to 42 months, found that the improvements in mood persisted (Wang et al., 2004). Investigation by McNicholas, Dean, Mulder, Carnegie, and Jones (2003) also found that administration of a T gel significantly improved mood ratings compared to baseline. It should be noted, however, that none of these studies employed a placebo-controlled design. Consequently, it is impossible to know if the mood improvements are due to the T replacement or would be equally detectable in a group of hypogonadal men who believed they were receiving T but were actually receiving placebo.

In a placebo-controlled study, Steidle et al. (2003) found that hypogonadal men randomized to receive either T replacement, through a transdermal gel or a patch, or placebo showed no significant differences during treatment on their self-reported mood ratings, assessed through the same questionnaire used in the studies described above. Schmidt et al. (2004) induced hypogonadism in 31 healthy eugonadal men between the ages of 23 and 46 by suppressing T secretion with a GnRH agonist (Lupron) for one

month, followed by a random assignment to receive either T replacement or placebo for a second month, and then a cross-over to the other condition for an additional month. They found that with the exception of hot flashes, libido, and feeling emotionally charged, mood, as assessed by a series of self-report questionnaires including the Beck Depression Inventory (BDI), did not significantly differ between the Lupron plus placebo group and either the Lupron plus T group or the baseline rating. While this does not provide much evidence of an androgenic influence on depressive affect, three men in the hypogonadal state did score in the clinically significant range on the BDI, which was reversed when they shifted to the T replacement state. In a study by O'Connor, Archer, Hair, and Wu (2002), eight hypogonadal men 23-40 years old receiving biweekly injections of 200 mg T enanthate had significantly improved mood ratings on the Profile of Mood States after one to two weeks of treatment compared to baseline, eugonadal men receiving exogenous T, and eugonadal men receiving placebo. It should be noted, however, that the T treatment effect was not significant for the "depression-dejection" subscale of the Profile of Mood States.

Other researchers have investigated the relationship between androgens and depressive affect in humans by administering supraphysiological doses of androgens to healthy non-depressed individuals and assessing their subsequent mood states. In a study by Tricker et al. (1996), the authors found that 43 healthy eugonadal men between 19-40 years of age who were randomized to receive either weekly T or placebo injections for 10 weeks showed no change in self-reported or observer-reported measures of mood during T administration. In a randomized, placebo-controlled, crossover experiment of 56 men between the ages of 20 and 50, Pope et al. (2000) administered increasing dosages of T

cypionate for six weeks and placebo for six weeks, with a wash-out period of six weeks in between the two treatment conditions. Based on participants' responses on a wide range of standardized mania and depression questionnaires and analyses of diary entries of their manic and depressive symptoms, they found that depression scores did not significantly change during either T treatment or withdrawal compared to baseline, but manic scores significantly increased during T administration. The changes in mania ratings were, however, mainly influenced by a few participants who displayed marked symptoms. In the previously cited O'Connor et al. (2002) study, healthy eugonadal men receiving exogenous sources of T did not show significant changes in affect over the treatment period. In an interesting double-blind, placebo-controlled, crossover study of 14 healthy, young women receiving a single dose of 0.5 mg T cyclodextrin, T administration, compared to placebo, did not significantly change self-reports of depression, anger, and anxiety on the Profile of Mood States, but did enhance functional connectivity between the left pre-frontal cortex and right parietal cortex hypothesized to be a "cortical depression circuit" (Schutter, Peper, Koppeschaar, Kahn, & van Honk, 2005).

A number of studies have also looked at the mood modifying properties of androgen supplementation in depressed individuals. For example, Seidman, Miyazaki, and Roose (2005) randomized 26 men with treatment-resistant depression who were currently on a serotonergic antidepressant to either T enanthate or placebo for six weeks. They found that Hamilton Depression Rating Scale (HAM-D) scores decreased significantly in both the T and placebo groups, though there were no significant differences between the groups. It should be mentioned, however, that a greater number

of participants randomized to the treatment group exhibited reductions in HAM-D scores of ≥ 50% than in the placebo group. In a study of 15 eugonadal men over the age of 50 with depression randomized to receive either a physiologic dose of T cypionate (100 mg/week) or a supraphysiologic dose (200 mg/week), the authors found that there was a 42% decrease on HAM-D scores across the groups. They also found that the improvement in depression scores was predominantly caused by the 10 participants who had been diagnosed with depression after the age of 45, who evidenced a 53% decrease in their HAM-D scores, rather than the five participants who had early-onset depression (i.e. diagnosis prior to the age of 45), who only evidenced an 18% decrease in their depression ratings (Perry et al., 2002). Wolkowitz et al. (1999) randomly assigned 22 subjects (12 men and 10 women) ranging in age from 33 to 53 years with diagnosed major depression to receive either dehydroepiandrosterone (a weak endogenous androgen that is a precursor to T) or placebo for six weeks. This treatment was on top of any antidepressant they were already taking. Dehydroepiandrosterone-treated subjects were found to have a significant decrease in their HAM-D scores compared to placebo. Also, 5 out of 11 dehydroepiandrosterone group members were deemed responsive to treatment compared to 0 out of 11 in the placebo group.

Some studies have gone a step further and assessed the effects of androgens on hypogonadal depressed men. Pope, Cohane, Kanayama, Siegel, and Hudson (2003) had hypogonadal men, who were currently taking antidepressants, randomized to receive either T or placebo gel for eight weeks and found that those receiving T showed significantly greater improvements in their depressive symptoms than those receiving placebo. A more recent study, but in men with low to low-normal T levels diagnosed

with dysthymia (a chronic depressive condition), found that participants randomized to receive injections of T for six weeks demonstrated a significantly greater decrease in their HAM-D scores after treatment than the participants randomized to receive placebo. In addition, 7 out of the 13 participants in the T group met criteria for remission while only 1 out of the 10 in the placebo condition did so (Seidman et al., 2009). While these results point to the possibility that T might be an effective antidepressant strategy in hypogonadal men there are, however, a number of studies that have not found the same effect. Notably, investigations by Seidman and Roose (2006), Orengo, Fullerton, and Kunik (2005), and Seidman, Spatz, Rizzo, and Roose (2001) have all found that T supplementation is indistinguishable from placebo in terms of its impact on depressive affect.

Investigation of the relationship between androgens and depressive affect has also been approached by measuring individual differences in androgen levels and mood ratings. An early study by Doering et al. (1975) assessed the endogenous serum T levels and affective states of 20 healthy, young males over a period of two months and found a small, but significant positive correlation between T values and depression ratings on the Multiple Affect Adjective Checklist. More recently, there have been a number of large-scale, population-based studies that have looked at current androgen levels and depressive symptoms in men. Massachusetts Male Aging Study was conducted in a sample of 1709 men between the ages of 40 and 70 who were asked to complete a self-report depression questionnaire, the Center for Epidemiological Studies Depression Scale (CES-D), and provide a blood sample for T measurement. Feldman et al. (2002) found that serum T levels were not significantly associated with CES-D diagnosed depression

(i.e. a score of > 16 on the CES-D). In a five-year longitudinal investigation of androgen levels, cognitive functioning, and depression in a sample of 247 men with a mean age of 75 years at study entry, Ponholzer et al. (2009) found that levels of total T and dehydroepiandrosterone sulphate were not significantly associated with the Diagnostic and Statistical Manual of Mental Disorder-IV criteria for depression at either baseline or after five years. It should be noted, however, that after five years there were a greater number of participants with diagnosed depression and lower T and dehydroepiandrosterone sulphate levels compared to at entry into the study. In a 10-year follow up to the Rancho Bernardo Study of heart disease risk factors, Barrett-Connor, Von Muhlen, and Kritz-Silverstein (1999) found that in a sample of 856 men with a mean age of 70 years there was a significant negative correlation between BDI scores and bioavailable, but not total, T levels. Thus lower T levels were associated with higher depression scores. Two other epidemiological studies have found nonlinear relationships between T and depressive affect. Kratzik et al. (2007) found that risk of depression, as assessed by the BDI, was significantly associated with bioavailable T levels only in hypogonadal and hypergonadal men. Interestingly, the researchers only found this relationship with underweight or obese men. In men of normal weight, T levels were negatively associated with depressive affect. Booth, Johnson, and Granger (1999) found that in a sample of Vietnam veterans with a mean age of 38, serum T levels above and below 600 ng/dl were significantly associated with depression, as assessed by the Diagnostic Interview Schedule.

The evidence from individual differences studies does not reveal a very clear pattern of relationship between androgens and negative affect. As a result, a very limited

number of studies have begun to look at the association between current androgen levels, CAG repeat length, and depressive symptoms. Seidman, Araujo, Roose, and McKinlay (2001) studied 1000 men between the ages of 48 and 79 who were already participating in the Massachusetts Male Aging Study described above. They assessed total T levels through blood samples, CAG repeat status, and self-report ratings on the CES-D and found that low total T levels together with shorter CAG repeat lengths were significantly associated with an enhanced risk of depression. Neither variable alone was significantly associated with CES-D defined depression. In addition, moderate and longer CAG repeat lengths were not associated with total T or risk of depression. Another investigation studied 236 healthy elderly men between the ages of 70 and 85 over a period of three years and found that neither serum free T levels nor CAG repeat length were significantly associated with scores on the Geriatric Depression Scale at either baseline or at assessment three years later. They did, however, find that Geriatric Depression Scale scores were significantly and positively correlated with dehydroepiandrosterone sulphate levels at baseline but not at time 2 testing (T'Sjoen et al., 2005). In a sample of 525 black and 721 Caucasian males below the age of 40 who were already participating in the Coronary Artery Risk Development in (Young) Adults Male Hormone Study, Colangelo et al. (2007) found that the interaction between total serum T levels and CAG repeat length in black men, white men, and in both groups combined was significantly associated with CES-D rated depressive symptoms, defined for their purposes by a score on the CES-D of ≥ 16. The interaction between bioavailable T and CAG repeat length was, however, only significantly associated with depressive affect in young black men, such that black men who had the shortest CAG repeat lengths and bioavailable T levels

that fell in the top 75% had a significantly lower risk for depressive symptoms. Finally, Geng et al. (2007) investigated the relationship between polymorphisms in the AR gene and the estrogen receptor gene and major depression in a sample of adolescent females. They found that young women with major depression had significantly shorter CAG repeat lengths in the AR compared to controls. These results should, however, be interpreted cautiously given the X-linkage of the AR gene and its repercussions for accurately characterizing ARs located inside the female central nervous system.

Based on the studies reviewed above, there is some evidence to suggest that T therapy in eugonadal, depressed men may be beneficial, but that exogenous administration of T in normal, healthy individuals may not have a modifying effect on mood. Some studies suggest that CAG repeat length might be associated with risk for depression. In spite of these observations, no conclusions about the relationship between androgens and negative affect can be made because the literature on the topic is not uniformly pointed in one direction. As was the case with the androgen and visual-spatial data, the inconsistencies in the androgen and depression research may be explained by a number of reasons. Many of the studies used blood sampling to determine T levels which, as outlined earlier, is an imprecise measure of the T that is biologically active in the body. While a few studies have looked at bioavailable T levels, the method of determining bioavailable T concentrations from blood sampling is still subject to imprecision as it is not measured directly but is only calculated from an equation involving total serum T concentrations and serum sex hormone binding globulin, and is an estimate at best. Another important issue to consider is that different studies employ different measures of depressive affect, each of which may differ in their methods of

administration (e.g. self-report vs. interview) and their diagnostic cut-points. Consequently, this makes it difficult to compare findings between studies.

Inconsistencies in the findings could also be explained by the fact that most of the studies have been conducted with older aged (i.e. $\geq$ 50 years) males, at the expense of studies with younger male samples. More focused investigation on a young adult male population, who are more likely to show a full range of androgen levels and are less likely to have age-related illness or be on medication which could alter androgen levels or act as additional sources of negative mood, could be helpful in trying to understand the true natural relationship between androgens and depressive affect. Finally, the research base may lack clarity because investigators have focused too heavily on the impact of androgen levels on diagnosable clinical depression, instead of also looking at negative mood states that do not meet the threshold for depression. It has been speculated by several reviewers of the depression literature that there may be an association between mild mood syndrome and low androgen levels (Seidman & Roose, 2000; Korenman, 2000). In fact, a study of HIV-positive men with depressive symptomatology who received exogenous sources of T, found that participants who had low mood at the baseline but did not meet the criteria for major depression or dysthymia still experienced significant decreases in their HAM-D scores over the study period (Rabkin, Rabkin, & Wagner, 1995). As a result, there could be a robust and potentially clearer association between androgens and milder mood syndrome; however, it has yet to be systematically investigated.

The purpose of the present study is to investigate whether individual differences in bioavailable T levels, and in CAG repeat length, are significantly associated with performance on tasks of SO and SV, or are associated with levels of negative affect in a sample of young, healthy, university-aged males. Considering the controversy in the literature surrounding the existence and direction of the relationship between androgens and visual-spatial cognition and the fact that the length of the CAG polymorphism in the AR gene influences the function of the AR, it would be important to investigate whether taking into account CAG repeat length along with bioavailable T levels is more predictive of performance on tests of spatial ability than either variable alone. Because the CAG repeat sequence shows substantial variability in length from one person to another, it may help to explain some of the inconsistency in the spatial literature. With regard to negative affect, investigating the associations between CAG repeat length and bioavailable T levels over a wider spectrum of mood states, including negative mood states that span the normal range and do not meet the criteria for clinical depression, could enable us to more clearly determine the nature of the relationship between CAG repeat length, T, and mood. By studying a population who is not selected on the basis of having depression, and thus is not on anti-depressant medication which can artificially alter androgen levels, the potential to detect associations between T and mood may be enhanced.

Based on the information reviewed above, it is hypothesized that visual-spatial ability will be influenced by both T levels and CAG repeat length and that levels of T in combination with CAG repeat length will predict levels of negative affect within a non-depressed population.

CHAPTER 2

METHODS

Participants

Participants were 106 male volunteers between the ages of 17 and 30 years from the University of Western Ontario (M = 18.77 yrs; SD = 1.69; range = 17-27 yrs). This age range was selected because T levels in males are at their highest concentrations across the life-span between these ages (Leifke et al., 2000). The group was of average intelligence, as estimated from the North American Adult Reading Test (NAART) (M = 106.13; SD = 6.72; range = 86-119). Only males were recruited because of the X-linked nature of the AR gene. A reimbursement was provided for their participation; during the summer through monetary compensation and in the fall-winter academic session through undergraduate course credits.

Procedure

Participants were tested individually at scheduled times between 1300 hrs and 1900 hrs. Testing was restricted to these hours in order to control for circadian variations in levels of bioavailable T, which are at their most stable in the afternoon and early evening (Gupta, Lindemulder, & Sathyan, 2000). Each test session was approximately 1.5 hrs. Participants were asked to provide three saliva samples; two at the beginning of the test session and one at the end. The first sample was used to collect DNA in order to determine each participant's AR genotype (i.e. CAG repeat length). The second sample, along with the third taken approximately 1.25 hrs later, were used to determine average T for each participant. Details of the specimen collection and analysis are described below.

A fixed order of testing was followed for all participants, all of whom were tested by the same experimenter. During the saliva collection at the beginning of the session participants completed a hand-preference inventory and the Positive and Negative Affect Schedule (Watson, Clark, & Tellegen, 1988) (not reported here). Following saliva collection, participants completed a test of word recognition. This was followed by a series of paper-and-pencil tests measuring spatial reasoning. Then participants' two hands were photocopied for calculation of the 2D:4D ratio (not reported here). Following this, two verbal fluency tasks were administered. The session ended with the collection of the third saliva sample, during which participants completed two standardized clinical screening scales to assess depressive symptoms. Finally participants completed a questionnaire pertaining to their current state of health, neurological history, dietary choices, and demographic characteristics that might affect either testosterone levels or cognitive performance.

Cognitive Tests

Participants completed a set of paper-and-pencil cognitive tests. Four tests assessed spatial reasoning and were chosen to represent the two major subtypes of spatial ability known as spatial visualization and spatial orientation (Ekstrom et al., 1976). Tests assessing both subtypes were used because T might conceivably be found to correlate more strongly with one subtype than the other. Thus two of the four tests were tests of spatial visualization and the other two were tests of spatial orientation. Two cognitive control tasks, tests of verbal fluency, were administered to participants to demonstrate the selectivity of any association observed between T levels and the spatial scores. In other studies, T either has failed to correlate with verbal fluency (Moffat & Hampson, 1996;

Yonker et al., 2006) or has even shown a reverse relationship relative to correlations seen for spatial tasks (Van Goozen et al., 1994; O'Connor et al., 2001).

Spatial Reasoning:

The Mental Rotations Test (Vandenberg & Kuse, 1978) was used to assess spatial orientation (Figure 2.1, top). The test consisted of two parts with four minutes allowed for each part; each part had 12 items. Each item consisted of a target image of a three-dimensional object made of cubes. Participants were asked to select the two correctly rotated images from four possibilities. The test was scored according to the criteria recommended by Vandenberg and Kuse (1978), which includes a correction for guessing. The maximum possible score was 48.

The Cube Comparisons Test (Ekstrom et al., 1976) was the second test used to assess spatial orientation (Figure 2.1, bottom). It consisted of two parts, each with 21 items. Three minutes were allowed to complete each part. This test measured participants' abilities to mentally rotate a line drawing of a three-dimensional cube which had different symbols (e.g. alphabetic letters, geometric shapes) on each of its six sides. Participants had to decide whether the two cubes shown in each of the 21 items represented the same cube or different cubes. The score was the number correct, adjusted for guessing. The maximum possible score was 42.

The Paper Folding Test (Ekstrom et al., 1976) assessed spatial visualization (Figure 2.2, top). It consisted of two parts, each with 10 items. Three minutes were allowed to complete each part. Each item showed a paper being folded before a hole was punched through it. Participants had to select one of the five alternatives that represented

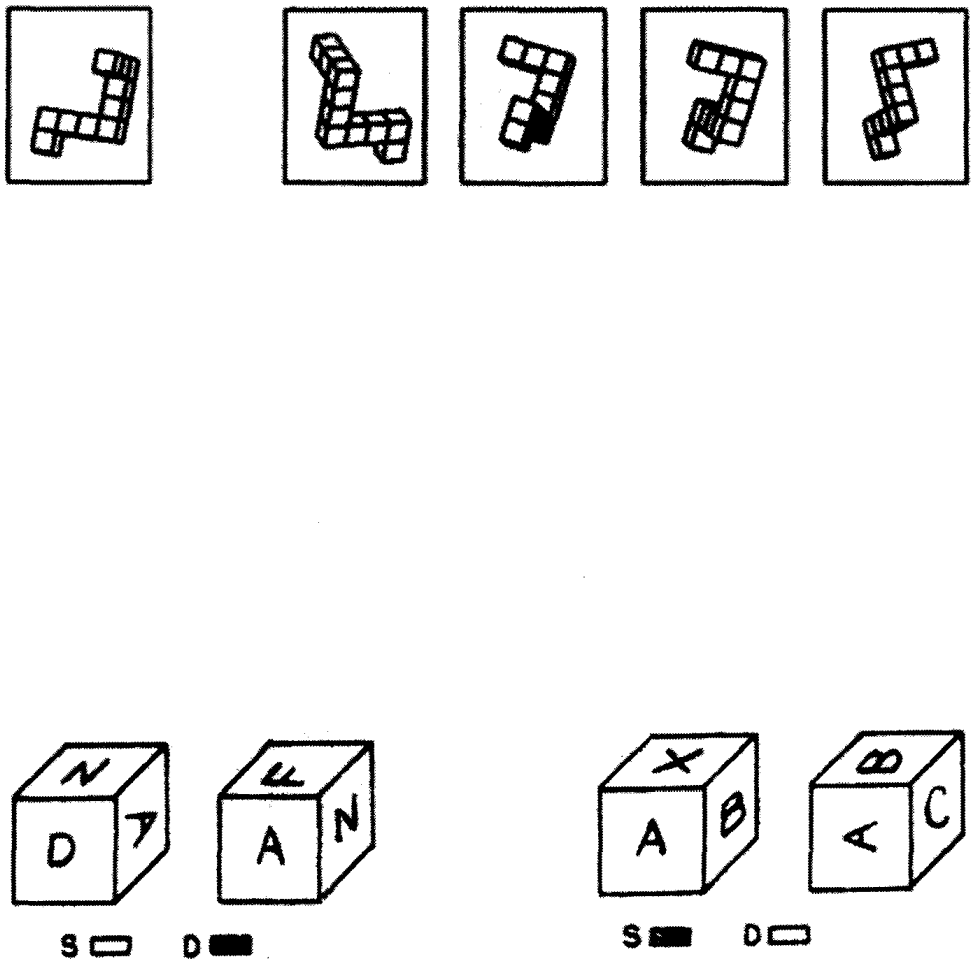
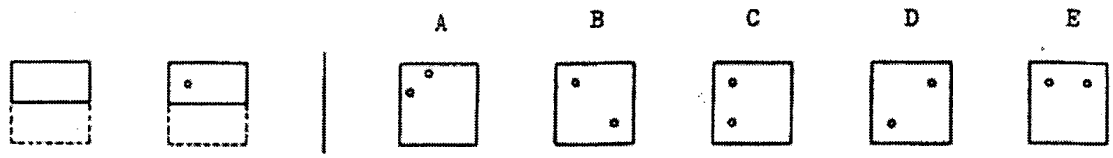


Figure 2.1. Example test items from the Mental Rotations Test (Vandenberg & Kuse, 1978) (top panel) and the Cube Comparisons Test (Ekstrom et al., 1976) (bottom panel).



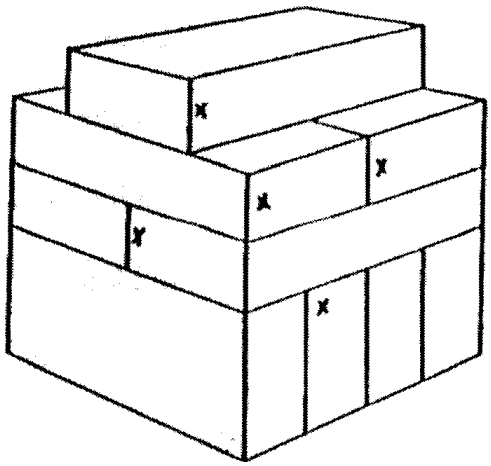


Figure 2.2. Example test items from the Paper Folding Test (Ekstrom et al., 1976) (top panel) and the MacQuarrie Blocks Test (MacQuarrie, 1953) (bottom panel).

the position of the holes in the paper after it had been unfolded. The score was the number correct, adjusted for guessing. The maximum possible score was 20.

The MacQuarrie Blocks test (MacQuarrie, 1953) was a second test of spatial visualization (Figure 2.2, bottom). It consisted of pictures of a pile of blocks, some of which were labelled with an "X". The task was to figure out how many blocks touched each block that had an X on it. Participants were given two and a half minutes to complete the test, which consisted of six piles of blocks. The score was the number correct; there was no adjustment for guessing. The maximum possible score was 30.

Verbal Fluency:

The Controlled Oral Word Association Test (COWAT) (Benton & Hamsher, 1976) asked participants to list aloud as many words as they could think of in one minute that started with each of three specified letters of the alphabet. The experimenter recorded the responses. The score for each participant was the number of correct words generated, summed over all three letters.

The Rhyme Fluency Test (Maki, Rich, & Rosenbaum, 2002) asked participants to list aloud as many words as they could think of that rhymed with a specified target word. One target word was used on each trial. A total of three trials were completed, with participants given 30 seconds to complete each of the first two trials and one minute to complete the third trial. The first two trials consisted of target words that were difficult to rhyme (e.g. lease), while the third trial consisted of a target word that was easy to rhyme (e.g. best). The experimenter recorded the responses. The score was the number of rhyming words generated summed over all three trials.

Other Control Tasks:

Hand preference was assessed using a modified version of the *Crovitz-Zener Handedness Inventory* (Crovitz & Zener, 1962). This 18-item inventory had a five-point range of response for each item, where "1" indicated a strong right-hand preference and "5" indicated a strong left-hand preference for a particular activity. A right-handed (RH) participant was defined as one who scored less than or equal to 30 on the handedness inventory; a left-handed (LH) participant scored above 30. The LH category thus included both strongly left-handed subjects and individuals with a weak or ambidextrous hand preference. Left-handedness has been associated in prior studies with differences in spatial ability and with differences in the association between T levels and spatial ability in males (Harshman, Hampson, & Berenbaum, 1983; Moffat & Hampson, 1996).

General ability was estimated using the *North American Adult Reading Test* (NAART) (Blair & Spreen, 1989); a validated measure of general intelligence (Uttl, 2002; Crawford, 1992; Blair & Spreen, 1989). The NAART was administered in order to ensure that there were no intellectual differences between subgroups of participants (e.g. handedness groups) that could potentially influence scores on spatial reasoning tasks. The NAART is a reading test of irregularly spelled words which vary in their frequency of use in the English language. The test was comprised of 61 words that participants were asked to read aloud. The experimenter recorded errors on a separate response sheet. Participants' raw scores were converted to a Full Scale IQ equivalent using the standard procedure set out by Blair and Spreen (1989). Performance on the NAART was not expected to be influenced by T levels; previous studies have failed to find correlations

between hormone levels and performance on such tests of crystallized intelligence (O'Connor et al., 2001; Van Goozen et al., 1994).

Depressive Symptoms

Participants were asked to complete two questionnaires that inquired about depressive symptoms, the *Center for Epidemiologic Studies Depression Scale* (CES-D) (Radloff, 1977) and the *Patient Health Questionnaire 9* (PHQ-9) (Spitzer, Kroenke, & Williams, 1999). Both scales can be used to measure levels of depressive affect and have been proven reliable in general population samples (Radloff, 1977; Kroenke, Spitzer, & Williams, 2001). They provided measures of individuals' trait tendencies to have negative interpretations of events. The CES-D is a 20-item scale that asked participants to rate the extent to which they felt a particular way in the past week (e.g. "I felt sad") on a four-point scale ranging from "rarely" to "most of the time". Participants' responses were summed across the 20 items, which included four reverse-scored items. The PHQ-9 is a nine-item scale that asked participants to rate the extent to which they had been bothered by problems over the last two weeks (e.g. "Feeling tired or having little energy") on a four-point scale ranging from "not at all" to "nearly every day". Responses to the nine test items were summed to yield a total score.

Saliva Collection

In order to ensure the purity of the saliva samples, participants were asked to avoid eating, drinking fluids other than water, smoking, chewing gum, or brushing their teeth for 30 min prior to testing. Prior to the first saliva sample, participants rinsed their mouths with water. Participants were then asked to expel about 2 ml of saliva by spitting into a sterile plastic Oragene®·DNA vial which contained 2 ml of Oragene®·DNA

stabilizing solution in its cap. Once collected, the vial was securely capped allowing for the cap contents to be released into the saliva and the DNA within the sample to be immediately stabilized for long-term storage at room temperature or in low-temperature freezers. Saliva sampling is a non-invasive method of DNA collection that produces a higher DNA yield than mouthwash or buccal swab methods, produces better quality DNA than buccal swab samples, and is easier to purify than DNA collection through blood sampling (Rogers, Cole, Lan, Crossa, & Demerath, 2007). The vials were stored at room temperature (21°C) until the end of the study, then were analyzed by a research lab specializing in DNA analysis.

Collection of the two saliva samples for hormone determination followed the DNA collection. Participants chewed a piece of inert sugarless gum in order to stimulate saliva flow, then expelled the saliva that accumulated in the mouth into a polystyrene culture tube pre-treated with sodium azide. About 4 ml of whole saliva was collected. Saliva contains only that fraction of T that is not bound to sex hormone binding globulin and, therefore, closely approximates the bioavailable fraction of T that is available to tissue for metabolic purposes (Pardridge & Demers, 1991; Sannikka, Terho, Suominen, & Santi, 1983). Following collection, the tubes were stored at room temperature for 18-24 hrs to allow separation to occur. The tubes were then frozen at – 20 °C until analysis.

Radioimmunoassays

The radioimmunoassays were performed by a lab technician blind to the purpose of the study, who had extensive experience in salivary radioimmunoassay. A direct assay of T was performed using a commercial kit, the ^{125}I Coat-A-Count kit for total testosterone (Diagnostic Products, Los Angeles, CA, USA), modified for use with saliva

according to a standard lab protocol (Moffat & Hampson, 1996). The antiserum was highly specific for T, showing cross-reactivity with DHT of less than 5% and negligible cross-reactivity with other steroids. The intra-assay coefficient of variation was 6.6% and the sensitivity was 10 pg/ml.

All saliva samples were assayed in duplicate. For each participant, the obtained values from the saliva collected at the beginning and end of the test session were averaged in order to provide the most reliable estimate of T. Results below refer to this mean concentration, unless otherwise stated.

DNA Analysis

The saliva samples collected for the purpose of determining the size of each participant's CAG repeat polymorphism in the AR gene were sent for analysis to The Center for Applied Genomics at the Hospital for Sick Children in Toronto. In brief, 50 ng of DNA was extracted from the saliva samples, and the CAG repeat region of the AR gene was amplified using polymerase chain reaction (PCR) with one primer labeled with 6-FAM dye for visualization (5'-CTTTCCAGAATCTGTTCCAG-3'), and a second unlabeled primer (5'-GAAGGTTGCTGTTCCTCATC-3'). Amplified fragments were run through capillary electrophoresis and read using an ABI3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) in order to separate the PCR products by size. Quantification of the length of the CAG repeat polymorphism from each sample was accomplished using the software GeneMapper v. 3.5.

Statistical Analyses

Descriptive statistics were calculated for each variable. Pearson's product-moment correlation coefficients were used to investigate the relationship between

bioavailable T levels and spatial abilities, bioavailable T levels and depressive symptoms, CAG repeat length and spatial abilities, and CAG repeat length and depressive symptoms. In order to determine if CAG repeat length contributed to either performance on spatial tests or level of negative affect above and beyond what could be accounted for by T levels alone, linear regression analyses were performed. All analyses were evaluated with a criteria of significance at the $p = .05$ level. Spatial data were analysed for the group as a whole, and separately by handedness group because previous literature suggests that hand preference may moderate the relationship between T and spatial abilities. All analyses were performed using SPSS software for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA).

CHAPTER 3

RESULTS

Testosterone Concentrations

Total T concentrations for the entire sample (n = 106) ranged from 51.98 pg/ml to 196.10 pg/ml (M = 104.67, SD = 28.14). For the right-handed (RH) group, T concentrations had a M = 102.40 pg/ml and SD = 27.12, while for the left-handed (LH) group, T concentrations had a M = 108.13 pg/ml and SD = 29.64. The difference in mean T between the RH and LH groups was not significant, $t(104) = -1.03$, $p = .307$ (Table 3.1).

CAG Repeat Length

CAG repeat length was available only for 86 participants. This was because the DNA within the saliva samples of 20 participants was not of sufficient quantity and/or quality to be analysed. CAG repeat length, measured in length of base pairs (bp), for the entire sample (n = 86) ranged from 253 bp to 296 bp. The difference in repeat length between the RH (M = 280.33 bp, SD = 8.95) and LH (M = 284.22 bp, SD = 7.04) groups was statistically significant $t(84) = -2.09$, $p = .040$ (Table 3.1).

Pearson correlations between CAG repeat length and T levels were calculated, both for the group as a whole and separately for each handedness subgroup, in order to determine if there was any association between CAG repeat length and bioavailable T concentrations. Correlations were non-significant in all three cases and the rs ranged from .03 to .11.

Table 3.1. Mean Concentrations of Testosterone (SD) and Mean CAG Repeat Length (SD) as a Function of Handedness

	RH (n = 64)	LH (n = 42)
Testosterone (pg/ml)	102.40 (27.12)	108.13 (29.64)
CAG Repeat length (bp) [a]	280.33 (8.95)	284.22 (7.04)**

[a] n = 55 in RH group, n = 31 in LH group

*** $p < .01$, ** $p < .05$, * $p < .10$

Spatial Reasoning

One participant whose T concentration was more than three SDs above the mean was excluded from all analyses between T and spatial tests. This might have been caused by blood contamination of the saliva. For the Cube Comparisons Test, eight participants who apparently did not understand the instructions and who scored at chance (less than or equal to 3), were excluded from the analyses as their data were deemed to be invalid. Thus the sample size for all analyses involving Cube Comparisons was reduced to 98 subjects.

Handedness Differences in Spatial Reasoning

Differences between the performance of the two handedness groups on spatial tests and the two control verbal fluency tests were assessed. In addition to examining each spatial test individually, an *a priori* unweighted composite score was computed for the SV tests and the SO tests. Such aggregates are generally considered more appropriate measures of a broad construct and generally exhibit higher reliability than the individual component tests (Epstein, 1980). The SV composite consisted of the Paper Folding Test and the MacQuarrie Blocks Test, while the SO composite consisted of the Mental Rotations Test and the Cube Comparisons Test. Composites were computed for each participant by converting the raw scores on each test to standard scores and then averaging. Results showed that RH males performed significantly better than LH males on nearly all the spatial tests. The only exception was the Cube Comparisons Test, where the difference did not reach significance. With regard to the cognitive control measures, no significant differences were found between RH and LH males in verbal fluency performance.

Table 3.2 summarizes the handedness differences in spatial reasoning.

Simple Correlations Between T and Spatial Reasoning

Pearson correlations between bioavailable T concentrations and spatial performance were evaluated in the two handedness subgroups independently. Salivary T concentrations were significantly and positively correlated with performance on the Paper Folding Test in RH males, such that participants with higher T levels had significantly better performance on this test of SV, $r(58) = .28$, $p = .032$. Amongst LH males, the correlation between T levels and the SO composite approached significance, $r(36) = -.31$, $p = .060$, as did the correlation between T and Mental Rotation performance, $r(40) = -.30$, $p = .054$. It should be noted, however, that for the Mental Rotation Test, there was a large discrepancy in the LH group between the coefficients obtained using T concentrations from the first saliva sample and those obtained from the second saliva sample. This was the only instance where results from the two saliva samples were so discrepant. Specifically, the correlation between Mental Rotation and T levels from the first saliva sample was significant, $r(40) = -.38$, $p = .013$, while T levels from the second saliva sample showed only a weak non-significant correlation of $r(40) = -.19$, $p = .221$. Salivary T was not significantly correlated with performance on the verbal fluency tasks.

A closer inspection of the data showed that salivary T concentrations were correlated with spatial measures in a pattern that was dependent on the hand preference of the participants. Specifically, the majority of obtained correlation coefficients were positive in RH males and were negative in LH males. Using Fisher's Z transformation, it was found that the correlations seen in right-handers and left-handers significantly differed on Paper Folding ($z = 2.29$, $p = .022$) and on the SV composite as a whole

Table 3.2. Mean Performance (SD) on Cognitive Tests as a Function of Handedness

Measure	RH ($n = 64$)	LH ($n = 42$)
Paper Folding Test	11.85 (3.27)**	10.02 (4.08)
MacQuarrie Blocks Test	17.62 (4.26)**	15.74 (5.01)
SV Composite	.18 (.76)***	-.30 (.85)
Mental Rotations Test	29.03 (9.27)**	24.28 (9.75)
Cube Comparisons Test	20.78 (7.83)	18.47 (8.57)
SO Composite	.17 (.82)**	-.23 (.93)
Rhyme Fluency	15.69 (4.06)	15.49 (4.69)
COWAT	38.20 (9.89)	36.33 (9.20)

*** $p < .01$, ** $p < .05$, * $p < .10$

($z = 2.26, p = .024$), and approached significance for Mental Rotations ($z = 1.72, p = .085$).

Table 3.3 summarizes the correlations between T concentrations and cognitive performance in RH and LH males, and in the total sample.

Simple Correlations Between CAG Repeat Length and Spatial Reasoning

Correlations between CAG repeat length and spatial performance were also evaluated for the group as a whole and in the two handedness subgroups. All obtained correlation coefficients were negative. CAG repeat length was significantly and negatively correlated with Paper Folding in LH males, $r(29) = -.40, p = .027$ and in the group as a whole, $r(83) = -.25, p = .022$. There was also a significant negative correlation between the SV composite and CAG repeat length in left-handers, $r(29) = -.41, p = .023$ and in the group as a whole, $r(83) = -.26, p = .018$. No other correlations in either right- or left-handers reached significance. Overall, CAG repeat length was not significantly correlated with performance on the verbal control tasks, although the correlation between CAG and performance on the COWAT approached significance, $r(29) = -.35, p = .055$.

Fisher's *Z* transformation showed that the correlations between spatial performance and CAG repeat length did not significantly differ between the two handedness groups.

Table 3.4 summarizes the linear correlations between CAG repeat length and cognitive performance in RH and LH males, and for the total sample.

Table 3.3. Correlations Between Testosterone and Cognitive Tests in LH, RH, and Total Sample

Measure	RH (n = 64)	LH (n = 42)	Total Sample (n = 106)
	r	r	r
Paper Folding Test	.28**	-.19	-.01
MacQuarrie Blocks Test	.14	-.11	-.01
SV Composite	.21	-.26	-.02
Mental Rotations Test	.04	-.30*	-.17*
Cube Comparisons Test	.01	-.26	-.17*
SO Composite	-.04	-.31*	-.19*
Rhyme Fluency	.01	.22	.11
COWAT	.04	.12	.06

*** $p < .01$, ** $p < .05$, * $p < .10$

Table 3.4. Correlations Between CAG Repeat Length and Cognitive Tests in LH, RH, and Total Sample

Measure	RH ($n = 55$)	LH ($n = 31$)	Total Sample ($n = 86$)
	r	r	r
Paper Folding Test	-.21	-.40**	-.25**
MacQuarrie Blocks Test	-.05	-.29	-.16
SV Composite	-.13	-.41**	-.26**
Mental Rotations Test	.13	-.19	-.00
Cube Comparisons Test	-.12	-.07	-.12
SO Composite	-.00	-.14	-.08
Rhyme Fluency	-.13	-.19	-.15
COWAT	-.02	-.35*	-.13

*** $p < .01$, ** $p < .05$, * $p < .10$

Multiple Regression of T and CAG Repeat Length on Spatial Reasoning

Table 3.5 summarizes the results of linear regressions of T and CAG repeat length on spatial reasoning in RH males. The data for LH males are shown in Table 3.6.

Multiple regression was used to determine whether CAG repeat length contributed to the variance in performance on tests of spatial ability beyond what was accounted for by bioavailable T concentrations. A forced entry regression model was applied, with T levels and CAG repeat length acting as predictors in the regression equation and performance on the spatial tests acting as the criterion variable. Among RH males, performance on the Paper Folding Test was significantly predicted by T concentration and CAG repeat length, $F(2,49) = 3.90$, $p = .027$. Higher T levels, $t(49) = 2.02$, $p = .049$, and shorter CAG repeat lengths, $t(49) = -1.85$, $p = .070$, both predicted better performance on Paper Folding. Together the two predictors accounted for approximately 14% of the variance. None of the other multiple correlations reached significance in the RH group. It is important to note, however, that the reduction in the sample size due to the missing CAG data may have influenced the ability of some of the multiple *R*s to reach significance, in particular the multiple *R* for the SV composite, $F(2,50) = 1.88$, $p = .164$.

In LH males, prediction of performance on Paper Folding by T levels and CAG repeat length approached significance, $F(2,28) = 3.06$, $p = .063$. The standardized regression coefficient for CAG repeat length, but not T, was significant, $t(28) = -2.21$, $p = .036$. Shorter CAG repeat lengths predicted better scores on Paper Folding. Together the two predictors accounted for 18% of the variance. The multiple *R* also approached significance for the SV composite, $F(2,27) = 3.15$, $p = .059$. Again, CAG repeat length

Table 3.5. Linear Regressions of T Concentrations and CAG Repeat Length on Spatial Tests for RH males

Measure	*R*	Beta Coefficient	*t*	*p*
Paper Folding				
T	.37	.27	2.02	.049**
CAG		-.25	-1.85	.070*
MacQuarrie				
T	.15	.13	.95	.344
CAG		-.07	-.49	.626
SV Composite				
T	.26	.20	1.49	.142
CAG		-.16	-1.18	.244
Mental Rotations				
T	.11	.05	.35	.728
CAG		.10	.75	.458
Cube Comparisons				
T	.18	.00	-.00	.996
CAG		-.18	-1.24	.222
SO Composite				
T	.06	-.05	-.33	.745
CAG		-.04	-.31	.760

*** $p < .01$, ** $p < .05$, * $p < .10$

Table 3.6. Linear Regressions of T Concentrations and CAG Repeat Length on Spatial Tests for LH males

Measure	*R*	Beta Coefficient	*t*	*p*
Paper Folding				
T	.42	-.15	-.86	.396
CAG		-.38	-2.21	.036**
MacQuarrie				
T	.30	-.08	-.46	.649
CAG		-.28	-1.55	.132
SV Composite				
T	.44	-.20	-1.14	.266
CAG		-.36	-2.02	.053*
Mental Rotations				
T	.34	-.28	-1.58	.125
CAG		-.16	-.87	.391
Cube Comparisons				
T	.27	-.26	-1.38	.179
CAG		-.07	-.38	.705
SO Composite				
T	.34	-.31	-1.66	.109
CAG		-.14	-.73	.472

*** $p < .01$, ** $p < .05$, * $p < .10$

contributed more to the prediction of SV composite scores than did levels of T as only CAG repeat length, but not T, was marginally significant, $t(27) = -2.02, p = .053$. Together the two predictors accounted for approximately 19% of the variance in the SV composite scores. As was the case for the RH group, the reduced sample size due to the missing CAG repeat data and the fact that we had a much smaller LH population may have influenced the ability of the multiple *R*s and by extension the standardized regression coefficients to reach significance.

Depressive Affect

PHQ-9 and CES-D data were analysed for the group as a whole. Analyses were not conducted separately for each handedness group because there was no theoretical or empirical reason to believe that hand preference influences mood states.

In order to investigate whether T or CAG length might differ in males with depressive *versus* normal affect, males were classified into depressive subgroups by using established clinical cut-points for the total scores obtained on the PHQ-9 and CES-D. In the case of the PHQ-9, a score of 0-4 corresponded to normal levels of depressive symptoms, a score of 5-9 corresponded to mild levels of depressive symptoms, and a score of 10 or higher corresponded to probable depression (Kroenke et al., 2001). With regard to the CES-D, a score of 0-15 corresponded to normal levels of depressive symptoms, a score of 16- 22 corresponded to mild levels of depressive symptoms, and a score of 23 or higher corresponded to probable depression (Radloff, 1977).

Because the construct of depression is made up of different symptom components (e.g. affective, somatic), most scales that assess levels of depressive symptomatology include questions that address these different components (Radloff, 1977). It is

conceivable that these components might relate differentially to T levels. Therefore, participants' responses to each of the individual items on the PHQ-9 and the CES-D were entered into a principal components analysis with varimax rotation. Four factors were extracted, based on the fact that the variance extracted by each of these factors, or their eigenvalues, was greater than 1. Based on the pattern of item loadings, these factors were identified as negative affect, social/evaluative, somatic/lethargic, and positive affect (Table 3.7). The four factors found in this study were the same as those that have been found in other factor analytic studies of the CES-D (Radloff, 1977; Rhee et al., 1999; Sheehan, Fifield, Reisine, & Tennen, 1995).

Simple Correlations Between T and Depressive Symptoms

Pearson correlations between T concentrations and total scores on the PHQ-9 and the CES-D were negative, approaching significance only for the PHQ-9, $r(103) = -.18$, $p = .060$. Pearson correlations between T concentrations and factor scores on each of the four components also were evaluated. These showed that higher T levels were significantly correlated with lower ratings on questions that corresponded to the somatic/lethargic symptoms of depression, $r(104) = -.22$, $p = .025$.

Table 3.8 summarizes the simple correlations between T and depressive symptoms for the total sample.

Simple Correlations Between CAG Repeat Length and Depressive Symptoms

Pearson correlations between CAG repeat length and total scores on the PHQ-9 and the CES-D respectively were evaluated for the total sample. Both correlations were low and neither correlation reached significance.

Table 3.7. Factor Loadings for Individual Items on the CES-D and PHQ-9

Item Number	Negative Affect	Social/ Evaluative	Somatic/ Lethargic	Positive Affect
PHQ-9 1	.32	.08	.28	.25
PHQ-9 2	.70	.19	.23	.18
PHQ-9 3	.14	-.07	.74	.10
PHQ-9 4	.31	.20	.58	.10
PHQ-9 5	.57	.17	.36	.04
PHQ-9 6	.72	.17	.12	.11
PHQ-9 7	.07	.22	.55	.45
PHQ-9 8	.16	.70	.26	.11
PHQ-9 9	.58	.57	-.05	-.06
CES-D 1	.15	.43	.31	.23
CES-D 2	.37	.50	-.02	.00
CES-D 3	.73	.24	.21	.23
CES-D 4	.36	.07	.21	.36
CES-D 5	.08	.28	.39	.48
CES-D 6	.71	.27	.07	.23
CES-D 7	.36	.28	.39	-.21
CES-D 8	.00	-.04	.01	.82
CES-D 9	.61	.50	.00	.00
CES-D 10	.36	.43	.06	.14
CES-D 11	.01	.21	.77	-.06
CES-D 12	.45	.22	.02	.66
CES-D 13	.30	.46	.24	.20
CES-D 14	.35	.59	.17	.19
CES-D 15	-.03	.82	.16	.09
CES-D 16	.46	.20	.15	.63
CES-D 17	.59	.08	.08	.08
CES-D 18	.73	.22	.16	.16
CES-D 19	.32	.74	.14	.06
CES-D 20	.16	.19	.47	.34

Table 3.8. Correlations Between Testosterone and Depressive Symptom Scores (n = 106) and CAG Repeat Length and Depressive Symptom Scores (n = 86) for Total Sample

Symptom Score	T (pg/ml)	CAG Length (bp)
	r	r
PHQ-9 Total	-.18*	-.10
CES-D Total	-.16	-.01
Negative Affect Factor	-.11	.02
Social/Evaluative Factor	.04	.08
Somatic/Lethargic Factor	-.22**	-.22**
Positive Affect Factor	-.15	.06

*** $p < .01$, ** $p < .05$, * $p < .10$

Scores on the somatic/lethargic factor were significantly and negatively correlated with CAG repeat length, $r(84) = -.22$, $p = .041$. Shorter CAG repeat lengths were associated with higher ratings on questions that corresponded to the somatic/lethargic symptoms of depression. None of the correlations between CAG length and the other depressive components were significant.

Table 3.8 summarizes the linear correlations between CAG repeat length and depressive symptoms for the total sample.

Depressive Subgroup Differences in T and CAG Repeat Length

Comparisons of mean T levels across the normal, mild, and depressed subgroups for each of the two depression scales were conducted using one-way analyses of variance. Results showed that the different subgroups on the PHQ-9 did not differ significantly from each other with respect to T concentrations. Similarly, mean T concentrations did not significantly differ between CES-D subgroups. If anything, mean T levels for both the PHQ-9 and the CES-D were lowest for the subgroup that had mild levels of depressive symptoms.

Comparisons of mean CAG repeat length across the subgroups were also conducted for each of the depression scales. The different subgroups on either the PHQ-9 or the CES-D did not differ significantly from each other with respect to CAG repeat length.

Table 3.9 summarizes the subgroup differences in T and CAG repeat length.

Multiple Regression of T and CAG Repeat Length on Depressive Symptoms

Table 3.10 summarizes the linear regressions of T and CAG repeat length on Depressive symptom scores.

Table 3.9. Mean Concentrations of Testosterone (SD) and Mean CAG Repeat Length (SD) as a Function of Level of Depressive Symptoms

Measure	T (pg/ml)	CAG Length (bp)
PHQ-9		
Normal ($n = 41$)	110.46 (32.00)	282.54 (7.69)
Mild ($n = 43$)	99.31 (21.58)	281.35 (9.70)
Depressed ($n = 21$)	101.20 (27.80)	280.61 (7.79)
CES-D		
Normal ($n = 74$)	105.83 (26.73)	281.58 (8.94)
Mild ($n = 19$)	95.24 (26.35)	282.31 (8.26)
Depressed ($n = 12$)	106.96 (33.48)	281.10 (6.77)

$^{***}p < .01$, $^{**}p < .05$, $^{*}p < .10$

Table 3.10. Linear Regressions of T Concentrations and CAG Repeat Length on Depressive Symptom Scores

Symptom Score	*R*	Beta Coefficient	*t*	*p*
PHQ-9 Total				
T	.20	-.18	-1.66	.100
CAG		-.09	-.84	.405
CES-D Total				
T	.16	-.16	-1.46	.149
CAG		-.00	-.05	.961
Negative Affect				
T	.12	-.11	-1.03	.305
CAG		.03	.24	.811
Social/Evaluative				
T	.09	.04	.38	.706
CAG		.07	.67	.505
Somatic/Lethargic				
T	.30	-.21	-1.97	.052*
CAG		-.21	-2.01	.048**
Positive Affect				
T	.17	-.16	-1.45	.151
CAG		.07	.62	.536

*** $p < .01$, ** $p < .05$, * $p < .10$

Multiple linear regressions were computed for the overall scores on the PHQ-9 and the CES-D and for each of the depressive symptom component scores to determine if CAG repeat length contributed to the variance in depressive symptoms. A forced entry regression model was applied, with T concentrations and CAG repeat length acting as predictors in the regression equation and depressive symptom scores acting as the criterion variable. Scores on the PHQ-9 and CES-D were not significantly predicted by T levels or CAG repeat length. With regard to the depressive component scores, results showed that responses to questions loading on the somatic/lethargic factor were significantly predicted by T concentration and CAG repeat length, $F(2,83) = 4.17$, $p = .019$. Lower T levels, $t(83) = -1.97$, $p = .052$, and shorter CAG repeat lengths, $t(83) = -2.01$, $p = .048$, both predicted higher ratings on questions that pertained to the somatic/lethargic symptoms of depression, and the standardized regression coefficients were equal in size for the two predictors. Together they accounted for approximately 9% of the variance in the somatic/lethargic factor scores. Scores on the other factors were not significantly predicted by either T or CAG repeat length.

CHAPTER 4

DISCUSSION

In a sample of 106 university-aged males, we found that CAG repeat length and bioavailable T concentrations significantly contributed to the prediction of performance on certain measures of spatial ability. Some of the associations differed depending on the hand preference of participants. Results also showed that mean spatial performance and CAG repeat length significantly differed between handedness groups. Within the depression data, regression analyses found that T levels and CAG repeat length accounted for a significant amount of the variance in the endorsement of certain types of depressive symptoms.

Visual-Spatial Cognition

Our results revealed differences in spatial ability as a function of handedness. Specifically, RH males performed significantly better than LH males on the majority of spatial tests. This finding is consistent with a number of studies that have looked at the association between spatial ability and handedness (e.g. Snyder & Harris, 1993; Gordon & Kravetz, 1991; McKeever, 1986; Harshman et al., 1983; Sherman, 1979; Yen, 1975; Levy, 1969), although the reverse pattern has been seen in some studies (e.g. Sanders, Wilson, & Vandenberg, 1982; Kocel, 1977; McGee, 1976). Harshman et al. (1983) suggested that these disparate findings could be reconciled by taking into consideration the sex and overall intellectual level of participants, and found support for that hypothesis in several large datasets. In high-functioning populations, like the current sample which was made up entirely of university students, RH males have typically been found to have better spatial abilities than LH males, whereas the opposite seems to be true in lower

functioning populations. This suggests that intellectual ability may be an important moderator variable. Our finding of better spatial performance in RH than LH males fits with the overall pattern of results found in the literature. In addition, the present study found no differences in performance on verbal fluency tasks between handedness groups. This is again consistent with the findings from other cognitively-stratified samples (Harshman et al., 1983; Moffat & Hampson, 1996).

The differences in spatial ability between handedness groups may be due to neurological factors. It has been well-documented that right-handers and left-handers differ in the functional organization of their brains (Hécaen, DeAgostini, & Monzon-Montes, 1981; Rasmussen & Milner, 1977). For example, 95-98% of RH individuals have their speech functions localized to the left hemisphere, while only 70% of LH individuals show this language asymmetry (Rasmussen & Milner, 1977). Consequently, if there are differences in the cerebral lateralization of language between right- and left-handers, then there might also be differences in the organization of the brain regions involved in visual-spatial processing. There is some evidence to suggest that this might be the case (Hécaen et al., 1981; Levy, 1969) and that these differences may have consequences for spatial ability. A social or environmental explanation of the findings can also be employed, albeit less convincingly. Such an explanation would suggest that right- and left-handers differ in the types of cognitive experiences they are exposed to and by extension perform differently on tasks of spatial ability. While there is no doubt that there are individual differences in cognitive experience, to say that right- and left-handers systematically differ in the experiences that are key to spatial performance is far more suspect.

A number of recent studies have looked at the associations between adult T levels and spatial skills (Yonker et al., 2006; O'Connor et al., 2000; Janowsky et al., 1994; Christiansen & Knussmann, 1987). Though correlations between T and spatial performance have been found, the direction of the correlation has been inconsistent across studies. In the present data, the direction of the correlations varied between handedness groups. The correlations we observed in the present sample were predominantly positive for RH males, with performance on Paper Folding being significantly correlated with T. For LH males, the correlations were negative with performance on the Mental Rotations Test and the SO composite closely approaching significance ($p \leq .060$). The correlation coefficients significantly differed between handedness groups for measures of SV (Paper Folding, the SV composite), and approached significance for the Mental Rotations Test.

To our knowledge, only one other study has investigated the relationship between T and spatial abilities as a function of handedness. Moffat and Hampson (1996), sampled bioavailable T and used two of the same tasks used in our study, Paper Folding and the Mental Rotations Test. As in the present study, Moffat and Hampson (1996) found that the correlations between T and spatial ability varied depending on hand preference. However, right-handers in the Moffat and Hampson (1996) study produced negative correlations between T and spatial ability, whereas the present study found a positive correlation on the Paper Folding Test. There is no clear reason as to why our findings differed from those of Moffat and Hampson (1996); however, there are a few potential explanations. One difference between the two studies is that we did not deliberately recruit left-handers for our study whereas Moffat and Hampson (1996) did. This may

have caused the males in the Moffat and Hampson (1996) study to have been more strongly LH than those in our study, but this explanation does not adequately account for the disparate RH data. It is possible that there might be some unknown variable that influences the relationship between T and spatial ability which ended up being sampled in systematically different ways in the two studies, thereby causing the conflicting findings. The fact that T sampling was done at different times of day in the two studies could also explain the different pattern of results seen in RH males. We tested subjects in the afternoon and early evening when T levels are low, whereas Moffat and Hampson (1996) tested subjects in the early morning when T levels are almost twice as high (Dabbs, 1990). If Moffat and Hampson's (1996) finding of an inverted-U shape relationship between spatial ability and T in right-handers is true, our results may fall on the same inverted-U shaped curve, but they might simply fall at the lower end of the curve where a positive association between T and spatial abilities is likely to be seen.

In spite of the divergent findings, the current investigation provides evidence that hand preference not only influences spatial ability independently, but also moderates the relationship between T and spatial performance. If the brains of RH and LH individuals are differently organized, as previously described, then it is possible that adult T may be acting on neural systems that are potentially different in right- and left-handers. As a result, the impact of T on spatial ability may differ between these two groups.

Investigation of the influence of the CAG polymorphism in the AR gene on spatial ability was a novel aspect to this study. To this end, we performed simple correlations between CAG repeat length and spatial ability within each handedness subgroup. CAG repeat length was found to be negatively correlated with spatial ability

in both right- and left-handers and the correlations reached significance for two of the spatial visualization measures, the Paper Folding Test and the SV composite, in LH males and in the group as a whole. Shorter CAG repeat lengths, and by extension greater AR activity, were associated with significantly better performance on measures of SV. Shorter stretches of CAG repeats are associated with increased AR transcription which in turn can modulate androgen action (Chamberlain et al., 1994). Finding a significant relationship between CAG repeats and spatial ability is revealing because it suggests that androgens are the basis for the observed association. This finding helps to substantiate the claim that there is a true relationship between T and spatial abilities. The conflicting nature of the results in the literature have called into question the veracity of this relationship, but our finding that CAG repeat length was significantly associated with performance on certain spatial tasks suggests that androgen action does influence spatial cognition in some sort of meaningful way.

A major limitation of our findings has to do with our sample size. We aimed to test and determine the CAG repeat length of over 100 participants, however, we did not anticipate being unable to analyse the DNA of 20 subjects. As a result, having such a substantially reduced sample may have influenced our ability to see statistically significant correlations between CAG repeat length and spatial performance. To go a step further, we had not accounted *a priori* for the fact that handedness might influence the relationship between these two variables, and by separating the analyses by hand group we further reduced our statistical power and the ability to see significant correlations in the data. For example, some of the correlation coefficients obtained between CAG repeat length and spatial ability within the two handedness groups were

the same size as correlations that reached significance in the T and spatial ability data. Another limitation, which is not specific to the CAG data but also extends to our findings between T and spatial ability, is that we did not expect such a large subset of our test population to be LH. The percentage of left-handers in the general population is between 10-13% (Connolly & Bishop, 1992; Gilbert & Wysocki, 1992). Since our sample was not selected for left-handedness, we would expect a similar percentage of left-handedness to be seen in our data. This was not the case, however, as our sample was comprised of approximately 40% left-handers. Having such a large number of left-handers meant that our RH sample was smaller than anticipated. Consequently, this might have reduced our ability to detect significant effects between T and spatial ability or CAG and spatial ability even within RH males.

Another finding relating to the length of the CAG sequence in this study was that CAG repeat length was significantly longer in LH males than RH males. This would suggest that the LH males in our sample had poorer AR function than RH males. These results are in contrast to those found by Medland et al. (2005), which suggested that longer CAG repeats were associated with reduced risk for left-handedness in males. It is possible that the reason for this difference was due to the fact that we used a much more thorough assessment of handedness, which differed in its criteria for determining left-handedness, than the one employed by Medland et al. (2005). Interestingly, our results are compatible with the hypothesis that lower levels of androgen action, prenatally, may contribute to the development of left-handedness (Witelson, 1991).

The main objective of this study was to determine whether CAG repeat length would contribute to the prediction of spatial ability independent of bioavailable T levels.

To this end, regression analyses were conducted in each handedness group independently. We found that for RH males, the multiple *R* was significant for Paper Folding, while for LH males, the multiple *R*s for Paper Folding and the SV composite closely approached significance ($p \leq .065$). In right-handers the standardized regression coefficients revealed that better Paper Folding performance was associated with higher levels of T and shorter CAG repeat lengths, while better SV performance in left-handers was significantly predicted only by shorter CAG repeat lengths. These results demonstrate that CAG repeat length is contributing to the prediction of spatial performance regardless of whether it works in concert with T to amplify androgenic effects on the body, as was seen in right-handers, or independently of T, as was seen in left-handers. By increasing the number of participants within each of the handedness groups we might improve our ability to see significant multiple *R*s for other spatial measures.

It is well known that T exerts many of its physiological effects after conversion to its metabolic derivatives estradiol or DHT. As a result, one might ask whether the observed relationships in this study are due to estradiol or DHT and not T. Based on our findings, it is unlikely that estradiol is driving this association. Most studies that have looked at the link between estradiol and spatial abilities have found a negative relationship between the two variables (Maki et al., 2002; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Güntürkün, 2000; Hampson, 1990). If estradiol is truly the critical hormone related to spatial cognition then we would expect to see that lower levels of T, which in turn would be metabolized into lower levels of estradiol, are associated with better spatial performance. This, however, was not the case, as we found a

significant positive association between T and spatial ability in RH males. In addition, the fact that we found significant associations between CAG repeat length and spatial performance suggests that androgens are the basis for the relationship, because only androgens and not estrogens can bind to the AR.

We cannot, however, rule out the possibility that DHT is driving the observed relationship. This is because DHT is an androgen and binds to the AR. Consequently, DHT could be the critical hormone mediating the relationship between CAG repeat length and spatial cognition. Also, a few studies have found that DHT is positively correlated with spatial ability in males (Christiansen & Knussmann, 1987; Christiansen, 1993), which parallels our findings in right-handers. By sampling T and DHT in the same sample of subjects, it should be possible for future studies to more accurately determine which hormone is driving the association or if both hormones are working together to produce the observed effects on visual-spatial cognition.

Depressive Affect

In order to assess the influence of T concentrations on depressive symptom scores, zero-order correlations were computed. Results showed that total scores on the PHQ-9 and the CES-D were not significantly correlated with T levels, although the correlation was negative and did approach significance in the case of the PHQ-9. Other studies have found significant negative associations between endogenous T levels and depressive affect in non-depressed, eugonadal men, albeit in elderly populations (Joshi et al., 2009; Morsink et al., 2007; Barrett-Connor et al., 1999). It is conceivable that with a larger sample we too would find a similar result.

A novel aspect to the present study was to look at the association between the underlying components (i.e. factors) of depression and T concentrations. We found a significant negative correlation between ratings of somatic/lethargic symptoms of depression and T concentrations, suggesting that lower T levels are associated with greater endorsement of the vegetative symptoms of depression. This finding somewhat parallels that of Rabkin, Wagner, and Rabkin (2000), who found that HIV-positive men with hypogonadal mood symptoms showed significant improvement, after T supplementation, only in the vegetative symptoms of depression, as assessed by the HAM-D. Taken together, this might mean that the effects of T on mood may be most evident in the somatic symptoms, at least within a population not selected for meeting the criteria for depression per se. Conversely, a study by Pope et al. (2003), which tested depressed men with low serum T levels, found that those randomized to receive T supplementation showed significant improvements in both the vegetative and affective symptoms of depression. This brings up the question of whether clinical depression and mildly depressive affect within a non-depressed population lie on the same continuum or whether they are separate entities. It might be the case that our findings differed from those of Pope et al. (2003) because T may have a different physiological mechanism of action on mood in clinically depressed individuals than in non-depressed individuals with negative mood states. The possibility still exists, however, that negative affect is also sensitive to T even in non-depressed populations. It might simply be that it is just less sensitive to T than the somatic symptoms, and that perhaps a larger change in T levels is needed before affective symptoms become apparent. In addition, it might be the case that changes in affective symptoms in the Rabkin et al. (2000) study were not detected even

though exogenous sources of T were administered to participants similar to the Pope et al. (2003) study, because changes in somatic symptoms may be more salient to HIV-positive patients than affective symptoms given the nature of their illness and the likelihood of having co-existing physical symptoms unrelated to mood.

Correlations were also computed between CAG repeat length and depressive symptom scores. A significant negative relationship between somatic/lethargic symptoms of depression and CAG repeat length was found. Shorter CAG repeat lengths, signifying higher AR activity, were associated with vegetative symptoms of depression. An association with the AR CAG polymorphism suggests that androgens are the basis for the observed relationship. It should be noted, however, that the direction of the association is in contrast to Härkönen et al. (2003) who showed that CAG repeat length was positively correlated with depressed mood and "wish to be dead"; symptoms which more closely relate to the negative affect component of depression. The difference in findings might be due to the fact that Härkönen et al. (2003) used the Aging Males' Symptoms Scale to assess depressive symptoms. This scale is not specific to depression, thus it is possible that it does not adequately tap into all of the factors underlying depression, including the somatic symptoms, making it difficult for associations between CAG repeat length and vegetative symptoms of depression to be seen. In addition, there is evidence that measures of depression for elderly populations tend to minimize questions about somatic and vegetative symptoms because they can overlap with symptoms of co-occurring medical illness in test takers (T'Sjoen et al., 2005).

Multiple regression showed that scores on the somatic/lethargic factor were significantly predicted by the combination of T concentrations and CAG length.

Specifically, lower levels of T and shorter CAG repeat lengths predicted higher ratings of the somatic/lethargic symptoms of depression on the two depression inventories. This would suggest that CAG repeat length is contributing something novel to prediction that cannot already be accounted for by knowledge of participants' T levels. Other studies that have looked at the influence of T concentrations and CAG polymorphism on depressive symptoms have not investigated the effect of these variables on particular symptoms of depression; therefore a direct comparison of the current findings to these studies cannot be made. However, Seidman et al. (2001) did find that T concentrations and CAG repeat length significantly predicted CES-D diagnosed depression in the same manner that T levels and CAG repeat length predicted somatic/lethargic factor scores in our data. That is, men who had both low total T levels and shorter CAG repeat lengths had a significantly greater likelihood of having a CES-D score greater than 16.

A final comment to be made about the depressive affect data has to do again with the issue of sample size. While the sample sizes for the depression analyses were larger than those for the spatial reasoning analyses, since there was no precedent to separate them by hand preference, the reduction caused by the missing CAG repeat data may still have influenced the ability to see significant findings, especially when performing the multiple regressions. A closer inspection of the multiple regression analyses revealed several instances where the standardized regression coefficients for T concentrations were in a range that approached significance. It is conceivable that by reaching our intended sample size some of these coefficients may become significant, making their contribution to the prediction of depressive affect something not to be overlooked. In addition, further investigation is merited on whether T concentration and CAG repeat

length differ depending on the classification of depressive affect as normal, mild, or depressed. Larger sample sizes may increase the number of subjects in each of the categories, enabling group differences to become evident.

Conclusion

This study was the first of its kind to investigate the contribution of T concentrations and CAG polymorphism in the AR gene to individual differences in visual-spatial cognition. To this end, we were able to find, even with constraints of sample size, that CAG repeat length provides novel information regarding the association between androgens and spatial abilities and that its contribution may vary as a function of hand preference. Consequently, the length of CAG repeat stretches should be taken into consideration in future studies. With regard to depressive affect, a novel aspect to this study was that it looked at the association between T levels and CAG repeat length and affective status in a young healthy population across the entire spectrum of depressive symptoms, including normal, mildly depressed, and depressed states. This analysis, too, showed that CAG repeat length could be an important variable in studies investigating the associations between androgens and negative affect.

While our findings are preliminary, they do suggest that androgens and their mechanisms of action are significant and noteworthy influences on complex behaviours such as visual-spatial cognition and mood. Performing a similar study, with a larger sample size will hopefully help to further elucidate these associations.

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APPENDIX A

ETHICS APPROVAL

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Use of Human Subjects - Ethics Approval Notice

Review Number	**08 06 02**	**Approval Date**	**08 06 04**
Principal Investigator	**Elizabeth Hampson/Janani Sankar**	**End Date**	**09 04 30**
Protocol Title	**Associations between testosterone, thinking, and mood**		
Sponsor	**n/a**		

This is to notify you that The University of Western Ontario Department of Psychology Research Ethics Board (PREB) has granted expedited ethics approval to the above named research study on the date noted above.

The PREB is a sub-REB of The University of Western Ontario's Research Ethics Board for Non-Medical Research Involving Human Subjects (NMREB) which is organized and operates according to the Tri-Council Policy Statement and the applicable laws and regulations of Ontario. (See Office of Research Ethics web site: http://www.uwo.ca/research/ethics/)

This approval shall remain valid until end date noted above assuming timely and acceptable responses to the University's periodic requests for surveillance and monitoring information.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the PREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of research assistant, telephone number etc). Subjects must receive a copy of the information/consent documentation.

Investigators must promptly also report to the PREB:
a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to the PREB for approval.

Members of the PREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the PREB.

[redacted]

Clive Seligman Ph.D.

Chair, Psychology Expedited Research Ethics Board (PREB)

The other members of the 2007-2008 PREB are: Mike Atkinson, David Dozois, Bill Fisher and Matthew Maxwell-Smith

CC: UWO Office of Research Ethics

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