GONADAL STEROIDS AND COGNITIVE FUNCTIONING IN MIDDLE-TO-OLDER AGED MALES

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TABLE OF CONTENTS

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STATEMENT OF ORIGINALITY AND AUTHENTICITY

I declare that this thesis contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made.

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Donel M. Martin _____Date_____.

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APOE	Apoliproprotein E
BDI	Beck Depression Index
BIMC	Blessed Information Memory Concentration test
BT	Bioavailable testosterone
BMI	Body mass index
CAH	Congenital adrenal hyperplasia
cEFT	Calculated free testosterone levels
COMT	Catechol *O*-methyltransferase
CRT	Choice reaction time
DHEA-S	Dehydroepiandrosterone sulphate
DHT	Dihydrotestosterone
DLPFC	Dorsolateral pre-frontal cortex
DT	Decision time
E2	Estradiol
FAMAS	Florey Adelaide Male Ageing Study
FOME	Fuld Object Memory Evaluation
FSH	Follicle stimulating hormone
FT	Free testosterone
FTI	Free testosterone index
fMRI	Functional magnetic resonance imageing

Gc	Crystallised intelligence
GFI	Goodness of fit index
Gs	General processing speed
HPG	Hypothalamic-pituitary-gonadal axis
IHH	Idiopathic hypogonadotrophic hypogonadism
ISD	Intra-individual reaction time standard deviation
IT	Inspection time
LH	Luteinising hormone
MRT	Mental rotation test
MWT	Morris Water Task
MT	Movement time
PD	Parkinson's disease
PET	Positron emission tomography
OMO	Odd-Man-Out test
PFC	Prefrontal cortex
PMA	Primary Mental Abilities
rCBF	Regional cerebral blood flow
RT	Reaction time
SALT	Spatial Array Learning Tests
SART	Sustained Attention to Response Test
SD	Standard deviation
SEM	Structural equation modelling
SHBG	Sex hormone binding globulin
SOA	Stimulus onset asynchrony
SOPT	Self ordered pointing task

SRT	Simple reaction time
T	Testosterone
TT	Total testosterone
VE	Virtual environment
VMWT	Virtual Morris Water Task
Vsp	Visualisation speed
WM	Working memory
WMH	White matter hyperintensities
WPR	Worse performance rule

PAPERS ARISING FROM THIS THESIS

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GONADAL STEROIDS AND COGNITIVE FUNCTIONING IN MIDDLE-TO-OLDER AGED MALES

Summary

The basis for sex differences in cognitive ability remains poorly defined and controversial both scientifically and politically. One of the biological hypotheses on sex differences, of particular relevance to this thesis, concerns the role of gonadal steroids, specifically testosterone (T) and oestrogen, and their relationship to individual differences in the performance of specific cognitive tasks. In addition, the role that age-related changes in these hormones play in relation to generalised and pathological cognitive ageing in males is studied. It is important to determine whether decreases in T levels that occur with ageing in males are associated with age-related decreases in cognitive performance because T levels can potentially be modified.

Males have consistently been found to outperform females on measures of visuo-spatial function; performance on the Vandenberg and Kuse Mental Rotation Test (MRT) shows the largest and most robust of sex differences. Gonadal steroids have both organisational and activational effects which contribute to both within-sex variability and between-sex differences in visuo-spatial cognition. As males age, endogenous plasma T levels decline gradually yet variably between individuals. Studies in older males show improvement in visuo-spatial cognition following T supplementation; however, it remains to be resolved whether decreases in endogenous T levels with ageing are associated with poorer MRT performance.

Some recent studies in older males have reported positive correlations between measures of plasma T levels and cognitive functioning, including processing speed and executive function measures. These data are inconsistent,

however, and important questions remain concerning, for example: the age at which the effect is strongest; whether there are different effects at different ages; whether there is an optimal level at which T levels affect particular abilities; and which abilities show the strongest association with endogenous plasma T levels.

Increased intra-individual variability in performance on Choice Reaction Time (RT) tasks has recently been shown to be a strong predictor of cognitive functioning in university students. Methodological advances in the analyses of RT distributions has allowed for the calculation of robust estimates of intra-individual RT variability. The association between these estimates and cognitive performance in middle and older aged males, however, remains to be determined. Further, the association between endogenous plasma T levels and intra-individual RT variability in aged males is unknown.

The thesis addresses these issues; firstly, through cross-sectional analyses of the associations between different measures of plasma T levels, learning and memory, processing speed, and executive function performance in a large population based sample of 1046 men aged between 35 and 81 years. Secondly, further cross-sectional analyses are reported from a subsequent study in a healthy sub-sample of 96 of these men on the associations between endogenous plasma T levels, MRT performance, constituent abilities related to MRT performance, and performance on composite measures of both processing speed and executive function. In a third study, these data are re-analysed in relation to intra-individual variability in RT performance.

In light of the results of these studies, the role that age-related declines in plasma T levels play in relation to generalised age-related cognitive decline in males is discussed.

PREAMBLE

The purpose of the research detailed in this thesis was to address two primary aims; firstly, to investigate the association between gonadal steroid levels and visuo-spatial ability in adult males; and secondly, to determine whether the changes in gonadal steroid levels that occur with ageing in males were associated with declines in visuo-spatial ability or in ability measures indicative of generalised age-related cognitive decline, or both of them. In order to introduce the research on these two aims, I have provided two introductory chapters reviewing background literature on each aim. In Chapter One, a detailed literature review is provided on the role of gonadal steroids in the male advantage on visuo-spatial ability. Evidence is reviewed for both the organisational and activational effects of gonadal steroids and their contribution to both within-sex variability and between-sex differences in visuo-spatial cognition. Methodological problems associated with this research are outlined and tentative conclusions are drawn. In Chapter Two, I have provided both the background to and a review of recent studies which have supported the suggestion that changes in gonadal steroid levels that occur with ageing in males may be associated with generalised age-related cognitive decline. In this chapter, I have outlined both the changes that occur in gonadal steroid levels as males age and the normal age-related changes in cognitive function. In addition, two dominant theories of cognitive ageing are introduced, namely, processing speed theory, and the theory of prefrontal decline. Recent research in support of the hypothesis that declines in T levels with ageing in males may be associated with generalised age-related cognitive decline is reviewed. Both general and specific hypotheses pertaining to the two primary aims of this thesis are then presented, followed by a brief outline of the research studies constituting this thesis.

CHAPTER ONE: GONADAL STEROIDS AND VISUO-SPATIAL ABILITY IN MALES

1.1. Summary

The basis for sex differences in cognitive abilities remains poorly defined and controversial. Despite the controversy, considerable evidence for the biological bases of sex differences in cognition has accumulated over the last 30 years, facilitated by technological innovations, which has allowed a greater understanding of how our brains develop and function. One of the biological hypotheses on sex differences, of particular relevance to the present thesis, concerns the role of sex hormones, specifically T and oestrogen, and their relationship to individual differences in the performance of specific visuo-spatial tasks. In this chapter the dominant role of T in the male advantage in both visuo-spatial and navigational behaviour is reviewed. Discussion is limited to adult males; however, comparable non-human animal research is described where evidence from human studies is lacking. Evidence for both organisational and activational effects of endogenous and exogenous gonadal steroids on visuo-spatial ability in adult males is reviewed and methodological limitations discussed.

1.2. Sex Differences in Cognition

The two major domains of cognitive function identified as exhibiting reliable sex differences are verbal abilities and visuo-spatial abilities. Females have consistently been found to outperform males on tests of verbal ability and specifically on tests of verbal fluency, vocabulary, and verbal memory. However, it must be noted that the effect size[1] for these sex differences is generally classified as

[1] Conventionally, the effect size parameter (*d*) indicates the magnitude of a difference between group means in standard deviation units (Cohen, 1988).

small, that is, in the vicinity of $d = 0.2$ (Kimura, 1999). Hyde and Linn (1988), in their extensive meta-analytic review of the literature on sex differences in verbal abilities, classified experiments according to the type of verbal ability assessed (e.g., general verbal ability tests, vocabulary, tests of reading comprehension) and the ages of participants. The majority of the effect sizes they reported were small with the largest effect sizes for female advantages in quality of speech production ($d = 0.33$) and for general verbal ability ($d = 0.20$). Other abilities that females have generally been found to outperform males on are tests of perceptual speed and perceptual motor tasks, which either require rapid matching of stimuli or rapid, fine motor movements, or both of them (Halpern, 2000). Perceptual speed tasks (also known as clerical speed tasks) are usually pencil-and-paper tasks, assessed by the number of items correctly completed within a specified time limit (Salthouse, 2000). An example of one of these tests is Digit Symbol (also known as Coding) from the Wechsler scales (see below). The female advantage on this test has been attributed to superior fine motor ability (Polubinski & Melamed, 1986) but this has not been unequivocally established.

Males, on the other hand, have been found to consistently outperform females on tasks assessing "visuo-spatial" abilities. Carroll (1993) provided the following definition of this broad category of cognitive ability:

'spatial and other visual perceptual abilities have to do with individual's abilities in searching the visual field, apprehending the forms, shapes and positions of objects as visually perceived, forming mental representations of those forms, shapes and positions, and manipulating such representations "mentally"…'(p.304).

The breadth of this definition is illustrative of the heterogeneity of tasks measuring visuo-spatial abilities. It is for this reason that some researchers have reduced the domain to a number of qualitatively different abilities, for example, tasks which require spatial visualisation or spatial perception (Linn & Peterson, 1986; Carroll, 1993; Halpern, 2000). Nonetheless, these classifications remain very general and fail to capture the specific aspects of task performance which may underlie sex differences in performance. In an analysis of 286 studies, Voyer, Voyer, and Bryden (1995) adopted a test-by-test approach and derived an overall mean weighted sex difference favouring males, $d = 0.37$. The largest effect sizes were reported for the Vandenberg and Kuse (1978) Mental Rotation Test (MRT), the Rod and Frame test (Witkin, 1954), and Spatial Relations from the Primary Mental Abilities (PMA) battery (Thurston, 1938); $d = 0.67$, $d = 0.48$ and $d = 0.44$, respectively. Moreover, when the effect size of the male advantage on the Vandenberg and Kuse MRT was partitioned by experimental procedure, the size of the effect was found to be even larger when the test was scored out of 20 ($d = 0.94$) than when out of 40 ($d = 0.70$).

The Vandenberg and Kuse (1978) MRT, therefore, shows the largest and most robust of all sex differences in visuo-spatial abilities, which according to two large meta-analytic reviews is in the vicinity of 1 SD (Voyer et al., 1995; Linn & Peterson, 1986). Accordingly, it appears that it is the cognitive processes required by this task that best represent the locus of sex-differences in visuo-spatial cognition. This task is examined in detail in the course of this thesis (see Chapter 5).

Recent studies using virtual environments (VEs), tasks thought to tap "real world" navigational skills, have also demonstrated moderate to large sex differences

favouring males (e.g., Sandstrom, Kaufman, & Huettel, 1998; Astur, Oritz, & Sutherland, 1998; Astur, Tropp, Sava, Constable, & Markus, 2004; Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005; Moffat, Hampson, & Hatzipantelis, 1998). Other studies, however, have not found sex differences (Rossano & Moak, 1998; Wilson, Foreman, & Tlauka, 1997; Moffat & Resnick, 2002). These discrepant results may be due to a number of possible mediating factors related to VE performance, including: the type of VE task employed (e.g., virtual maze or simulated "real world" environment); task performance measures; or individual differences in computer interface proficiency (Waller, 2000), strategy utilisation, or prior experience with VEs (e.g., computer game experience). Notwithstanding, one VE task in particular, the Virtual Morris Water Task (VMWT), has demonstrated reliable and robust sex differences favoring males (Sandstrom et al., 1998; Astur et al., 1998; Astur et al., 2004; Driscoll et al., 2005). These differences are highly comparable to those observed using naïve rodents with the Morris Water Task (MWT; Morris, 1981)(Jones & Watson, 2005; Veng, Granholm, & Rose, 2003; Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996; Roof & Havens, 1992), thus providing a basis for cross-species comparisons.

Performance measures on the VMWT are correlated, although only to a limited extent, with performance on the Vandenberg and Kuse MRT (Astur et al., 2004; Driscoll et al., 2005). The magnitude of these correlations suggests that although the tasks share some common cognitive processes, the two types of visuo-spatial tasks are distinct and hence recruit different skills. Nonetheless, it is the cognitive processes tapped similarly by both these tasks which presumably form the loci of human sex differences in visuo-spatial abilities. Accordingly, data from both these tasks is considered in the following discussion.

1.3. Gonadal Steroids and Visuo-Spatial Cognition

The effects of gonadal steroids during early development are termed 'organisational' because they are thought to involve the permanent alteration of patterns of neural organisation, whilst later effects are termed 'activational' because they involve circulating hormonal levels (e.g., during puberty, or due to diurnal rhythms, or exogenous hormone administration) which activate existing steroid-responsive neural circuits (Duff & Hampson, 2001). Both the organisational and activational effects of gonadal steroids, namely, T and oestrogen, are thought to contribute to within-sex variability and to between-sex differences in sexually dimorphic behaviour and cognition (Collaer & Hines, 1995).

1.3.1. *Organisational Effects*

There are three main lines of evidence which implicate the role of organisational effects of pre- and post-natal levels of gonadal hormones on adult cognition: Animal studies; congenital disorders involving abnormal hormone exposure; and trans-sexual studies. These are now discussed in turn.

Animal Studies

T exerts its effects directly, via the androgen receptor, and also through its metabolites dihydrotestosterone (DHT) and estradiol (E2) (MacLusky & Naftolin, 1981). In rats, nuclear receptors for gonadal steroids are located in the hypothalamus, hippocampus, and in parts of the cerebral cortex, regions that support the differentiation of sexual behaviour, working memory, and reference memory, respectively (Williams, Barnett, & Meck, 1990). These cortical regions have been found to be structurally different in males and females, differences known to be determined by early exposure to gonadal steroids (Kimura, 1999). Female rodents treated with androgens in the last week of gestation perform like normal males in

the MWT; moreover, their hippocampal CA1 and CA3 pyramidal cell field volumes are male-like. Similarly, male rodents prenatally treated with flutamide, an androgen receptor antagonist, and castrated upon delivery perform like females in the MWT and have female-like CA1 and CA3 pyramidal cell field volumes (Isgor & Sengelaub, 1998). Postnatal organisational effects of gonadal steroids on rodent spatial behaviour have also been reported. For example, adult male rats castrated at birth showed navigation performance more like normal female rats than uncastrated rats on a radial arm maze test (Williams et al., 1990). Conversely, neonatal female rats treated with T for 90 days outperform both female and male controls on the MWT (Roof & Havens, 1992). Together these results implicate both the pre- and post-natal periods as critical for the organisational effects of gonadal steroids on adult spatial behaviour and hippocampal morphology in rodents.

The extent to which these observations can be generalised to humans is still unclear, although two lines of research have helped to shed some light on the organisational effects of sex hormones on the human central nervous system and their effects on cognition. These are now discussed.

Disorders involving Abnormal Hormone Exposure

In humans, males have elevated levels of T prenatally during gestational weeks 8-24 and post-natally until about 5 months of age (Kimura, 1999). Two clinical patient groups with disorders resulting in abnormal gonadal hormone exposure during these critical periods are individuals with idiopathic hypogonadotrophic hypogonadism (IHH) and congenital adrenal hyperplasia (CAH).

Men with IHH (which may be congenital or acquired) have a deficiency in the hypothalamic gonadotrophic hormone responsible for regulating the production

and release of sex hormones and this results in abnormally low levels of T (Kimura, 1999). Of interest in relation to the organisational effects of T, are men with congenital IHH (i.e., Kallmann's syndrome) because they have low T levels during the critical periods of development. Hier and Crowley (1982) investigated the visuo-spatial performance of 19 IHH men compared to 19 control men and 5 men with acquired hypogonadotropic hypogonadism. They found the IHH men to be significantly impaired on three tests of spatial ability compared to both control groups, implying a permanent organising influence of androgens. Cappa et al. (1988) failed to replicate this finding in 13 IHH males compared to controls. A weakness in Hier and Crowley's (1982) argument, as Kimura (1999) pointed out, is the fact that IHH is a long-term condition, which, therefore, renders the interpretation of organisational effects problematic. In addition, due to the apparent difficulty for researchers in obtaining large enough clinical samples to allow for studies with adequate statistical power to produce reliable results, it is unlikely this issue will be easily resolved.

CAH results in an overproduction of adrenal androgens beginning prenatally. CAH has been found to occur in both males and females and is generally identified neonatally, particularly in females, because of the occurrence of somewhat masculinised genitals (Kimura, 1999). In their review of the CAH literature on females, Collaer and Hines (1995) concluded that CAH females show performance superior to controls only on visuo-spatial tasks known to show large sex differences (e.g., mental rotation tasks and PMA Spatial Relations). In a recent study, a relatively large number of patients with CAH (40 females and 29 males) were compared to controls on two mental rotation and two targeting tasks. Females with CAH did not significantly outperform controls on the mental rotation tasks;

however, the results were in the predicted direction. Females with CAH also outperformed controls on both targeting tasks. Conversely, males with CAH performed worse than controls on both mental rotation tasks and similarly to controls on both targeting tasks (Hines, Fane, Pasterski, Mathews, Conway, & Brook, 2003). These data were consistent with the findings of a smaller study which compared the performance of five preadolescent boys with CAH to four controls on the PMA Spatial Relations test (Hampson, Rovet, & Altmann, 1998). In contrast, other researchers have not found differences between males with CAH and controls in spatial performance (McQuire, Ryan, & Omenn, 1975; Resnick, Berenbaum, Gottesman, & Bouchard, 1986). Due to the fact that for males with CAH, prenatal T levels are generally not elevated (Wudy, Dorr, Solleder, Djalali, & Homoki, 1999), Hines and colleagues interpreted these data as suggesting that mental rotation ability develops primarily during the first six months of postnatal life when androgen levels in males are elevated. Consistent with this interpretation are the findings of a large scale study which failed to find any relationship between pre-natal levels of T, as indicated by the 2d:4d finger ratio, and mental rotation ability (Coolican & Peters, 2003). The 2d:4d ratio, or the ratio of the lengths of the second and fourth fingers in adults, has been found to be related to prenatal gonadal steroid levels by correlating negatively with T and positively with oestrogen levels (Manning, Scutt, Wilson, & Lewis-Jones, 1998)

Since CAH females are generally treated at an early stage with corrective hormone therapy, thereby modifying the effects of the excess androgens post-natally, and CAH males have abnormally high androgen levels post-natally, the results of Hines et al. (2003) are consistent with the possibility of a quadratic,

inverted U shaped relationship between T and visuo-spatial ability (discussed later), a fact the authors failed to consider.

Transsexual Studies

Another line of research which relates to the organisational effects of T and visuo-spatial abilities is the studies of male and female transsexuals.

Van Goozen, Slabberkoorn, Gooren, Sanders, and Cohen-Kettenis (2002) investigated both the organising and activating effects of sex hormones on visuo-spatial performance in male and female homosexual transsexuals. Nineteen female to male (FM) transsexuals, 22 male to female (MF) transsexuals, 20 heterosexual male controls (MC) and 23 heterosexual female controls (FC) were administered a battery of visuo-spatial ability tasks, including three mental rotation tasks (i.e., RF-2D, Ekstrom, et al., 1976; MRT, Vandenberg & Kuse, 1978; RF-SD, Shepard & Metzler, 1971). Analyses of pre-treatment cognitive functioning of all the groups revealed a statistically significant linear increase from FCs to FMs to MFs to MCs on four out of the five visuo-spatial tests. These data suggest an organising effect of sex hormones on cognitive performance in homosexual transsexuals.

It is possible that a number of psychosocial factors, for example, core sexual identity, may have affected the performance of these individuals on the spatial ability tasks tested. For example, an investigation into the role of gender trait possession and performance on two visuo-spatial tasks, including a 3-D mental rotation task (i.e., Phillips, 1979, version of the Shepard & Metzler, 1971, task), demonstrated that gender trait possession, in particular, androgyny (i.e., relatively high self rating of masculinity and femininity), added significantly to the overall explanation of performance on the 3-D mental rotation task (Hamilton, 1995). Psychological gender (M-F) has also been reported to account for 9% of the

variance in Vandenberg and Kuse MRT performance in 60 heterosexual males, 60 homosexual males, 60 heterosexual females and 60 homosexual females (Rahman, Wilson, & Abrahams, 2004).

In summary, these convergent research paradigms have demonstrated a variable and somewhat inconsistent relationship for an organisational effect of sex hormones on visuo-spatial ability and the exact nature of this relationship remains unclear. The evidence from human studies, in particular, is not strong due to small sample sizes and other confounding effects.

Other Confounding Variables

Two variables (related to organisational effects of sex hormones) found to influence visuo-spatial ability performance are handedness (e.g., Moffat & Hampson, 1996) and sexuality (e.g., Neave, Menaged, & Weightman, 1999). Thus, in order to control for these potentially confounding variables, only right-handed, heterosexual males were studied in some of the research reported in this thesis (see Chapters Five and Six).

1.3.2. *Activational Effects*

Endogenous Gonadal Steroid Levels and Visuo-Spatial Ability in Males

Studies into the activational effects of endogenous sex hormones on visuo-spatial abilities in males, while numerous, have yielded conflicting results. The inconsistencies plausibly arise because of differing research methodologies, the many factors which co-vary with the hormonal factors of interest, and the difficulties in discriminating cause from effect. The following discussion is limited to studies involving adult males and performance on the most relevant visuo-spatial tasks (i.e., those requiring mental rotation or virtual navigation).

Several studies have reported medium sized positive linear relationships between activational levels of T and performance on visuo-spatial tests requiring mental rotation (Hooven, Chabris, Ellison, & Kosslyn, 2004; Silverman, Kastuk, Choi, & Phillips, 1999; Errico, Parsons, Kling, & King, 1992; Moffat, Zonderman, Metter, Blackman, Harman, & Resnick, 2002; Gordon & Lee, 1986). T was recently found to explain more variance in VMWT navigational performance than either sex or age (Driscoll, et al., 2005). Conversely, inverse relationships (Gouchie & Kimura, 1991; Moffat & Hampson, 1996; Neave et al., 1999), and a complete absence of any relationship have also been reported (Wolf & Kirschbaum, 2002; Kampen & Sherwin, 1996; McKeever, Rich, Deyo, & Conner, 1987; Fonda, Bertrand, O'Donnell, Longcope, & McKinlay, 2005; Kempel, Gohlke, Klempau, Zinsberger, Reuter, & Hennig, 2005; Halari et al., 2005; Falter, Arroyo, & Davis, 2006; Hassler, Gupta, & Wollmann, 1992). Methodological issues, including methods for measuring T, lack of control of factors known to affect endogenous T levels, and differing measures of mental rotation, or differences in test administration, or some combination of factors, may be responsible for the inconsistent findings.

Three measures of T generally employed in these studies are, Total Testosterone (TT), Bioavailable Testosterone (BT) and Free Testosterone (FT). Whilst TT, BT, and FT are measured in serum, FT can be also measured in saliva. TT is the sum of both the unbound (free) and bound T (i.e., the fraction bound to either sex hormone binding globulin (SHBG) or non-specific (albumin) binding proteins). BT is the sum of both FT and the fraction bound to albumin, and is generally believed to be the best indicator of tissue exposure to androgens (Morley, Kaiser, Raum, Perry III et al., 1997). FT measured in serum or saliva is considered

to reflect unbound T, that is, the proportion not bound to either SHBG or albumin. The FT measure is typically employed because, unlike bound T, it is argued that only FT can pass the blood-brain barrier and hence potentially influence cognitive function (Shute, Pellegrino, Hubert, & Reynolds, 1983). Although the measurement of FT from saliva offers advantages for researchers, it is important to note that methodological factors related to sample collection and storage procedures recently have been demonstrated to affect measurement accuracy (Whembolua, Granger, Singer, Kivlighan, & Marguin, 2006; Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004; Goncharov et al., 2006). These factors may have affected the results of previous studies.

Additional methodological issues include timing of samples, assay variability, and other factors affecting T production or measurement. Examples include smoking, daily alcohol intake, body mass index (BMI), depression, and years of education (Barrett-Connor, Goodman-Gruen, & Patay, 1999).

T levels are highest in the early morning and decline throughout the day (Kimura, 1999) and it has been shown that in males, T variation due to the diurnal cycle influences visuo-spatial ability (Moffat & Hampson, 1996). In males living in the northern hemisphere, T levels are higher in autumn than in spring (Kimura, 1999). Based upon the analyses of composite scores derived from three visuo-spatial tasks (Hidden Figures; Ekstrom, French, & Harman, 1976: Paper Folding; Ekstrom, French, & Harman, 1976: and Mental Rotations) and samples of salivary T, it has been shown that a male group studied in spring outperformed a male group studied in autumn (Kimura & Hampson, 1994). Taken together, these studies (Moffat & Hampson, 1996; Kimura & Hampson, 1994) suggest that high FT levels in young adult males may be associated with poor visuo-spatial performance. It is

important to note, however, that this effect was found only in right-handed individuals in one of these studies (Moffat & Hampson, 1996), and that both studies utilised a between rather than a within-subjects experimental design and that in neither study were other variables known to influence T levels, such as obesity, smoking, alcohol, sexuality, age and presence of chronic disease controlled for, or reported.

To illustrate the influence of potential confounds, two conflicting studies, which were very similar methodologically, are compared. Silverman et al. (1999) and Moffat and Hampson (1996) both conducted studies using male university students. Both studies used salivary samples to measure FT, both utilised the Vandenberg and Kuse MRT and both used the same laboratory for their saliva sample assays. Additionally, both studies took saliva samples in the early morning and reported highly comparable mean T levels. Despite these methodological similarities, Silverman et al. (1999) reported a statistically significant positive correlation between T levels and visuo-spatial ability ($r = .28$, $p < .05$) whilst Moffat and Hampson (1996) reported a statistically significant negative correlation ($r = -.44$, $p < .01$). Silverman and colleagues wrote that the only methodological difference between the two studies in relation to this outcome was the fact that they used different versions of the same visuo-spatial test. Additionally, however, 21 out of the 40 male participants in Moffat and Hampson's study were left-handed; Silverman and colleagues failed to screen participants for neurological and psychiatric conditions; and both studies failed to control for obesity, smoking, daily alcohol intake, and sexuality. Further, methodological differences related to both the transport and storage of the salivary samples could have affected the T

measurements (Granger et al., 2004) and may account for the discrepancies in the results.

A proposed quadratic (i.e., inverted-U shaped) relationship between endogenous gonadal steroids and visuo-spatial performance (Petersen, 1976) has been used to explain contradictory results between various studies. The essence of this theory, based upon data from both males and females, is that there is an optimal level at which gonadal steroids induce peak visuo-spatial performance. Deviations from these, that is, levels either higher or lower than this optimal range, result in decreased performance. Despite the popularity of this theory, the empirical evidence typically cited in its favour is rather weak (i.e., Gouchie & Kimura, 1991; Moffat & Hampson, 1996; Neave et al., 1999; Shute et al., 1983). Individually, none of these studies has actually obtained the proposed quadratic function in males (Silverman et al., 1999). Additionally, the contrary results of the two studies just described seem inconsistent with the theory on logical grounds: Whilst both studies reported similar mean FT levels, one reported a negative and the other a positive relationship. Other weaknesses in the theory include the lack of discrimination between organisational and activational effects of gonadal steroids and the lack of consensus as to whether it is T itself, or its derivatives (Nyborg, 1983), which are responsible for the proposed relationship (Moffat & Hampson, 1996). It is interesting to note that non-linear relationships have been reported between endogenous T levels and performance on non-spatial cognitive tests in males (Barrett-Connor et al., 1999). Additionally, several studies have reported significant impairments in visuo-spatial performance following the exogenous administration of supraphysiological doses of T, both in adult men (O'Connor, Archer, Hair, &

Wu, 2001) and in male rodents (Roof & Havens, 1992; Naghdi, Nafisy, & Majlessi, 2001; Naghdi, Majlessi, & Bozorgmehr, 2005).

One approach to resolving these dilemmas is through the investigation of the effects of exogenously administered hormones.

Exogenously Administered Androgens

The reported effects of T supplementation on visuo-spatial ability are contradictory and the effects appear dependent on the methodology used and the age groups studied. For the purposes of the present discussion, they are grouped according to the age group sampled, that is, either young or older adult males.

Supplementation in young adult males.

The investigation into the effects of hormone supplementation, specifically, effects on cognition in young adult males, has involved three different clinical subject groups: hypogonadal, transsexual, and healthy males.

Four studies which have investigated the effects of T supplementation on visuo-spatial abilities in young hypogonadal males reported no effect (Hier & Crowley, 1982; Alexander et al., 1998; O'Connor et al., 2001; O'Carroll, 1984). Three of these studies (Hier & Crowley, 1982; O'Connor et al., 2001; O'Carroll, 1984) had small sample sizes (six, eight, and seven hypogonadal men, respectively) and failed to include a mental rotation task. Alexander et al. (1998) also failed to observe an effect despite employing a much larger sample of 33 young hypogonadal adult males and utilising four visuo-spatial tasks, including a mental rotation task. Nonetheless, the potential for longer-term effects of T supplementation cannot be ruled out on the basis of these studies.

The effects of androgen antagonists and oestrogens on visuo-spatial ability in young adult MF transsexuals have also yielded contradictory results. Van

Goozen, Cohen-Kettenis, Gooren, Frijda, and Van de Poll (1995) reported, for example, deterioration in performance on a 2-D mental rotation task in 15 MFs after three months of treatment. This finding was not replicated in 20 MF transsexuals who completed three visuo-spatial tasks (i.e., RF-2D; RF-3D; and Hidden Figures), after three months of similar treatment (Slabbekoorn, Van Goozen, Megens, Gooren, & Cohen-Kettenis, 1999). Taken together, the results of these studies were interpreted as demonstrating of a 'preventative learning effect' on performance on these visuo-spatial tasks in comparison to controls (Slabbekoorn et al., 1999). This refers to the effect, purportedly caused by the experimental treatment, which negates the expected improvement in performance on the respective tasks expected from practice. This particular effect, however, was not replicated in 22 MF transsexuals after 14 weeks of the same treatment by the same researchers using the same visuo-spatial tasks (Van Goozen et al., 2002).

As with the studies in young hypogonadal adult males, the evidence for an effect of exogenously administered sex hormones on visuo-spatial ability using MF transsexuals is weak and inconsistent. Treatment and psychosocial confounding effects specific to this subject group, in addition to the factors listed previously, may have affected the results making definitive conclusions impossible. Nevertheless, longer-term organisational effects of gonadal hormones on transsexual MF's cognition cannot be discounted.

The effects of supraphysiological doses of T on visuo-spatial ability have also been investigated in normal healthy eugonadal young males (i.e., males with normal physiological T levels). Four studies reported that, in comparison to control groups, supraphysiological doses of T had no effect on visuo-spatial performance (Alexander et al., 1998; O'Carroll, 1984; Cherrier et al., 2002; Bhasin et al., 2001),

whilst a fifth study reported negative effects (O'Connor et al., 2001). After six weeks of supplementation with high-dosage T injections no effect was observed in eight young eugonadal adult males (O'Carroll, 1984) on the Revised Minnesota Paper Form Board Test-Form AA (Likert & Quasha, 1970). Similarly, after six weeks of supplementation with supraphysiological doses of T, no statistically significant effects, beyond practice effects, were observed in 10 young eugonadal adult males on a battery of tests of cognitive ability including four visuo-spatial tasks (i.e., Mental Rotations, Surface Development, Paper Folding, and Hidden Patterns; Alexander et al., 1998). Consistent with these data two studies reported that supraphysiological doses of T had no statistically significant effects on spatial memory (Cherrier et al., 2002; Bhasin et al., 2001). Interestingly, O'Connor et al., (2001) observed that performance declined significantly on the WAIS Block Design task in 14 young eugonadal adult males who received four weeks of supraphysiological doses but performance returned to baseline levels after eight weeks of treatment.

Due to the relatively small sample sizes employed in these studies and their heterogeneous visuo-spatial measures, it is difficult to draw definitive conclusions from the data. Nevertheless, it appears that supraphysiological T doses in young adult males neither improve nor significantly impair spatial performance, at least over the intermediate term.

Supplementation in older adult males.

There is considerable interest in the effects of T supplementation on cognitive function in older adult males because of the progressive decline in plasma T levels across the lifespan (discussed later). The majority of studies have reported a beneficial effect of T supplementation on visuo-spatial ability in aged men

(Cherrier et al., 2001; Cherrier et al., 2004; Cherrier et al., 2005a; Janowsky, Chavez, & Orwoll, 2000; Janowsky, Oviatt, & Orwoll, 1994; Cherrier et al., 2005b; Gray et al., 2005; Tan & Pu, 2003; Cherrier et al., 2007; Vaughan, Goldstein, & Tenover, 2007), although four studies have failed to detect any effect (Haren, Wittert, Chapman, Coates, & Morely, 2005; Lu et al., 2006; Kenny, Fabregas, Song, Biskup, Bellantonio, 2004; Sih et al., 1997). Four studies have reported positive effects following T supplementation in older adult males on the Block Design test, whilst only one study has reported no effect (see Table 1.). The Block Design test is a timed test of visuo-spatial ability that measures a person's ability to analyse and construct abstract figures from their component parts (Wechsler, 1981). Interestingly, it appears that the ratio of serum T to E2 levels following T supplementation may play an influential role in these observed improvements in test performance. Cherrier et al. (2004), for example, found that E2 followed TT as a significant predictor of Block Design performance, explaining an additional 26% of the variance in a regression analysis. Janowsky et al. (1994) also reported a statistically significant improvement in Block Design performance but only after correcting for baseline E2 levels. Similarly, a multiple regression analysis of the treatment group's performance revealed E2 but not T as a significant contributor to the model. Two recent failures to replicate this effect on the Block Design test (Haren et al., 2005; Lu et al., 2006) may have been because the effect of E2 levels were not accounted for. For the Self-Ordered Pointing Task (SOPT; Petrides & Milner, 1982), which is considered to be both a measure of executive function and visuo-spatial working memory (WM), T supplementation improved older adult males' performance to levels approximately equivalent to a younger adult male control group (Janowsky et al., 2000). Consistent with the pattern of results

outlined, better performance was related to higher FT to E2 ratios. Interestingly, in aged male rodents a similar pattern of results pertaining to improvements in spatial WM performance following T supplementation has also been observed (Bimonte-Nelson et al., 2003).

A recent study investigated the effect of the conversion of T to E2 following T supplementation by using anastrozole, an aromatase inhibitor which inhibits the action of the enzyme responsible for the conversion of androgens to oestrogens within the brain (Cherrier et al., 2005a). Sixty healthy, community-dwelling males aged from 50 to 90 years were randomly assigned to one of three groups: A T only group (T); a T and anastrozole group (AT); and a placebo group (P). Participants were tested on a battery of cognitive tests, including a 3-D spatial memory route test (Cherrier et al., 2001) and the SOPT, at baseline and then at weeks three and six of treatment. A statistically significant improvement from baseline performance was observed on the 3-D spatial memory route task for the AT group but not for either the T or P groups. No treatment effect was observed with the SOPT, although these researchers used a short version of the task. These data suggest that T but not E2 was responsible for the observed improvement in 3-D spatial memory performance. Consistent with these results, Cherrier and colleagues also showed that supplementing older hypogonadal men with DHT improved their performance both on their 3-D spatial memory task and the Spatial Array Learning Tests (SALT; Cherrier et al., 2001) when compared to baseline levels (Cherrier, Craft, & Matsumoto, 2003). Together these data implicate the role of non-aromatizable androgens in the improvement of visuo-spatial performance with androgen supplementation in aged adult males.

It is also interesting to note that two of the studies which found no effect on tests of visuo-spatial ability (i.e., Haren et al., 2005; Kenny et al., 2004), specifically employed aged men with lower T levels. These results are consistent with studies in young hypogonadal adult males, which similarly reported no effect (O'Connor et al., 2001; Alexander et al., 1998; Hier & Crowley, 1982; O'Carroll, 1984). This pattern of results suggests that baseline gonadal status, specifically T levels around the lower limit for normal young adults, is an important factor which mediates the effect of exogenous T supplementation on visuo-spatial ability in aged adult males.

Despite the fact that there have been relatively few studies which have investigated T supplementation effects on visuo-spatial cognition in aged men and that the majority of these studies have utilised small sample sizes and only two visuo-spatial measures at most, a consistent pattern of results is apparent. T supplementation in aged males appears to improve Block Design performance and visuo-spatial WM/executive function as measured by the SOPT; visual memory and learning do not appear to be affected. However, the question of whether this effect is transferable to other visuo-spatial tasks, such as mental rotation tasks, remains unanswered at present.

1.4. Testosterone and Mental Rotation Ability in Ageing Males

In addition to the possibility of a direct association between T and mental rotation ability in adult males, it is also plausible to suggest that the association between T and mental rotation ability in males may be mediated by relationships between circulating gonadal steroid levels and cognitive abilities constituent to mental rotation performance, such as, for example, processing speed or executive function performance (discussed in Chapter 2). Hooven et al. (2004) concluded that there was no evidence indicating that actual rotation performance was related to

salivary FT levels in college aged males, as determined from correlating FT levels with both the slope and intercepts of the functions relating performance to angular disparity. Instead, the authors concluded that T levels appeared to be related to component aspects of the task, hypothesising potentially mediating roles for other distinct constituent cognitive processes of the task, such as, the ability to discriminate between novel stimuli, processing speed, and focussed attention/decision processes (i.e., executive functions).

1.4.1. *Research Aim*

The first aim of the research detailed in this thesis was, therefore, to determine the association between endogenous plasma T levels and mental rotation performance in middle-to-older aged males.

1.4.2. *Specific Hypotheses*

1) Higher T levels are related to better mental rotation performance.
2) The relationship between T and mental rotation performance is mediated by associations between T levels and cognitive abilities constituent to mental rotation performance, such as, processing speed and executive function (discussed in Chapter 2).

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Table 1. Summary of effects of T supplementation on tests of visuo-spatial ability in older adult males

Visuo-spatial ability test	Improvement	No Effect
Block Design	Cherrier et al. (2005b)	Haren et al. (2005)
	(*M* (*SD*) = 76(5), *N* = 32)	(*M* (*SD*) = 68.5(6), *N* = 76)
	Cherrier et al. (2004)	Lu et al. (2006)
	(range 50-80 years, *N* = 25)	(over 50 years, *N* =38)
	Cherrier et al. (2001)	
	(range 50-80 years, *N* = 25)	
	Janowsky et al. (1994)	
	(range 60-75 years, *N* = 56)	
Self-Ordered Pointing Task	Janowsky et al. (2000)	Cherrier et al. (2005a)
	(range 61-75 years, *N* = 19)	(*M* (*SD*) = 65(11), *N* = 60)
3-D Spatial Memory	Cherrier et al. (2007)	Cherrier et al. (2003)
	(*M* (*SD*) = 67(11), *N* = 57)	(*M* (*SD*) = 57(9), *N* = 12)
	Cherrier et al. (2005a)	
	(*M* (*SD*) = 65(11), *N* = 60)	
	Cherrier et al. (2005b)	
	(*M* (*SD*) = 76(5), *N* = 32)	
	Cherrier et al. (2001)	
	(range 50-80 years, *N* = 25)	
Visuo-Spatial Memory	Gray et al. (2005)	
	(*M* (*SD*) = 65.5(0.7), *N* = 44)	
The Clock Drawing Test	Tan & Pu (2003)	Kenny et al. (2004)
	(range 68-80 years, *N* = 10)	(*M* (*SD*) = 80(5), *N* = 11)
Visual Reproduction		Janowsky et al. (1994)
		(range 60-75 years, *N* = 56)
JOLO	Vaughan et al. (2007)	Lu et al. (2006)
	(range 65-83 years, *N* = 46)	(over 50 years, *N* =38)
SALT		Cherrier et al. (2001)
		(range 50-80 years, *N* = 25)
RVDLT		Sih et al. (1997)
		(*M* (*SD*) = 68(6), *N* = 15)
RVDTR		Sih et al. (1997)
		(*M* (*SD*) = 68(6), *N* = 15)
BVRT		Vaughan et al. (2007)
		(range 65-83 years, *N* = 46)

Note: The Clock Drawing Test (Tuokko, Hadjistavropoulos, Miller, & Beattie, 1992); Visual Reproduction (Wechsler, 1987); JOLO is Judgment of Line Orientation (Benton, Hamsher, Varney & Spreen, 1983); SALT is Spatial Array Learning Tests (Cherrier et al., 2001); RDVLT is Rey Visual Design Learning Test (Spreen & Strauss, 1991); RVDTR is Rey Visual Design Test-Recall (Spreen & Strauss, 1991); BVRT is Benton Visual Retention Test (Benton, 1974).

CHAPTER TWO: GONADAL STEROIDS AND COGNITIVE FUNCTIONING IN AGEING MALES

2.1. Summary

In this chapter studies are reviewed which have provided support for the suggestion that changes in plasma gonadal steroid levels that occur with ageing in males may play a role in mediating generalised age-related cognitive decline. Specifically, studies in aged males are reviewed which have shown positive associations between measures of endogenous plasma T levels and either processing speed or executive function measures, or both of them. A positive association between endogenous plasma T levels and processing speed, executive function, or both of them, implies that age-related changes in gonadal steroid production in males may be related to generalised or pathological age-related cognitive decline, or both of them. Further, these associations may mediate the relationship between T levels and males performance on visuo-spatial tasks, such as those requiring mental rotation, as discussed in Chapter One. General and specific hypotheses pertaining to the studies detailed in this thesis are summarised.

2.2. Endogenous Plasma Testosterone Levels and Cognitive Functioning in Ageing Males

Plasma T levels decrease progressively but variably as men age (Tan, Pu, & Culberson, 2003). The factors other than age that determine this decline remain fully to be determined. In a large scale longitudinal study of 1,156 community dwelling men aged between 40 and 70 years at baseline, for example, it was reported that endogenous levels of TT, BT, and dehyroepiandrosterone sulphate (DHEA-S) declined with age at a rate of 1.6, 2.5, and 5.2% a year, respectively (Feldman et al., 2002). Although there is great inter-individual variability in

endogenous hormonal levels in males, this rate of decline is such that 7% of 40-60 year old males, 20% of 60-80 year old males, and 35% of males older than 80 years have TT levels below the 'normal' lower limit of 350ng/dL, or 12.1 nmol/L (Vermeulen & Kaufman, 1995). Due to the concomitant increase in SHBG levels with ageing (Feldmen et al., 2002), age-related declines in both FT and BT levels are even greater.

2.3. Ageing and Cognition

In his review, Salthouse (2004) reported that whilst vocabulary (verbal ability) increases with age until the mid-50s, or later, before declining, other major domains of cognitive function, such as mental speed, reasoning, and memory, decrease linearly with age (i.e., r = -0.47, r = -0.48, and r = -.43, respectively). In relation to visuo-spatial ability, a similar rate of decline with age has been reported (r = -0.36; Meinz & Salthouse, 1998). For mental rotation ability, older adults are consistently outperformed by younger adults and experience greater slowing as a function of the required angle of rotation (Inagaki, Meguro, Shimada, Ishizaki, Okuzumi, & Yamadori, 2002; Dror & Kosslyn, 1994; Puglisi & Morrell, 1986; Berg, Hertzog, & Hunt, 1982).

The rate of cognitive decline with increasing age is influenced by a number of variables, including: health, education level, marital status, socio-economic status, participation in intellectually stimulating environments and life satisfaction (Schaie, 1994). The extent of the influence of these variables depends on the cognitive ability in question; that is, whether the basis for performance is primarily verbal or non-verbal. Non-verbal cognitive abilities, such as visuo-spatial ability, for instance, are not affected by lifestyle factors such as socio-economic status, or intellectual activities (Gold et al., 1995). In addition, age-related declines in visuo-

spatial performance have been demonstrated to occur relatively independently from spatial visualisation experience, as indicated by employment type and by frequency of ability utilisation (Salthouse et al., 1990). Hence, the variables which are influential on the rate of age-related decline of visuo-spatial ability are, primarily, health and education levels.

Although the general trends of cognitive ageing in respect to the questions of 'what' and 'when' have been fairly well documented in the literature, current theoretical debate and research has been primarily concerned with determining the actual causes and factors which might modify cognitive decline. This issue is of particular relevance to this thesis.

Two theoretical approaches, which attempt to address this issue, are processing speed theory and the theory of pre-frontal decline. These are now discussed in turn.

2.4. Processing Speed and Endogenous Testosterone Levels in Men

Processing-speed theory (e.g., Salthouse, 1996a) proposes to account for age-related declines in fluid abilities in terms of general cognitive slowing. Fluid abilities are considered to be biologically based and are generally interpreted as fundamental to reasoning and novel problem-solving abilities and are not dependent upon culturally attained knowledge (Cattell, 1971). These fluid abilities include varieties of memory, reasoning, and spatial ability.

The central hypothesis of this theory is that a major contributor to the age-related decline of fluid abilities is the general slowing of the speed of execution of cognitive operations, or speed of information processing. Processing speed, according to this theory, is gauged by measures, which are: relatively simple and not dependent upon knowledge or other cognitive abilities (e.g., general verbal

ability); not merely representative of input or output sensory and motor processes (i.e., they reflect the quickness of execution of cognitive processes); multiple, so as to minimise task specific variance and to emphasise common, construct related variance (Salthouse, 1996a).

Critics of this theory have questioned the validity of the concept "speed of processing" and the measures used to assess it (see Deary, 2000). This criticism is justified by the fact that there are at least six different types of variables (i.e., decision speed, perceptual speed, psychomotor speed, reaction time, psychophysical speed, and the time course of internal responses) used to assess "processing speed" (Salthouse, 2000). Nevertheless, some of the variables used to gauge processing speed, such as measures of perceptual speed (i.e., tasks which require matching, search, or substitution), have consistently demonstrated a moderate to strong relationship with age (Salthouse, 1998; Sliwinski & Buschke, 1999; Schaie, 1989). Furthermore, when speed is statistically controlled it has been found to account for about 75% of the age-related variance in a wide variety of memory and other cognitive measures (Salthouse, 1996a,b).

Two cognitive abilities of particular relevance to the present research and which have been found to be particularly sensitive to age-related decline in processing speed, are Baddeley's WM construct, and mental rotation ability. WM tasks are generally classified as either verbal or spatial in nature and are characterised by their dependence upon the ability to store and actively process several stimuli simultaneously (Salthouse, 1994). They have been found to be particularly susceptible to age-related decline, sharing between 71% and 96% of their age-related variance with processing speed measures (Salthouse, 1994).

Mental rotation ability is also susceptible to age-related decline in processing speed, with the speed requirements of the task and the time required to process the complex stimuli identified as responsible for age-related deficits in performance (Dror & Kosslyn, 1994; Berg et al., 1982). Taken together, these data suggest that processing speed plays a key role in the decline of performance with ageing of both WM and mental rotation abilities.

The mechanism by which changes in processing speed occur in the ageing brain remains unclear. Changes in myelination, changes in the concentrations of neurotransmitters, weakened inhibitory circuits, and changes in various neurotransmitter-receptor systems have all been hypothesised to contribute to decline in processing speed (Deary, 2000). Whether any of these changes are associated with age-related declines in the levels of endogenous gonadal steroids is yet to be determined, although some recent research has provided support for a relationship between endogenous sex hormone levels and processing speed (see Table 2). For example, it was recently reported that men younger than 72 years with higher TT levels performed better on a composite processing speed measure, whilst for older age groups higher TT levels were associated with poorer performance (Hogervorst, De Jager, Budge, & Smith, 2004). This trend remained in the analyses even after adjusting for potential confounds such as SHBG levels, years of education, depression, BMI, current smoking and alcohol use. Consistent with these data, Moffat et al. (2002) reported that in 254 men, faster Trails A performance was associated with a higher free testosterone index (FTI) after adjusting for health and disease-related variables. Similarly, in 310 community dwelling older men aged over 50 years, those with higher levels of BT performed better on the Digit Symbol test from the Wechsler scales, after adjusting for age and

education (Yaffe, Lui, Zmuda, & Cauley, 2002). Further, in 84 men aged between 71 and 80 years, those with both higher TT and BT levels scored better on a composite processing speed measure, which included the Digit Symbol test. Quintile analyses revealed differences between the highest and lowest extremes of about 1 SD of the Z- score distribution No associations were found, however, in the younger age groups (Muller, Aleman, Grobbee, de Haan, & van der Schouw, 2005).

In contrast to these findings, a recent large-scale epidemiological study of 981 middle- to old-aged men found that log FT and TT scores were not predictive of Digit Symbol performance in adjusted models (Fonda, Bertrand, O'Donnell, Longcope, McKinlay, 2005). Similarly, Lessov-Schlaggar et al. (2005) failed to find a relationship between TT and either Digit Symbol or Trails A performance in 349 twins; however, in this study the cognitive tests were administered 10 to 16 years after the blood samples were collected and analyses were not adjusted for BMI.

Due to differences in the hormone and outcome measures and the statistical methods utilised, it is difficult to draw conclusions from these data. Nevertheless, both an effect of age and of endogenous gonadal hormone levels on processing speed is apparent in men, and further research to determine definitive relationships is required.

Table 2. Summary of the effects of endogenous T levels on processing speed in older adult males

Authors	Tests	Testosterone Measure	Age Group Or Mean (years ± SD)	Effect
Hogervorst et al. (2004)	Pattern and Letter Comparison Test	TT	61 - 72	Positive
			72 - 87	Negative
Moffat et al. (2002)	Trails A	FTI	64.1 ± 9.4	Positive
Yaffe et al. (2002)	Digit Symbol	BT	73.0 ± 7.1	Positive
Muller et al. (2005)	Composite: (Digit Span Forward Digit Span Backward Digit Symbol and Trails A)	TT and FT	71 - 80	Positive
Fonda et al. (2005)	Digit Symbol	TT and FT	62.7 ± 8.2	No Effect
Lessov-Schlaggar et al. (2005)	Trails A	TT	63.1 ± 2.1	No Effect
	Digit Symbol	TT	63.1 ± 2.1	No Effect

Note: TT refers to total testosterone; FTI, free testosterone index; BT bioavailable testosterone; FT, free testosterone; Pattern and Letter Comparison Test (Salthouse, 1996b); Trails A (Reitan, & Wolfson, 1993); Digit Symbol (Wechsler, 1981); Digit Span Forward (Wechsler, 1987); Digit Span Backward (Wechsler, 1987).

2.5. Pre-Frontal Decline and Endogenous Testosterone Levels in Men

The theory of pre-frontal cognitive decline with ageing is of relevance to the present study due to the relationship between visual-spatial ability and so-called "executive functions".

Executive functions are best conceptualised as having four key components: volition; planning; purposive action; or, self-regulation (Lezak, 2004).

All of these 'executive' mental capacities are intrinsic to the performance of complex behaviours and, based upon neuropsychological evidence, are generally considered functions of the brain's frontal lobes (Lezak, 2004). It has been noted by a number of researchers that executive functions are intrinsic to the performance of visuo-spatial abilities (e.g., Miyake, Friedman, Rettinger, Shah, & Hegarty, 2001; Libon et al., 1994).

The theory of prefrontal decline proposes that pronounced atrophic changes in the brain's frontal lobes, which accompany ageing, are responsible for a disproportionate decline in executive functions (West, 1996; West, 2000). Recently, however, it has been argued by several different researchers that these changes do not produce global decreases in executive frontal lobe function (e.g., Tisserand & Jolles, 2003; MacPherson, Phillips, & Della Sala, 2002; Wecker, Kramer, Wisniewski, Delis, & Kaplan, 2000). In relation to age-related changes in function, these authors have indicated that distinctions should be made between specific subregions within the prefrontal cortex (PFC) and grosser neural networks of interconnected brain regions in relation to age-related changes in function, thereby suggesting the need for a more refined theoretical approach.

A subregion within the frontal lobes, the dorsolateral pre-frontal cortex (DLPFC), has been reported by several researchers to be particularly susceptible to age-related decline in function (MacPherson et al., 2002; Esposito, Kirkby, Van Horn, Ellmore, & Berman, 1999). The DLPFC lies within the frontal lobes and is thought to support several key mental abilities, including retrospective memory, prospective memory, and inhibition of prepotent responses (Fuster, 1989). Evidence from Positron Emission Tomography (PET), functional Magnetic Resonance Imaging (fMRI), and lesion studies has demonstrated that the DLPFC,

specifically Brodmann's area 46, which lies within it, is extensively involved in visuo-spatial WM (Kessels, Postma, Winjnalda, & de Haan, 2000). According to the so-called 'two-stage' model of WM, the ventrolateral PFC principally supports the maintenance of information in WM, whilst the DLPFC is more involved with the active manipulation of stored items (Owen, Evans, & Petrides, 1996). Due to the fact that the ability to manipulate mental images is a key factor when performing both visuo-spatial WM and mental rotation tasks and that the DLPFC is postulated as supporting the ability to manipulate stored items, it could be that age-related decline of the DLPFC may contribute to the age-related decline in performance of both these abilities.

Raz, Briggs, Marks, and Acker (1999) investigated the neural substrates of age-related decline in the performance of several mental imagery tasks, including several involving mental rotation, by measuring the volume of the DLPFC and the volume of other cortical regions associated with modality-specific visual information processing. These researchers reported statistically significant relationships between the volume of the DLPFC and performance on their visuo-spatial mental imagery tasks; this relationship did not hold for the other recruited cortical regions. This observation is consistent with the predictions of the theory of pre-frontal decline and highlights the importance of measures that isolate frontal lobe functions (i.e., executive abilities), in order to control for age-related decline in the integrity of PFC, or more specifically, DLPFC functions, when investigating factors (for example gonadal steroids) which may influence the age-related decline in visuo-spatial WM or mental rotation performance.

Apart from neuronal volume, pronounced age related neurobiological changes of the frontal lobes potentially related to impaired executive function with

ageing include: decreases in synaptic density; the concentration, synthesis, and number of receptor sites for some neurotransmitters, specifically dopamine; and increases in senile plaques (West, 1996).

There is some data to support a mediating role of endogenous gonadal steroids on the rate of pre-frontal decline. For example, in 547 community-dwelling men aged between 59-89 years, men with higher levels of BT and lower levels of E2 performed better on the Blessed Information Memory Concentration test (BIMC; Blessed, Tomlinson, & Roth, 1968), a measure of mental control, after adjusting for age and education (Barrett-Connor et al., 1999). Perry et al. (2001) also reported that in 81 community-dwelling males aged over 55, declining BT was associated with impairment in executive function, as assessed by the Executive Interview measure (EXIT; Royall, Mahurin, & Gray, 1992). Recently, in 395 men aged between 40 and 80 years, there was a significant linear relationship reported between TT and composite executive function scores after adjustments for confounds (Muller et al., 2005). Consistent with these data, T supplementation in older males improved performance on the SOPT to levels almost equivalent to those of a young adult male control group (Janowsky et al., 2000). The SOPT is widely used as a test of executive function and has proven an effective measure of frontal lobe dysfunction (Bryan & Luszcz, 2001; Shimamura & Jurica, 1994). Similarly to the findings of Barrett-Connor et al. (1999), better performance in this study was related to higher FT to E2 ratios.

2.6. Are the Processing Speed and Pre-Frontal Decline Theories Complementary Accounts of Cognitive Ageing?

The age-related effects associated with slower processing speed and prefrontal decline, or both of them, could well be complementary in leading to age-

related decreases in performance of visuo-spatial tasks, such as those involving mental rotation. These effects and their relationship to age-related declines in fluid-spatial abilities were investigated in a recent study using MRI to estimate frontal lobe volume. A perceptual speed composite measure to gauge processing speed and a battery of cognitive ability tests to derive a fluid-spatial intelligence measure were administered (Schretlin et al., 2000). Multiple regression analyses were performed on data obtained from 112 participants to derive a model of cognitive ageing. The findings demonstrated that fluid-spatial intelligence is dependent on both frontal lobe volume and executive ability independent of the effects of perceptual speed. However, most of the variance (67%) in executive ability related to frontal lobe volume was mediated by perceptual speed. These data are important in relation to the work presented in this thesis because they further emphasise the influence of processing speed and prefrontal decline singly and together in explaining age-related decline in visuo-spatial performance, and serve as a basis for constructing my overall hypothesis. Accordingly, the relationship between both processing speed and prefrontal decline theories needs to be taken into account when attempting to determine the factors which may relate to age-related decline in both visuo-spatial WM and mental rotation performance.

2.7. Study Rationale

The majority of correlational studies conducted in younger adult males have reported positive linear relationships between endogenous T levels and mental rotation performance. Moreover, T supplementation in older adult males has been demonstrated to improve performance on tests of visuo-spatial ability. Together these data suggest that age-related declines in endogenous T levels in males may be associated with poorer mental rotation performance.

Hooven et al. (2004) suggested that the relationship between endogenous T levels in males and performance on the Vandenberg and Kuse (1978) MRT may be mediated by relationships between T levels and distinct constituent cognitive abilities necessary to task performance, namely; perceptual discrimination, processing speed, and focussed attention/decision processes (i.e., executive functions). There is some indication from recent large population based studies that lower endogenous T levels in older adult males may be associated with poorer processing speed and executive function performance. A positive association between endogenous T levels and processing speed and/or executive function performance in middle-to-older aged men would suggest that declines in T levels that occur with ageing in males may be associated with generalised age-related cognitive decline.

2.8. Research Aim

The second aim of the research detailed in this thesis was to determine whether changes in gonadal steroid levels that occur with ageing in males were associated with age-related declines in mental rotation performance, or in ability measures indicative of generalised age-related cognitive decline, such as processing speed and executive function, or both of them.

2.9. General Hypothesis

Decreased endogenous plasma T levels in aged males are associated with poorer mental rotation performance. This association is mediated by positive associations between endogenous T levels and both processing speed and executive function, respectively. Endogenous plasma T levels will be identified in males as a potentially modifiable factor for the amelioration of generalised age-related cognitive decline, pathological cognitive decline, or both of them.

2.10. Specific Hypotheses

3) Higher T levels are related to better mental rotation performance.
4) This relationship is mediated by relationships between T levels and both processing speed and executive function.
5) Higher T levels are related to faster processing speed performance.
6) Higher T levels are related to better executive function performance.
7) Lower T levels are related to poorer cognitive functioning with ageing.

2.11. Significance of Project

The present research sought to determine whether decreased plasma T levels in aged males were associated with both poorer visuo-spatial performance and generalised age-related cognitive decline. The associations between different measures of plasma T (i.e., TT, FT, and BT) and neuropsychological measures of learning and memory, processing speed, and executive function were initially examined in a large community-residing sample of middle-and-older aged men. Following this, a healthy sub-sample of these men was recruited to participate in a second study where they completed extensive cognitive testing. In this second study, associations were examined, cross-sectionally, between endogenous plasma T levels, mental rotation performance, cognitive abilities hypothesised to be constituent to mental rotation performance, and measures of generalised age-related cognitive decline, such as, processing speed and executive function. From these data, statistical models were examined which described the associations between chronological age, plasma T levels, processing speed, executive functioning, and mental rotation performance in middle-to-older aged men. In a subsequent study, these associations were then re-analysed in light of new research which suggested that intra-individual variability in reaction time (RT) task performance was a strong

predictor of cognitive functioning. Together this research determined, firstly, the association between plasma T levels and visuo-spatial functioning in middle-to-older aged men, and secondly, helped to elucidate the relationship between endogenous plasma T levels and age-related cognitive decline in males.

2.12. Studies Detailed in this Thesis

This thesis first reports cross-sectional analyses of associations between gonadal steroid levels and neuropsychological measures of learning and memory, processing speed, and executive function in a large randomly recruited sample of 1195 community-residing males aged between 35 and 80 years (Chapter 3). Secondly, from a sub-sample of 96 of these men, cross sectional analyses are reported on the associations between chronological age, endogenous plasma T levels, mental rotation performance, abilities hypothesised as constituent to mental rotation performance, and measures of generalised age-related cognitive decline, such as, processing speed and executive function (Chapter 5). Finally, in light of recent intelligence research, further analyses of these data are reported on the corresponding associations between chronological age, plasma T levels, intra-individual variability in RT performance, and processing speed performance (see Chapter 6).

CHAPTER THREE: TESTOSTERONE AND COGNITIVE FUNCTION IN MIDDLE-TO-OLDER AGED MEN: CROSS-SECTIONAL ANALYSES OF DATA FROM THE FLOREY ADELAIDE MALE AGEING STUDY (FAMAS)[1]

3.1. Summary

Recent evidence suggests that declining T levels in ageing males may be associated with both normal and/or pathological cognitive ageing. In remains to be determined, however, whether endogenous gonadal steroid levels in males either mediate or moderate the associations between age and performance on cognitive ability measures, such as, learning and memory, executive function, and processing speed.

These questions were initially investigated by cross-sectional analyses of the baseline data from 1,046 community-dwelling men aged 35-81 years participating in the FAMAS. Multiply adjusted analyses included participants' history of medical conditions, anthropometric measurements, medication use, smoking status, alcohol use and mood. Hormone measurements included TT, BT, calculated free testosterone (cEFT), E2, SHBG, follicle stimulating hormone (FSH), and luteinising hormone (LH). Cognitive measures included the Fuld Object Memory Evaluation (FOME), Trails A and Trails B.

Results showed that higher cEFT and TT levels were associated with both poorer learning and memory and executive function performance and faster processing speed in multiply adjusted analyses. cEFT levels were found to moderate the relationship between age and learning and memory performance quadratically and to mediate the relationship between age and processing speed.

[1] This work has been published (see appendix 1) and material is referenced in subsequent chapters.

3.2. Introduction

The domains of cognitive function most susceptible to the effects of age include psychometric measures of processing speed, reasoning, memory, and spatial abilities (Salthouse, 2004; Schaie, 1994; Verhaeghen & Salthouse, 1997). The decline in these domains begins as early as the mid-twenties and continues almost linearly into old age (Salthouse, 2004). Many factors, for example, health status, demographic, life-style, and genetics contribute to inter-individual differences in the rate and severity of decline.

One factor identified as potentially influential to both normal and/or pathological cognitive ageing in males is T. Plasma T levels decline progressively but variably as men age. The rate and extent of decline is moderated primarily by physical and psychological health and life-style factors (Tan & Pu, 2001; Muller, den Tonkelaar, Thijssen, Grobbee, & van der Schouw, 2003; Vermeulen & Kaufman, 1995; Orwoll et al., 2006). Recently, several large population-based studies of aged community-dwelling males have reported positive associations between T levels and various neuropsychological measures, including those assessing learning and memory (Morley et al., 1997; Moffat et al., 2002; Barrett-Connor et al., 1999; Thilers, Macdonald & Herlitz, 2006), processing speed (Hogervorst et al., 2004; Yaffe et al., 2002; Muller et al., 2005), and executive function (Barrett-Connor et al., 1999; Muller et al., 2005; Perry et al., 2001). These data are not consistent, however, and a number of important questions remain to be resolved. For example, it remains unclear at which age range the greatest effect of T occurs, or whether there is an optimal level at which T affects specific cognitive abilities. In addition, it remains unresolved which T measure best predicts cognitive functioning in males, and whether T levels either mediate or moderate the

relationship between age and performance on measures of cognitive function. A mediational model would suggest that endogenous T levels, at least to an extent, account for the relationship between age and cognition. A moderational model, however, would suggest that T levels directly affect the direction and/or strength of the relationship between age and cognition. To address these questions, I conducted cross-sectional analyses on the baseline data collected for FAMAS, a large population-based cohort study. In this study, three T measures were included, and multiple regression analyses were conducted to control for physical health and life-style factors known to co-vary with both cognition and endogenous T levels.

3.3. Method

Subjects

FAMAS is a cohort study of 1195 males aged 35-81 years, randomly selected from households in the northern and western suburbs of Adelaide using the Electronic White Pages (EWP).

Selection criteria

Participants were excluded from these analyses if they were currently taking T supplements ($N = 6$), had prostate cancer or a history of prostate cancer ($N = 23$), a diagnosed neurodegenerative disorder (i.e., AD, $N = 1$; Parkinson's Disease, $N = 1$), or abnormal thyroid stimulating hormone (TSH) levels (i.e., TSH < 0.10 and TSH > 4, $N = 33$). Only participants with complete data for all relevant measures were included in the analyses reported here ($N = 1046$).

The project was approved by the Royal Adelaide Hospital Research Ethics Committee and by the Aboriginal Health Research Ethics Committee of South Australia, in accord with the Declaration of Helsinki. Written informed consent was obtained from all FAMAS participants.

Questionnaires

Questionnaire A

Questionnaire A included standard demographic questions regarding ethnicity, income, education, and work status, in addition to, health information, such as, medical conditions, prior surgery, medication use and cigarette smoking. It also included the 36-item short-form survey (SF-36; Ware & Sherbourne, 1992), the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), the frequency of symptoms of obstructive sleep apnoea, physical activity level, and the International Prostate Symptom Scale (IPSS; Barry et al., 1992).

Questionnaire B

Questionnaire B assessed sexual desire and erectile dysfunction and comprised the Sexual Desire Inventory 2 (SDI-2; Spector, Carey, & Steinberg, 1996), the International Index of Erectile Function (IIEF; Rosen, Riley, Wagner, Osterloh, Kirkpatrick, & Mishra, 1997), and the Global Impotence Rating (GIR; Feldman, Goldstein, Hatzichristou, Krane, & McKinlay, 1994).

Dietary Questionnaire

Participant's daily alcohol intake was calculated (in grams) from information obtained using the Australian Cancer Council of Victoria's (ACCV) electronically scored dietary questionnaire (Hodge, Patterson, Brown, Ireland, & Giles, 2000).

Cognitive Measures

The Fuld Object Memory Evaluation (FOME)

The FOME (Fuld, 1980) was used to evaluate different component abilities of memory functioning. The test was designed as a screen for dementia; it uses a procedure which minimises cultural, linguistic and educational bias for detecting memory impairments when screening patients for possible dementia. Participants

were initially required to reach into a bag with one hand, then the other, and to try to identify ten common objects by touch alone. The objects were familiar household items: a ball, button, bottle, card, cup, key, matches, nail, ring and scissors. Each item was named out loud following its removal from the bag and then hidden from sight until all 10 objects had been removed. Participants were then asked to verbally repeat the names of the objects and were reminded if any were forgotten. Following a distraction task, which was the Trail Making Test Parts A and B (Reitan & Wolfson, 1993)(see below), participants were asked to again recall the 10 objects. Four more selective reminding trials were then administered without distraction. After a 30-minute delay, participants were asked to recall the 10 objects once again. The task has four outcome measures: Total Recall refers to the total number of items remembered across all five trials; Total Storage refers to the accumulated total of different items recalled across the five trials; Total Memory Repetitions refers to the total number of items recalled on successive trials without reminding; and Total Memory Interference refers to the accumulated total of ineffective reminders across the five trials.

The Trail Making Test

Trails A, from the Trail Making Test, was used as a measure of processing speed. The task is comprised of a page scattered with circled numbers ranging between 1-15. After a practice trial, participants were required to trace a line starting from circle 1 and ending at circle 15 in as little time as possible. If an error was committed at any time during the task, participants were required to restart from the last correct move. Trails B, from the Trail Making Test, was used to assess both processing speed and the ability to alternate between sets of stimuli. The task consists of a similar array of circles containing both numbers (1-13) and

letters (A-L) on a page. Participants were required to draw a line alternating between the circled numbers and letters sequentially in as little time as possible. The Trails B to Trails A ratio (B/A) was used as an index of executive function; scores reflect the attentional capacity necessary to rapidly alternate between two sets (Arbuthnott & Frank, 2000).

Physical Measurements

Finger Tapping Test

The finger tapping test (Reitan & Wolfson, 1993) is a measure of subtle motor and other cognitive impairment. The device measures rapid alternating muscle movements and is comprised of a counter with a lever (Psychological Assessment Resources Inc, Odessa, FL, USA). Participants were required to begin the test with their preferred hand palm down and fingers extended with the index finger placed on the lever. Following this, participants were instructed to depress and release the lever as quickly as they could for 10 seconds moving only the index finger and not the whole hand or arm. Prior to beginning the test, participants had two practice trials for each hand. The test consisted of five trials of 10 seconds duration, first with the preferred hand then the other. The outcome measure was the mean finger tapping score for each hand over the five trials which were within a range of five taps.

Anthropometry

Anthropometrical measurements (i.e., height, body weight, waist circumference, and hip circumference) were performed using the methodology outlined by Norton and Olds (1996).

Height was measured using a wall mounted stadiometer and the stretch stature method. At the end of a deep inhalation, the measurement was taken with the measurer applying a gentle upward lift through the mastoid processes.

Body weight was measured using portable electronic scales which incorporated a load cell (accurate to 100g). These measurements were taken in the morning following an overnight fast with the participant's barefoot and wearing only light clothing.

Waist circumference was measured from the level of the narrowest point (or midway) between the lower costal border and the top of the iliac crest and read in the midaxillary line. The mean of three measurements was used in subsequent analyses.

Hip circumference was measured from the level of the greatest posterior protuberance of the buttocks with the tape maintained in a horizontal plane. The mean of three measurements was used in subsequent analyses.

Handgrip Strength

Bilateral handgrip peak force was determined from maximal isometric contraction using a grip dynamometer (Smedley, Chicago, Illinois, USA) and the maximal voluntary contraction protocol (ACHPER, 1987). Participants were required to complete three repetitions on each hand. Prior to actual measurement, participants each completed at least three repetitions at 50% of maximum, three at 75%, and one at maximum. Subjects had two minutes rest before starting the actual test.

Hormone Assays

Total Testosterone (TT)

Plasma TT was measured by chemiluminescent immunoassay using Elecsys (ROCHE, Indianapolis, USA). The coefficient of variation (CV) for this assay was 9.3% at a concentration of 10.7 nmol/L.

Bioavailable Testosterone (BT)

Serum BT was measured using the ammonium sulphate precipitation method based upon a previously established methodology (O'Connor, Baker, Dulmanis, & Hudson, 1973). The inter-assay CV's for these measurements were 6.15% at 4.99 nmol/L and 14.17% at 0.18 nmol/L and intra-assay CVs were 3.02% at 8.13 nmol/L and 3.32% at 1.38 nmol/L.

Sex Hormone Binding Globulin (SHBG)

SHBG was measured with a solid-phase, two-site chemiluminescent, immunometric assay DPC IMMULITE SHBG (Diagnostic Products Corporation, Los Angeles, CA) following the dilution of samples to 1:21 with SHBG sample dilutent (CV 4.0% at 32.3 nmol/L).

Calculated Free Testosterone (cEFT)

cEFT was calculated using recently validated calculations based on empirical measurements from an Australian sample (Ly & Handelsman, 2005). The equations for low TT (TT < 5nmol/L) and high TT (TT > 5nmol/L) levels were as follows (TT and SHBG in nmol/L):

$$\text{cEFT (low)} = -6.593 + 19.304TT + 0.056SHBG - 0.0959TT \times SHBG$$

$$\text{cEFT (high)} = -52.65 + 24.4TT - 0.704SHBG - 0.0782\ TT \times SHBG - 0.0584\ TT^2$$

Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH), and Estradiol (E2)

FSH, LH, and E2 were measured using an automated enzyme immunometric assay with CV's of 3.1% at 7.0 IU/L for FSH, 4.0% at 7.7 IU/L for LH and 14.0% at 155 pmol/L for E2, respectively.

Statistical Analyses

All tests and transformations were performed with SPSS 12.0 statistical package. Log transformed hormone levels and logged Body Mass Indices (BMI) were used in all analyses to reduce skew. All analyses were adjusted for the following covariates: education, alcohol intake in grams per day, current smoking status, depressive mood as assessed by the Beck Depression Index (BDI), and each participant's history or presence of angina, anxiety, diabetes, cancer, enlarged prostate, high blood cholesterol, high blood pressure, insomnia, osteoarthritis, rheumatoid arthritis, epilepsy, or active thyroid disease. In addition, all analyses were adjusted for the use of medication to treat mood disorders, medications which interact directly with the hypothalamic-pituitary-gonadal (HPG) axis, and medications which contain opiates or opiate derivatives. Participants' average finger tapping score for the dominant hand was also included in the analyses for the Trail Making Tests to control for fine motor ability.

Following Baron and Kenny's three-step model (Baron & Kenny, 1986), linear regression analyses were used to test the mediational hypotheses. First, I regressed hormones onto age to test whether age was associated with hormone levels after adjusting for all covariates. Second, I regressed cognition on age to determine whether age was related to the cognitive outcome variables after

adjusting for all covariates but excluding hormones. Third, I regressed cognition on age and hormones adjusting for all covariates. According to the model, mediation is established if: 1) age is associated with hormone levels; 2) age is associated with cognitive functioning; 3) hormone levels are associated with cognitive functioning and the association between age and cognition is attenuated when it is compared with the outcome at step two. Significance testing for the indirect effect of age on the cognitive outcome variables via mediating hormonal levels was conducted using Sobel's equation (Sobel, 1982).

Moderation effects were tested with the addition of interaction terms (i.e., age*hormone level) to the multivariate model. Non-linear relationships and quadratic moderation effects were tested with the addition of quadratic terms to hierarchical regression analyses. Analyses of covariance using quintiles of hormone levels were used to graph the data.

Procedure

Upon recruitment into FAMAS, participants were mailed a comprehensive questionnaire (Questionnaire A), a dietary questionnaire, a participant information sheet and clinic appointment details. Both Questionnaire A and the dietary questionnaire were completed by all FAMAS participants prior to their first clinic visit. After an overnight fast, participants attended one of two regional clinics where plasma was collected between 0800 and 1000 hrs, anthropometric measurements obtained (i.e., height, weight, waist and hip measurements), blood pressure, hand grip strength, and finger tapping assessed and neuropsychological tests administered. At this first clinic visit all participants also completed Questionnaire B. All questionnaires were carefully checked for completeness, clarity, and consistency at the clinic visits.

3.4. Results

Descriptive Data

The descriptive characteristics of the FAMAS cohort are shown in Table 3. The mean age of the men was 54 years which is slightly younger than men in comparable studies (e.g., Thilers et al., 2006; Hogervorst et al., 2004; Muller et al., 2005; Fonda et al., 2005).

Relationships between Age, Hormones, and Cognitive Functioning

All zero order correlations between age, log transformed hormone levels and cognitive functioning outcome variables are shown in Appendix 2. The results of the first step of the regression analyses for testing the mediational hypotheses are shown in Table 4. After adjusting for covariates, increased age was associated with lower TT, BT, and cEFT levels and with increased E2, SHBG, FSH, and LH levels.

For the second step of the regression analyses, after adjusting for all covariates increased age was associated with poorer performance on Total Recall (b = -0.138, p <0.001), Total Storage (b = -0.046, p <0.001), and Total Memory Repetitions (b = -0.173, p <0.001) from the FOME. Also on the FOME, age was associated with increased scores for Total Memory Interference (b = 0.051, p <0.001). For the Trail Making Tests, increased age was associated with poorer performance (i.e., longer durations) for both Parts A (b = 0.194, p <0.001) and B (b = 1.30, p <0.001) and higher Trails B to Trails A ratios (b = 0.021, p =0.002).

The results of the third step of the regression analyses are presented in Table 5. In multiply adjusted models, higher TT and cEFT levels were negatively associated with performance across all four FOME variables (see Models 1-14.). In contrast, higher BT levels were negatively associated with Total Memory

Interference (see Models 15-21.) Higher TT and cEFT levels were also associated with faster Trails A performance. After adjusting for covariates, no hormone measure significantly predicted Trails B performance, however, both higher TT and cEFT levels were associated with higher ratios of Trails B to Trails A performance. Similarly, higher SHBG levels were associated with lower Trails B to Trails A ratios.

Consistent with a model of partial mediation, the un-standardised regression coefficients for the effect of age on Trails A were attenuated after adjusting for cEFT levels. Similarly, the effect of age on Trails A was also attenuated after adjusting for SHBG levels. After adjusting for BT levels, the un-standardised regression coefficient for the effects of age was attenuated for Total Memory Interference from the FOME. Similarly, adjustment for LH levels attenuated the effects of age on both Total Recall and Total Memory Interference from the FOME. The results of the significance testing for indirect mediational effects are presented in Table 5. The relationship between quintiles of cEFT, TT and BT levels with Trails A performance is shown in Figure 1.

Table 3. Descriptive data of study variables (N = 1046)

Variable	Mean (S.D.) or %	*Range* Lower	Higher
Age (years)	54.3 (11.3)	35	80
Hormones:			
TT (nmol/L)	14.1 (5.32)	0.20	45.7
BT (nmol/L)	5.10 (1.84)	0.45	14.4
cEFT (pmol/L)	212 (95.1)	-24.2	735
E2 (pmol/L)	74.6 (40.2)	24	584
SHBG (nmol/L)	35.1 (16.1)	6	180
FSH (IU/L)	8.47 (9.40)	1	93
LH (IU/L)	6.01 (6.10)	1	88
Cognitive Measures:			
FOME			
Total Recall	38.9 (5.51)	14	50
Total Storage	45.4 (2.60)	31	50
Total Memory Repetitions	25.2 (7.07)	1	40
Total Memory Interference	1.65 (2.37)	0	18
Trails A (secs)	15.9 (6.59)	5	61
Trails B (secs)	81.8 (36.5)	25	480
TrailsB/Trails A	5.38 (1.91)	1.84	19.9
Physical Measures:			
Average Waist Circumference (cm)	101 (12.2)	71.0	163
Body Mass Index (kg/m^2)	28.5 (4.59)	16.8	54.9
Average Dominant Finger Tap	48.4 (7.87)	20.6	76.4
Health Measures:			
Angina	5.35 %	0 (No)	1 (Yes)
Anxiety	8.41 %	0 (No)	1 (Yes)
Diabetes	8.80 %	0 (No)	1 (Yes)
High Blood Cholesterol	31.5 %	0 (No)	1 (Yes)
High Blood Pressure	27.7 %	0 (No)	1 (Yes)
Insomnia	9.18 %	0 (No)	1 (Yes)
Osteoarthritis	7.84 %	0 (No)	1 (Yes)
Rheumatoidarthritis	3.92 %	0 (No)	1 (Yes)
Thyroid Problems	0.96 %	0 (No)	1 (Yes)
Enlarged Prostate	7.84 %	0 (No)	1 (Yes)
Other Cancers	8.32 %	0 (No)	1 (Yes)
Epilepsy	0.38 %	0 (No)	1 (Yes)
Current Smoking Status:	1.42 (0.79)	1	3
No = 1	77.5 %		
Occasionally = 2	3.15 %		
Yes = 3	19.3 %		
Alcohol intake (grams/day)	19.9 (21.8)	0	111
Education:	3.64 (1.69)	1	6
Did not finish High School = 1	21.8 %		
High School = 2	7.00 %		
Other Post High School Qualification =3	4.11 %		
Trade Certificate = 4	32.7 %		
Certificate/Diploma = 5	22.8 %		
Bachelor Degree + = 6	12.0 %		
Dysphoria:			
Beck Depression Inventory (BDI)	5.45 (6.66)	0	128
Medication use:			
Mood Disorder Drugs	5.74 %	0 (No)	1 (Yes)
HPG Axis Drugs	1.05 %	0 (No)	1 (Yes)
Opiate Drugs	6.79 %	0 (No)	1 (Yes)

Notes: TT indicates total testosterone; BT, bioavailable testosterone; cEFT, calculated free testosterone; E2, estradiol; SHBG, sex hormone binding globulin; FSH, follicle stimulating hormone; LH, luteinising hormone; FOME, Fuld Object Memory Evaluation; HPG Axis, hypothalamic-pituitary-gonadal axis.

Table 4. The effects of age on hormone levels (N = 1046).

Hormones	Unstandardised regression coefficients	*P*
TT	-0.002	0.001
BT	-0.006	<0.001
cEFT	-0.004	<0.001
E2	0.001	0.022
SHBG	0.006	<0.001
FSH	0.008	<0.001
LH	0.004	<0.001

Notes: Logarithmically transformed values used in analyses.
All analyses are adjusted for covariates (see text).
TT indicates total testsosterone; BT, bioavailable testosterones; cEFT, calculated free testosterone; E2, estradiol; SHBG, sex hormone binding globulin; FSH, follicle stimulating hormone; LH, luteinising hormone.

The moderational hypotheses were tested by repeating these analyses with the addition of interaction terms. Significant interaction effects were observed for TT and cEFT on all FOME performance variables and for Trails B/Trails A performance. Interactions between SHBG levels and age on Total Recall, Total Memory Repetitions, and Trails B/Trails A performance, however, were not significant. Stepwise hierarchical multiple regression analyses with the addition of quadratic terms revealed significant quadratic moderation terms for the interaction effects of TT levels and age and cEFT levels and age on all FOME variables in multiply adjusted models. The partial correlation coefficients for these terms from these analyses are presented in Table 6. The relationship between quintiles of cEFT levels, age, and Total Recall performance is shown in Figure 2.

Table 5. Un-standardised regression coefficients (b) and *p* values for the effects of both hormone levels and age on cognitive functioning (*N* = 1046).

	TotRec	TotStor	TotMRep	TotMInt	TrailsA	TrailsB	TrailsB/TrailsA
Models 1-7.							
TT	-7.52	-2.25	-9.70	1.61	-3.89	-7.17	0.989
p values	<0.001	<0.001	<0.001	0.003	0.001	0.235	0.008
Age	-0.151	-0.050	-0.190	0.053	0.187	1.29	0.023
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Models 8-14.							
cEFT	-6.54	-2.02	-8.38	1.39	-3.90**	-6.90	0.956
p values	<0.001	<0.001	<0.001	0.002	<0.001	0.136	0.001
Age	-0.167	-0.055	-0.211	0.057	0.176	1.27	0.025
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Models 15-21.							
BT	2.28	0.993	2.57	-1.24*	0.542	-5.00	0.016
p values	0.053	0.080	0.090	0.016	0.674	0.468	0.970
Age	-0.124	-0.040	-0.157	0.043	0.198	1.27	0.021
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
Models 22-28.							
LH	-1.60*	-0.421	-2.20*	0.347	-0.396	1.88	0.309
p values	0.018	0.195	0.011	0.236	0.591	0.635	0.205
Age	-0.132	-0.045	-0.165	0.049	0.196	1.30	0.020
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
Models 29-35.							
SHBG	2.39	0.909	3.02	-0.539	4.01**	2.20	-0.987
p values	0.018	0.061	0.020	0.218	<0.001	0.710	0.007
Age	-0.153	-0.052	-0.192	0.054	0.170	1.29	0.027
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Models 36-42.							
E2	-1.06	-0.055	-1.40	0.729	1.29	-0.960	-0.272
p values	0.224	0.897	0.215	0.054	0.176	0.851	0.388
Age	-0.137	-0.046	-0.171	0.050	0.192	1.30	0.021
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Models 43-49.							
FSH	0.043	0.088	-0.111	-0.081	-0.126	5.73	0.364
p values	0.941	0.752	0.883	0.746	0.842	0.091	0.082
Age	-0.139	-0.047	-0.173	0.051	0.195	1.26	0.018
p values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007

Notes: Logarithmically transformed values used in analyses. ** Indicates significant mediation effect, $p < 0.01$ * Indicates significant mediation effect, $p < 0.05$; TotRec indicates total recall; TotStor, total storage; TotMRep, total memory repetitions; TotMInt, total memory interference; TT, total testosterone; cEFT, calculated free testosterone; BT, bioavailable testosterone; LH, luteinising hormone; SHBG, sex hormone binding globulin; E2, estradiol; FSH, follicle stimulating hormone. All models are adjusted for covariates.

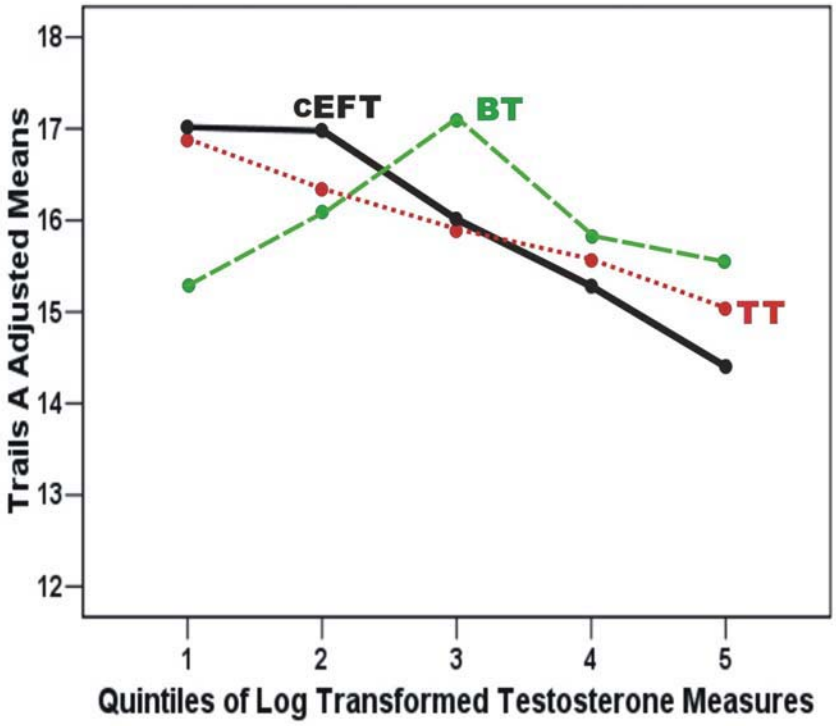

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Figure 1. Represents the effects of log transformed quintiles of T levels on Trails A from the Trails Making Test. Means for cEFT and TT levels are adjusted for age, thyroid problems, cancer, and dominant hand average finger tapping score. Means for BT levels are adjusted for age, thyroid problems and dominant hand average finger tapping score (N = 1046).

Table 6. Partial correlation coefficients and significance levels from the regression analyses for the effects of T and age on FOME performance (N =1046)

	Total Recall		Total Storage		Total Mem Rep		Total Mem Int	
Interaction Term	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
$(Age*LogTT)^2$	-0.369	<0.001	-0.245	<0.001	-0.364	<0.001	0.279	<0.001
$(Age*LogcEFT)^2$	-0.353	<0.001	-0.241	<0.001	-0.348	<0.001	0.121	<0.001

Notes: All analyses are adjusted for covariates.
Total Mem Rep indicates total memory repetitions; Total Mem Int, total memory interference; LogTT, logarithmically transformed total testosterone; LogcEFT, logarithmically transformed calculated free testosterone.

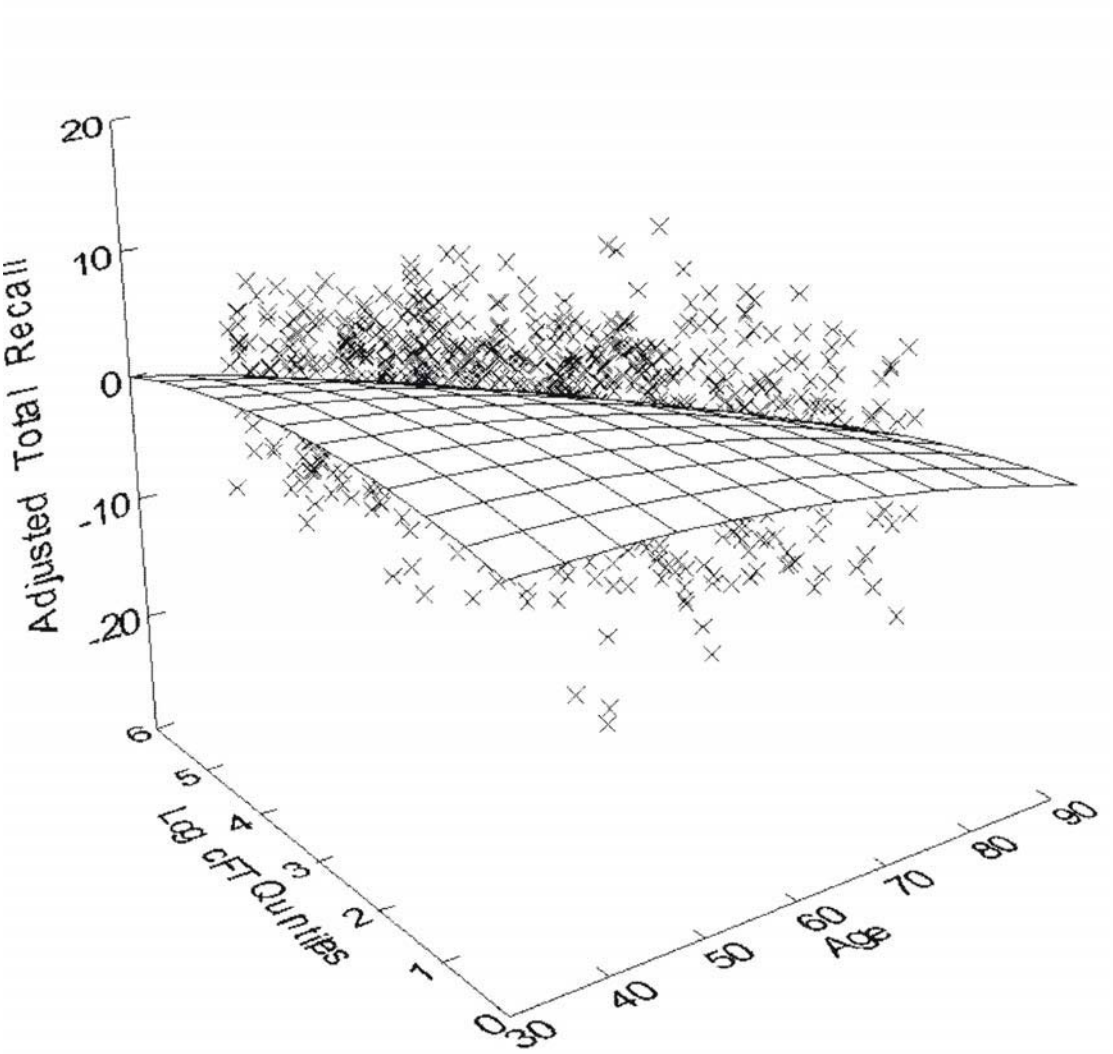

Figure 2. Represents the quadratic moderation effect of log transformed quintiles of cEFT levels and age on Total Recall from the Fuld Object Memory Examination (FOME). Means are adjusted for current smoking status, insomnia, dysphoria, epilepsy, and log transformed BMI (N =1046).

3.5. Discussion

The results of this study showed that higher TT and cEFT levels were associated with better performance on a measure of processing speed and poorer performance on measures of both learning and memory and executive function after adjustment for age, and salient health and lifestyle variables in a large sample of community dwelling middle-to-older aged males.

The finding that cEFT levels mediated the relationship between age and processing speed was consistent with other studies relating higher FT levels to faster processing speed (Hogervorst et al., 2004; Yaffe et al., 2002; Muller et al., 2005). In contrast, a recent study reported no such relationship using the Digit Symbol Substitution Test in a similarly sized sample of 981 men (Fonda et al., 2005). In this study, however, the researchers did not control for fine motor ability, which affects performance on these types of tasks (Kreiner & Ryan, 2001). The observation that none of the hormone measures predicted Trails B performance after controlling for confounds was consistent with similar studies with similar study samples (Barrett-Connor et al., 1999; Yaffe et al., 2002; Perry et al., 2001). In contrast to other studies which have reported positive relationships between T and executive function (i.e., Barrett-Connor et al., 1999; Muller et al., 2005; Perry et al., 2001), in the present study, both higher TT and cEFT levels were associated with higher ratios of Trails B to Trails A, or poorer executive function performance. Importantly, however, none of these other studies used executive function tests specifically sensitive to set-shifting ability. The ratio of Trails B to Trails A performance predicts poorer set-shifting ability (Arbuthnott & Frank, 2000), an executive function demonstrated by neuro-imaging studies to be modulated by the DLPFC (Smith, Taylor, Brammer, & Rubia, 2004; Nagahama et al., 2001). The

DLPFC is similarly employed during the performance of both WM (D'Esposito et al., 1999; D'Esposito, Postle, & Rypma, 2000; Barde & Thompson-Schill, 2002) and long-term memory tasks (Rossi, Cappa, & Babiloni, 2001; Rossi, Miniussi, Pasqualetti, Babiloni, Rossini, & Cappa, 2004; Simons & Spiers, 2003), which has lead some researchers to postulate that, in addition to modulating set-shifting ability, the DLPFC both organises material prior to encoding (Fletcher, Shallice, & Dolan, 1998; Blumenfeld & Ranganath, 2006) and monitors and verifies information during memory retrieval (Simons & Spiers, 2003; Dobbins, Foley, Schacter, & Wagner, 2002; Achim & Lepage, 2005). Consistent with these observations, in the present study it was shown that higher T levels were similarly associated with both higher Trails B to Trails A ratios and poorer FOME performance. The question of whether shared mechanisms are responsible for these associations, however, remains to be determined.

This is the first study to show that both higher TT and cEFT levels are associated with poorer learning and memory functioning in middle-to-older aged males. The quadratic moderation effects found for both TT and cEFT levels on the relationship between age and performance on all the FOME measures, further suggests that higher T levels may amplify the effect of ageing on memory functioning in men. In contrast to these results, higher BT levels were found to partially mediate the relationship between age and Total Memory Interference performance. This result highlights the importance of different T measures and methods of T measurement on hormone-cognition associations.

The difference between the associations found for both TT and cEFT, compared to BT, on FOME Total Memory Interference performance may stem from circadian variation in morning levels of serum T fractions. Previously it has been

shown that during the period between 0330-1000 hrs the BT fraction of serum T declines by 15-45% relative to both the TT and cEFT fractions (Cooke, McIntosh, & McIntosh, 1993), an effect which occurs concurrently with the rise in cortisol from its nocturnal nadir (Cooke et al., 1993; Cooke, McIntosh, & Murray-McIntosh, 1996). Because this effect coincided with the period of cognitive testing in the present study, it is possible that during this time the BT measure may have been incorrect and therefore, did not represent the biologically active fraction of T within the brain as precisely as TT or cEFT.

The present findings are consistent with a small randomised control study in healthy older eugonadal males which found that three months of T treatment had negative effects on verbal memory (Maki, Brandt, Dobs, Mordecai-Strom, & Resnick, 2004). Similarly, three smaller studies have reported negative but non-significant relationships between TT and verbal memory in elderly men after controlling for known confounds (Hogervorst et al., 2004; Wolf & Kirschbaum, 2002; Aleman et al., 2001). In contrast, other studies have either reported positive linear correlations (Moffat et al., 2002; Barrett-Connor et al., 1999), or found no relationship at all between T and verbal memory performance (Muller et al., 2005; Aleman et al., 2001; Carlson & Sherwin, 1998). For example, Moffat et al. (2002) found that in 407 healthy elderly males (mean age = 64.07, *SD* = 9.4 years), higher free testosterone indices (FTI) (i.e., the ratio of TT to SHBG levels) were associated with better verbal memory performance as assessed by the California Verbal Learning Test (CVLT; Delis, Kramer, Kaplan, & Ober, 1987). In contrast, Muller et al. (2005) failed to find a relationship between verbal memory performance and either TT or FT in multivariate models. In the present study, the analyses were conducted with a larger sample size (i.e., *N* = 1046; versus *N* = 193, Moffat et al.,

2002; and $N = 395$, Muller et al., 2005) and a different method to calculate FT (i.e., Ly & Handelsman, 2005). In addition, Moffat et al. (2002) analysed longitudinal data and had both an older and healthier study sample, which also excluded subjects who had either cerebrovascular disease, non-skin cancer, or who met criteria for probable or possible Alzheimer's Disease (AD). Therefore, neither men with high FT levels (i.e., men < 50 years), nor men with poor memory performance (i.e., men with dementia, or at risk for dementia), were represented in their data. Although Muller et al. (2005) reported no relationship and utilised a similar sample of subjects to the present study, both in age range and health status, in their analyses of T quintiles, higher levels were associated with poorer memory performance for both T measures, which is consistent with the present results.

A potential limitation to the present findings was the likely possibility that there were men in the FAMAS cohort who had either subtle pre-existing cognitive impairments or were in the prodromal preclinical phases of pathological neurodegenerative disorders, such as AD. Given this possibility, it is important to note that this 'noise' in the data was presently unaccounted for and hence may have influenced the reported associations between T levels and cognition. Alternatively, it is also plausible that this 'noise' in the data may actually be integral to the reported effects and, therefore, capture an important aspect of the progression of AD and the possible influence of changes in endogenous T levels. In Section 7.4 'Study Limitations' a genetic marker for AD is discussed which should be taken into account in future studies to help to elucidate this issue.

Another limitation to the present results was our assay's lack of sensitivity to detect E2 in the lower concentrations typically found in older men. This limitation may account for the lack of associations presently found between E2 levels and

cognitive functioning in this sample. Future studies may overcome this issue through the use of high-sensitivity liquid chromatography tandem mass spectrometry assays (e.g., Kushnir et al., 2008).

In conclusion it was found that higher TT and cEFT levels were associated with faster processing speed and both poorer executive and learning and memory functioning in a large sample of community residing middle-to-older aged males. Furthermore, cEFT levels were found to both quadratically moderate the relationship between age and learning and memory functioning and mediate the relationship between age and processing speed.

CHAPTER FOUR: COMMON METHODOLOGIES

4.1. Summary

In this chapter the methodologies common to the research presented in Chapter's Five and Six are detailed.

4.2. Introduction

The following methods detail the selection criteria, materials and apparatus, and study procedure common to the analyses presented in Chapter's Five and Six. Please refer to these chapters for further information pertaining to data cleaning and statistical analyses.

4.3. Participants and Selection Criteria

Participants ($N = 96$) were recruited from the 1195 men aged 35 – 80 years participating in FAMAS (see Chapter 3). Participants were recruited to form three age groups: 38-49 years ($N = 29$), 50-59 years ($N = 37$), and 60-69 years ($N = 30$).

Only right-handed, exclusively heterosexual men, without any self-reported history of anxiety, depression, diabetes, thyroid problems, or prostate cancer, and with an average daily alcohol consumption of below 40 grams per day were included in this study. This information was obtained from the FAMAS database (see Chapter 3). Men were excluded if they were currently taking hormone supplements or medication likely to affect mental performance, had a history of psychiatric illness or drug abuse, suffered from a neuropsychological condition, or had recently suffered a head injury. This information was based on self-report.

4.4. Materials and Apparatus

Questionnaires

Handedness Questionnaire

An English translation of The Dutch Handedness Questionnaire (Van Strien, 2002) was used as a measure of handedness. The questionnaire consists of 10 questions which are scored +1 for 'right', 0 for 'both', and –1 for 'left'. Scores vary from –10 for extremely left-handed to +10 for extremely right-handed.

Cognitive tests

Mental Rotation

The Vandenberg & Kuse Mental Rotation test: I used a computerised version of the Vandenberg and Kuse (1978) MRT, which assesses three-dimensional mental rotation ability. Each item consisted of a target drawing and four test drawings. Participants were required to designate which two of the four test drawings correctly depicted the target drawing in rotated positions. To select an item, participants were required to use the computer mouse to click numbered boxes displayed under each alternative. To answer each item, participants had to select two items then click a button displayed at the bottom of the screen to finalise the response. Alternatively, participants could skip the question by selecting no items and clicking the button at the bottom of the screen. The test had 20 items which were administered in two separate sections comprised of 10 items each. Participants had five minutes per section to complete as many of the questions as possible. Participants were instructed to work as quickly as possible without sacrificing accuracy. In addition, they were told that their score would refect both correct and incorrect responses and that it would not be to their advantage to guess. Prior to commencing the actual test, participants were required successfully to

complete three practice items. One point was awarded only if both choices per question were correct and the measure for the test was the total number of points (maximum score 20).

Tests of Executive Function

The Self-Ordered Pointing Task (SOPT): The SOPT is a test of executive function which has been demonstrated as sensitive to frontal lobe dysfunction (Petrides & Milner, 1982); it assesses the capacity to initiate and execute a sequence of responses with constant monitoring of performance (Bryan & Luszcz, 2001). I used a computerised version of the test where participants were presented with four levels consisting of arrays of 6, 8, 10, or 12 abstract designs all of which were different. The level with 6 designs was presented first, followed by the levels with 8, 10, then 12 designs. Each level had three separate trials. For each trial an equal number of screens was presented as there were designs on the screen. For each screen the array of designs was reshuffled into different spatial positions on a grid. Participants were instructed to use the mouse to click on one design per screen and to continue the task by always clicking on a design that they had previously not clicked on until the end of each trial. Participants were also instructed that the test was not timed and that they could spend as much time as needed to complete the task. An error was recorded whenever participants clicked on a previously selected design. If participants selected a particular spatial location four times in succession, a warning was presented on the screen notifying the use of an illegal strategy. Consistent with the previous study which utilised this test (i.e., Cherrier et al., 2005), the outcome measure for this task was the total number of errors committed across the levels with 10 and 12 designs.

The Sustained Attention to Response Test (SART): The SART (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997) is a computer-administered test of executive function which has been demonstrated via fMRI to recruit regions of the PFC, including the right ventral PFC and the left DLPFC (Fassbender et al., 2004). The stimuli consisted of the individual presentation of randomly selected digits between 1 and 9, displayed in one of five randomly assigned fonts (48 point, 72 point, 94 point, 100 point, and 120 point). Each digit was presented for 245 msec and was followed immediately by a mask, which was presented for 900 msec. The mask consisted of a 29mm ring with a diagonal cross in the middle. Participants were required to depress the left mouse button for all digits except the digit '3'. There were 225 trials and the digit '3' was presented randomly either 24 or 25 times. The measure for this task was the percentage of the total number of trials where the participant did not 'hit' the mouse when the digit '3' was presented. Higher scores indicated better performance.

Processing Speed Tests

The Digit Symbol: The Digit Symbol is a pen-and-paper administered test from the Wechsler Adult Intelligence Scale Revised (WAIS-R; Wechsler, 1981) and is an excellent index of general processing speed (Gs; Burns & Nettelbeck, 2003). The task was administered following the instructions in the manual (Wechsler, 1981). Participants had 90 seconds to complete as many items as possible. The measure for the task was the total number of items answered correctly.

Cross Out: The Cross Out is a pen-and-paper administered subtest taken from the Woodcock-Johnson Psycho-Educational Battery-Revised (WJ-R; Woodcock & Johnson, 1989) which measures Gs. Participants were required to cross out the exact copy of a target symbol amongst four alternatives as quickly and

accurately as possible. Participants had three minutes to complete as much of the task as possible. The measure for the task was the total number of items answered correctly.

Visualisation Speed/Perceptual Discrimination Tasks

Inspection Time (IT): Inspection Time is a computer-administered task, which measures Visualisation Speed (Vsp): the "time needed to perform tests requiring somewhat complex visualisation of stimuli…"(O'Connor & Burns, 2003). The target stimulus consisted of two vertical lines 22 mm and 27 mm long joined at the top by a horizontal line, 12 mm long. The shorter of the two lines appeared on the left or right of the screen equiprobably. Prior to the target stimulus presentation, a small white circle acted as a warning cue. This was presented for approximately 520 ms. Following immediately after the target stimulus presentation was a "flash mask" (Evans & Nettelbeck, 1993), consisting of two vertical lines 37 mm in length shaped like lightening bolts. The flash mask was presented for 290 ms. Participants were instructed to indicate, via the keyboard, which of the two vertical lines was shorter, the left or right. Prior to commencing the test, participants had three sets of 10 practice trials to familiarise themselves with the task; the stimulus onset asynchronies (SOA)s for the three sets of practice trials were 976 ms, 512 ms and 304 ms, respectively. IT was estimated using an adaptive staircase algorithm (Wetherill & Levitt, 1965). Each participant commenced with an SOA of 256 ms. Following this, three correct responses for a given SOA corresponded with a decrease in the SOA by 16 ms, whilst an incorrect response corresponded with an increase in the SOA by 16 ms. After eight reversals in direction, the average SOA was calculated with an associated probability of .79 for a correct response.

Odd Man Out (OMO)- Decision Time (DT): The OMO-DT task (Frearson & Eysenck, 1986) is another measure of VSp (O'Connor & Burns, 2003). The apparatus for the task was based on the display and response panel described by Jenson and Munro (1979) but scaled down to a smaller panel (15 X 24 cm) tilted at 30º with eight response buttons (each with a corresponding red light) arranged in a semi-circle around a home button. Every red light was spaced 3.5cm apart and equidistant (10cm) from the home key. For each trial, three of the eight lights were illuminated such that the distance between the left light and the centre light was different from the distance between the right light and the centre light. Participants were required to indicate, by pressing the response key corresponding to the target light, which of the lights was the furthest away (i.e., the "odd-man-out"). The task consisted of 60 trials. Prior to commencing the task each participant had completed 20 practice trials. The measures for the task were DT, which was measured from the onset of the stimulus presentation until the home button is released, and movement time (MT), which was measured from the release of the home button until the target button is pressed. Both measures were recorded for each correct response. The outcome measure for this test was the median DT for all correct responses.

Reaction Time Tasks

Simple Reaction Time (SRT): The same apparatus from the OMO task was used to measure SRT. Participants were required to hold down the home button with their preferred finger and, immediately following the light 'switching on' directly above the button, to release their finger as quickly as possible. For each trial the light above the home button was presented after a pseudo-random period of 1-8 seconds. The task consisted of 60 trials. Prior to commencing the actual test

participants had completed 10 practice trials. The outcome measure of this task was the median DT measured between the stimulus presentation and the release of the home button.

Choice Reaction Time (CRT): The same apparatus was used to measure CRT. Participants were required to hold down the home button with their preferred finger and, immediately following one of the other eight lights 'switching on', to release the home button and press the corresponding response key as quickly as possible. The task had three conditions (2 lights, 4 lights or 8 lights) which differed on the number of lights that needed monitoring. For each condition there were 60 trials. Participants had completed 20 practice trials prior to commencing each condition. Only the DT measurements from this task were used subsequent analyses.

Working Memory Tasks

Dot Matrix Task: I used a computer-administered version of the Dot Matrix Task (Law, Morrin, & Pellegrino, 1995), a measure of visuo-spatial WM. The task required participants to verify a series of simple matrix equations whilst simultaneously remembering dot locations on a 5 X 5 grid. Each matrix equation to be verified was either an addition or subtraction equation presented as lines drawn on 3 X 3 dot matrices. Participants had 10 seconds to verify each equation by using the mouse to click on either the "True" or "False" buttons displayed on the screen. If the time expired without a response, a warning was displayed on the screen indicating that a response was required. If an incorrect response was selected, the message "No, look again closely" was displayed. Immediately following a correct response a 5 X 5 grid was displayed for 1500 ms with a dot presented in one of the squares. After each trial, a 5 X 5 blank grid was displayed on the screen.

Participants were required to select with the mouse the spaces on the blank grid which had contained the dots. The test had four levels (i.e., 2, 3, 4, and 5 equation-grid pairs) with four items per level (total 16 items). The level with two equation-grid pairs was presented first, followed by the levels with three, four, and five equation-grid pairs. For each item participants were not allowed to select more grid spaces than there were equation-grid pairs but they could select fewer grid spaces than required. To enter their selection and move to the next question, participants had to click a button displayed at the bottom of the screen. Prior to commencing the actual test participants had to successfully complete three practice items consisting of two equation-grid pairs. The measure for the task was the total number of dot positions correctly recalled.

Picture Swaps: I used a computerised adaption of the Swaps test (Stankov, 2000), a measure of WM. Each item consisted of three different cartoon pictures of animals and everyday objects (e.g., rocket, glove, elephant), which were simultaneously presented on the computer screen. Each picture was referred to as being in a position, either 1, 2 or 3. Accompanying each item were instructions which told participants to conduct a series of interchanges or "swaps" of the positions of the pictures mentally. For example, if the objects 'rocket glove elephant' were presented accompanied with the instructions "Swap 2 and 3" the answer would be 'rocket elephant glove'. Participants could proceed to the response screen by clicking a button displayed at the bottom of the screen whenever they were ready. Displayed on each response screen were all six possible re-orderings of the three objects from which participants were required to select with the mouse the order they thought was correct. The test had three levels: The first required two swaps, the second three swaps, and the third four swaps. There were

24 items in total with items 1-4 requiring two swaps, items 5-13 requiring three swaps, and items 14-24 requiring four swaps. Participants had successfully to complete two practice items with one swap and two with two swaps prior to commencing the actual test. Participants were instructed to perform the swaps 'mentally' as quickly and accurately as possible. The test allowed 10 minutes for the completion of as many items as possible. The measure for the task was the total number of correct responses.

Crystallised Intelligence (Gc)

Information: I used a computerised multiple-choice general knowledge test adapted from various versions of the Information test, a sub-test from the Wechsler scales, as a measure of Gc. This test consisted of 30 items which tapped general knowledge about common events, objects, places and people. For every item there were four response alternatives. Participants were required to use the mouse to click on the letter next to the answer they thought correct (a, b, c, or d). One point was awarded for every correct response and the final score was the total number of items answered correctly.

Laboratory Measurements

Plasma Total Testosterone (TT)

TT was determined by a solid-phase, competitive chemiluminescent enzyme immunoassay, IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, CA). At a concentration of 14.3 nmol/L, the intra and inter-assay coefficients of variation (CV) for this assay were 7.2% and 8.2%, respectively. This assay had an analytical sensitivity of 0.5 nmol/L.

Sex Hormone-Binding Globulin (SHBG)

A solid-phase, two-site chemiluminescent immunometric assay (IMMULITE 2000) was used to determine SHBG (intra and inter-assay CVs were 2.5% and 5.2%, respectively, at 21 nmol/L). The analytical sensitivity of this assay was 0.02 nmol/L.

4.5. Study Procedure

Information regarding participant's history of anxiety, depression, prostate cancer, current average daily alcohol consumption, and sexuality was obtained from the FAMAS database (see Chapter 3) and used as a basis for selection. Initial handedness evaluations for participant selection were based on the dominant hand(s) from the handgrip strength assessment (see Chapter 3). Letters were sent introducing the study with an information sheet attached to those participants who met these initial selection criteria ($N \approx 600$). The letter and information sheet explained the purpose of the study, what was required, and the exclusion criteria. The letters also informed potential participants that they could expect to be contacted by telephone.

The study took place at the Royal Adelaide Hospital between 09:30-12:00 hrs. Prior to the commencement of the study each participant was required to complete the Dutch Handedness Questionnaire. Informed consent was obtained from all participants.

Between 09:30 and 10:00 hrs participants each had approximately 10mL of blood drawn from a forearm vein by venipuncture. Samples were processed then stored at –70 °C until the study's completion. Participants were tested either individually or in pairs, in sessions approximately two hours in duration conducted under quiet conditions. The battery of tasks was administered in the following

order: Cross Out, Digit Symbol, Dot Matrix, Vandenberg and Kuse MRT, Information, Swaps, SOPT, SART, SRT, CRT, OMO, then IT. This order was maintained for all participants. Both the Cross Out and Digit Symbol tests were administered following verbally presented instructions in accordance with their corresponding manuals. For both tasks speed and accuracy were emphasised as important. A stopwatch was used to time both tasks which together took about five minutes to complete. Next, the Dot Matrix task, the Vandenberg and Kuse MRT, Information, Swaps and the SOPT were administered on computers following both visually and verbally presented instructions. For the Dot Matrix task, the Vandenberg and Kuse MRT, and the Swaps test, participants were required to correctly complete three practice trials prior to beginning each task. Following the completion of the SOPT, participants were asked to complete a short questionnaire which enquired about strategy use. Together these four tasks took approximately 90 minutes to complete. The SART was then administered following individually presented verbal instructions. Participants were informed that they should use their preferred hand to respond with the mouse. This task took approximately five minutes to complete. The SRT, CRT, and OMO were then administered following individually presented verbal instructions. For all three RT tasks participants were instructed to use the same finger. CRT was administered following the instructions of Jensen and Munro (1979). For the OMO task, participants were instructed not to release the home button with their finger until after they had made their decision. Together the RT tasks took approximately 20 minutes to complete. Finally, IT was administered following both visually and verbally presented instructions relayed individually to all participants. The IT task took approximately eight minutes to administer.

CHAPTER FIVE: ENDOGENOUS TESTOSTERONE LEVELS, MENTAL ROTATION PERFORMANCE, AND CONSTITUENT ABILITIES IN MIDDLE-TO-OLDER AGED MEN[2]

5.1. Summary

Evidence from both human and animal studies suggests that gonadal steroids, such as T, exert activational effects on adult spatial behaviour. Endogenous T levels decline gradually yet variably as men age; however, it remains to be shown whether these decreases are associated with age-related declines in visuo-spatial performance or constituent abilities indicative of generalised age-related cognitive decline.

Ninety-six healthy, community dwelling men aged between 38 and 69 years were recruited from FAMAS and completed the Vandenberg and Kuse MRT together with a battery of tests assessing processing speed, executive function, perceptual discrimination, WM, and RT measures. Significant main effects of tertiles of cEFT levels were found on composite measures of processing speed, executive function, and perceptual discrimination in men aged over 50 years in both age and Gc controlled analyses; higher cEFT levels were associated with poorer performance. Additionally, hierarchical multiple regression and path analyses on the whole data set showed that cEFT levels negatively moderated processing speed performance, which in turn predicted both poorer WM and MRT performance. Together these data suggest that age-related declines in endogenous T levels in healthy middle-to-older aged men are not associated with generalised age-related cognitive decline. Instead, the present results together with the results detailed in

[2] This work has been published (see appendix 2) and material is referenced in subsequent chapters.

Chapter Three, suggest that higher cEFT levels in middle-to-older aged males may be associated with poorer cognitive functioning.

5.2. Introduction

T supplementation studies in older males have consistently demonstrated improvements in performance on tests of spatial ability (Cherrier et al., 2001; Cherrier et al., 2004; Cherrier et al., 2005; Cherrier et al., 2007; Janowsky et al., 1994). Together these data suggest that androgens have activational effects which facilitate spatial behaviour in males and that these effects are most evident in older men. It remains to be resolved, however, whether age-related declines in circulating T levels are associated with poorer performance on tests of spatial ability as males age or whether these changes are associated with generalised age-related cognitive decline.

Evidence from studies in animals (Williams et al., 1990; Williams and Meck, 1991; Isgor and Sengelaub, 1998) and from studies in humans (Imperato-McGinley et al., 1991; Masica, Money, Ehrhardt, & Lewis, 1969) shows that androgens exert early organisational effects on spatial ability which, in turn, affect adult performance. Furthermore, evidence from animal studies suggests that the activational effects of T may also facilitate spatial abilities in adult males (Daniel, Winsauer, & Moerschbaecher, 2003; Kritzer, McLaughlin, Smirlis, & Robinson, 2001; Sandstrom, Kim, & Wasserman, 2006). Some correlational studies with younger men (Errico et al., 1992; Hooven et al., 2004; Silverman et al., 1999) and supplementation studies in older men (Cherrier et al., 2001; Cherrier et al., 2004; Cherrier et al., 2005; Cherrier et al., 2007; Janowsky et al., 1994) support this hypothesis; however, the results are far from consistent, with many studies finding either negative associations (e.g., Yonker Eriksson, Nilsson, & Herlitz, 2006;

Gouchie and Kimura, 1991; Moffat and Hampson, 1996), or no relationship at all (e.g., Fonda et al., 2005; Kempel et al., 2005; Falter et al., 2006; McKeever et al., 1987). Factors which may account for these discrepancies between studies include: the age of the male participants; the use of heterogeneous tests of visuo-spatial ability; the confounding influence of T's organisational effects on brain lateralisation and sexuality; the influence of health and life-style variables on both T levels and cognition; and differences in T measurement and determination.

The Vandenberg and Kuse MRT exhibits the largest and most robust of all sex differences in visuo-spatial ability (Voyer et al., 1995; Linn and Peterson, 1986). This difference is robust with ageing (Halpern, 2000; Salthouse, 1998) and cannot be accounted for by either socialisation or environmental factors (e.g., computer game experience; Quaiser-Pohl, Geiser, & Lehmann, 2006; Terlecki and Newcombe, 2005: gender role socialisation; Saucier, McCreary, & Saxberg, 2002; Voyer, Nolan, & Voyer, 2000), or performance factors, such as time limits or scoring procedures (Peters, 2005; Masters, 1998).

Whilst previous studies on endogenous T levels and MRT performance have tended to use university aged males (e.g., Hooven et al., 2004; Falter et al., 2006; Kempel et al., 2005; Gouchie & Kimura, 1991; Silverman et al., 1999), very little data exist on the association between plasma T and MRT performance as men age. Wolf and Kirschbaum (2002), for example, found no relationship between either T or FT and mental rotation performance in a study involving 30 healthy elderly men (aged: 69.0 ± 1.3 years). Similarly, a T supplementation study in 17 healthy elderly males (aged: 68.7 ± 1.9 years) found no statistically significant improvement on the same test of mental rotation, although T levels were increased to three times those of a young control group (Wolf, Preut, Hellhammer, Kudielka, Schurmeyer, &

Kirschbaum, 2000). It is important to note that both of these studies involved relatively small samples of elderly men (i.e., Wolf and Kirschbaum, (2002), N = 30; Wolf et al., (2000), N = 17), and that both studies used a test of mental rotation (Horn, 1983) which involved letters and numbers instead of the 3D geometric figures used in the Vandenberg and Kuse MRT. Importantly, no study to date has specifically examined associations between T and MRT performance in healthy middle- to-older aged males whilst controlling for confounds known to affect both T and cognition.

Research into associations between T and performance on cognitive abilities, in particular in older and elderly men, has similarly generated equivocal results across a broad spectrum of both cognitive and neuropsychological tests. Generally, these studies have employed a wide variety of cognitive ability measures (e.g., verbal memory, WM, processing speed, and executive function) in an attempt to determine whether higher T levels in older males are neuroprotective, and several have reported positive associations between both T and processing speed (Martin, Wittert, Burns, & Sugarman, 2007 (see Chapter 3); Hogervorst et al., 2004; Yaffe et al., 2002; Muller et al., 2005) and between T and executive function (Barrett-Connor et al., 1999; Muller et al., 2005; Perry et al., 2001). These associations are of particular interest because of the corresponding theories that relate processing speed (e.g., Salthouse, 1996a) and executive function (e.g., West, 1996) to generalised age-related cognitive decline (see Chapter 2).

To further illustrate the inconsistencies in the data pertaining to associations between endogenous T levels and both processing speed and executive function performance, in Chapter Three, for example, it was shown that cEFT levels were positively associated with processing speed but negatively associated with executive

function. In the present study, several methodological improvements were made on the previous study to control for further potential confounding factors which may have influenced my results. These are detailed below.

In a recent investigation into the relationship between T and the cognitive processes underlying MRT performance it was concluded that T may facilitate MRT performance through its effect on constituent cognitive processes performance (Hooven et al., 2004). In the present study, this hypothesis was investigated by the inclusion of multiple cognitive ability tests, including measures of both processing speed and executive function, so as to examine potential mediating relationships.

The aims of this study were, therefore, firstly: to determine whether T levels were directly associated with performance on the Vandenberg and Kuse MRT in middle-to-older aged men; secondly, to determine whether T may facilitate MRT performance by modulating abilities constituent to task performance; and, thirdly, to determine whether changes in T levels with ageing were associated with generalised age-related cognitive decline. To investigate these three aims, several methodological improvements were made both on the previous study and previous research: firstly, strict selection criteria was imposed to control for confounds related to the organisational effects of gonadal steroids on spatial performance (i.e., handedness, sexuality), health and disease factors known to affect hormone levels and/or cognition, and other factors negatively related to cognitive performance; secondly, multiple tests of processing speed, executive function, and WM were administered in order to create composite cognitive measures and to control for task specific variance; and thirdly, a test of Gc was used instead of educational attainment to control for individual differences in culturally attained knowledge.

5.3. Method

Participants

Ninety-six participants were recruited from FAMAS to form three age groups: 38-49 years, 50-59 years, and 60-69 years (see Chapter 3).

Materials and Apparatus

Details of the study's procedure and the administration of cognitive tests are described in Chapter Four. Briefly, participants attended a testing session conducted at the Royal Adelaide Hospital between 0930 and 1200 hrs. After completing the Dutch Handedness Questionnaire, each participant had blood drawn from a forearm vein prior to completing twelve cognitive tests in the following order: Cross Out, Digit Symbol, Dot Matrix, Vandenberg and Kuse MRT, Information, Swaps, SOPT, SART, SRT, CRT, OMO, then IT.

Statistical Analyses

One-way analyses of variances (ANOVAs) were firstly conducted to examine age group differences on the descriptive variables. The effect of age group on individual cognitive test performance was then examined using analyses of covariance (ANCOVAs) with Information scores as a covariate to control for Gc. Data were missing for two participants on the OMO and for one participant on the SWAPS test. In addition, one participant did not complete the SART, SRT, Choice RT, OMO or IT tasks. Group differences were examined using post hoc tests with the Bonferroni correction for multiple comparisons. Composite cognitive variable constructs were calculated for processing speed, perceptual discrimination, RT, and WM ability using the mean Z- scores derived from the relevant raw scores obtained from individual tests. The processing speed composite consisted of the mean standardised Digit Symbol and Cross Out scores; the executive function composite,

the mean of standardised SART total percentage correct scores and the total errors from 10 and 12 stimuli trials of the SOPT; the WM composite, the mean of standardised Swaps and Dot Matrix scores; the RT composite, the mean of standardised SRT, and 2, 4, and 8 Choice RT scores; the perceptual discrimination composite, the mean of standardised IT and OMO-DT scores. Tertiles of cEFT levels derived from the entire subject sample were used to examine the effect of FT group on cognitive ability performance. Main effects of cEFT levels and potential interactions between age group and cEFT group on both MRT scores and each composite cognitive construct were examined with separate ANCOVAs using Information scores as a covariate. On the basis of these results additional ANCOVAs were conducted to examine age-group specific effects. In each of these analyses, MRT raw scores and performance on each of the composite cognitive constructs were the dependent variables, tertiles of cEFT levels the independent variable, and both age and Information scores entered as covariates. Predictors of MRT performance were then determined using hierarchical multiple regression, following which, path analyses were conducted to model associations between the predictors of MRT performance with ageing. On the basis of previous research, processing speed (e.g., Dror and Kosslyn, 1994; Berg et al., 1982), executive function (e.g., Raz et al., 1999; Kemps and Newson, 2005), and WM (e.g., Raz et al., 1999; Kemps and Newson, 2005) were each hypothesised to independently predict MRT performance with ageing. In addition to chi-square (χ^2), I used the goodness of fit index (GFI) as an absolute index of overall model fit. GFI is a measure of the relative amount of observed variances and covariances accounted for by the model (Hoyle & Panter, 1995).

5.4. Results

Age Group Effects on Descriptive Data

One-way ANOVAs were conducted to examine the effect of age group on the descriptive data (see Table 7.). A significant main effect of age group was found for SHBG levels, whereby SHBG levels were found to increase with age. No other significant main effects were found on any of the other measures.

Age Group Effects on Cognitive Test Scores

ANCOVAs were conducted to test for main effects of age group on the cognitive test scores using Information scores as a covariate, to control for Gc (see Table 8.). Significant main effects of age group were found on the Vandenberg and Kuse MRT, Digit Symbol, Cross Out, Dot Matrix, Swaps, SOPT and IT. Post hoc testing showed that the youngest age group outperformed both the middle and older age groups on the Vandenberg and Kuse MRT test ($p = 0.009$, $p = 0.004$), the Digit Symbol ($p = 0.003$, $p = 0.003$), the Cross Out ($p < 0.001$, $p < 0.001$), Swaps ($p < 0.001$, $p < 0.001$), and on IT ($p = 0.025$, $p < 0.001$). On the Dot Matrix test the youngest age group outperformed the oldest age group ($p = 0.001$). On the SOPT the younger also outperformed the oldest age group ($p < 0.001$), and the middle age group outperformed the older age group ($p = 0.043$). All other comparisons were not statistically significant.

The Effects of Endogenous T Levels on Cognition

Six separate 3 X 3 ANCOVAs were conducted using MRT scores and each of five composite cognitive variables as dependent variable with tertiles of cEFT levels and the three age groups as the independent variables, and Information score as a covariate. There was no main effect of cEFT levels on MRT performance or on any of the other five cognitive variables.

Table 7. Age group differences in descriptive data.

Variable	Age Group(years) 38-49 *M* (SD) Or %	50-59 *M* (SD) Or %	60-69 *M* (SD) Or %	*F* value (2, 93)	η_p^2
Age (years)	44.4 (3.2)	54.8 (3.1)	63.5 (3.0)		
Information Score	22.9 (2.7)	21.6 (3.4)	21.9 (3.3)	1.56	0.03
TT (nmol/L)	14.7 (5.3)	14.0 (4.3)	14.0 (5.4)	0.16	0.00
SHBG (nmol/L)	23.6 (9.7)	29.2 (11.3)	31.2 (12.3)	3.71*	0.07
cEFT (pmol/L)	246 (88.6)	222 (74.5)	217 (88.6)	1.01	0.02
BMI (kg/m^2)	28.0 (4.0)	28.7 (3.6)	27.7 (4.7)	0.62	0.01
Av. Waist Circum. (cm)	97.1 (10.7)	100 (8.1)	98.1 (11.9)	0.78	0.02
BDI Score	3.7 (4.4)	4.3 (4.4)	4.3 (4.7)	0.17	0.00
Alcohol intake (grams/day)	15.1 (15.7)	9.7 (10.1)	9.6 (10.4)	2.00	0.04
Smoking:					
No = 1	79.3%	75.7%	86.7%		
Occasionally = 2	10.3%	2.7%	0.0%		
Yes = 3	10.3%	21.6%	13.3%		
Education:					
No High School = 1	6.9%	13.5%	6.7%		
High School = 2	3.4%	10.8%	0.0%		
Other Certificate = 3	0.0%	0.0%	6.7%		
Trade Certificate = 4	34.5%	35.1%	36.7%		
Certificate/Diploma = 5	27.6%	27.0%	36.7%		
Bachelor Degree (+) = 6	27.6%	13.5%	13.3%		
Medication:					
Mood Disorder Drugs	0.0%	2.7%	3.3%		
HPG Axis Drugs	0.0%	0.0%	0.0%		
Opiate Drugs	0.0%	2.7%	10.0%		
Handedness Score	9.8 (0.7)	9.8 (0.7)	10.0 (0.2)	0.57	0.01
N	29	37	30		

Notes: * $p < 0.05$

Information Score indicates mean total score (30 possible); TT, total testosterone; SHBG, sex hormone binding globulin; cEFT, calculated free testosterone; BMI, mean body mass index; Av. Waist Circum., average waist circumference; BDI score, Beck Depression Inventory mean score (63 possible); Handedness Score, mean Dutch Handedness Questionnaire score (10 possible).

Table 8. Age group differences in cognitive measures controlling for Information scores.

Variable	Age Group (years) 38-49 *M* (SEM)	50-59 *M* (SEM)	60-69 *M* (SEM)	*F* value (2, 92)	η_p^2
V and K MRT	11.1 (0.8)	7.9 (0.7)	7.4 (0.7)	7.06**	0.13
Digit Symbol	56.4 (1.7)	48.5 (1.5)	47.7 (1.6)	8.35***	0.15
Cross Out	26.3 (0.6)	22.3 (0.5)	21.9 (0.6)	16.1***	0.26
Dot Matrix	40.3 (1.4)	36.8 (1.3)	32.7 (1.4)	7.3**	0.14
Picture Swaps	14.0 (0.8)	9.5 (0.7)	7.9 (0.8)	16.2***	0.26
SOPT§	7.4 (0.9)	10.4 (0.8)	13.3 (0.8)	12.0***	0.21
SART (correct %)	57.1 (4.5)	51.5 (4.0)	49.1 (4.5)	0.83	0.02
IT (msec.)§	87.5 (7.0)	113 (6.2)	127 (6.9)	8.39***	0.16
OMO-DT (msec.)§	766 (32.8)	728 (28.0)	761 (31.4)	0.49	0.01
SRT (msec.)§	256 (5.4)	254 (4.8)	247 (5.3)	0.77	0.02
2-Choice RT (msec.)§	356 (8.3)	351 (7.3)	340 (8.2)	0.96	0.02
4-Choice RT (msec.)§	359 (8.2)	359 (7.3)	344 (8.2)	1.17	0.03
8-Choice RT (msec.)§	393 (10.6)	388 (9.3)	377 (10.4)	0.61	0.01

Notes: *** $p < 0.001$
** $p < 0.01$
§ denotes lower values indicate better performance
V and K MRT indicates the mean score from the Vandenberg and Kuse mental rotation test (20 possible); Digit Symbol mean score (133 possible); Cross Out mean score (30 possible); Dot Matrix mean score (56 possible); Picture Swaps mean score (24 possible); SOPT, self ordered pointing task mean errors from 10 and 12 design conditions (60 possible); SART, sustained attention response to task; IT, inspection time; OMO-DT, odd man out decision time; SRT, simple reaction time.

A significant cEFT level X age group interaction effect was found for perceptual discrimination (F (4, 82) = 3.65, p = 0.009, η_p^2 = 0.151). This result indicated that age group modified the association between cEFT levels and adjusted perceptual discrimination performance. In the youngest age group a positive association was apparent between tertiles of cEFT levels and perceptual discrimination performance, whereby higher cEFT levels were associated with better performance, whilst in both the middle and older age groups (i.e., the men aged over 50 years), higher cEFT levels were associated with poorer performance. The consistent effect of cEFT group on performance in these two older age groups

relative to the younger aged group prompted me to collapse the these groups and conduct separate analyses in only the men aged over 50 years ($N = 65$). These analyses are reported next.

Significant main effects of cEFT group were found on perceptual discrimination (F (2,60) = 6.03, p = 0.004, η_p^2 = 0.167), processing speed (F (2,60) = 3.67, p = 0.031, η_p^2 = 0.109), and executive function performance (F (2,60) = 3.90, p = 0.026, η_p^2 = 0.115). On each of these composite measures higher cEFT levels were associated with poorer performance in men aged between 50 –70 years (see Figure 3). Table 9 shows the partial correlations between age, cEFT, TT, and the cognitive variables controlling for Information scores in only those men who were aged over 50 years. In these analyses, both cEFT and TT levels were significantly associated with poorer performance on processing speed, executive function, perceptual discrimination, and RT composite measures.

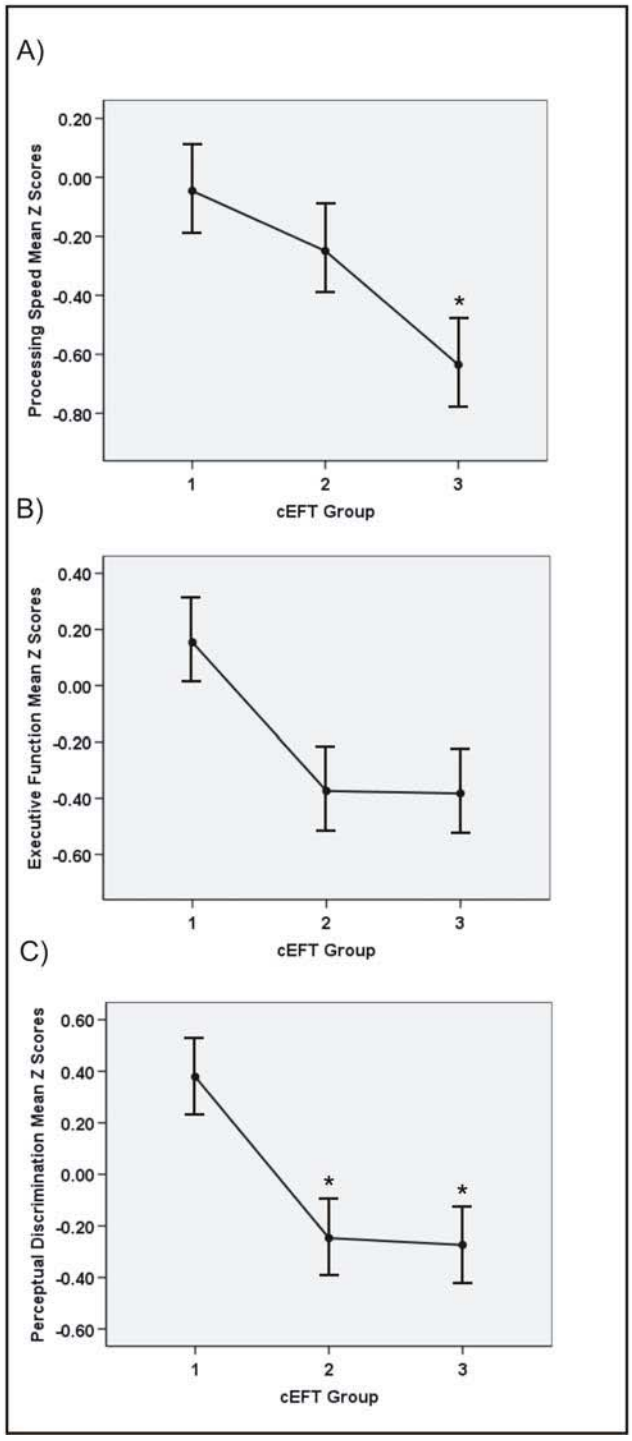

Figure 3. The relationship between tertiles of cEFT levels and (A) Processing Speed, (B) Executive Function, and (C) Perceptual Discrimination in men aged 50-70 years ($N = 65$). All values are expressed as mean z-scores ± SEM, and adjustments have been made for age and Information scores. The signs on the Perceptual Discrimination composite have been reversed. Men in the highest cEFT tertile performed significantly worse than the lowest cEFT tertile on the Processing Speed composite (*$p < 0.05$). Similarly, men in the second and third cEFT tertiles performed significantly worse than the lowest cEFT tertile on the Perceptual Discrimination composite (*$p < 0.05$). Limits for cEFT levels were as follows: first tertile 72.52 to 178.67 pmol/L, second tertile 184.99 to 259.07 pmol/L and the third tertile 270.80 to 455.73 pmol/L.

Table 9. Partial Pearson correlation coefficients between Vandenberg and Kuse mental rotation test performance, age, cEFT, TT, and composite cognitive performance measures controlling for Information scores for men aged over 50 years ($N = 65$).

	Age	CEFT	TT	1	2	3	4	5
1. V and K MRT	-0.06	-0.14	-0.13	-	-	-	-	-
2. Processing Speed Composite	-0.11	-0.35**	-0.36**	0.18	-	-	-	-
3. Executive Function Composite	-0.12	-0.28*	-0.34**	0.06	0.24	-	-	-
4. Perceptual Discrimination Composite§	0.01	-0.42***	-0.43***	0.12	0.41**	0.25*	-	-
5. Reaction Time Composite§	0.25*	-0.33**	-0.28*	0.02	0.30*	0.12	0.47***	-
6. Working Memory Composite	-0.30*	-0.16	-0.21	0.39**	0.47***	0.51***	-0.32**	-0.20

Notes: *** $p < 0.001$
** $p < 0.01$
* $p < 0.05$
§ denotes the signs for these composite scores have been reversed.
V and K MRT indicates Vandenberg and Kuse Mental Rotation Test.

Predictors of Mental Rotation Performance with Ageing

The extent to which processing speed, executive function, WM, and cEFT levels, might predict MRT performance with ageing was investigated by hierarchical multiple regression analyses across the entire data set. For each of these analyses, age group was recoded into two dichotomous dummy variables and entered simultaneously into the regression models. In addition, Information scores

were entered at the first step for each of the models. In separate models, WM and processing speed, but not executive function and cEFT levels, each significantly predicted and mediated the age-related variance in MRT performance (see Table 10.). WM was the best predictor of MRT performance, accounting for 75.2% of the age-related variance, whilst processing speed accounted for 61.2%.

A series of path analyses were then conducted to further examine the associations amongst these six variables in predicting MRT performance with ageing. Age was placed as the only exogenous variable in the hypothesised model due to its association with decreased cEFT levels. In the men aged 50 years and over, cEFT levels were associated with poorer processing speed and executive function, but not WM performance. Because previous research has shown that the majority of age-related variance in WM performance is accounted for by processing speed (e.g., Salthouse, 1994), processing speed was placed causally prior to WM in the model. Executive function was also placed antecedent to WM on the basis of previous research (e.g., Owen et al., 1996; D'Esposito, Postle, Ballard, & Lease, 1999; Rypma & D'Esposito, 2000). WM was postulated to be the major source of age-related variation in MRT scores on the basis of the hierarchical multiple regression results described above. Figure 4 (A) shows this hypothesised model with standardised path coefficients, $\chi^2(7, N = 94) = 13.4$, $p = 0.064$, GFI = 0.96, RMSEA = 0.099. In this model the coefficient for the path from age to cEFT levels approached significance, $p = 0.067$. To test whether the model fit the data better without the variable executive function and its associated path to WM, an alternative model was fitted with only processing speed and age as predictors of WM performance. This resulted in the reduced model Figure 4 (B), with

corresponding lower chi-square and higher GFI values, $\chi^2(4, N = 94) = 2.99$, $p = 0.559$, GFI = 0.99, RMSEA = 0.00, respectively.

Table 10. Predictors of Vandenberg and Kuse mental rotation test performance ($N = 94$)

(Step) Predictor	Multiple R	Multiple R^2	R^2 change	F (change)	F (model)
Model 1.					
(1) Information	.246	.060	.060	F (1,92) = 5.91*	F (1,92) = 5.91*
(2) Age	.425	.181	.121	F (2,90) = 6.62**	F (3,90) = 6.63***
Model 2.[1]					
(1) WM	.488	.238	.178	F (1,91) = 21.3***	F (2,91) = 14.2***
(2) Age	.518	.269	.030	F (2,89) = 1.84	F (4,89) = 8.17***
Model 3.[1]					
(1) Speed	.405	.164	.104	F (1,91) = 11.3**	F (2,91) = 8.93***
(2) Age	.459	.211	.047	F (2,89) = 2.63	F (4,89) = 5.94***
Model 4.[1]					
(1) EF	.307	.095	.034	F (1,91) = 3.44	F (2,91) = 4.75*
(2) Age	.431	.186	.091	F (2,89) = 4.98**	F (4,89) = 5.07**
Model 5.[1]					
(1) cEFT	.246	.060	.000	F (1,91) = .011	F (2,91) = 2.93
(2) Age	.429	.184	.124	F (2,89) = 6.75**	F (4,89) = 5.02**

Notes: *** $p < 0.001$
** $p < 0.01$
* $p < 0.05$
[1] Indicates Information scores were entered at the first step in these models.
Information indicates total score on the Information test; Age, the three age groups dichotomised into two dummy variables; WM, mean working memory Z- score; Speed, mean processing speed Z- score; EF, mean executive function Z- score; cEFT, calculated free testosterone levels.

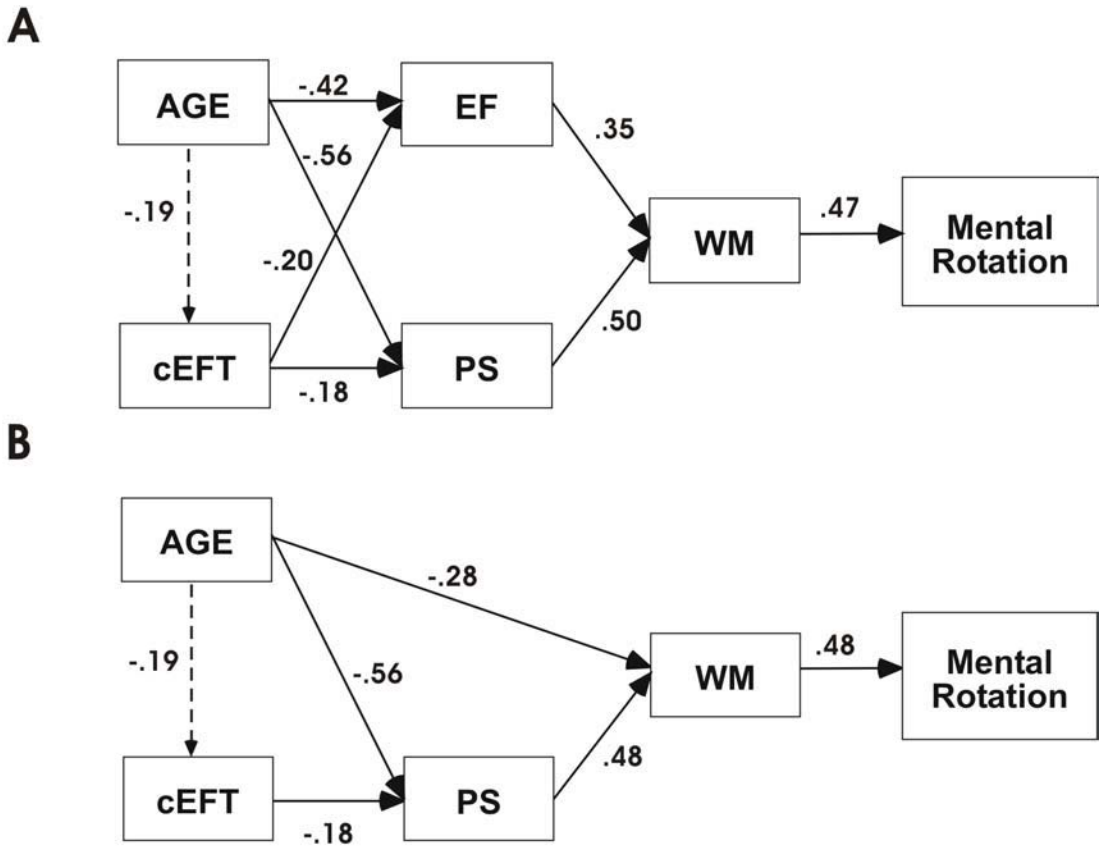

Figure 4. Path diagrams. (A) Hypothesised Model 1. (B) The final reduced Model 2. Standardised path coefficients are indicated at the respective paths. Solid and dotted lines represent paths which were significant and not significant, respectively. PS is processing speed composite; EF is the executive function composite; WM is the working memory composite; mental rotation is standardised Vandenberg and Kuse Mental Rotation Test scores.

5.5. Discussion

In this study endogenous FT levels were not found to be directly associated with MRT performance in a sample of healthy middle-to-older aged men after controlling for known confounds. Instead it was found that in men aged between 50- 70 years, cEFT levels in the lower part of the normal range were associated with faster processing speed, better executive function, and perceptual discrimination performance. Although these associations did not hold across the entire sample, hierarchical regression and path analyses showed cEFT levels moderated processing speed performance, which in turn accounted for age-related declines in both WM

and MRT performance. Together these results did not support the hypothesis that changes in T levels in middle-to-older aged men are associated with generalised age-related cognitive decline.

The negative association found between cEFT levels and executive functioning is consistent with the results from the cross-sectional analyses of the entire FAMAS cohort at baseline, which showed that higher cEFT and TT levels were associated with poorer set-shifting ability (see Chapter 3). Set-shifting ability has previously been demonstrated by neuro-imaging studies to be a function of the DLPFC (e.g., Smith et al., 2004; Nagahama et al., 2001). In the present study the SART, which similarly recruits DLPFC function (Fassbender et al., 2004), was used as a measure of executive function. In addition, the SOPT, which is a test of frontal lobe function previously shown to be sensitive to T modulation (Janowsky et al., 2000), was also used to comprise the composite executive function measure. By contrast, Janowsky et al. (2000) found that T supplementation improved SOPT performance in 10 men aged 61 to 75 years. Interestingly, both the mean and range of FT levels post T supplementation in their study (i.e., M = 45.2 pg/mL or 156.8 pmol/L, range 53.1 - 187.7 pmol/L, N = 10) were comparable to the lowest tertile cEFT group in the present study (i.e., M = 141.7 pmol/L, range 72.5 – 178.7 pmol/L, N = 25) which was also associated with the best performance. Although it is difficult to directly compare values from different T assays, it is possible that the men in Janowsky et al.'s study had very low FT levels at baseline and that this range may represent the optimal level for prefrontal cortical function.

In similarly aged men, two other studies have reported significant positive correlations between BT levels, which represent the T fraction not bound to SHBG, and executive function measures (Barrett-Connor et al., 1999; Perry et al., 2001). In

Chapter Three, the BT measure was shown to be a poor predictor of cognitive function and to sometimes exhibit hormone-cognition associations in directions opposite to both TT and cEFT measures. It is important to note that in both these studies the correlations between BT and executive function measures were the only significant hormone-cognition associations found in males and that neither showed significant relationships with TT. In the present study, however, similarly sized effects were found for both cEFT and TT measures across three different composite measures.

The theory of prefrontal decline proposes that pronounced atrophic changes in the brain's frontal lobes which accompany ageing are responsible for a disproportionate decline in executive functions (West, 1996). The DLPFC has been reported by several researchers to be particularly susceptible to age-related decline in function (MacPherson et al., 2002; Esposito et al., 1999). Furthermore, the DLPFC is thought to support several key mental abilities, including retrospective memory, prospective memory, and inhibition of prepotent responses (Fuster, 1989). Taken together, the present findings in conjunction with those detailed in Chapter Three suggest that frontal lobe function, in particular DLPFC function, is modulated by endogenous T levels such that cEFT levels in the lower part of the normal range for younger men are associated with better cognitive performance in older men.

The negative association found between cEFT levels and processing speed is in contrast to three other studies in similarly aged men which have reported positive associations (Martin et al., 2007 (see Chapter 3); Hogervorst et al., 2004; Moffat et al., 2002), and one study which reported no effect (Fonda et al., 2005). In Chapter Three, cEFT levels were found to mediate the relationship between age and processing speed, as measured by Trails A. It is interesting to note that a sub-

sample of men from this previous study also participated in the present study and that the same measures of T, that is cEFT and TT levels, were used in both analyses. Several key methodological differences between these studies, however, may explain the discrepant results. Firstly, strict exclusion criteria were imposed on participation in the present study to control for confounds relating both to test performance and T's organisational effects, such as, handedness and sexuality. Secondly, composite measures of cognitive abilities were used instead of single measures to minimise task specific variance and to emphasise common, construct related variance. Thirdly, a measure of Gc together with age were used as covariates in the analyses of the men aged over 50 years, instead of educational attainment, age, and a plethora of other health and disease related variables (see Chapter 3).

Positive associations between T and measures of processing speed in healthy aged males have also been reported in two other studies (i.e., Hogervorst et al. 2004; Moffat et al. 2002. In addition to the differences in exclusion criteria between these and the present study, it is also important to note that there were differences in T measures between studies. Whilst Hogervorst et al. (2004) used TT controlling for SHBG levels, Moffat et al. (2002) used the FTI. The physiologically inactive fraction of T consists of approximately 50-70 % of TT levels which are tightly bound to SHBG (Morris et al., 2004). As SHBG levels show substantial inter-individual variation between men, primarily due to health related factors, it is important to account accurately for this fraction when determining the bioavailable, physiologically active fraction of T (BioT). The FTI has been shown to be the worst predictor of BioT in aging men (Ho et al., 2006; Morris et al., 2004; Ly and

Handelsman, 2005). The use of TT controlling for SHBG levels would, therefore, similarly inaccurately represent BioT in hormone-cognition associations.

Processing speed theory (Salthouse, 1996a) attempts to account for age-related declines in fluid abilities in terms of a general slowing of the speed of execution of cognitive operations, or speed of information processing. Consistent with this theory, path analyses in the present study showed that processing speed, but not executive function, accounted for the majority of the age-related variance in WM performance, which in turn predicted poorer MRT performance.

In addition, a significant interaction effect was found between age group and cEFT tertiles on perceptual discrimination ability, which indicated that in the youngest age group (i.e., 38-49 years) higher cEFT levels were associated with better performance. A possible explanation for this age group effect may be due to deleterious effects of age on neurotransmitter systems that underlie cognitive function, such as, the cholinergic (Terry and Buccafusco, 2003) and dopaminergic systems (Backman, Nyberg, Lindenberger, Li, & Farde, 2006). Importantly, both these neurotransmitter systems have been shown to be modulated by T levels in adult male rats (i.e., Cholinergic: Leonard, Moerschbaecher, & Winsauer, 2007; Daniel et al., 2003; Nakamura et al., 2002; Dopaminergic: Kritzer, 2000; Kritzer, 2003; Kritzer, Brewer, Montalmant, Davenport, & Robinson, 2007).

In conclusion, the data of the present study showed that in healthy middle-to-older aged men aged over 50 years, cEFT levels within the low normal range for young adults were associated with better cognitive function across three domains, namely, perceptual discrimination, processing speed, and executive function. Moreover, path analyses on the entire sample showed cEFT levels to moderate

processing speed, which in turn predicted age-related declines in both WM and MRT performance.

CHAPTER SIX: FREE TESTOSTERONE, ATTENTIONAL CONTROL, AND PROCESSING SPEED PERFORMANCE IN MIDDLE-TO-OLDER AGED MEN

6.1. Summary

Psychometric measures of processing speed are strong predictors of cognitive functioning with ageing; however, the neurobiological mechanisms underlying this association remain unclear. In Chapter Five a negative association was found between cEFT levels and processing speed performance in men aged between 50-70 years. Moreover, consistent with the processing speed theory of cognitive ageing, path analyses across the entire data set showed that cEFT levels negatively moderated processing speed, which in turn predicted both WM and MRT performance with ageing. Methodological advances in the analysis of RT distributions have allowed for the calculation of robust estimates of intra-individual variability, thought to reflect aspects of attentional control. Therefore, using data from this study, associations between chronological age, cEFT levels, estimates of intra-individual RT variability, processing speed, WM, and executive function performance were examined. Ex-Guassian distributions were used to provide estimates of intra-individual variability from four RT measures. Results showed that cEFT levels were significantly associated with the exponential portion of the curve but not with the estimates derived from the Gaussian portion of the curve across three out of the four RT measures. Further, path analyses across the entire data set showed that cEFT levels predicted the slower responses associated with the exponential portion of the curve, which in turn predicted declines in processing speed performance with increased age.

6.2. Introduction

Both cross-sectional (e.g., Bryan & Luszcz, 1996; Salthouse 1996a, b; Sliwinski & Buschke, 1999) and longitudinal (e.g., Sliwinski & Buschke, 1999; Zimprich & Martin, 2002) studies show that psychometric measures of processing speed account for a large proportion of the age-related variance in fluid abilities**,** including, memory, reasoning, and spatial ability. Conceptually, processing speed accurately describes age-related changes in cognition; however, there is a lack of an adequate explanatory account which bridges the gap between behaviour and neurobiological mechanisms (Deary, 2000). Although the neurobiological mechanisms underlying this association remain unclear, potential associations between cEFT levels and RT measures, including estimates of intra-individual RT variability, may help to elucidate whether cEFT levels moderate processing speed performance via speed or via attentional control processes.

In intelligence research, analyses of the association between processing speed and psychometric performance have commonly involved measures of central tendency, such as the mean or median. Measures of intra-individual variability in RT performance (e.g., intra-individual RT standard deviation, ISD), however, have also been a long established but generally neglected correlate of intelligence (e.g., Jensen, 1982; Jensen, 1987). In a landmark study conducted by Larson and Alderton (1990), this association was further investigated by decomposing ISDs: Firstly, by ranking an individual's RTs from fastest to slowest and then grouping them into consecutive RT bands. After correlating each RT band's median with intelligence and WM measures, the authors reported that the slowest RT bands were the best predictors of performance. Larson and Alderton (1990) referred to this as the "worst performance rule" (WPR), which states, "The worst RT trials reveal

more about intelligence than do other portions of the RT distribution…". A comprehensive review (Coyle, 2003) found overwhelming support for the hypothesis across differing varieties of tasks and participants; furthermore, additional analyses showed that the WPR could not be attributed to either statistical or data artefacts.

It has been argued that the use of separate RT bands in this form of analysis reduces the reliability of estimates, particularly when the total number of RT trials is limited (Schmiedek et al., 2007). Because outliers bias skewness estimates of a given RT distribution (Ratcliff, 1979), Schmiedek et al. (2007) proposed that more accurate estimates of RT skew could be gauged from using the entire RT distribution. One method to obtain such estimates is by fitting an explicit density function, the Ex-Guassian (Hohle, 1965). Good fit of the Ex-Guassian to empirical RT data has been demonstrated in a variety of research settings (e.g., Heathcote, Popiel, & Mewhort, 1991; Hockley, 1984; Leth-Steensen, Elbaz, & Douglas, 2000; McAuley, Yap, Christ, & White, 2006; Schmiedek et al., 2007; Spieler, Balota, & Faust, 2000).

The Ex-Guassian, or the convolution of a Guassian and an exponential distribution, is characterised by three parameters; mu (μ) and sigma (σ), which represent the mean and standard deviation of the Guassian, respectively; and tau (τ), which represents both the mean and standard deviation of the exponential. In relation to the WPR, the skewness of an individual's RT distribution is best characterised by τ, because for a Guassian, $\tau = 0$. Recently, τ was demonstrated to be the strongest unique predictor of performance on WM, reasoning, and processing speed measures in a large sample of university students (Schmeidek et al., 2007). Consistent with the WPR, these findings showed that intra-individual variability in

RT performance estimated by τ, is a highly important determinant of cognitive performance. The extent to which τ predicts cognitive performance in middle-to-older aged adults, however, remains to be determined.

In two studies, West and colleagues (West, 1999; West et al., 2002) investigated age effects on Ex-Guassian parameters in task conditions which required either minimal or higher levels of executive control. In the first of these studies, age-related differences in Ex-Guassian parameters from a continuous WM task (i.e., 1-back; West, 1999) were examined and it was found that the response time costs measured by τ were greater for older adults than for the younger adults on the more demanding 1-back condition. In a subsequent study, this effect was replicated and found to remain after controlling for processing speed (West et al., 2002). Together these data were interpreted to reflect age-related decreases in executive control processes which were independent from generalised slowing (i.e., processing speed; West et al., 2002). If changes in intra-individual variability are both predictive of but independent to age-related changes in processing speed, the identification of the factors associated with increased intra-individual variability could potentially facilitate the amelioration of cognitive decline with ageing.

In Chapter Five a negative association was found between cEFT levels and processing speed in the men aged between 50 and 70 years. Additionally, this association was found in turn to predict age-related declines in WM and MRT performance across the entire data set. In the present study, Ex-Gaussian parameters were calculated from four RT tasks. Because cEFT levels were previously found to moderate processing speed performance, associations between cEFT levels and Ex-Guassian parameters were examined, therefore, as potential mediators to this relationship. Specifically, the present study sought to determine

whether cEFT levels moderated processing speed performance via either speed (i.e., μ or σ) or via attentional control processes (i.e., τ).

6.3. Method

Participants

Ninety-six participants were recruited from FAMAS to form three age groups: 38-49 years, 50-59 years, and 60-69 years (see Chapter 4).

Materials and Apparatus

Details of the study's procedure and the administration of cognitive tests are described in Chapter Four. Briefly, participants attended a testing session conducted at the Royal Adelaide Hospital between 0930 and 1200 hrs. After completing the Dutch Handedness Questionnaire, each participant had blood drawn from a forearm vein prior to completing twelve cognitive tests in the following order: Cross Out, Digit Symbol, Dot Matrix, Vandenberg and Kuse MRT, Information, Swaps, SOPT, SART, SRT, CRT, OMO, then IT.

Data Preparation and Statistical Analyses

All RT data were cleaned of all incorrect and outlier responses prior to the statistical analyses to prevent errors, accidental key presses, and task interruptions, from influencing the Ex-Guassian parameters. Outliers were defined as Choice RT's and OMO-DT's faster than 200ms or four standard deviations (SDs) slower than the individual's mean RT on a given task. On the basis of these criteria, 3.2% of 2-Choice, 3.3% of 4-Choice, 3.7% of 8-Choice, and 4.4% of OMO-DT trials were excluded from further analysis. Ex-Guassian parameters were then estimated using QMPE v2.18 (Cousineau, Brown, & Heathcote, 2004; Heathcote, Brown, & Mewhort, 2002). In all subsequent analyses these estimates were treated as missing if the total number of valid trails were less than 50 out of a possible 60. Similarly,

estimates were treated as missing if greater than 4 SDs above the mean on a given RT task. For the composite cognitive ability measures, mean Z-scores were calculated from the raw scores derived from the constituent ability tests (see Chapter 4). Tertiles of cEFT levels were calculated across the entire sample to examine main effects of cEFT group.

One-way ANOVAs were used to examine potential main effects of age group on all descriptive, hormonal, and cognitive measures. Group differences were examined with post hoc analyses using the Bonferroni correction for multiple comparisons. ANCOVAs were then used to examine the potential main effects of age group on the individual Ex-Guassian parameters whilst adjusting for individual differences in Gc (i.e., Information scores). Separate ANOVAs were also conducted to examine the effect of task complexity on individual Ex-Guassian parameter estimates (i.e., differences between RT tasks) and post hoc analyses conducted to examine differences between estimates. Further ANCOVAs were then conducted to examine potential main effects of cEFT group on the Ex-Guassian parameters. In these analyses, both age and Information scores were entered as covariates to adjust for potential confounds. Raw correlations between chronological age, Ex-Guassian parameters, cEFT levels, and composite cognitive measures were then examined for potential associations between the Ex-Guassian parameters and the composite cognitive functioning measures. Following this, hierarchical multiple regression analyses were conducted to investigate each of the Ex-Guassian parameters as predictors of cognitive performance. In each of these analyses, Information scores were entered in the first step. Each composite cognitive measure was then regressed on each individual Ex-Guassian parameter whilst partialing out the variance predicted by the other two remaining parameters.

Finally, path analysis was used to model associations between chronological age, cEFT levels, the Ex-Guassian parameter estimates, and cognitive function.

6.4. Results

Age Group Effects

Age group effects on demographic, hormone, and composite cognitive measures are shown in Table 11. Further demographic data on this sample are reported in Chapter Five. On the composite cognitive outcome measures of processing speed, WM, and executive function, the oldest age group performed worse than the younger age groups ($p < 0.001$; $p < 0.001$; and $p < 0.001$). No age group effects were found on Information scores.

Ex-Guassian parameters derived from the 2-, 4-, and 8-Choice RT and OMO tasks are shown separately for each age group in Table 12. There was no effect of age group on any of the Ex-Guassian parameters.

Table 11. Age group effects on demographic, hormone, and cognitive measures

Variable	Age Group (years) 38-49 *M*	(SD)	50-59 *M*	(SD)	60-69 *M*	(SD)	*F* value (2,92)	η_p^2
Age (years)	44.4	(3.2)	54.8	(3.1)	63.7	(3.0)		
BMI (kg/m^2)	28.0	(4.0)	28.8	(3.6)	27.6	(4.8)	0.68	0.02
Handedness Score	9.8	(0.7)	9.8	(0.7)	10.0	(0.2)	0.54	0.01
cEFT (pmol/L)	246	(88.6)	222	(74.5)	219	(89.5)	0.91	0.02
TT (nmol/L)	14.7	(5.3)	14.0	(4.3)	14.2	(5.4)	0.14	0.00
SHBG (nmol/L)	23.6	(9.7)	29.2	(11.3)	31.8	(12.1)	4.17*	0.08
Processing Speed	0.71	(0.8)	-0.27	(0.9)	-0.35	(0.6)	17.2***	0.27
Working Memory[1]	0.64	(0.9)	-0.11	(0.8)	-0.49	(0.7)	16.3***	0.26
Executive Function	0.41	(0.7)	-0.04	(0.8)	-0.32	(0.7)	7.52**	0.14
Information	22.9	(2.7)	21.6	(3.4)	22.0	(3.2)	1.50	0.03
N	29		37		29			

Notes: *** $p < 0.001$
** $p < 0.01$
* $p < 0.05$
[1] $N = 94$
Mean Z-scores were used for the cognitive function measures; BMI indicates mean body mass index; Handedness Score, mean Dutch Handedness Questionnaire Score (10 possible); cEFT, calculated free testosterone; TT, total testosterone; SHBG, sex hormone binding globulin.

Differences between the Ex-Guassian Parameters as a Function of Task Demands

To examine whether the Ex-Guassian parameters differed across the RT tasks, separate ANOVAs were conducted using μ, σ, and τ as dependent variables and RT task as a within subjects independent variable. For each Ex-Guassian parameter there was a main effect of RT task, whereby estimates increased with increasing task demands across the 2-, 4-, and 8-Choice RT and OMO tasks (i.e., μ, F (3, 364) = 457, p <0.001; σ, F (3, 364) = 68.8, p <0.001; τ, F (3, 363) = 166, p <0.001). Post hoc analyses showed that for each Ex-Guassian parameter, the OMO task had higher values than each other CRT task (all $p < 0.001$). No other

comparisons between tasks were significant. Figure 5 shows the mean Ex-Guassian parameters derived from each RT test.

Table 12. Age group effects on Ex-Guassian parameters

Measure (msec)	Age Group (years)			*F value (df)*	η_p^2
	39-50 *M (SEM)*	51-60 *M (SEM)*	61-70 *M (SEM)*		
2 Choice RT					
μ	313 (7)	311 (7)	300 (8)	0.83 (2,87)	0.02
σ	22.8 (3)	23.5 (2)	23.5 (3)	0.03 (2,87)	0.00
τ	59.5 (4)	52.7 (4)	59.7 (4)	1.08 (2,87)	0.02
4 Choice RT					
μ	317 (7)	315 (7)	301 (8)	1.34 (2,86)	0.03
σ	26.4 (3)	24.0 (2)	24.5 (3)	0.22 (2,86)	0.01
τ	55.5 (5)	60.6 (4)	63.8 (5)	0.73 (2,86)	0.02
8 Choice RT					
μ	329 (7)	332 (7)	316 (8)	1.34 (2,88)	0.03
σ	23.7 (3)	24.6 (2)	22.2 (3)	0.24 (2,88)	0.01
τ	92.2 (10)	81.0 (9)	82.2 (10)	0.40 (2,87)	0.01
OMO DT					
μ	562 (16)	558 (14)	546 (16)	0.25 (2,87)	0.01
σ	56.7 (7)	62.0 (6)	62.5 (7)	0.22 (2,87)	0.01
τ	303 (30)	248 (25)	317 (28)	1.91 (2,87)	0.04

Note: All values are adjusted for Information Scores.

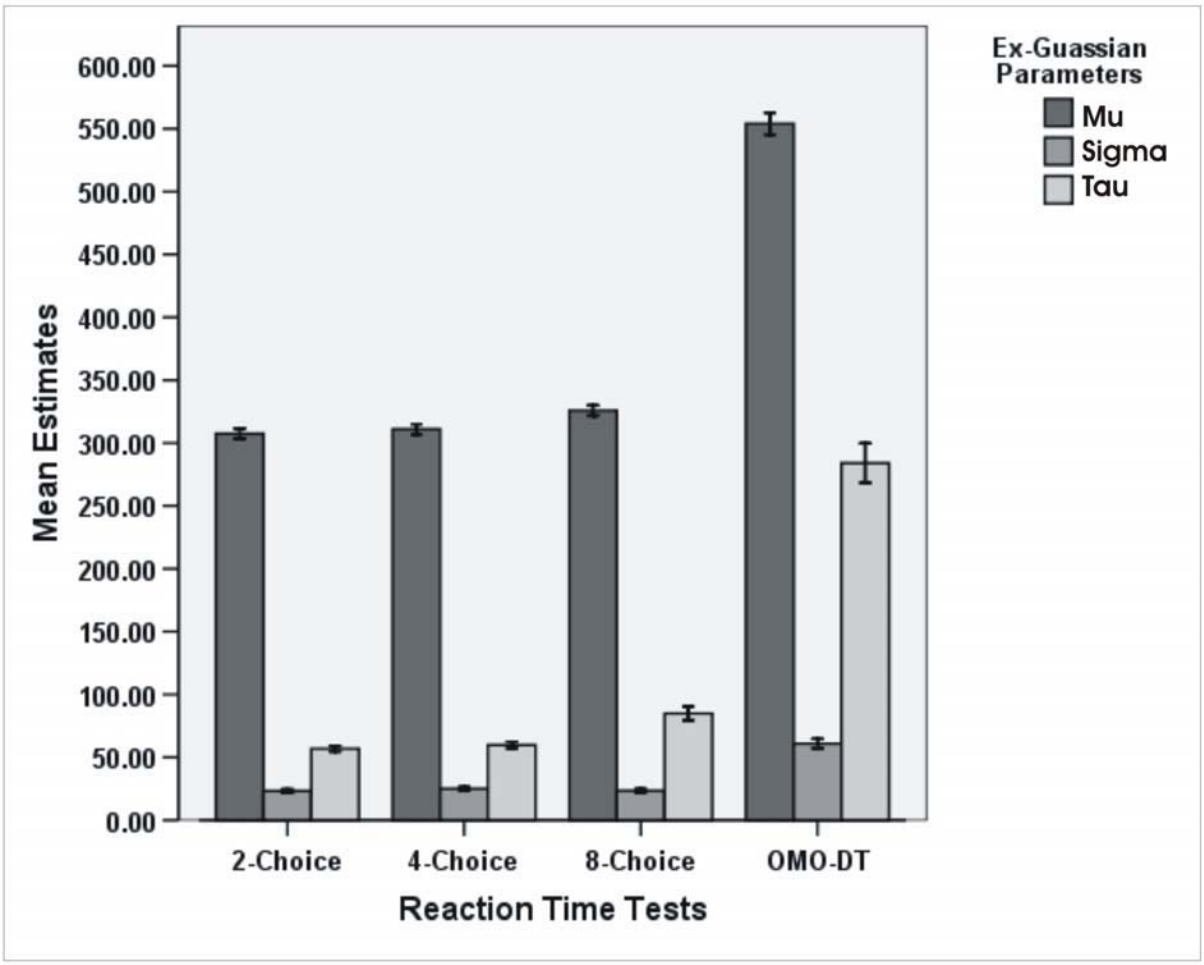

Figure 5. Mean Ex-Guassian parameter estimates derived from the different reaction time tasks. Error bars represent ± 1 SEM.

Free T Effects on Ex-Guassian Parameters

Effects of cEFT group (i.e., tertiles of cEFT) on Ex-Guassian parameters are shown in Table 13. A significant main effect of cEFT group was found on τ from the 4-Choice RT test, whereby the highest cEFT group performed worse than either the low or medium cEFT groups. Similarly, there was a trend towards a significant main effect of age group on τ from the 2-Choice RT test.

Table 13. The effect of cEFT tertiles on Ex-Guassian parameters

Measure	cEFT Tertiles			*F value (df)*	η_p^2
	Low *M (SEM)*	Medium *M (SEM)*	High *M (SEM)*		
2 Choice RT					
μ	311 (7)	311 (7)	303 (7)	0.39 (2,86)	0.01
σ	27.1 (3)	22.7 (3)	20.5 (2)	1.79 (2,86)	0.04
τ	49.5 (4)	57.8 (4)	62.8 (4)	2.81[1] (2,86)	0.06
4 Choice RT					
μ	304 (7)	321 (7)	309 (7)	1.54 (2,85)	0.04
σ	21.4 (3)	26.6 (3)	26.6 (3)	1.27 (2,85)	0.03
τ	54.4 (5)	55.4 (5)	69.5 (4)	3.58* (2,85)	0.08
8 Choice RT					
μ	324 (8)	334 (7)	321 (7)	0.87 (2,87)	0.02
σ	22.8 (3)	27.9 (3)	20.1 (2)	2.61 (2,87)	0.06
τ	71.5 (10)	86.9 (9)	95.5 (9)	1.53 (2,86)	0.03
OMO DT					
μ	554 (16)	549 (15)	562 (15)	0.18 (2,86)	0.00
σ	57.3 (7)	64.4 (7)	60.2 (7)	0.28 (2,86)	0.01
τ	255 (29)	277 (28)	320 (27)	1.41 (2,86)	0.03

Notes: * $p < 0.05$
[1] $p = 0.07$
All values are adjusted for Age and Information Scores. Limits for cEFT tertiles were as follows: first tertile 72.52 to 178.67 pmol/L, second tertile 184.99 to 259.07 pmol/L and the third tertile 270.80 to 455.73 pmol/L.

Ex-Guassian Parameters as Predictors of Cognitive Function

Hierarchical multiple regression analyses were conducted to determine the extent to which the individual Ex-Guassian parameters uniquely predicted variance in cognitive performance. For processing speed, τ from the 2-, 4-, and 8-Choice RT tasks significantly predicted performance over and above both μ and σ, uniquely accounting for between 35.2 - 51.5 % of the total variance in the models. In addition, σ from 2-Choice RT test was a significant predictor, accounting for 31.9% of the total variance. For WM, τ from the 4-Choice RT test was the only significant predictor, accounting for 31% of the total variance. No other Ex-Guassian

parameters were found to predict cognitive performance. The results of these analyses are shown in Table 14.

Table 14. Ex-Guassian parameters as predictors of cognitive functioning

	Multiple R^2	Partial Multiple R^2		
Variable	Total	μ	σ	τ
Processing Speed				
2 Choice RT	0.163**	0.003 (1.8)	0.052 (31.9)*	0.084 (51.5)**
4 Choice RT	0.190**	0.000 (0.0)	0.034 (17.9)	0.090 (47.4)**
8 Choice RT	0.122*	0.003 (2.5)	0.001 (0.8)	0.043 (35.2)*
OMO DT	0.081	0.000 (0.0)	0.009 (11.1)	0.001 (1.2)
Working Memory				
2 Choice RT	0.123*	0.009 (7.3)	0.010 (8.1)	0.012 (9.8)
4 Choice RT	0.184**	0.011 (6.0)	0.027 (14.7)	0.057 (31.0)*
8 Choice RT	0.148**	0.000 (0.0)	0.001 (0.7)	0.025 (16.9)
OMO DT	0.111*	0.003 (2.7)	0.001 (0.9)	0.011 (9.9)
Executive Function				
2 Choice RT	0.053	0.000 (0.0)	0.000 (0.0)	0.008 (15.1)
4 Choice RT	0.062	0.001 (1.6)	0.012 (19.4)	0.005 (8.1)
8 Choice RT	0.063	0.005 (7.9)	0.012 (19.1)	0.001 (1.6)
OMO DT	0.070	0.005 (7.1)	0.017 (24.3)	0.011 (15.7)

Notes: $^{**}p < 0.01$
$^{*}p < 0.05$
Values in parentheses represent percentage total R^2. All analyses were adjusted for Information scores.

Associations between Age, cEFT Levels, Tau, and Processing Speed Performance

Path analyses were conducted to model the associations between chronological age, cEFT levels, and processing speed performance. Because the regression analyses showed that τ uniquely predicted processing speed performance over and above both μ and σ on three out of the four RT tasks, a common latent factor for τ was used as a predictor in the models. This latent factor was defined by the τ parameter estimates derived from all four RT tasks to gauge an overall index

of intra-individual variability across all the available RT data. Further, a common latent factor for processing speed was defined from the raw scores from both the Digit Symbol and Cross Out tests. Chronological age was included in the models on the basis of its negative association with processing speed performance (see Table 11). Missing data points were calculated in the models by Amos 5 (Arbuckle, 1995) using maximum likelihood estimation. The first model, which excluded cEFT levels as a mediator between age and τ, fit the data adequately, χ^2 (12, N = 95) 17.6, p = 0.13, CFI = 0.95, RMSEA = 0.070. In the second model, cEFT levels were included on the basis of the significant positive correlations with τ on three of the RT tasks (see Table 15). Figure 6 shows this model with standardised path coefficients. The overall fit of this model improved χ^2 (18, N =95) 21.4, p = 0.26, CFI = 0.97, RMSEA = 0.045. In this model, the path between age and cEFT levels approached significance, p = 0.072.

Table 15. Correlations between age, cEFT levels, Ex-Guassian parameters, and cognitive function measures

	cEFT	2Chμ	4Chμ	8Chμ	OMOμ	2Chσ	4Chσ	8Chσ	OMOσ	2Chτ	4Chτ	8Chτ	OMOτ
2Chμ	-.04 (92)												
4Chμ	.09 (91)	**.80** (90)											
8Chμ	.01 (93)	**.65** (90)	**.40** (89)										
OMOμ	.10 (92)	**.37** (89)	**.46** (88)	**.49** (90)									
2Chσ	-.14 (92)	**.48** (92)	**.30** (90)	**.30** (90)	-.01 (89)								
4Chσ	.17 (91)	**.28** (90)	**.53** (91)	**.35** (90)	.11 (88)	**.31** (90)							
8Chσ	-.17 (93)	**.18** (90)	**.26** (90)	**.54** (93)	.11 (90)	.14 (90)	**. 25** (90)						
OMOσ	.05 (92)	-.00 (89)	.12 (88)	.16 (90)	**.43** (91)	-.03 (89)	. 07 (88)	-.01 (90)					
2Chτ	**.23** (92)	-.03 (92)	**.18** (90)	.15 (90)	**.30** (89)	**-.37** (92)	**.24** (90)	**.19** (90)	.14 (89)				
4Chτ	**.22** (91)	-.09 (90)	-.06 (91)	-.02 (90)	.14 (88)	-.09 (90)	-.14 (91)	-.10 (90)	.13 (88)	**.41** (90)			
8Chτ	.12 (92)	.07 (89)	.14 (89)	.05 (92)	**.32** (90)	.06 (89)	.07 (89)	-.17 (92)	.15 (89)	.15 (89)	**.40** (89)		
OMOτ	**.21** (92)	.13 (89)	.15 (88)	.13 (90)	**.43** (91)	**-.23** (89)	.16 (88)	.04 (90)	.10 (91)	**.38** (89)	**.20** (88)	.15 (89)	
Age	**-.18** (95)	**-.23** (92)	**-.25** (91)	**-.18** (93)	-.09 (92)	-.00 (92)	-.13 (91)	-.03 (93)	.09 (92)	-.02 (92)	**.18** (91)	-.05 (92)	.01(92)
Processing Speed	-.08 (95)	-.04 (92)	-.11 (91)	-.08 (93)	**-.17** (92)	-.13 (92)	**-.18** (91)	-.03 (93)	**-.19** (92)	**-.24** (92)	**-.27** (91)	**-.19** (92)	-.10 (92)
Working Memory	-.03 (94)	.06 (91)	.04 (90)	.03 (92)	-.07 (91)	-.04 (91)	-.07 (90)	.04 (92)	-.08 (91)	-.12 (91)	**-.23** (90)	-.14 (91)	-.13 (91)
Executive Function	-.12 (95)	.00 (92)	-.04 (91)	. 01 (93)	-.07 (92)	.02 (92)	-.08 (91)	-.10 (93)	-.15 (92)	-.12 (92)	-.07 (91)	.01 (92)	-.12 (92)
Information	.06 (94)	.01 (91)	.01 (90)	-.00 (92)	- .13 (91)	-.01 (91)	.09 (90)	-.14 (92)	-.11 (91)	-.13 (91)	-.02 (90)	.08 (91)	.03 (91)

Notes: Bold values indicate $p < 0.05$.Values in parentheses indicate *n*; cEFT indicates calculated free testosterone levels; 2-, 4-, 8Chμ and OMOμ indicates 2, 4, and 8 Choice Reaction Time and Odd-Man-Out Mu parameter estimates; 2-, 4-, 8Chσ and OMOσ indicates 2-, 4-, and 8 Choice Reaction Time and Odd-Man-Out Sigma parameter estimates; 2-, 4-, 8Chτ and OMOτ indicates 2-,4-, and 8 Choice Reaction Time and Odd-Man-Out Tau parameter estimates.

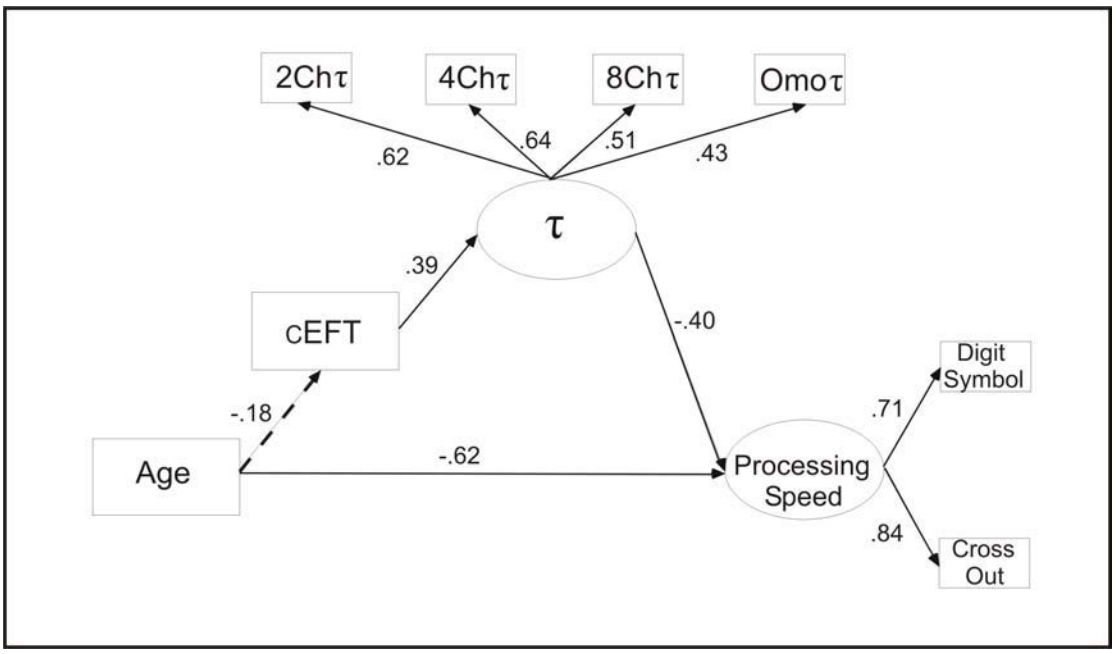

Figure 6. Model of the associations between age, cEFT levels, tau, and processing speed performance ($N = 95$).

Note: 2-, 4-, 8Chτ, and Omoτ indicate the tau parameter estimates from the 2-,4-, and 8 Choice Reaction Time, and Odd-Man-Out tasks, respectively; cEFT, calculated free testosterone levels. Circles denote latent variables.

6.5. Discussion

In the present study it was found that RT intra-individual variability predicted processing speed performance. Previously, consistent with the WPR, Schmiedek et al. (2007) examined Ex-Guassian parameters derived from eight RT tasks as predictors of cognitive functioning in a college aged sample, and found τ to be the strongest unique predictor of WM, reasoning and processing speed performance. Similarly, in the present study, τ was found to be the strongest predictor of processing speed performance for three out of the four RT tasks and, additionally, for WM performance on one task. In contrast to Schmiedek et al.'s (2007) findings, however, in the present study τ was found to be a stronger predictor of processing speed instead of WM performance. Several methodological

differences between studies, such as, the participants' ages, the measures used to derive the Ex-Guassian parameter estimates, and the statistical analyses undertaken, may account for this discrepancy. For example, in the present study middle-to-older aged subjects were used instead of college-aged students. External validity is generally compromised when using college-aged students in intelligence research due to restrictions in both age-range and overall ability levels. Schmiedek et al. (2007) also used a battery of 2-Choice RT tasks as indicators for common latent Ex-Guassian parameter estimates in their structural equation modelling (SEM). In contrast, in the present study four RT tasks of increasing complexity were used to derive separate Ex-Guassian parameters for each test. Because the RT tasks varied in task demands, the average RT distribution for each task also differed, which corresponded with differences in the Ex-Guassian parameters between tasks. For this reason, associations between the individual Ex-Guassian parameters and measures of cognitive functioning were examined separately presently.

In Chapter Five it was shown that cEFT levels moderated processing speed performance in healthy middle-to-older aged men. In the present study, path analyses showed that τ mediated this association such that higher cEFT levels were associated with increased intra-individual variability in RT performance, or higher values for τ, which in turn predicted poorer processing speed performance. This finding of a positive association between cEFT levels and intra-individual RT variability, reflecting poorer attentional control, suggests a potential neural mechanism by which endogenous FT levels may moderate cognitive functioning in ageing men. In addition, the failure to find an association between τ and our composite executive function measure further suggests that the aspects of attentional control captured by τ are independent to frontal lobe mediated executive

functions. This result is consistent with a recent study which similarly reported that ISDs derived from 2, 4, and 8 Choice RT tasks were not associated with a composite executive functioning measure in 48 healthy older adults (mean age = 71.5 years; De Frias, Dixon, Fisher, & Camicioli, 2007). In contrast, in a neuro-imaging study which investigated associations between white matter hyperintensities (WMH) (i.e., the presence of white matter lesions as detected by MRI) within specific brain regions in a large sample of older adults, it was found that increased WMHs within the frontal cortex were associated with greater intra-individual variability, but not overall slowing, on a Choice RT task (Bunce, Anstey, Christensen, Dear, Wen, & Sachdev, 2007). The lack of behavioural associations found between intra-individual RT variability and executive functions, in contrast to the neuro-imaging work, suggests that although the integrity of the frontal lobes subserves intra-individual variability to a small extent, other mechanisms are also involved.

In terms of age-group effects on the Ex-Guassian parameters, it was interesting to note that there were no significant effects found across any estimates derived from the RT tasks. However, inspection of the raw correlations between the individual parameters and chronological age revealed significant negative correlations with μ on three of the four RT tasks, and a significant positive correlation with τ on one test. The significant negative correlations between age and μ are inconsistent with previous research in large population samples which has found increasing age to be associated with slower mean RTs (Der & Deary; 2006; Verhaeghen & Salthouse, 1997). This surprising result may be an artefact of separating the fastest from the slowest RTs by fitting the Ex-Guassian distribution.

Typically, positive correlations between mean RTs and age are interpreted to reflect evidence of generalised slowing, consistent with the processing speed theory of age-related cognitive decline (e.g., Verhaeghen & Salthouse, 1997). More recently, however, Rabbitt, Osman, Moore, and Stollery, (2001) argued that mean performance indices coarsely reflect intra-individual performance variability, and that the effects of both age and intelligence on the fastest correct responses on RT tasks are relatively small compared to these same effects on an individual's total number of slower responses, reflected by measures of performance variability. Consistent with this hypothesis, Hultsch, MacDonald, and Dixon, (2002) computed residualised ISDs for both the 20^{th} and 80^{th} percentiles of 99 younger and 763 older adults' RT distributions across four RT tasks, adjusted for age group, gender, trial and their corresponding interactions, and conducted a hierarchical regression for each task using both age and the 20^{th} percentile ISDs as predictors of the 80^{th} percentile ISDs. For all tasks, age was a significant predictor of the 80^{th} percentile ISDs; moreover, this effect was found to slightly increase after partialing out variability in the 20^{th} percentile ISDs. This result was interpreted to imply that intra-individual variability in RT performance is positively skewed with increasing age. Similarly, Williams, Hultsch, Strauss, Hunter, and Tannock, (2005) reported a quadratic relationship between age and intra-individual variability in performance on a 2-Choice RT task across 273 participants aged between 6 - 81 years. In the adults, age was associated with increasing intra-individual variability in performance independent of mean response speed and other confounds. Consistent with these data, age was found to be significantly positively correlated with τ on the 4-Choice RT task; in contrast, on the same task, age was also significantly negatively correlated with μ. The lack of further significant positive associations

found between age and τ derived from the other three RT tasks may be due to the mediating effects of cEFT levels (see Fig. 5).

The significant negative correlation found between μ and age on the 4-Choice RT task and similarly for both the 2- and 8-Choice RT tasks, may have occurred as an artefact of the statistical dependencies between the Ex-Guassian parameters caused by an increase in the number of slower responses with increasing age. Schmiedek et al. (2007) reported in their analyses substantial and highly significant correlations between the Ex-Guassian parameters, such that τ was negatively correlated with both μ and σ, and μ and σ were positively correlated. These compensatory relationships between parameters occur as a consequence of the requirement that the sum of μ and τ must fit the overall mean of the observed distribution. Recently, a similar pattern of effects was reported in an investigation which used Ex-Guassian parameters to compare the performance of children with Attention Deficit Hyperactivity Disorder (ADHD) with controls on a Go/No-Go test (Connors Continuous Performance Test; Connors, 1994). Corresponding with a larger number of slower responses during task performance, the ADHD children were shown to have a larger mean τ than controls; however, they were also found to have a smaller mean μ (Hervey et al., 2006). Future research is needed to further elucidate age effects on the different Ex-Guassian parameter estimates derived from RT tasks.

Taken together these data extend the findings detailed in Chapter Five by showing that in healthy middle-to-older aged men endogenous FT levels moderated processing speed performance via attentional control processes. Specifically, path analyses showed that higher cEFT levels were associated with increased τ, or intra-individual performance variability, which in turn predicted poorer processing speed

performance with increased age. Consistent with the WPR, hierarchical multiple regression analyses showed that τ significantly predicted both processing speed and WM performance independent to the other Ex-Guassian parameters.

CHAPTER SEVEN: GENERAL DISCUSSION

7.1. Gonadal Steroids and Cognitive Functioning in Middle-to-Older Aged Men

The purpose of the research detailed in this thesis was to examine the effects of gonadal steroid levels on cognitive functioning in men. Initial cross-sectional analyses of these associations across 1046 males aged between 35-81 years showed that cEFT levels were the strongest predictor of cognitive performance when compared with two other commonly used measurements of plasma T levels (see Chapter 3). This was also found to be the case in the studies reported in Chapters Five and Six. Furthermore, in this initial study, cEFT levels were found to moderate the associations between age and both learning and memory and executive function performance after adjustment for confounds. Moreover, a quadratic moderation effect of cEFT levels on the association between age and learning and memory performance showed that the negative effects of high-normal cEFT levels increased with age. Consistent with these data, analyses of the associations between cEFT levels and cognitive functioning measures in the second study similarly showed negative effects of high-normal cEFT levels but only in oldest men in the study sample (i.e., those aged between 50-70 years). In this second study, the largest effects of cEFT levels were found on perceptual discrimination and processing speed measures.

Contrary to initial expectations, no association was found between plasma T levels and mental rotation performance in the second study. This finding was consistent with two smaller studies, one correlational (Wolf & Kirschbaum, 2002), and one involving T supplementation (Wolf et al., 2000), which similarly reported no association in their slightly older participants. Furthermore, the current result was consistent with Hooven et al.'s (2004) suggestion that T's effect on MRT

performance may be moderated via associations with underlying cognitive processes. Thus, it was shown using path analyses that cEFT levels moderated MRT performance indirectly, via associations with both executive function and processing speed. The best fitting model in these analyses, however, was obtained after excluding the executive function variable, so that MRT performance was best predicted by the negative association between cEFT levels and processing speed (see Chapter 5). Together, these data replicated the findings of Schretlen et al. (2000), which similarly found that both processing speed and executive function each independently contributed to explaining age-related variance in fluid-spatial ability but that processing speed was the stronger predictor of the two. In the present research these findings were extended to show that cEFT levels indirectly moderated MRT performance in middle-to-older aged men via an association with processing speed.

Together the results from these studies suggest that the maintenance of high-normal FT levels in middle-to-older aged men, or levels within the normal range for young men, may adversely affect performance on fluid cognitive abilities, such as, learning and memory, executive function, perceptual discrimination, and processing speed. Furthermore, these results show that the largest effects of FT levels were found on the performance of relatively simple speeded measures (i.e., perceptual discrimination and processing speed), which were shown to be strong predictors of age-related test performance on more complex, higher order cognitive abilities.

7.2. Free Testosterone Levels and Generalised Age-Related Cognitive Decline in Men

The second major aim of this thesis was to determine whether declines in plasma T levels with ageing in men were associated with generalised age-related

cognitive decline. To address this aim, associations were examined between FT levels and performance on both processing speed and executive function measures (see Chapter 2).

Across the entire FAMAS cohort, higher cEFT levels were associated with poorer executive function performance. Similarly, the second study showed that in 65 men aged between 50-70 years, higher cEFT levels were associated with poorer performance on a composite measure of executive function. Whilst these results appear to convey a consistent message, it is important to note that neuropsychological measures of executive function are notoriously heterogeneous and almost always invoke non-executive processes (e.g., Baddeley, Della Sala, Papagno, & Spinnler, 1997; Lamar, Zonderman, & Resnick, 2002). This limits the interpretation of the present results in relation to determining specific mechanisms by which FT levels may modulate generalised age-related cognitive decline in men. However, as noted in Chapter Five, there exists some commonality between the neural processes recruited by the executive function measures used in both studies of this thesis; both the ratio of Trails B to Trails A and SART are measures that index recruitment of the DLPFC. Potential modulation of DLPFC function by FT levels, therefore, stands as a reference point for future research into the effects of gonadal steroid levels on executive functioning in males.

Whilst poorer executive functioning was shown to predict both WM and MRT performance with increased age, path analyses showed that processing speed was a stronger predictor of WM performance; moreover, it was age-related decrements in both processing speed and WM abilities which best predicted MRT performance. This result was consistent with the predictions of the processing speed theory of cognitive ageing (Salthouse, 1996a). In order to elucidate how

cEFT levels moderate processing speed performance in men, this association was further examined in Chapter Six.

The results of the subsequent analyses showed that cEFT levels were associated with increased intra-individual RT variability and that this association predicted poorer processing speed performance with increased age. Importantly, these findings indicated a potential neural mechanism by which FT levels moderate cognitive functioning in men. Furthermore, estimates of intra-individual RT variability were not found to be predictive of executive functioning, nor to correlate with executive function performance. Therefore, these data further suggest dissociation between the neural mechanisms by which FT levels moderate both processing speed and executive function performance. Potential mechanisms and avenues for future research are discussed below.

7.3. Potential Mechanisms by which Free Testosterone Levels may Moderate Cognitive Function in Men

The hippocampus is fundamental to learning and memory and is considered to be modulated by gonadal steroids because of the presence of high concentrations of androgen receptors within it. Studies in adult male rats have shown that intrahippocampal T injections impair learning and memory performance (Naghdi et al., 2001; Naghdi & Asadollahi, 2004; Moradpour, Naghdi, & Fathollahi, 2006; Naghdi et al., 2005). A recent study investigated the mechanism by which androgens affect learning and memory as mediated by hippocampal function, by the administration of T and anisomycin, a protein synthesis inhibitor used to prevent T's genomic effects. Whilst both T and anisomycin were each shown independently to impair spatial learning and memory performance, when administered together, the amnesic effects were reversed (Naghdi et al., 2005).

Together these data suggest that both T's genomic and non-genomic mechanisms are important for hippocampal function. It remains to be determined, however, exactly how supraphysiological T concentrations within the hippocampus may cause memory impairments.

In human research on older males, the data from T supplementation studies in older males into the effects of T on verbal memory is equivocal; whilst five studies have reported beneficial effects (Cherrier et al., 2001; Cherrier et al., 2003; Cherrier et al., 2005; Cherrier et al., 2007; Vaughan et al., 2007), four studies have reported no effect (Wolf et al., 2000; Janowsky et al., 1994; Lu et al., 2005; Sih et al., 1997), and two studies a negative effect (Maki et al., 2004; Maki et al., 2007). In contrast to studies in animals, the mechanisms by which T either enhances or impairs memory function in humans are less clear; however, there is evidence which suggests that the reported improvements may be due to T's aromatisation to E2 (Cherrier et al., 2003; Cherrier et al., 2005; Beer et al., 2006). Methodological problems associated with delineating the cognitive effects of E2 aromatised from T in males are discussed below.

In a recent study, changes in regional brain glucose metabolic rates using positron emission tomography were measured following supraphysiological T doses in elderly men (Maki et al., 2007). In this study, decreased short-term verbal memory performance was associated with widespread increases in frontal activity, including within the DLPCF, and a trend towards increased activity in the hippocampus. Cherrier et al., (2007) recently reported improvements in both spatial and verbal memory performance following moderate but not supraphysiological T doses. Previously, higher FTI levels in elderly men have been shown to predict increased regional cerebral blood flow (rCBF) within both hippocampal and frontal

areas (Moffat & Resnick, 2007). In primates, androgen receptors have been located both in the hippocampus (Beyenburg et al., 2000) and the frontal cortex (Finley & Kritzer, 1999). Taken together, these data suggest that T levels in elderly men affect cerebral metabolism in the regions within the brain known to subserve learning and memory functioning, and that these effects may be caused either by the direct genomic effects of T, or of E2 aromatised from T, or both. Further, these data also suggest that learning and memory improvements/decrements following T supplementation are dependent on the age of the subjects and, more importantly, the dose. Future T supplementation studies should therefore attempt to isolate specific mechanisms, through the use of both aromatase antagonists and neuro-imagery techniques (e.g., PET or fMRI), to elucidate how different T levels moderate memory function in men. In these studies attempts should also be made to control for genetic variation in the CYP19 gene, which regulates aromatase expression in the brain.

In relation to potential mechanisms by which T levels moderate executive functioning, the dopaminergic neurotransmitter system has been identified as playing an integral role in modulating performance on higher order cognitive abilities, including measures of mental flexibility, response inhibition, and attention (Volkow et al., 1998). Recent studies in adult male rodents have shown that long-term gonadectomy increases dopamine innervation within the PFC (Kritzer, 2000; Kritzer, 2003; Kritzer et al., 2006), an effect subsequently attenuated by T and DHT but not E2 replacement (Kritzer, 2003; Kritzer et al., 2006). Together these studies show that T but not E2 levels directly moderate frontal functions in adult males. Further animal research is required, however, to determine the behavioural

outcomes of this effect in older animals with lower pre-treatment prefrontal dopamine innervation.

In a neural network model of cognitive aging it has been proposed that increased performance inconsistency (i.e., intra-individual RT variability) may result from age-related deficits in dopaminergic, or other neurotransmitter, neuromodulation causing an increase to neurons' signal to noise ratio (Li, Lindenberger, & Sikstom, 2001). However, a recent study in Parkinson's disease (PD) patients with varying disease severity found no association between intra-individual performance variability and dopaminergic function as measured by finger tapping speed and gait (De Frias et al., 2007). Because frontal lobe functions (including executive functions) are primarily modulated by the dopaminergic system (e.g., Volkow et al., 1998; Backman et al., 2006), these data, together with the finding reported in Chapter Six of no association between intra-individual variability and executive function performance, suggest that intra-individual variability in Choice RT tasks, in contrast to more frontal-cortex-mediated executively demanding tasks (e.g., MacDonald, Nyberg, & Backman, 2006), may not be primarily modulated by dopaminergic pathways.

Alternatively, there is evidence from studies in both animals and humans that the basal forebrain cholinergic system subserves early signal driven, or 'bottom up', processing of novel, salient, or unexpected stimuli in attention demanding contexts (Sarter, Givens, & Bruno, 2001; Sarter, Hasselmo, Bruno, & Givens, 2005; Sarter, Gehring, & Kozak, 2006; Erskine et al., 2004). In adult male rats, changes in T levels have been found to modulate cholinergic neurotransmission (Leonard et al., 2007; Daniel et al., 2003; Nakamura et al., 2002). On the basis of this research, it seems reasonable to speculate, therefore, that endogenous FT levels may moderate

intra-individual variability in men via an interaction with the cholinergic system such that high normal levels are associated with higher distractibility or increased attentional fluctuations. Further, as a result of compromised attentional processes, processing speed performance is decreased, which therefore decreases performance on higher order fluid abilities. Future research in aged male animals, which evaluates both how different T levels affect the integrity of the cholinergic neurotransmitter system and the resulting behavioural outcomes, will help to further elucidate how FT levels modulate cognitive functioning in men.

In summary, recent neuro-imaging studies into the effects of endogenous and exogenous T levels have shown that T levels are related to cerebral blood flow and glucose metabolism within the regions of the brain known to subserve both spatial ability, learning, and memory. Furthermore, animal studies involving the manipulation of gonadal steroid levels, the analysis of behavioural outcomes, and measurement of physiological changes, have demonstrated that T levels interact with neurotransmitter systems pertinent to cognitive functioning. Future research in both human and animals is required, however, to determine specific neural mechanisms responsible for the hormone-cognition associations reported in this thesis.

7.4. Study Limitations

Important limitations to the results detailed in this thesis include the use of cross-sectional analyses, which disallowed for direct inferences of causality between the hormone and cognitive measures, and the lack of control for genetic factors, for example, possession of the apoliproprotein E (APOE) ε4 allele (Parasuraman, Green wood, & Sunderland, 2002) or allelic variants of Catechol *O*-

methyltransferase (COMT; Mattay et al., 2003), which may have influenced cognitive performance.

Possession of the APOE ϵ4 allele has been found to be associated with both increased risk and earlier onset of AD in a dose-dependent manner, such that risk increases from non-carriers, to heterozygotes (one ϵ4 allele), to homozygotes (two ϵ4 alleles). For a review see Corder, Lannfelt, Bogdanovic, Fratiglioni, and Mori (1998). Non-demented carriers of the APOE ϵ4 allele have been found to perform poorer relative to non-carriers on tests of delayed memory (Flory, Manuck, Ferrell, Ryan, & Muldoon, 2000; Burkhardt et al., 2005; Bondi et al., 1995; Bondi, Salmon, Galasko, Thomas, & Thal, 1999), executive function (Wetter et al., 2005; Rosen, Bergeson, Putnam, Harwell, & Sunderland, 2002; Greenwood, Lambert, Sunderland, & Parasuraman, 2005), visual memory (Flory et al., 2000), and visuo-spatial attention and WM (Greenwood et al., 2005). There is also evidence which suggests that T levels and APOE status may interact in their effect on cognitive ageing. For example, Hogervorst, Lehmann, Warden, McBroom, and Smith (2002) found that the presence of AD was associated with both low testosterone levels and the APOEε4 X T interaction. In addition, Burkhardt et al. (2005) found that in 16 elderly male APOEε4 carriers higher FT levels were associated with poorer performance on a composite measure of executive functioning, WM and attention, whilst in 29 non-ε4 carriers higher FT levels were associated with better general cognitive functioning. Future investigations into hormone-cognition relationships should, therefore, control for APOE genotype status.

Another important limitation to these findings is the possibility that the reported associations between T and cognitive performance may have been due to either the effects of E2, or the ratio of T to E2. In the second study of this thesis E2

levels were not accounted for because of my assay's lack of sensitivity to accurately detect levels in the lower range typical of older men. Recently, in a large study of 2,623 men aged 65 years and older, free E2 levels were reported to be modestly positively correlated with FT levels ($r = 0.20$, $p < 0.001$), but also to vary substantially between individuals (Orwoll et al., 2006). Given that the majority of free E2 available to the male brain is known to be produced locally through the aromatisation of T (Simpson, 2000) and that polymorphisms of the CYP19 gene regulate aromatase expression in the brain, it is difficult, therefore, to discriminate between the effect of T on cognition associations in males without accounting for genetic variation. Future investigations in this field utilising androgen supplementation or suppression treatments with aromatase antagonists may overcome this issue and help to elucidate specific associations between free E2 levels and performance on cognitive abilities in males.

In relation to the generalisability of the present findings it is also important to emphasise that the sub-sample of FAMAS participants studied in Chapters Five and Six was atypical to a randomly selected sample taken from the general population. Neither unhealthy, left-handed, homosexual, nor men with current or previous mood disorders were represented in the sample. These exclusion criteria therefore limited the generalisability of the hormone-cognition associations reported in these chapters. Furthermore, the findings reported in these chapters were specific to middle-to-older aged men: the question of whether similar associations or otherwise hold in either younger (i.e., < 39 years) or older (i.e., >70 years) men remains to be determined.

7.5. Conclusions

Contrary to initial expectations, endogenous plasma T levels were not found to be associated with visuo-spatial ability in middle-to-older aged men. Furthermore, declines in plasma T levels with ageing in males were not found to be associated with generalised age-related cognitive decline. Instead, FT levels were found to be associated with some cognitive ability measures but these associations were in the opposite direction than initially hypothesised. To summarise these findings briefly, high-normal FT levels, in particular in older men, were found to be associated with poorer learning and memory, executive function, processing speed, perceptual discrimination, and intra-individual RT variability performance. Moreover, analyses of the associations between chronological age, FT levels, intra-individual RT variability, and processing speed performance showed that higher cEFT levels were associated with increased intra-individual RT variability, and that this association was predictive of poorer processing speed performance. Together these findings show that in middle-to-older aged men, FT levels moderate performance on executive function and intra-individual RT variability measures and it is these associations which predict poorer cognitive functioning on higher order fluid abilities. To conclude, these data do not support the proposition that T replacement therapy will protect against age-related cognitive decline in older men with endogenous T levels within the 'normal' range for younger men. In contrast, these data suggest that the maintenance of high T levels into old age may be related to poorer cognitive functioning.

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Appendix 2. Zero order correlations between age, log transformed hormone levels and cognitive functioning outcome variables ($N = 1046$)

Variable	Age	LogcEFT	LogTT	LogBT	LogSHBG	LogLH	LogFSH	LogE2	TrailsA	TrailsB	TrailsB/A	TotRec	TotMemRep	TotStor
LogcEFT	-.27**													
LogTT	-.16**	.94**												
LogBT	-.45**	.68**	.64**											
LogSHBG	.34**	.04	.33**	-.06*										
LogLH	.23**	-.03	.07*	-.05	.24**									
LogFSH	.33**	-.21**	-.12**	-.21**	.21**	.62**								
LogE2	.10**	-.01	.04	-.05	.14*	.08*	.03							
TrailsA	.44**	-.23**	-.15**	-.18**	.25**	.11**	.15**	.08*						
TrailsB	.48**	-.16**	-.10**	-.23**	.19**	.14**	.22**	.05	.61**					
TrailsB/A	.10**	.07*	.06*	-.05	-.03	.07*	.09**	-.01	-.31**	.50**				
TotRec	-.30**	-.12**	-.13**	.21**	-.02	-.13**	-.10**	-.07*	-.13**	-.28**	-.20**			
TotMRep	-.29**	-.13**	-.13**	.20**	-.02	-.13**	-.10**	-.07*	-.10**	-.25**	-.19**	.97**		
TotStor	-.22**	-.08*	-.08*	.16**	-.02	-.09**	-.07*	-.03	-.14**	-.27**	-.16**	.79**	.68**	
TotMInt	.26**	.03	.04	-.19**	.04	.09**	.08**	.08**	.20**	.33**	.16**	-.77**	-.66**	.-.80**

Notes: ** $p < 0.01$
* $p < 0.05$

Log cEFT indicates log transformed calculated free testsosterone; LogTT, log transformed total testosterone; LogBT, log transformed bioavailable testosterone; LogSHBG, log transformed sex hormone binding globulin; LogLH, log transformed luteinising hormone; LogFSH, log transformed follicle stimulating hormone; LogE2, log transformed estradiol; TrailsA, Trail making test part A; TrailsB, Trail making test part B; TotRec, total recall; TotMRep, total memory repetitions; TotStor, total storage TotMInt, total memory interference.

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