# COGNITION IN PROSTATE CANCER PATIENTS BEFORE UNDERGOING ANDROGEN DEPRIVATION THERAPY AND ELDERLY MALES.

**By**

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# Abstract

Research conducted in the past has shown the deleterious effects of androgen deprivation therapy (ADT) on cognitive impairment in prostate cancer (PC) patients. However, many studies have been limited due to small sample sizes and due to unmatched control samples. Many previous undertakings have also lacked the comprehensive assessment of patients before treatment leading to mixed outcomes of affected cognitive domains during treatment which have been found to be in executive function and spatial reasoning. The current study therefore aimed to characterise cognitive function in patients compared to age matched healthy controls.

Thirty ageing prostate cancer patients before ADT and twenty-nine ageing older matched healthy control participants underwent behavioural cognitive measures and psychosocial measures which were analysed to assess differences between groups cross-sectionally. Covariates (anxiety, depression and testosterone) were also added to analysis models to assess their effects. No significant differences on neuropsychological measures or mood scales were found between groups suggesting that executive function and spatial reasoning were intact in both groups at baseline. However, a significant difference was found in testosterone levels between patients and controls in which patients had higher testosterone levels.

Brain imaging was undertaken of the controls and patients using magnetic resonance imaging techniques. Groups underwent fMRI tasks on imaging paradigms (stops signal task and mental rotation task) plus task free functional resting state functional connectivity and arterial spin labelling. No activation, resting state or arterial spin labelling differences were found between groups. However, a significant difference in scanner behavioural performance was found in which patients had better accuracy on sub components of the mental rotation task.

A longitudinal study was subsequently undertaken to assess behavioural cognitive performance in healthy ageing participants only and their neural correlates in relation to testosterone, anxiety and depression. This was to assess the usefulness of the sample as a control sample that was undertaken before. Furthermore, the study was conducted to explore the reliability and stability of cognition and neural function over a longitudinal follow-up period. Outcomes showed no significant differences were found between baseline and six month follow-up. However, testosterone was shown to covary with two subcomponents of executive function. Neuroimaging revealed testosterone covaried with one subcomponent of spatial reasoning.

In conclusion, patients and healthy controls were similarly matched for cognitive and neural function showing patients had intact executive and spatial reasoning function before they began ADT. Patients may have implemented a compensative mechanism that required testosterone to gain similar performance to healthy controls 81on tasks of spatial reasoning. However, this has implications when patients begin therapy as testosterone will be deprived. Therefore, these compensatory mechanisms may not be at play. This informs further research and facilitates the development and management of cognition in prostate cancer patients before and during therapy to prevent side-effects before therapy so they are not exacerbated during therapy. The second study of longitudinal performance in healthy controls showed that cognitive and neural; function was reliable and stable across a period of six months. This confirmed that the sample was well matched to patients for age and intelligence and provides better robustness for the cross-sectional study which assessed patients and controls.

# CHAPTER 1

## 1.0 Introduction

Androgen deprivation therapy (ADT) is an effective treatment for more locally advanced prostate cancer (PC) and acts by moderating hormone levels to prevent cancer metastasis thereby prolonging PC survival. However, adverse effects of ADT have been reported, particularly in relation to reductions of testosterone. There is also evidence that changing hormone levels can moderate cognitive functions, however, existing literature exploring the cognitive side effects of ADT is inconsistent and the number and scope of investigations is limited. The present study aimed to investigate the nature and basis of cognitive changes that occur before ADT using multi-model behavioural and neuroimaging techniques.

This chapter presents the current literature around prostate cancer and its treatment; the effects of androgen deprivation therapy (ADT) among men with prostate cancer and a broader picture of how changes in testosterone affect cognition. Finally, the chapter presents the current literature around cognitive changes in men with prostate cancer who receive ADT. An underexplored area of past literature is the comprehensive assessment of cognition and underlying brain changes that occur in PC patients before commencing ADT. Therefore, the current investigation was conducted with comprehensive cognitive measures and neuroimaging methods before PC patients began ADT. Additionally, past assessments have suffered from unmatched control cohorts. Therefore, a further exploration of healthy controls was undertaken over a longitudinal period to identify factors that may affect cognition over time and to validate that healthy controls were matched in the comparison to patients and controls.

### 1.1 Background to PC and its treatment

The prostate is a gland found in the male reproductive system responsible for secreting an alkaline fluid that makes up almost 30 percent of the volume of semen, sperm and seminal vesicle fluid (Huggins, Scott, & Heinen, 1942). The alkalinity of semen thereby allows neutralization of acidic content of the vaginal tract to prolong the lifespan of sperm (Huggins et al., 1942). The prostate has been classified into five zones including the; peripheral zone, central zone, transition zone and anterior fibro-muscular zone. Out of these five zones, the peripheral zone is located in the posterior portion of the prostate gland that surrounds the urethra and has been found to be the most prone to cancer where almost 70-80% of prostate cancers are found (Haffner et al., 2009).

The incidence of PC has been rising. There were 576 males with PC per 100,000 males between the ages of 65-69 in the UK in 2013 (Cancer Research UK, 2013). By the ages of 75-79 years this incidence rate rose to 815.2 per 100,000 (cancer Research UK, 2013). The total number of new PC cases diagnosed in 2013 was 47,300 cases (Cancer Research UK, 2013). This increased from the year 2011 when the number of PC cases diagnosed was 41,736 (Cancer Research UK, 2011). On average, more than half (54%) of males over 70 are diagnosed with PC (Cancer Research UK, 2013). This may be because males over the age of 50 have been shown to have an increasing number of microscopic focus adenocarcinomas compared to younger samples in the prostate gland that eventually become larger glandular carcinomas (Scardino, 1989). Increased incidence and prevalence rates may reflect in part, improvements in the detection of prostate cancer including prostate specific antigen tests (PSA) and transurethral re-sectioning surgeries that reveal tumour formations (Mayor, 2012). Ninety four percent of PC patients have been shown to survive after one year of diagnosis, 85% after five years and 83.8% after ten years. Therefore, individuals are more likely to survive a PC diagnosis due to early discovery (Scardino, 1989). Consequently, due to

increased survival rates, current research has now moved focus to the side-effects of treatments.

Cancer occurs due to epigenetic and genetic changes to normal cells (Prendergast, Metz, & Muller, 2010). This variation in cell structure may lead to abnormal cell formations and then to tumorigenesis (Mills, Ferguson, & Alt, 2003). Cell augmentations may be evident in the prostate gland which progress to PC through tumorous developments (Butcher & Boland, 2012). Diagnosis of cancer is accompanied by a Gleason histologic score or grade that has been acquired though tissue biopsy of the prostate gland (Draisma, Postma, Schröder, van der Kwast, & de Koning, 2006). This grading is a dominant predictor of biologic behaviour and has an important role in patient treatment pathways (Draisma et al., 2006). The Gleason system is used to evaluate PC prognosis from microscopic tissue samples acquired during a biopsy of the gland by appearance of the microscopic prostate tissue. The Gleason score ranges from 1 (well segregated small identical circular glands where tumour formation does not invade into healthy prostatic tissue) to 5 (poorly segregated glands with loss of architecture and tumour incursion into the majority of the gland). The Gleason score (GS) is a combination of a primary and secondary score (Freeman & Coard, 2004). The primary pattern is assigned to the main trend of the tumour which typically forms more than 50% of the total pattern (Pierorazio, Walsh, Partin, & Epstein, 2013). The secondary grade is given to the next most prevalent pattern recognised which forms less than 50% of the overall pattern seen (Pierorazio et al., 2013). Therefore, the lowest possible score is 2 (non aggressive) and highest possible score is 10 (very aggressive) (Freeman et al., 2004; Huang et al., 2014). A final grade component may be recognised forming a tertiary part of the pattern which is generally more aggressive (Pierorazio et al., 2013). Many studies have showed that patients with a GS of more than 7 are at greater risk for extra-prostatic adenocarcinoma growth or

biochemical recurrence (Green, Hanlon, Al-Saleem, & Hanks, 1998). The utility of the GS has been assessed in the grading of tumours at grade 7. This is because a primary score of four plus a secondary score of three may be more likely to increase the chances of mortality compared to a primary score of three plus a secondary score of four as the more prominent pattern is the primary pattern. A study by Stark et al. (2009) found that 4+3 cancers were associated with a three-fold increase in aggressive PC (measured by PC mortality rates) compared to 3+4 (95% CI, 1.1 to 8.6). This indicates that primary scores should be given more attention in PC diagnosis if the score is high especially in GS 7 diagnosed cancers. Inter-observer concordance of Gleason score ratings between pathologists show discrepancy between distinguishing prostatic gland abnormalities identified as being either levels six or seven (Freeman & Coard, 2004). This may have implications on either over or under-grading of tumour severity. This may lead to treatment which may be damaging to healthy tissue if under-grading occurs or over administration of treatment if under grading. This may inevitably affect mortality and therefore consensus between physicians is required. This effect has been attributed due to low biopsy volumes and the Will Rogers phenomenon in which there is a systematic upgrading in GS known as a Gleason shift (Freeman & Coard, 2004; Stark et al., 2009). This has been found to occur because researchers have recommended that Gleason scores from 2-4 should not be identified from biopsy of the prostate (Epstein, 2000). The assignment of the Gleason score is based on the predominant tumour grade and secondary tumour grade. This results in an increase in the Gleason score for many patients which may falsely increase the Gleason score due to the lack of biopsy availability. This may lead to upgrading of 2 and 3 Gleason scores to higher levels that lead to intra-observer discrepancies and incorrect treatment options.

### 1.1.2 Treatments and management

Treatment choices for PC depend on the grading of cancer at the time of diagnosis. Methods to treat PC vary and come with advantages and disadvantages. A common and longstanding form of treatment for PC includes radical prostatectomy that involves the complete removal of the prostate gland (Anandadas et al., 2011; Petry et al., 2004). This approach has been used in forms of localised and metastatic PC in the primary phase and if cancer reoccurs after an initial treatment for localised PC (Metcalfe, Smaldone, Lin, Aparicio, & Chapin, 2017). The effectiveness of prostatectomy has been demonstrated when PC is locally advanced and aggressive requiring a rapid solution to prevent metastatic spread (Graefen & Schlomm, 2012; van Poppel, 2014). Orchiectomy is another technique that has been used to surgically castrate patients to depress the hormonal production of testosterone (Eisenberger et al., 1998). Surgical castration lowers the ability of cancer cells to metastasise through testosterone binding mechanisms. Prostatectomy on the other hand removes the chances for tumour manifestations to be acted upon due to site removal, leading to retardation of prostate cell growth (Eisenberger et al., 1998). The effectiveness of surgical ablation has been investigated and found to reduce metastasis, and mortality from any cause (Bill-Axelson et al., 2008; Holmberg et al., 2002). A study assigned 166 of 347 males with localised PC for radical prostatectomy and 201 patients to a watchful waiting condition with follow-up over 12.8 years (Bill-Axelson et al., 2008). Researchers found significant differences ($p<0.01$) in mortality rates in which 55 (14.6%) men assigned to surgery died and 81 (20.7%) men assigned to watchful waiting died due to PC (Bill-Axelson et al., 2008). Furthermore, patients with low grade tumours benefitted more from radical prostatectomy. A shortcoming was that the average age of participants was 65, therefore it is not known whether these findings extend to older patients. Bilateral orchiectomy although uncommon was considered the gold standard for androgen surgical suppression (Gomella, 2009). The half-life of native

testosterone was found to be reduced to 45 minutes (Gomella, 2009) with castrate levels of testosterone reached in only 8.6 $\pm$ 3.2 hours (Lin, Chen, Chen, & Chang, 1994). Improvements in patients with metastasis were moreover seen in the first 24 to 48 hours (Gomella, 2009). A negative aspect of orchiectomy was of the irreversible nature of the procedure and has therefore been associated with a significant psychological impact (Boccon-Gibod, 2005). Mood disorders such as anxiety and depression have been commonly reported in orchiectomy patients and found to stem from a number of factors including impotence and the feeling of loss of manhood (Louda et al., 2012). Psychosocial side-effects may be partially resolved by the use of testosterone replacement therapy (TRT) but plasma testosterone levels may never fully recover to pre surgical levels. Moreover, TRT is not viable in PC patients since it has been linked to PC stimulation and may lead to rising PSA or cancer recurrence (Rhoden & Morgentaler 2004). As a result, orchiectomy may induce lifelong psychosocial and cognitive impairments that are never fully resolved but can only be partially managed (see section 1.3 for the effects of testosterone on cognition). However, ADT allows testosterone suppression without surgical intervention to orchiectomy levels with a better prospect to resume daily living and quality of life, as testosterone levels return to normal after 18-24 weeks of the deprivation period (Murthy et al., 2006; Sun, Choueiri, Hamnvik, & et al., 2016).

Another form of treatment for PC is brachytherapy which is the technique of surgically inserting a radiated seed in the cancerous area (Blasko, Grimm, & Ragde, 1993). Theoretically, brachytherapy has been found to be advantageous as it can be administered in a highly confined area of cancer growth (Blasko et al., 1993). The effectiveness of brachytherapy has been demonstrated when administrated in temporary dosages combined with external beam therapy or as a permanent implant that loses strength over an extended period of time (Blasko et al., 1993; Yu et al., 1999). Moreover, the efficacy of high dose rate

brachytherapy (HDR-BT) has been evaluated compared to patients undergoing combined HDR-BT and external beam radio therapy (EBRT) in patients with tumours graded between Gleason score 2 to 10 (Smolska-Ciszewska et al., 2015). A further treatment option found to be effective in the management of PC is cryotherapy which is administered as a primary treatment or as an adjuvant therapy (Nguyen, Allen, & Pow-Sang, 2013). Cryotherapy is administered at extremely low cryoprobe temperatures to initiate ablation to the prostate gland tissue, thereby causing it to lose function (Shinohara, 2003). The above-mentioned treatments for PC have been effective in remission and reduced cancer recurrence when applied correctly. The recurrence free rates detected through rising PSA levels >20ng/ml (suggests major risk of PC) of PC after prostatectomy has been found to be more that 80 percent over a period of five years (Han et al., 2003; Walsh, 2000). Long term biochemical relapse survival has been shown to be 74% after 15 years of bracytherapy treatment in one study (Sylvester et al., 2007). Cryotherapy has also been found to prevent biochemical relapse by up to 84% after 8.9 months of the therapy (De La Taille et al., 2000). Another study showed biochemical free prostate recurrence using cryotherapy as being 64% after 10 years (Siddiqui et al., 2016). Although the treatments described have low to medium recurrence rates, they involve the removal of the prostate or source organs involved in supplying androgens to the prostate. Whilst these methods are effective in halting cancer progression, they have been known to cause irreversible tissue damage to source organs and to healthy surrounding tissue which are not involved in cancer. This can lead to lifelong consequences that affect patients psychologically, physiologically and are difficult to manage. These side-effects include urinary incontinency, rectum pain, bowel dysfunction, erectile dysfunction, impotence and feeling of loss of masculinity (Bill-Axelson et al., 2014; Oliffe, 2005; Steineck et al., 2002). Many of these adverse effects can be treated but may require permanent assessment, medication or therapy.

A cornerstone treatment of advanced PC is androgen deprivation therapy (ADT). Androgen deprivation therapy has been used to down-regulate testosterone production and decrease the likelihood of the tumour microenvironment gaining access to testosterone for growth (Butcher & Boland, 2012). It was first initiated as a treatment method for PC in 1959 as a drug called diethylstilbestrol (DES) and worked by suppressing luteinizing hormone-releasing hormone (LHRH) (Byar, 1972). This led to reductions in bony metastases (Byar, 1972). Moreover, researchers showed that orchiectomy combined with DES was not cumulatively beneficial compared to standalone DES and that 1mg DES was comparable to 5mg DES on PC survival (Byar, 1972). However, findings also showed an association of DES with cardiovascular toxicity and was therefore not a favoured form of ADT (Malkowicz, 2001). The LHRH was purified in later years in which chronic exposure to LHRH supressed testosterone by desensitising pituitary cells (Schally et al., 1971) with a lower incidence of cardiovascular risk (The Leuprolide Study Group, 1984). The above literature demonstrates that ADT was a more viable option to treat PC without causing irreversible damage to the prostate gland and to surrounding tissue.

Many forms of testosterone suppression treatment have been documented. The main forms recognised in past research are oestrogens, steroidal anti androgens, non-steroidal anti androgens, 5α reductase inhibitors and the most commonly administered luteinizing hormone releasing hormone (LH-RH) agonists and antagonists (e.g. goserelin acetate) (Miyamoto, Messing, & Chang, 2004). Administration techniques include subcutaneous patches, intramuscular injection or in oral pill form (Nyman, Andersen, Lodding, Sandin, & Varenhorst, 2005; Perlmutter & Lepor, 2008). Additionally, treatment conventionally ranges between three to four months continuous or one to twelve months intermittent treatment durations (Tunn et al., 1998). Approximately 50 percent of PC patients are reported as receiving ADT as an induction therapy before the main treatment or as a standalone therapy

(Green et al., 2004). Only one study has compared different LHRH agonists (Heyns, Simonin, Grosgurin, Schall, & Porchet, 2003). Findings revealed higher survival rates with triptorelin pamoate compared to leuprolide acetate (90% vs. 90.5%) in a nine month retrospective randomised controlled study (Heyns et al., 2003). Although this was not significant at a pharmaceutical level, there was a trend for triptorelin to maintain superior castrate levels over nine months. Whilst these findings point to similarities between LHRH subcategories on survival and testosterone suppression, a limitation to the study was that a variety of LHRH variants exist that could be compared.

The effectiveness of ADT has been evaluated in a number of studies. One study compared the effects of LHRH to anti-androgen monotherapy in patients with localised PC (Raina, Pahalajani, Agarwal, & Zippe, 2007). One group of patients received LHRH agonist monotherapy and the other group received anti-androgen monotherapy over the course of four years. Findings verified that LHRH was more effective in suppressing PC metastatic growth compared to anti-androgen therapy, measured by prostate specific antigen (PSA) levels (1.6% vs. 57.1% respectively) (Raina et al., 2007). Furthermore, patients undergoing LHRH reached nadir levels of PSA (<0.1 ng/ml) more quickly compared to the antiandrogen group. Outcomes indicate that patients undergoing anti-androgen therapy required continuous monitoring of PSA levels, and if increases in PSA were present then a switch was required to other anti-androgens. Nevertheless, a study assessing the effectiveness of ADT in prolonging survival for older men with localised PC, found that ADT was not associated with increased survival benefits (Holmes, Chan, Jiang, & Du, 2007). Crude overall mortality was in fact higher in the ADT group compared to non-ADT group (hazard ratio =1.54; 95%) (Holmes et al., 2007). However, this mortality risk reduced in the non-ADT group after controlling for socio-demographic factors (education, percentage living below the poverty line and median annual income), tumour features and chemotherapy regimens over the 10 year period.

Shortcomings to the study included that patients were older than 65 years of age therefore the findings may not be transferable to younger patients. Moreover, medical profiles were not provided for patients who had had surgical interventions (orchiectomy, prostatectomy) in non-ADT patients or any combined treatments such as additional anti-androgens (bicalutamide lead ins etc.), which could be more effective in preventing mortality. Finally, males in the age range were more likely to have higher Gleason scores, comorbid conditions and be of an older age, which could significantly contribute to age advancing mortality and morbidity. For example, ageing when combined with ADT has been associated with a greater risk of developing Alzheimer's disease (Nead et al., 2016). One study assessed 16,888 subjects with PC with the Kaplan Meiger age stratified analyses to quantify the number of patients undergoing ADT that also developed Alzheimer's disease. Patients who were 70 years of age and undergoing ADT were found to have a 2.9% probability of developing Alzheimer's disease, over five years compared to younger ADT PC (1.9% probability) and age matched non-ADT PC (0.5%) patients calculated with hazards ratio (Nead et al., 2016). This suggests that ADT may play a role in the development of Alzheimer's disease in especially ageing participants leading to cognitive impairment (Baudic et al., 2006). The development of Alzheimer's disease may also have a larger role to play in post ADT medication regimens, cancer recurrence and mortality rates in geriatric populations.

Although ADT provides a significant clinical response in PC it has been shown that PC often becomes independent of androgens and inevitably leads to metastatic growth regardless of androgen deprivation (Miyamoto et al., 2004). LHRH in combination with anti-androgen medications appears to be the most effective hormonal treatment method for treating PC in the primary treatment stages (Boccardo et al., 1993; Crawford et al., 1989; Janknegt et al., 1993). However, alterations to medications are recommended when PC becomes independent

of androgens. Combined androgen blockade with anti-androgens has been hypothesised to lead to a better quality of life as it has been shown to preserve gonadal function compared to LHRH monotherapy that has been known to incapacitate gonadal function (Miyamoto et al., 2004). Despite the reported efficacy of ADT, a myriad of life affecting adverse effects are reported in an estimated 80-90 percent of patients (Green et al., 2004). These are presented in the next section.

### 1.2 Side effects and consequences of ADT use in men with prostate cancer

Physical side-effects of ADT have been described as being the most immediate and noticeable of the effects. The initial flare effect reported after LHRH agonist administration has been described being the most distressing and serious physiological outcome (Thompson, 2001). Past research has shown that approximately 4-11% of patients are reported to experience the flare effect and report side effects of the initial flare (Bubley, 2001; The Leuprolide Study Group, 1984). This effect occurs in the first one to two weeks when LHRH agonists attempt to hamper the normal release of LHRH from the hypothalamus (Thompson, 2001). Naturally occurring luteinizing hormones (LH) are known to peak at three times the baseline level during this period (Waxman et al., 1985) resulting in LH stimulation of leydig cells in the testes which exacerbates testosterone production (Chen, Hardy, & Zirkin, 2002). Patient have reported that this is uncomfortable and is often associated with pain during the first 36 hours of administration (Thompson, 2001). Nevertheless, researchers have shown that testosterone levels typically return to normal levels three weeks after LHRH administration (Thompson, 2001). Complications from the initial flare effect include lymphedema (8.7%), urinary obstruction and retention (2.7%), spinal compression (4.7%) and death (1.56%) (Kahan, Delrieu, Amor, Chiche, &

Steg, 1984; Kreis, Ahmann, Jordan, de Haan, & Scott, 1988; Thompson, Zeidman, & Rodriguez, 1990; Waxman et al., 1985). Blockade of flare has been explored through the administration of specific additional drugs during the initial lead in period ranging from of one week to one month. Agents such as cyproterone acetate (CPA) (Boccon-Gibod, Laudat, Dugue, & Steg, 1986), flutamide (Crawford et al., 1989), nilumide (Kuhn et al., 1989) or ketoconazole (Allen et al., 1983) have been found to blunt the flare effect.

Other adverse effects of ADT include loss of libido, erectile dysfunction, and hot flashes. Amelioration of these side effects requires additional medications such as Venlafaxine, gabapentin (Prostate Cancer, 2006; Sountoulides & Rountos, 2013). Non-pharmacological interventions have also been found to be effective for hot flashes and erectile dysfunction (Stefanopoulou, Yousaf, Grunfeld, & Hunter, 2015). Further treatments such as calcium and vitamin D supplements can help reduce side-effects such as osteoporosis (Sountoulides & Rountos, 2013). Anaemia is another effect reported in 90% of patients and can occur due to a multitude of factors. One important variable to consider is that androgens have been known to stimulate erythropoiesis which is a process that allows the formation of red blood cells (Crafts, 1946). Therefore deprivation of androgens could reduce this effect leading to lower blood counts and anaemia may result. Due to low haemoglobin levels anaemia may lead to fatigue - reported in 14 percent of patients undergoing three months of ADT (Prostate Cancer, 2006). Insulin resistance is a serious metabolic side effect reported of ADT which could progress to diabetes and increased cardiovascular problems (Sountoulides & Rountos, 2013). Diabetic medications and statin therapies are commonly prescribed to treat these conditions (Prostate Cancer, 2006). A further side effect reported in the vast majority (50%) of patients is gynecomastia which can be treated by prophylactic radiation (Viani, Bernardes da Silva, & Stefano, 2012).

Past literature has shown that participants may experience behavioural changes such as problems with cognition in domains of executive functioning, spatial reasoning and memory. These changes may occur due to many comorbid factors or treatments used to control comorbidities. Comorbid variables in ADT patients may include diabetes, high cholesterol levels, fatigue or mood changes. Patients undergoing ADT are 60% more likely to develop type 2 diabetes compared to healthy males in the general population (Tsai et al., 2015). Whilst diabetes in itself has been associated with cognitive impairment (impaired executive function and motor speed) even at early stages of discovery (Kodl & Seaquist, 2008; Ruis et al., 2009; Yeung, Fischer, & Dixon, 2009) treatment of it could intensify this effect. One study assessing the usage of metformin among diabetes sufferers found participants with diabetes had worse cognitive performance compared to those without diabetes. Additionally, diabetics medicated with metformin, had lower general cognitive status (mini-mental state exam) scores compared to unmedicated participants. (Moore et al., 2013). Mechanisms by which metformin may lead to cognitive impairment include through its downregulation of vitamin B12 which can lead to cognitive instability (Lachner, Steinle, & Regenold, 2012). This is important since metformin is a first line medication, prescribed initially due to its efficacy when type 2 diabetes is diagnosed to lower blood glucose levels (Rojas & Gomes, 2013). Thus it could lead to cognitive deficits in patients undergoing ADT (Rojas & Gomes, 2013). Limitations of the study included that durations of metformin use were not reported, severity of diabetes was not reported and only one cognitive assessment was implemented to assess cognition. Another study examined the effects of metformin on β-amyloid precursor protein (APP) in neurons of mouse models (Chen, Zhou, et al., 2009). Outcomes revealed that metformin administered at the levels of activating AMP-activated protein kinase (AMPK) increased the generation of Aβ proteins

(Chen, Zhou, et al., 2009). This was found to be due to the upregulation of β-secretase (BACE1) resulting in raised protein levels and enzyme activity (Chen, Zhou, et al., 2009). In conclusion, metformin did not exert an effect on Aβ degradation which is in contrast to the observations made when insulin monotherapy is administered. Thus, metformin monotherapy may lead to harmful consequences in geriatric patients with diabetes and could initiate cognitive impairments in ADT patients with diabetes.

Other areas of research show that patients undergoing ADT are susceptible to developing high cholesterol levels and are prescribed statin therapies (Mohamedali, Breunis, Timilshina, & Alibhai, 2011). Statins have been widely associated with cognitive deficits (Mohamedali et al., 2011). The food and drug administration (FDA) have received reports that some widely used statins (atorvastin, fluvastin, simvastin etc.) can lead to symptoms of memory loss and confusion but which after cessation are reversed and resolved (Administration, 2014). In contrast to this warning, some reviews have shown that no cognitive impairments were associated with statins (Gauthier & Massicotte, 2015; Gracias, Garrison, & Allan, 2014; Richardson et al., 2013).

Finally, mood disorders are commonly diagnosed in patients undergoing ADT. Rates of depression in ADT patients are eight times higher than in the general population (Prostate Cancer, 2006). Longitudinal studies exploring depression in ADT patients have shown differences in depression and anxiety levels across treatment periods. One study reported that patients (mean age=68.9, SD=2.5) undergoing ADT experienced adverse effects including confusion and depression compared to age matched patients undergoing oestrogen therapy and healthy controls at four weeks into ADT (Beer et al., 2006). However, a limitation was that mood assessments were not made after longer durations of

ADT. Another study also showed that ADT PC patients (mean age=62.05, SD=7.19) had greater levels of self-rated irritability, anxiety, moodiness and fatigue at nine months compared to age matched healthy controls (Cherrier, Aubin, & Higano, 2009). A further experiment by Almeida, Waterreus, Spry, Flicker, and Martins (2004) supports these findings showing that ADT patients (mean age=72.4, SD=7.5) had significantly higher anxiety and depression levels over a period of nine months compared to baseline. A limitation however was that no comparison groups were assessed in the study. As illustrated, mood factors are a prevalent side-effect of androgen blockade therapies. However, medications used to treat these conditions may be more harmful than beneficial. Anti-depressant medications are commonly administered to combat depression however, they have side effects of their own (NICE - National Institute for Health and Clinical Excellence Pathways, 2015). Researchers tested the effects of anti-depressant regimens including diothiepin and fluoxetine with outcomes showing that patients had lower performance on tests of sustained attention, concentration and memory with increased fatigue (Ramaekers, Muntjewerff, & O'Hanlon, 1995). As a result, anti-depressants may inadvertently lower quality of life and affect cognition (Stevanovic, Tadic, & Knez, 2014). On the other hand, a recent study by Greer, Sunderajan, Grannemann, Kurian, and Trivedi (2014) revealed that the antidepressant Duloxetine significantly improved cognition across several domains of psychomotor function, mental processing, and visual and verbal learning. The above literature demonstrates that many factors such as comorbities can affect cognition in PC patients undergoing ADT. Furthermore, treatments used to counteract these simultaneous side-effects have impacts on cognition that may not necessarily be beneficial.

As is illustrated, many physical and behavioural comorbidities that develop as a result of ADT that can lead to cognitive impairment. Moreover, medications used to treat

comorbidities may have negative impacts on cognition. These impacts may lead to long term complications or improvements, depending on whether variables are considered risk or resilience factors. Despite reports of extraneous factors having an effect of PC patients, the direct effects of ADT on cognition have been reported in a past literature review. One meta-analysis and systematic review collated data from 14 studies (McGinty et al., 2014). An analysis of effect sizes showed PC patients undergoing ADT had impairments on tasks that required spatial reasoning with a reaction time motor component. Further support for the review comes from a recent meta-review in which 28 previously conducted reviews with 20 primary studies were statistically assessed (Treanor, Li, & Donnelly, 2017). The researchers' analysis of effect sizes showed that the prevalence of cognitive impairment ranged from 10% to 69% in domains of executive function, spatial reasoning ability and verbal memory. This suggests there is a link between ADT and cognitive impairment. However, before exploring studies more specifically, the relationship between testosterone and cognition in other and similar contexts will be explored.

## 1.3 The Relationship between Testosterone and Cognition

### 1.3.1 Data in Healthy Humans

Testosterone has been found to modulate cognition in domains of memory, executive function, spatial reasoning and processing speed. The relationship between testosterone and these domains has been investigated in healthy participants. One study found that neither high nor low extremes of testosterone were associated with cognition whilst controlling for age (Muller, Aleman, Grobbee, de Haan, & van der Schouw, 2005). The study aim was to find the optimal level of sex hormone that benefitted cognition in healthy participants (Muller et al., 2005). Researchers revealed an inverse relationship showing a negative linear relationship between decreasing testosterone levels and processing speed and executive function. Conversely, a positive relationship was found between increasing testosterone levels and cognitive function in the oldest males (70-80 years) (Muller et al., 2005). However, when quadratic terms were added to the multivariate model, a curvilinear relationship was found in males between the ages of 40 – 70. This relationship showed that processing speed and executive function performance increased up to the third quintile z-score distribution but then decreased as a function of testosterone level (Muller et al., 2005). Researchers therefore showed that higher total and bioavailable testosterone levels were effective to an optimal level of between 16.8 to 19.4 nmol/L and 8.4 to 9.9 nmol/L respectively but that levels beyond this point did not contribute to better processing speed and executive functions. No explanations can clarify the curvilinear relationship. However, the linear relationship observed in the oldest cohort may have been due to very low testosterone levels that left elderly participants vulnerable to cognitive deficits while cognitive imbalances in younger males were due to other factors.

Further support for a curvilinear trend between testosterone and cognition comes from two studies which found a similar pattern of relationships between spatial scores and androgen levels in both healthy males and females (Gouchie & Kimura, 1991; Shute, Pellegrino, Hubert, & Reynolds, 1983). Another study found that bioavailable testosterone levels were positively correlated with verbal and non-verbal learning (Morley et al., 1997). This suggests that mid-range testosterone levels could enhance cognition whereas lower levels that are similar to those of ADT patients, lead to cognitive declines.

The relationship between testosterone and cognition in ageing individuals is not well understood. However, due to the known decline of testosterone as a function of ageing, it may be reasonable to infer that there are age related decreases in cognition that reflect declining testosterone levels. Research has shown that domains of executive function and spatial reasoning are especially affected as a function of testosterone decline in normal ageing males who are transitioning between middle age and elderly. One study assessed 310 older men (mean age 73) and acquired serum and bioavailable testosterone levels (Yaffe, Lui, Zmuda, & Cauley, 2002). Outcomes showed higher scores in domains of general cognitive function (mini-mental state exam), executive function (trails B test) and working memory (digit symbol test) in participants with higher bioavailable testosterone levels compared to those with low levels (Yaffe et al., 2002). Serum testosterone and sex hormone binding globulin were not associated with any performance differences on any of the tests (Yaffe et al., 2002). A shortcoming was that groups were not matched by education as participants identified as having cognitive impairment were older and less educated. Another investigation in 981 middle aged and older males (40-80 years) initially found significant differences between testosterone and at least one task of either working memory, spatial reasoning ability, processing speed and attention (Fonda, Bertrand, O'Donnell, Longcope, &

McKinlay, 2005). However, after co-varying for age, education level and physical fitness, any significant differences found disappeared (Fonda et al., 2005). The studies show that executive function and spatial ability specifically may be impaired as a function of testosterone decline in ageing. A confine to the study was that it was cross sectional and could not determine causation of cognitive decline through measurement of testosterone levels over time.

Nevertheless, longitudinal studies have been conducted and revealed cognitive impairments in areas of memory and spatial abilities in elderly males. One study by Moffat et al. (2002) assessed the effects of normal endogenous serum testosterone levels and normal free testosterone levels in older males (51-91 yrs) on a battery of cognitive tests over an average of 10 years. Outcomes revealed better cognitive performance on tests of visual memory, verbal memory, spatial reasoning and visuo motor scanning which were associated with higher free testosterone levels. Moreover, greater mean free testosterone levels and upper longitudinal free testosterone levels were concomitantly associated with a decreased rate of visual memory decline (Moffat et al., 2002). Significantly worse scores on spatial reasoning measures were also correlated with visual memory degeneration in hypo-gonadal males (Moffat et al., 2002). The study showed a possible beneficial effect of free testosterone on cognition in contrast to the findings above (Moffat et al., 2002). A shortcoming was that participants classified as hypo-gonadal were on average eight years older than those participants with normal testosterone levels. Older participants are known to have cognitive difficulties due to older age and age may have been a confounding factor (Lunenfield, 2003). The study was followed up with a positron emission tomography (PET) study in 40 older males over a period of 14 years to assess regional cerebral blood flow (rCBF) (Moffat & Resnick, 2007). Researchers found that free testosterone positively correlated with rCBF in the left putamen, bilateral thalamus and left inferior frontal gyrus (Moffat & Resnick, 2007).

These findings show one method by which testosterone could exert an effect on cognition through cerebral blood flow. Moreover, this provides support for the hypothesis of the mediating effects of free testosterone and cerebral blood flow in facilitating certain cognitive functions. Additionally, as the nature of the studies were longitudinal, they allowed inferences to be made on causation of cognitive decline with age being co-varied into regressor models. Cerebral blood flow may be a factor to take into account in androgen deprivation patients. This is because testosterone is depleted during ADT leading to reduced cerebral blood flow in certain areas of the brain. This could impair cognition due to regional hypo-perfusion.

However, some studies have not found an effect of declining testosterone levels on cognition. A study by Emmelot-Vonk et al. (2008) tested the effects of testosterone supplementation on hypo-gonadal males (aged 60-80) without cognitive impairment over a six month period. In a double-blind placebo-controlled study, participants receiving testosterone showed no differences on measures of cognitive functioning (Emmelot-Vonk et al., 2008). However, a shortcoming to the study was that participants were assessed over a short period of time. Nevertheless, the study was comparable to experiments conducted in the past with shorter follow up durations, where significant cognitive changes were reported. A further experiment also found no significant differences or correlations in testosterone substituted participants compared to a placebo hypo-gonadal group (Sih et al., 1997).

Many contradictions in testosterone literature have been found regarding its role in cognitive modulation. These differences may occur because of variations in participant age, cognitive ability, level of hypogonadism, individual differences in testosterone mechanisms, duration of treatment and administration techniques (injection, gel, patch). Several studies also had small sample sizes. The delineation of hypo-gonadal varies and rejection of participants with low psychosocial function, cognitive impairment or depression varies widely among studies.

Any of these factors may be influential on the varying effects of testosterone findings, but together they suggest the effects of testosterone are subtle and specific to memory and spatial cognitive domains. Moreover, causes of cognitive decline in healthy ageing males have been attributed to a gradual decline in testosterone as a function of age in healthy ageing individuals (Wu, Yu, & Chen, 2000). The studies above show that affected areas are analogues to those areas affected in PC patients undergoing ADT. Therefore, these domains require careful consideration to separate out affected areas that are a function of normal ageing and those that are related to ADT.

### 1.3.2 Data in Neurodegenerative Conditions Associated with Unhealthy Ageing.

Studies conducted in cohorts with disorders and disease that are known to lead to cognitive impairments were examined to assess the effects of testosterone on these conditions. This may assist in clarifying the role of testosterone substitution in cognition independent of cancer and allows hypotheses to be formed in patients with PC. One study measured the effects of testosterone substitution in hypo-gonadal participants diagnosed with Alzheimer's disease or mild cognitive impairment (Cherrier et al., 2005). Findings revealed that subjects substituted with testosterone for six weeks had better spatial memory scores, and constructional ability scores compared to a placebo group (Cherrier et al., 2005). Moreover, verbal memory scores of the placebo Alzheimer's group declined compared to those who were administered testosterone which stayed the same (Cherrier et al., 2005).

Indicators of the mechanisms behind testosterone and cognition from past literature of neurodegenerative disease suggest that testosterone may increase concentrations of nerve growth factor (NGF) (Freeman et al., 2004). This gene is commonly associated with maintenance, survival, and growth of neurons in areas such as the hippocampus (Freeman et

al., 2004). Testosterone has been demonstrated to especially upregulate NGF receptors in the forebrain area (Freeman et al., 2004). One study explored the effects of testosterone on brain derived neutrophic factor (BDNF) which is a protein that is upregulated by NGF (Gold, Chalifoux, Giesser, & Voskuhl, 2008). Researchers investigated the protein in patients with multiple sclerosis (MS) (Gold et al., 2008) as they are known to have deficits of NGF (Acosta, Cortes, MacPhee, & Namaka, 2013). Researchers administered 100mg of testosterone in 10 human males with MS, which was applied through gel application once per day for six months. This increased T levels to above normal levels (Gold et al., 2008). Clinical assessments were undertaken every three months including blood samples and tissue samples from the forebrain area. Findings showed a significant increase in BDNF in forebrain tissue samples after six months of T administration (Gold et al., 2008). This suggests a potential neuroprotective role of testosterone supplementation. A limitation of the study was that a specific mechanism could not be identified behind this effect. This is because any effect observed may be due to the conversion of testosterone to other hormones such as oestrogen through aromatization after passing the blood brain barrier. This conversion is debated but is also known to be neuroprotective (Brann, Dhandapani, Wakade, Mahesh, & Khan, 2007). The posterior medial amygdala (MePD) is a further region that has been associated with testosterone modulating effects that may mediate some aspects of cognition. The area is important for olfactory and pheromonal information and is dependent on hormones for function (Zuloaga, Puts, Jordan, & Breedlove, 2008).

In other literature, androgens have been found to prevent cell death and toxicity. Androgens have been shown as being protective against N-methyl-D-aspartate (NMDA) agonist receptor excitotoxicity apoptosis in hippocampal neurons. This allows synapse actions to resume as cell death from excitotoxicity is prevented by androgens (Morse, DeKosky, & Scheff, 1992). Hippocampal synaptic activity has been found to occur when glutamate neurotransmitters

bind to NMDA receptors. This leads to synaptic transmission and potentiation in hippocampal pyramidal cells. (Pouliot, Handa, & Beck, 1996).

## 1.4 Findings in PC patients Undergoing ADT

### 1.4.1 Cognitive domains affected by testosterone depletion associated with ADT

The testosterone studies and mechanisms highlighted above show how androgen levels may modulate cognition. However, contradictory outcomes have been found in studies evaluating the effects of ADT on cognition directly. There is mixed evidence on the specific domains of cognition that may be affected. The association between cognition and ADT is largely based on cross-sectional studies that have shown cognitive impairments in domains of verbal, memory, recognition memory, executive function and spatial reasoning (Bussiere, Beer, Neiss, & Janowsky, 2005; Jim, Small, Patterson, Salup, & Jacobsen, 2010) or no impairment in one study (Clay et al., 2007). However, due to the cross-sectional nature of the studies, they lack the ability to make temporal associations between risk factors and outcomes. Therefore, they are not as robust as investigations assessing patients over a period of time. Longitudinal studies have been conducted and are advantageous for the purpose of comparing treatment effects over time. This is because power is increased due to reductions in unsystematic error variance. This allows researchers the ability to detect systematic variance (Barrett, 2013). Moreover, it allows temporal associations to be made between risk factors and outcomes. Within subjects designs also allow individual change to be measured and requires smaller sample sizes to fulfil sample size requirements making studies less susceptible to type one and two errors.

Longitudinal assessments in PC patients undergoing ADT have found cognitive impairments in specific domains such as spatial reasoning. A nine-month prospective pilot study conducted by Jenkins, Bloomfield, Shilling, and Edginton (2005) assessed 32 (mean age=67.5, SD=4.7) PC patients undergoing LH-RH therapy (ADT) compared to age matched healthy controls. Findings initially demonstrated no cognitive group differences, however, analysis conducted with the reliable change index showed cognitive decline in the spatial reasoning domain compared to healthy controls. Although researchers did not find any group level interactions, outcomes showed an association between testosterone deprivation and spatial reasoning deficits. Shortcomings of the study were that it was not adequately powered therefore chance of errors were high.

A further prospective 12 month investigation assessing a larger sample of 77 PC patients undergoing ADT (mean age = 69.3, SD=6.9) revealed that patients had worse spatial reasoning performance compared to age matched healthy controls. Moreover, patients had worse performance over time in domains of attention (immediate span) and working memory over time compared to controls although no group differences were found in these domains (Alibhai et al., 2010). Confines to the study were that the older ADT sample previously had more years of education and were in generally good health apart from PC (Alibhai et al., 2010). This may constrain findings as the sample was not characteristic of the general PC older male population and hide any true group differences that would be apparent if the sample was from a normal distribution. Secondly, assessments of performance in some cognitive domains were measured with only one cognitive instrument which limits the robustness of findings by domains. Finally, a limitation of both studies above was that a healthy age matched sample was used rather than another PC group which was not administered any form of ADT. Another PC group may match more closely to ADT patients

as they are more likely to have comorbidities that reflect those of ADT patients. This thereby allows the isolation of ADT if all other factors are matched in both samples.

Other assessments have found higher levels of complex processing are affected in ADT patients which could severely impact on daily functioning. One prospective study assessed the impact of ADT on cognition in 20 patients (mean age=62.05, SD=7.19) who had rising PSA levels without evidence of metastasis between baseline, three, nine and 12 month intervals (Cherrier et al., 2009). A group by time interaction revealed significant impairments in spatial reasoning and executive function domains in ADT patients at three months compared to baseline levels compared to age matched PC patients that were not administered ADT. However, cognitive performance measured between 9 and 12 months when treatment had ended was almost back to baseline levels. Nevertheless, some marginal discrepancies were evident in verbal ability, spatial ability and executive function domains at 9 and 12 months. The study demonstrated that cognition deteriorated in ADT patients over the course of the ADT treatment period and marginal effects were present after cessation of therapy. However, a shortcoming was that patients were not followed up at longer post-treatment periods to assess if cognition returned to baseline levels. Researchers also recruited a small PC sample from a specialized oncology clinic which may be uncharacteristic of a normally distributed patient sample. Another larger investigation assessed 82 male patients with extra prostatic PC (mean age=73.3, SD=6.4) at baseline and six month follow up (Green et al., 2002). A group by time analysis revealed that those patients administered LH-RH had significantly reduced executive functioning performance at six months compared to an age matched PC group administered cyproterone (anti-androgen steroid) (Green et al., 2002). Further, calculation using the reliable change index (RCI) showed the LH-RH group had declines on at least one cognitive task and seven participants in at least two cognitive tasks of

executive function (Green et al., 2002). A constraint of the study was that the close monitoring group had significantly higher baseline IQ scores compared to the LH-RH treated participants which led to biased differences. Therefore, lack of cognitive performance change may be due to higher baseline cognitive performance in the close monitoring group (Green et al., 2002). However one noteworthy aspect of both studies was that they included other PC groups that were administered other forms of treatment and matched ADT samples more closely compared to healthy controls. This suggests that the finding that executive function was affected in both studies was more robust since outcomes were discovered in a longitudinal study and groups were closely matched suggesting that ADT was the cause of these deficits.

In contrast to the findings above, a study by Salminen et al. (2003) assessed cognition in LHRH administered PC patients compared to healthy controls between baseline, six and nine months (Salminen et al., 2003). Contrary to earlier studies, researchers found impairments in patients at baseline but improvements at six and twelve month follow-ups in domains of working memory, semantic memory and recognition memory (Salminen et al., 2003). No declines in any other cognitive domains or mood were found (Salminen et al., 2003). A constraint to the study was that assessments were not undertaken in controls at six and nine months (Salminen et al., 2003). Interpretations of the results were therefore restricted because the impact of practice effects could not be accounted for and patients could not be compared to normal distribution samples. This limits the interpretation of outcomes as normal ageing cognitive decline could not be separated from impairments due to the therapy. However, it does demonstrate that some cognitive deficits were present from baseline which warrants further investigation.

Outcomes from the studies reviewed above show mixed effects of ADT on cognition through cross sectional and longitudinal assessments. Furthermore, limitations of these studies such as participant attrition rates during longitudinal follow-up may have confounded outcomes. Nevertheless, a conducted systematic review and meta-analysis combining findings from previously conducted systematic reviews and meta-analyses assessing the effects of ADT on cognition has shown that ADT patients were most affected in domains of verbal fluency, verbal memory, spatial reasoning and executive functioning (Treanor et al., 2017). This lends support to an effect of ADT on spatial reasoning and higher level functions that are sub domains of executive function. Further research is therefore required to assess these domains in more depth with comprehensive neuropsychological batteries to limit shortcomings of past literature. This may also facilitate characterisation of cognition before patients begin ADT to allow researchers to assess cognition and develop strategies to prevent further cognitive decline during treatment.

### 1.4.2 Neural changes in ADT

The above literature shows that testosterone does modulate cognition in some specific domains suggesting that specific brain regions are susceptible to the effects of testosterone depletion. Researchers examining neural changes in ADT patients have found mixed effects of the therapy in human cohorts. A study by Cherrier, Borghesani, Shelton, and Higano (2010) assessed 12 non-metastatic PC patients (mean age=65) treated with ADT for nine months (Cherrier et al., 2010). Functional magnetic resonance (fMRI) imaging was used to measure brain activation on a comprehensive battery of tasks that measured spatial reasoning ability at baseline and nine months (Cherrier et al., 2010). Findings showed decreased BOLD activation in the right parietal occipital region in ADT patients compared to 12 age matched healthy males, who had no such decreases at nine months compared to baseline (Cherrier et al., 2010). A shortcoming to the study was that task related BOLD performance differences were not subjectively or behaviourally assessed. Moreover, patients were highly educated which may not be true of the general population of PC patients. However, an attempt was made to match participants by age and education.

A further fMRI study found that other cognitive domains were affected that required higher order processing. Researchers tested 15 non-metastatic PC patients undergoing ADT (mean age=69, SD=5.3) against 15 age matched non-metastatic PC patients who were not administered ADT (Chao et al., 2012). Researchers implemented an n-back working memory task and stop signal inhibition task to assess working memory and executive function (Chao et al., 2012). No performance differences were found at six months compared to baseline suggesting that both domains remained unaffected as a result of ADT (Chao et al., 2012). Nevertheless, a significant group by time interaction showed decreased BOLD activation on the stop signal task in the medial prefrontal cortex, right insula, and right inferior frontal gyrus at follow up (six months) vs. baseline (Chao et al., 2012). Resting state fMRI revealed

differences in resting state networks showing decreased connectivity in ADT patients between the right dorsolateral prefrontal cortex, insula and rostral anterior cingulate cortex when contrasted to the control group (Chao et al., 2012). The study therefore demonstrates differences in brain activity and task related BOLD activation of the ADT group compared to controls.

In a second publication, Chao et al. (2013) used voxel based morphometry (VBM) to track altered brain and tissue composition in the same sample as in their 2012 study. Researchers also administered a verbal working memory n-back task. Participants were tested again as before in a prospective six month study (Chao et al., 2013). Decreased gray matter volume in the primary motor cortex, fronto polar cortex, and dorsolateral prefrontal cortex were found (Chao et al., 2013) in ADT patients compared to controls, despite there being no performance differences between groups. The above studies were valid due to ADT patients being closely matched to PC patients that were not undergoing ADT suggesting that ADT was a causal factor that affected PC patients specifically. This makes findings of executive function deficits more robust than those of spatial functioning (Cherrier et al., 2010). Nonetheless, the studies employed a less comprehensive battery of assessments to measure working memory and executive function (Chao et al., 2013; Chao et al., 2012). This may explain the lack of behavioural differences found compared to neural impairments that were not explained by functional task neural activity but resting state and structural assessments. Moreover, patients were on a restricted duration of ADT and a small sample size was investigated (Chao et al., 2013; Chao et al., 2012). The impact of this is that the reliability and validity of the study may be compromised and chances of errors made were high as the study was not powered. On the other hand, Cherrier et al. (2010) had a small sample size but employed a comprehensive battery of assessments to assess spatial functioning which made outcomes more consistent across various aspects of spatial cognition.

Brain imaging literature of ADT patients suggests that ADT may be a causal factor that affects putative executive functioning and spatial reasoning areas of neural architecture. Whilst the studies showed that there were underlying associations between testosterone deprivation and cognition, they lacked the comprehensive assessment of cognition across domains. Nevertheless, the studies show that spatial reasoning and executive functioning networks may be affected in some way but require further investigation to assess if these adverse effects are present before ADT to later treatment periods. Both neuropsychological and neural literature show that spatial reasoning and executive functions were affected in patients undergoing ADT. Therefore, further assessments are required to assess these domains in more depth with comprehensive neuropsychological batteries and with some complementary techniques such as brain imaging to measure underlying cognitive constructs. This may facilitate in reflecting neural correlates of behavioural cognition but may also reveal further components that affect cognition which are not overtly visible through behavioural assessment alone in patients and ageing cohorts. Moreover, if these factors can be identified at baseline, then physicians may be able to create strategies to prevent further cognitive decline during therapy. This will allow patients to make informed decisions before ADT and to know if adverse effects occur that can be controlled. This could affect further treatment uptake and continuation.

### 1.5 Effects of Normal Ageing on Cognitive Domains Affected

The literature above shows deleterious effects of ADT in PC patients. However, these adverse effects may be age related and experienced as a normal function of ageing. Cognitive areas known to be affected by age related andropause include executive function, working memory and spatial reasoning (Janowsky, Oviatt, & Orwoll, 1994; Starkstein &

Kremer, 2001; Yogev-Seligmann, Hausdorff, & Giladi, 2008) which are closely linked to impairments found in PC patients undergoing ADT (Treanor et al., 2017). These domains are known to be involved in higher level multidimensional constructs involving short-term memory, longitudinal memory, problem solving, decision making, mental manipulation and language. Although literature exists maintaining their limited capacity in older samples, these aspects of cognition are important in preserving daily function and quality of life that warrants further investigation (Barrios et al., 2013; Vaughan & Giovanello, 2010; Williams & Kemper, 2010).

Numerous explanations behind age related deficits have been hypothesised. However, some researchers suggest that age related inhibition failure may be the source of cognitive deficits. Inhibition refers to the failure to suppress irrelevant stimuli in order to maintain or manipulate relevant stimuli essential for cognition (Alichniewicz, Brunner, Klünemann, & Greenlee, 2013; May, Hasher, & Kane, 1999). However, the overarching inhibition hypothesis may be oversimplified in attempting to explain the myriad of factors that are known to affect cognition in elderly samples. A more complete view of inhibitory functions may include executive function which incorporates many facets of higher level cognitions that are known to deteriorate in ageing cohorts. One study assessing executive function employed a zoo map test which requires formation and planning and is an ecological planning subtask with two variants (Allain, Nicoleau, Pinon, Etcharry-Bouyx, Barré, et al., 2005). The task was split into two parts and had a high demand component that required advanced planning. The second task was low demand in which participants were required to go to destinations from an externally made plan. Findings revealed a significant difference in the high demand formulation task in which older adults had problems in forward planning compared to young adults (Allain, Nicoleau, Pinon, Etcharry-Bouyx, Barré, et al., 2005). However, there were no significant differences in the second

component task (Allain, Nicoleau, Pinon, Etcharry-Bouyx, Barré, et al., 2005). This suggests that older adults had difficulty in planning and problem solving but were able to implement intricate pre-set plans.

Another area commonly affected in ageing is verbal ability which has generally been sub-categorised as a hybrid domain of executive function (Shao, Janse, Visser, & Meyer, 2014). Researchers assessing verbal ability administered a multilingual aphasia task to healthy older (mean age = 81) participants in one study (Schum & Sivan, 1997). A significant age related impairment was found in the sentence repetition sub test. A limitation to the study was that the sentence repetition test used high memory demands therefore besides testing verbal ability, it may also have assessed short-term and serial auditory information processing. This could be a sub domain of working memory rather than executive function and verbal ability. However, working memory may also be categorised under executive functioning (McCabe, Roediger, McDaniel, Balota, & Hambrick, 2010). A confine of the task implemented to measure verbal ability was that it lacked validity and that amalgams of domains were measured in the study. This limited the utility of the verbal ability test to assess the domain in question (Shao et al., 2014).

While the studies above show behavioural impairments in participants with normal healthy ageing, they are descriptive. To gain an insight into the mechanisms, brain imaging has been used to investigate executive function in ageing populations. Regional brain investigations in the past have largely been conducted in the pre-frontal cortex (PFC) as these areas are known to be associated with high level cognitive operations (Yogev, Hausdorff, & Giladi, 2008). One study investigating inhibition in ageing reported activations in the ventrolateral and dorsolateral prefrontal cortex that were larger in area in older adults compared to younger adults when they completed a stroop task (Kumakura et al., 2005). This may be due to reductions in neural efficiency in older adults resulting in an

overcompensation on the task. However, it is more likely that older adults were unable to suppress irrelevant stimuli on the task resulting in surplus activation. This is evidenced by the increased number of interference effects in older adults on the task compared to young adults (Kumakura et al., 2005). This is supported in other studies of executive function assessing verbal ability that show greater activation in the right inferior frontal gyrus of older adults compared to younger adults (Persson et al., 2004).

Spatial reasoning has been researched in healthy ageing populations due its role in daily living, orientation and could also influence social activities (Craik & Dirkx, 1992). However, previous literature does not point to an overwhelming age related decline in spatial cognition with healthy ageing (Klencklen, Després, & Dufour, 2012). Nevertheless, there is a general consensus that there are spatial processing, speed (motor), and neural functional differences in the processing of spatial stimuli as a function of age. Elderly cohorts have been found to specifically have self-reported trouble with tasks that involve mental rotation. One study found significantly reduced reaction time speed in older (mean age = 67.3) participants compared to younger (mean age = 21.2) participants (Band & Kok, 2000). Researchers suggest there was a trade off in which older participants were less willing to sacrifice accuracy for speed. Nevertheless, a significant difference was found in which young adults had better accuracy compared to older adults (Band & Kok, 2000). Errors were assessed and found to be a product of hesitant decision making in older adults. This may explain the observed post error slowing in the elderly indicating that they were aware of errors made and therefore, sacrificed reaction time for accuracy but were less successful in doing so (Band & Kok, 2000). This was more apparent when degrees required to mentally rotate shapes were smaller rather than larger (Band & Kok, 2000). These outcomes are supported by mental rotation task experiments with variations in stimuli such

as faces, body parts and geometric shapes (Adduri & Marotta, 2009; De Beni, Pazzaglia, & Gardini, 2006; Saimpont, Pozzo, & Papaxanthis, 2009).

Neural correlates of spatial ability and sub components such as mental imagery have been investigated in ageing adults. The parietal cortex and precuneous regions are areas that are regularly activated on spatial tasks in healthy samples (Burgess, 2008; Piefke, Onur, & Fink, 2012). Neural links of the mental rotation task have been explored in ageing samples showing recruitment of additional regions. One study showed activation of the bilateral premotor cortex, superior parietal lobe (bilateral), right insula, left thalamus, and bilateral cerebellum in both old (mean age = 70.9) and young (mean age = 30.4) participants (Van Impe et al., 2013) However, additional areas including the bilateral frontal poles and frontal operculum were also activated in older subjects during rotation activity but did not survive thresholds for multiple comparisons (Van Impe et al., 2013). Authors speculate these additional activation areas may have been recruited in older participants as compensation mechanisms. This is evidenced by non-significant behavioural differences in mental rotation task performance between groups with increasing task load (Van Impe et al., 2013). This has been a consistent theory aforementioned in elderly participants to successfully compensate for and execute spatial transformations.

The aforementioned studies show that participants of old age have reduced executive and spatial reasoning performance compared to younger samples. Furthermore, older adults may have neural functional changes as a function of increasing age. This may alter putative regional and neural cerebral mechanisms found in past research. Further research must be undertaken to establish and characterise neural maps in the elderly which account for these

changes during healthy ageing. This may thereby allow reliable neuroimaging comparisons to be made in geriatric clinical populations.

### 1.5.1 Possible Causes of Cognitive Decline in Normal Ageing

*Volumetric Changes*

Examination of the aetiology behind executive and spatial function deficits in normal ageing samples have been conducted through post mortem and structural imaging investigations. This has generally shown reduced gray matter volumes in elderly adults compared to younger adults (Kearney, Harwood, Gladman, Lincoln, & Masud, 2013). Uniform whole brain changes have not been found but the PFC has been found to reduce in volume to a greater extent than other regions such as the occipital cortex (Mirelman et al., 2012) with a 5 percent loss of gray matter per decade after the age of 20 (Schum & Sivan, 1997). Furthermore, diffusion tensor imaging studies report loss of white matter integrity in anterior tissue matter as a function of increasing age (Shao et al., 2014).

*Cerebral perfusion changes*

Additionally, declines in blood perfusion of elderly participants have been found compared to younger participants in positron emission tomography studies (Persson et al., 2004). Several studies have shown smaller voxel area activations in old compared to younger adults. Nevertheless, when brain regions activated are corrected for multiple comparisons, these differences in area do not exist (Erixon-Lindroth et al., 2005; Monsell, 2003; Schipper et al., 2014; Smith et al., 2001). Moreover, the hemodynamic response function (hrf) of regions such as the prefrontal, parietal or striatal regions, have rarely been researched and

require characterisation due to their association with higher level cognition (Volkow et al., 2000).

*Cerebral Biochemical changes*

In addition to changes in the PFC, other areas such as various neurotransmitter systems may undergo age associated changes. The dopamine system is shown as being an integral chemical pathway between fronto-parietal networks important for executive and spatial functioning. Declines in dopamine have been estimated at 8% per decade after the age of 40 (Volkow et al., 2000) which has been associated with impairments on tasks of executive control, episodic memory and attentional control (Backman et al., 2000; Erixon-Lindroth et al., 2005; Kumakura et al., 2005; Volkow et al., 2000). This indicates that dopamine substitution in normal healthy participants could reduce the undesirable effects of cognitive ageing on executive functions.

*Environmental and Genetic Factors that affect Ageing*

Other research points to environmental and genetic factors that could impact on cognition in ageing subjects. Executive function has been assessed in ageing populations. One study evaluating male twin pairs demonstrated that executive function decline was due to genetic factors at follow-up after 10 years (Lessov-Schlaggar, Swan, Reed, Wolf, & Carmelli, 2007). However, any declines at 18 years were determined to be due to environmental factors (Lessov-Schlaggar et al., 2007). This suggests genetic factors may underlie executive function impairments that were more evident at longitudinal follow-up rather than in the short-term. The study was advantageous because twin pairs were genetically matched and environmental variables were covaried into statistical models.

*Mood Factors as a function of Ageing*

A complication in the assessment of cognition in healthy ageing is of comorbid psychosocial variances. Due to the advancement of medicine and scientific discoveries, the ageing population is growing. Past literature suggests a 1-5% prevalence of depressive disorder in adults 65 and over at any one time in the United States and internationally (Hasin, Goodwin, Stinson, & Grant, 2005). Presentation of depressive disorders different to those of younger cohorts. Sleep disorders, fatigue, loss of interest, hopelessness and loneliness are all common features among depressed older adults (Blazer, 2003). The aetiology of depression in late adulthood may be due to late life stressful events, cardiovascular changes, neurological changes, role changes, spousal bereavement, self-negative evaluations, genetic risks etc. (Fiske, Wetherell, & Gatz, 2009). More importantly, slower cognitive information processing speed, spatial and executive function impairments are commonly found among adults with objective assessments (Butters et al., 2004). This is in line with findings previously aforementioned in slower reaction time speeds on spatial reasoning tasks and executive function impairments in healthy ageing adults. Some variance may therefore be explained by mood disorders.

In conclusion, the majority of literature in ageing participants demonstrates that older cohorts often experience cognitive limitations. Healthy ageing samples have been shown to have deficits compared to young samples in executive functions (inhibition, verbal ability and working memory) that also seem to reflect affected fronto-parietal networks detected through neuroimaging techniques. Spatial reasoning has also been shown to decline in ageing healthy participants with correlating neuronal activity that has been suspected to act as compensatory mechanisms in areas including the dorsolateral prefrontal cortex, parietal regions and frontal poles. Causes of these declines have been hypothesised to be due to reductions of underlying gray and white matter volume, reductions in cerebral blood flow,

reductions in dopamine receptors, environmental factors and increases in prevalence of mood disorders as a function of ageing. Likewise, prostate cancer patients undergoing ADT were found to have deficits in executive function assessed through neuropsychological measures. Investigations of neural architecture showed decreases in prefrontal cerebral volume and reductions in fronto-parietal connectivity as assessed through resting state connectivity and morphometry methods. Spatial reasoning declines were also shown through neuropsychological assessments however, no in scanner behavioural performance differences were found. Nevertheless, neuroimaging has revealed decreases in activation and volume in parietal occipital, primary motor cortex and fronto-polar cortices which have been related to spatial ability functions. The aetiology of these declines in ADT patients requires careful consideration to separate cognitions perturbed due to deprivation of testosterone and those affected as a normal function of ageing. This may impact on treatment side effects and management of these effects during ADT.

### 1.6 Limitations of current evidence base

Brain imaging studies showed consistent adverse effects of ADT on cognition. However, the studies lacked power and findings may not be reliable or have missed significant effects due to the acceptance of the null hypothesis (type II error) as a result of small sample sizes. Previous behavioural research has generally used a limited set of two to three tests over a six to twelve month period, and many studies have not adjusted for practice effects. Whilst researchers have reported declines in verbal ability, verbal memory and spatial reasoning abilities of ADT patients to controls (Beer et al., 2006; Green et al., 2002; Jenkins et al., 2005), other research has reported no such difference or improvements in cognitive ability (Alibhai et al., 2010; Almeida et al., 2001; Cherrier, Rose, & Higano, 2003; Salminen et al.,

2003). Some studies have reported inferior processing speed (Green et al., 2002; Jenkins et al., 2005) but these findings have not been replicated in subsequent studies. Another study reported improvements in verbal fluency (Salminen et al., 2003) but a subsequent experiment reported a decline in verbal fluency scores of ADT patients (Jenkins et al., 2005). A difficulty in comparing publications is that there is a lack of congruity across the research in cognitive domains and measures employed. Moreover, numerous measures have been employed to measure psychosocial function in ADT patients. This is problematic when comparing findings between studies because sensitivities and precisions of assessments vary according to the population being assessed (Tombaugh & McIntyre, 1992). Another difficulty in comparing studies includes that cut off scores vary to categorise participants as being abnormal or normal per measure therefore these boundaries are ambiguous among multiple studies (Tombaugh & McIntyre, 1992). Furthermore, some studies have reported no correlations of psychosocial function with cognitive measures. Nevertheless, numerous aspects of psychosocial function were not addressed such as roles of patients, family cohesion after ADT, prostate specific QOL or return to work especially in younger ADT patients. This may allow the comprehensive assessment of psychosocial function on cognition that can be identified and rectified before or during therapy periods.

In view of past literature, a longitudinal study was considered between patients and healthy controls to assess cognition before, during and after ADT. However, the data acquired in patients at follow-up, were not available for analysis during the time frame of the study. Therefore, the present study aimed to use a robust cognitive battery to assess cognitive function before PC patients underwent ADT. This was undertaken to resolve shortcomings of past research and to assess the nature of any deficits at baseline in the patient sample. Researchers may thereby be able to identify if any cognitive declines found at follow-up were carried over from baseline. The measures of the current project were selected through past

investigations indicating that verbal memory, verbal fluency, spatial reasoning and executive function were significantly affected in patients undergoing ADT. Moreover, brain imaging was undertaken to investigate cognition before ADT more thoroughly through neural examinations between groups. These differences were explored as past literature has shown that behavioural measures alone may not be sufficient to fully uncover the multitude of possible mechanisms by which cognition may be affected (Chao et al., 2012). Therefore, neuroimaging facilitates the neural correlates of neuropsychological outcomes. In this respect, brain imaging may be a more sensitive instrument to measure cognitive differences between groups.

The initial part of this thesis reviews research regarding the role of ADT in affecting cognition and psychosocial function in men with PC. The second section presents the main studies, which examined cognitive change using behavioural and brain imaging measures in PC patients eligible to receive ADT. The purpose of this investigation was to determine the nature of brain changes that occur in PC patients before ADT, so that physicians can improve treatment decisions. Furthermore, knowledge of potential adverse effects of such therapy can help facilitate treatment choices and therapy continuation in patients.

## 1.7 Thesis aims and hypothesis

**Global Aim:** To characterise the nature of cognition in prostate cancer patients before undergoing androgen deprivation therapy (ADT compared to healthy controls and to assess cognition in healthy controls over a longitudinal time frame of six months).

The following sub aims will be used to address the global aim:-

**Aim 1:** Use a comprehensive battery of measures to identify cognitive and psychosocial domains, affected due to prostate cancer diagnosis.

This aim was addressed in chapter two in which PC patients were assessed in a cross-sectional comparison before commencing ADT. Patients were assessed on a battery of comprehensive neuropsychological measures that evaluated cognition in domains of executive function and spatial reasoning compared to healthy ageing controls. Psychosocial function and testosterone were measured to assess their effect on cognition. Screening measures were put into place which measured intelligence and the presence of dementia in patients and controls that may confound outcomes.

**Aim 2:** To characterise the neural basis of cognitive change in patients before receiving ADT.

Chapter three addressed aim two in which patients were compared to healthy controls using brain imaging paradigms (fMRI), resting state functional connectivity (rfMRI) and arterial spin labelling (ASL). This allowed cerebral architecture to be measured in both groups that facilitated the understanding of neural mechanisms that underpinned neuropsychological

performance. Moreover, it allowed the identification of additional factors that could not be gauged through neuropsychological tests alone. Testosterone levels and psychosocial outcomes were added to all neuroimaging outcomes to assess its effect on brain imaging outcomes.

**Aim 3:** Validate healthy controls do not have any underlying cognitive deficits and to characterise cognition in the sample to compare to PC patients.

Aim three was investigated in chapter four. Healthy controls were assessed using neuropsychological assessments pertaining to executive function and spatial reasoning over six months. Neuroimaging fMRI, rfMRI and ASL were also measured at baseline and six months. This allowed the assessment of behavioural performance and underlying neural function in healthy controls alone when combined between time points to characterise cognition in ageing older samples.

**Aim 4:** Map the time course of cognitive, neurological and psychosocial changes in healthy controls over a period of six months.

Aim four was assessed in chapter four. Healthy participants were investigated using executive function and spatial reasoning neuropsychological paradigms at baseline and six months. Neuroimaging (fMRI, rfMRI and ASL) was undertaken at both time points. Psychosocial changes and testosterone level changes were analysed to assess their effect on performance differences between time points. This allowed the exploration of cognitive and neural reliability in controls and identification of factors that led to abnormal cognition and

underlying neural outcomes. Neuroimaging allowed the identification of additional factors not overtly apparent through behavioural measures alone.

**Global Hypothesis:**

**a.** No significant cognitive or neurological differences will be found between patients and controls before patients begin therapy in domains of executive function and spatial reasoning.

**b.** No significant changes in cognitive or neurological function will be evident in healthy controls at baseline compared to six months follow-up in domains of executive function and spatial reasoning.

The next chapters present data and analysis (behavioural and neural) of PC patients before undergoing ADT compared to healthy controls. This will help to characterise cognition in patients before they begin treatment. This may allow physicians to resolve side-effects before patients commence ADT to prevent any adverse effects during therapy. Moreover, this will inform patient treatment choices, uptake and continuance. Subsequent chapters characterised cognition using longitudinal behavioural and neuroimaging methodologies in the healthy controls that were assessed and compared to PC patients. This was to validate that the sample was representative of a normal ageing healthy cohort.

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# CHAPTER 2

Study one: Comparison of cognitive function between pre-treatment prostate cancer patients and healthy age-matched controls.

## 2.1 Introduction

There is emerging evidence that treatment for PC is associated with cognitive decline. A recent systematic review of 28 reviews containing primary studies revealed impaired cognition in domains of verbal memory, executive functions and spatial reasoning ability in PC patients undergoing ADT (Treanor et al., 2017). These findings have also been supported by numerous findings from past research (Bussiere et al., 2005; Cherrier et al., 2009). These studies assume that adverse effects were due to ADT, however impaired cognition may already be present before treatment. Thus, there is a need to characterise cognition in PC patients before commencing anti-androgen therapy and to consider additional explanatory variables which might account for differences in men with PC. Furthermore, several previous studies employed healthy controls that had higher cognitive levels and higher education levels at baseline (Green et al., 2002; Green et al., 2004). This may bias outcomes as healthy controls had an advantage over patients thereby producing false differences between groups. In the next sections, data on relevant confounding variables is presented.

Although with some mixed findings of the domains affected, numerous studies assessing cognition in prostate cancer (PC) patients before ADT have revealed comparable performance of patients to controls in the domains of executive function and spatial reasoning ability (Alibhai et al., 2010; Beer et al., 2006; Cherrier et al., 2009). However, other studies show that these domains may be affected prior to patients commencing treatment. One study assessing patients undergoing androgen deprivation therapy (ADT) found lower levels of

cognition before antiandrogen therapy compared to age and education matched healthy controls (Salminen et al., 2003). Patients had lower scores on the digit span, visuomotor digit symbol and word fluency task (Salminen et al., 2003). Interestingly, these tasks correspond to executive function and spatial reasoning domains, which were found to deteriorate in patients after commencing ADT compared to healthy controls and PC patients that were not receiving ADT (McGinty et al., 2014; Treanor et al., 2017). This could indicate that cognitive impairments were mediated by disease specific factors rather than to more specific effects such as age, intelligence, education or testosterone levels which were matched. Mixed findings of the domains affected and time period when patients were affected in previous research may be due to the methodological or non-comprehensive nature of batteries implemented in past assessments which the current study aimed to address. This allowed a detailed characterisation of cognition in patients before ADT that may be more valid to assess than during treatment. Identification of any side-effects or preventative strategies before therapy may allow physicians to avoid or prevent adverse effects worsening during therapy. This could permit patients to continue therapy with better treatment outcomes.

There are a number of potential risk factors for cognitive decline in men with PC that might be independent of treatment *per se.* A possible relationship has been found between the apolipoprotein (APo) gene and PC (Lehrer, 1998). Research shows greater expressions of apolipoprotein epsilon in patients with PC of which apolipoprotein epsilon 4 (APoE4) is known to be associated with an increased risk for Alzheimer's diseases and PC mortality rates (Grant, 2010). Males diagnosed with non-aggressive PC have been shown to carry copies of the genotype APoE3/APoE4 isoforms while patients diagnosed with aggressive PC have been shown to be carriers of APoE2/APoE4 isoforms (Ifere, Desmond, Demark-Wahnefried, & Nagy, 2013). While APoE2 has been associated with a neuroprotective effect

on Alzheimer's (Rebeck, Kindy, & LaDu, 2002) and APoE3 with neither an increase nor decreased risk on Alzheimer's, when combined with E4, the risk for Alzheimer's disease has been shown to escalate by 2-4 fold (Michaelson, 2014). Moreover, a higher prevalence (85%) of older males are diagnosed with non-aggressive low grade PC suggesting that these males could carry the APoE3/ApoE4 isoform (Ballentine Carter, 2012; Thompson et al., 2004). This puts PC males with the APoE4 isoforms at a higher risk for Alzheimer's which could be influential in characterising any cognitive impairments of PC patients at the pre-treatment stage.

Another important potential confounding variable to consider in the context of the diagnosis and treatment of cancer is mood. Cognitive changes found in patients before ADT could relate to anxiety and depression. The diagnosis of cancer is a stressful event for patients as evidenced by increased risk of cardiovascular deaths and suicides after PC diagnosis (Fang et al., 2010). Anxiety and depression in patients identified with PC have been explored in a meta-analysis and systematic review. The review found 27 journals with 4494 patients diagnosed with PC (Watts et al., 2014). The analysis revealed clinically diagnosed depression in 17.27% of patients and anxiety in 27.04% of patients before any treatments began (Watts et al., 2014). The article also found similar levels of anxiety and depression during and after treatment however, anxiety was more prevalent before treatment onset.

Anxiety has been associated with cognitive deficits. A group of researchers assessing subjects with clinically diagnosed anxiety revealed that even normal ageing led to poor performance as assessed through an intelligence test (Wechsler Adult Intelligence Scale synonyms test) and spatial reasoning domain assessments that heavily depended on central executive functioning (Koh's Block test, Card Rotations test, Visual Learning test and Analogies test)

(Wetherell, Gatz, & Pedersen, 2001). However, anxiety (State–Trait Personality Inventory (STPI)) assessments were taken at the participant's homes where subjects were in familiar environment. This may confound outcomes by reducing validity and power. This is because assessments of anxiety in unfamiliar situations may have produced scores with higher variability because vulnerable subjects may experience more anxiety in novel situations before undergoing cognitive measures. This higher variability in anxiety scores and cognitive performance may facilitate the discovery of many more relationships if both assessments were conducted in novel settings. Nevertheless, the study did show that higher level executive functions requiring the central executive system, phonological loop and visuo-spatial sketchpad (spatial reasoning) were affected by anxiety.

The relationship between executive function and depression is well documented. For example, Alves et al. (2014) conducted a systematic review of 28 articles and showed that patients with depression had deficits in sub-components of executive function. These areas included planning, shifting, inhibition, working memory, integration of information, fluency and organisation (Alves et al., 2014). However, numerous measures were employed to assess executive functions by researchers in the studies included in the review, making it difficult to compare outcomes. Moreover, researchers did not control for disorder subtypes, level of severity, drug use or comorbidity which may lead to misinterpretation of findings (Alves et al., 2014). Another study assessing depression evaluated spatial reasoning in patients with melancholic subtype depression (Rogers et al., 2002). Results indicated motor slowing on the mental rotation task compared to non-melancholic participants (Rogers et al., 2002).

Despite research showing associations between poor psychosocial function and cognitive impairments, the relationships are not well understood. Some research points to environmental factors affecting cancer patients after diagnosis. Multilevel modelling has found that family functioning surrounding cancer diagnosis is important. This is because

psychosocial function of the family can impact on the on the patient's quality of life and mood (Edwards & Clarke, 2004). Moreover, researchers have illustrated that families able to openly communicate and express their feelings, have lower levels of depression and anxiety in both the patients and family members (Edwards & Clarke, 2004). Taken together, this suggests that clinicians and researchers could take a family focused approach to cancer diagnosis and illness. This in turn may improve the patient's quality of life to help preserve cognitive function before and during treatment.

Mood disorders may additionally lead to sleep deprivation and insomnia that lead to cognitive problems (Nutt, Wilson, & Paterson, 2008; Staner, 2003). Adverse effects of sleep deprivation have been extensively explored with findings generally showing that fatigue from total sleep deprivation can lead to neurocognitive slowing (Klumpers et al., 2015). This may in turn create abnormal brain patterns to compensate for cognitive slowing (Klumpers et al., 2015). Furthermore, medications could create additional behavioural impairments. Experimenters investigating the effects of selective serotonin reuptake inhibitors in fifty patients with clinically diagnosed depression found a gradual decline in cognition over a period of eight weeks (Sayyah, Eslami, AlaiShehni, & Kouti, 2016). However, outcomes were limited to the mini-mental state exam which has been shown to measure many aspects of cognition to determine the presence of dementia. However, it is not known to thoroughly or comprehensively measure individual domains. Other medications that are anti-anxiolytic, such as benzodiazepines, may lead to cognitive impairments - affecting visuo-spatial ability, processing speed and verbal learning skills (Stewart, 2005). Moreover, a causal link between benzodiazepines and cognitive deterioration has been supported since cognition is known to return to normal when patients are removed from the therapy (Stewart, 2005). Therefore, identification of mood disorders were required so that the extent to which it impacts on cognition is accounted for in patients.

In summary, a number of factors affect cognition in patients diagnosed with PC, however, the majority of research employed samples of patients undergoing ADT and at least one study has shown that cognition may be affected before ADT. Moreover, some assessments implemented healthy control samples that were not matched compared to patients leading to naturally higher levels of baseline cognition in controls. This illustrates the need to identify whether cognitive impairments are present before the administration of ADT so that side-effects during and after actual treatment periods can be evaluated. This may facilitate patients in making an informed decision on the type of treatment options available to them with their various adverse effects.

### 2.1.1 Aim

The aim of this chapter was to characterise cognitive function in PC patients prior to commencing ADT and compare with healthy age-matched controls using a comprehensive battery of neuropsychological, testosterone and mood assessments.

### 2.1.2 Hypothesis

a. There will be no significant differences in pre-treatment PC patients and healthy age-matched controls.
b. There will be no significant differences between the scores of pre-treatment PC patients and healthy age-matched controls in areas of intellectual functioning, verbal memory, executive function and spatial reasoning
c. There will be no significant differences between the testosterone levels of pre-treatment PC patients and healthy age-matched controls.
d. There will be a significant difference in the levels of APoE4 carried by PC patients and healthy age matched controls.
e. There will be no significant differences in anxiety and depression scores between pre-treatment PC patients and healthy age-matched controls.

## 2.2 Material and Methods

**Design:** A cross-sectional between subjects design was used to investigate cognitive performance in PC patients prior to commencing ADT compared with healthy age-matched controls.

### 2.2.1 Sample

**Control sample:**

Participants were included if they were (1) between the ages of 50-80; (2) able to speak and write English; (3) had high blood pressure if controlled; (4) diabetes if controlled. Participants were excluded if they were (1) unable to provide informed consent; (2) had a history of treatment for neurological impairments; (3) had a history of stroke; (4) had a history of epilepsy or neurodegenerative conditions; (5) had a serious past medical history (e.g. heart failure, aneurysm, congestive heart failure etc.); (6) had scores below 24 on the MMSE; (7) had a history of any illicit psychoactive drug use; (8) or failed to meet any MRI screening requirements (refer to Appendix 1). Participants expressing interest in the study were sent an email or telephoned to explain the nature of the study and of the time commitments required from them. If participants were still interested in participating then a date and time was set for them to come to the University of Birmingham.

Patients:

PC patients were included if they were (1) aged between 50 and 80; (2) had rising PSA levels; (3) diagnosed with PC; (4) prescribed ADT and; (5) were proficient in English. Patients were excluded if (1) they were unable to provide informed consent; (2) were already receiving ADT; (3) had metastatic cancer; (4) history of systemic cancer treatment; (5) any past treatment history affecting cognition; (6) neurodegenerative conditions; (7) dementia or

Alzheimer's or scores below 24 on the mini mental state exam (MMSE); (8) failed to meet any magnetic resonance imaging (MRI) screening requirements.

**Table 2.1** Inclusion and Exclusion criteria

| Inclusion criteria | |
|---|---|
| **PC patient group** | **Community based control group** |
| -Proficient in spoken and written English<br>-Diagnosis of PC and prescribed ADT<br>-Aged between 50 - 80 | -Proficient in spoken and written English<br>-Aged between 50 - 80<br>-Similar age profile to PC patients |
| **Exclusion criteria** | |
| **PC patient group** | **Community based control group** |
| -Unable to provide informed consent<br>-Already started ADT<br>-Receiving treatment for cognitive difficulties (inc dementia and Alzheimer's treatment)<br>-History of stroke, epilepsy or any neurodegenerative condition<br>-Diagnosis of dementia or Alzheimer's disease<br>-Score below 24 on the mini-mental state exam (MMSE).<br>-Any form of diabetes<br>-Illicit psychoactive drug use<br>-Failing to meet requirements in the fMRI screening sheet | -Unable to provide informed consent<br>-Receiving treatment for cognitive difficulties (inc dementia and Alzheimer's treatment)<br>-History of stroke, epilepsy or any neurodegenerative condition<br>-Diagnosis of dementia or Alzheimer's disease<br>-Score below 24 on the mini-mental state exam (MMSE).<br>-Any form of diabetes<br>-Illicit psychoactive drug use<br>-Failing to meet requirements in the fMRI screening sheet. |

Abbreviations: PC, prostate cancer; ADT, androgen deprivation therapy; fMRI, functional magnetic resonance imaging.

### 2.2.2 Ethical approvals

Controls: A protocol was developed and presented to the ethics and approval committee at University of Birmingham. The project was given a favourable decision by the ethics committee at the University of Birmingham to recruit and assess healthy controls behaviourally and through neuroimaging methods (Appendix 2: Reference number: ERN_11-0429AP26; Science, Technology, Engineering and Mathematics Review Committee).

### 2.2.3 Recruitment

Patients: were recruited through multidisciplinary meetings at University hospitals Birmingham Queen Elizabeth Hospital, England, UK. Research and development (R&D) ethical approval was gained through the integrated research application system (IRAS) form and submission to the NHS national research ethics service (NRES). Patients that were awaiting ADT were specifically recruited. This is because the treatment is commonly administered in cases where the cancer is localised thereby being less aggressive. These patients are more predisposed to having genotypes and genes (APoE4) that have been associated with non-aggressive cancers and can lead to Alzheimer's disease especially when testosterone is deprived (Ifere et al., 2013).

Healthy controls: were recruited from community based organisations which catered towards the needs of healthy elderly populations, including gym facilities, old age and elderly care homes, sports facilities (e.g. Birmingham bowls, golf establishments etc.) and religious organisations (churches, temples, mosques etc.) (Appendix 2: Reference number: ERN_11-0429AP26; Science, Technology, Engineering and Mathematics Review Committee).

### 2.2.4 Saliva Sample Collection

Saliva samples were collected to measure testosterone levels through salivary concentration assays. Testosterone was selected in the unbound form and because it has been shown to correlate highly with serum saliva in males (Vining & McGinley, 1987). Saliva samples were obtained through a Salimetrics Enzyme Immunoassay kit. Participants were instructed not to eat anything within one hour of saliva collection and not to drink any alcohol 12 hours before collection as this could affect collection accuracy (Vining & McGinley, 1987). They were

also asked to drink water 10 minutes prior to collection so that any deposits affecting collection were eradicated in the mouth. Subsequently, subjects were asked to passively collect approximately 18 ml of saliva in a polypropylene vial without saliva stimulation. Once samples were collected, they were stored at -20$^{o}$C to avoid bacterial contamination. Concentrations were assayed and measured in picograms per millilitre (pg/ml). Testosterone levels were acquired using a competitive assaying technique. This was undertaken by allowing testosterone in saliva samples to compete with testosterone amalgamated horseradish peroxidase to bind to antibody sites on a microtiter dish. Unbound components were washed away after an incubation period. Testosterone was measured by the reaction of horseradish peroxidase to tetramethylbenzidine which elicited a blue colour. A yellow colour was observed when the reaction had ceased. Testosterone enzyme conjugate was measured as being inversely proportional to the testosterone present in the sample (Chard, 1990).

Testosterone concentrations were calculated by first subtracting raw pg/ml values from background values and then averaging any duplicates found. The non-specific binding wells were then subtracted from each reading. The percent bound value (Bo) was divided and multiplied by 100 to derive percentage bound value. These values were input into a PRISM (version 4.0) software to perform a nonlinear regression analysis using data reduction methods.

## 2.3 Outcome measures

### 2.3.1 Neuropsychological measures

Table 2.2 summarises key properties of the measures used in the thesis for cognitive assessment. A full rationale for the selection and scoring is provided in Appendix 3.

**Table 2.2:** Key Properties of Neuropsychological Measures

| **Neuropsychological Measures** | **key properties of the measures** |
|---|---|
| Mini Mini-Mental State Exam - II (MMSE-II) (Folstein, Folstein, & McHugh, 1975). | A quick test to assess multiple cognitive domains to detect surface level cognition to assess symptoms of Dementia. |
| Wechsler Abbreviated Scale of Intelligence (WASI-II) (Wechsler, 2011). | Rapid and thorough measure of IQ using a range of verbal comprehension and perceptual reasoning sub-tests |
| Delis Kaplan Executive Function System (D-KEFS) test (Delis, Kaplan, & Kramer, 2001a). | Comprehensive measure of executive function<br>Subtests from the measure employed included:<br>D-KEFS Trail making test<br>D-KEFS verbal fluency test<br>D-KEFS Design Fluency test<br>D-KEFS Colour word interference test<br>D-KEFS Tower test |
| Wechsler Adult Intelligence scale (WAIS-IV) Working Memory Index (WMI) (Wechsler, 2009). | An assessment of working memory with tasks comprising:<br>Digit span task: digits forward, backward and sequential<br>Arithmetic: simple mental arithmetic |
| Cambridge Neurological test Automated Battery (CANTAB) (Cambridge Cognition, 2006). | A spatial working subtest of the computerized battery was employed to assess spatial reasoning skills by assessing the ability to retain spatial information for later manipulation in working memory. |

Abbreviations: IQ, intelligence quotient

### 2.3.2 Psychosocial measures

Table 2.3 summarises key properties of the measures used in the thesis for psychosocial assessment. A full rationale for the selection and scoring is provided in Appendix 3.

**Table 2.3:** Key Properties of Psychosocial measures

| | |
|---|---|
| Behaviour Rating Inventory of Executive Function – Adult version (BRIEF-A) (Gioia, Isquith, Guy, & Kenworthy, 2000). | Meta-cognitive questionnaire that assess self-report of constructs relating to executive function components included inhibition, attentional shift, emotional control, self-monitoring, initiation of tasks, working memory, planning and organisation, task monitor and organisation of materials. |
| Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983). | Self-rating instrument used to detect any clinical or borderline levels of anxiety or depression |

## 2.4 Procedure

Patients were identified through a consultant at University hospitals Birmingham Queen Elizabeth Hospital, England, UK. Researchers from the University of Birmingham were present at the appointment and collected the relevant contact details (phone number and/or email address) required to contact interested patients at a later date. Patients were also given a research pack with participant information sheets with details of what the study involved (refer to Appendix 4). A researcher called the patient by phone call or by email if available to arrange a phone call appointment. The phone call was firstly to consent patients into the study if they were still interested after reading the information packs. Secondly, the call was to screen patients before study commencement on questions pertaining to the inclusion exclusion criteria in table 2.1 with a screening form (Appendix 5). Finally, a time was arranged for the patient to attend the University to take part in the study if successful at the initial screening stage. When participants arrived for the study, they were consented into the study by a trained researcher through written consent (Appendix 6). General practitioner (GP) details were also acquired from the participants in case the patient was to be referred for concerns of dementia. A similar procedure was undertaken with healthy controls that were recruited from various facilities including gym facilities, old age and elderly care homes, sports facilities and religious organisations. Information packs were either handed to participants or emailed. A phone call was made to interested participants to screen and consent them into the study. Participants were excluded if they did not meet the requirements of table 2.1. When participants arrived at the University, they gave written consent if interested and GP details were similarly acquired should they present with signs of dementia.

Assessments commenced after screening and consent was obtained. An attempt was made to complete all assessments in one day and at the same time in each participant to control for confounding effects of tiredness and fatigue. However, participants who requested to

complete assessments over two to three days were assessed on separate days. Neuropsychological assessments were split into sessions in these cases.

Exclusions were made if participants did not meet the inclusion criteria (table 2.1). Assessments were conducted in a small room with the examiner sitting opposite the participant at a table. Saliva samples were acquired from participants after 12pm to measure testosterone through assay and analysis. An attempt was made to collect saliva samples at the same time from all subjects. This was for consistency and to avoid variations in diurnal testosterone levels (Brambilla, Matsumoto, Araujo, & McKinlay, 2009). Duration of neuropsychological assessments were 2 hrs 45 minutes (Appendix 7). Fatigue and alertness confounds were controlled for by prohibiting any caffeine related beverages or foods during testing periods.

## 2.5 Statistical Analysis

The main question was to assess if there were significant differences between PC patients prior to commencing ADT and healthy age-matched controls using neuropsychological and psychosocial measures. The primary outcome measures were scores obtained on a battery of executive function and spatial reasoning measures. A p value of either 0.05 or less (two-tailed) was accepted as statistically significant. Firstly, independent samples t-tests (or Mann Whitney Whitney U tests were conducted where variance assumptions were violated) to assess the level of change that occurred in anxiety, depression and testosterone levels between healthy controls and patients. Subsequently, an independent samples t-test was conducted on the WASI-II FSIQ-4 subscale. Next, a MANOVA was employed to assess differences between groups on contrast scaled scores of the D-KEFS as dependent variables (TMT-A, VF, DF, CWI) and tower test scaled scores. The fixed factor again was group (healthy controls or patients). A similar MANOVA was conducted on the spatial working memory CANTAB task with number of between and double errors in the three conditions as dependent variables (4 boxes, 6 boxes and 8 boxes errors made). CANTAB strategy scores were compared in an independent samples t test.

Further MANCOVAs were conducted where anxiety, depression and testosterone were added as covariates only if any group differences were found. However, testosterone level was added as a covariate to all models as it was relevant to the current investigation between patients and controls. The working memory Index from the Wechsler Adult Intelligence scale (WAIS) was added as a covariate in a separate MANCOVA so that it could be controlled for in the spatial working memory index CANTAB task. This was to control for working memory so that spatial reasoning performance of participants could be the assessed on the CANTAB. Testosterone was added to the model to assess the effects of testosterone on spatial reasoning performance whilst controlling for working memory.

The BRIEF-A assessment was modelled using similar MANOVAs where the dependent variables were all scaled scores on the self-report questionnaires (Inhibition, self-shift, self-emotional control, self-organisation, ability to initiate, working memory, monitor, behavioural regulation index, metacognition and global executive function scores) and the grouping variable was patient and healthy controls (group) to model an interaction. Covariates (anxiety and depression) were added to the MANOVA in a MANCOVA if group differences were found to gauge if covariates covaried with any differences between cohorts. Testosterone was added to the model regardless of group change to assess if it contributed to any variance.

The use of MANOVAs and MANCOVAs allowed the entry of many dependent variables in unison which benefited the management of family-wise error rates and reduced type 1 errors. Moreover, MANOVA analyses are known to be resilient to violations of homogeneity which is not the case with other statistical methods including ANOVAs etc. (O'Brien & Kaiser, 1985).

Significant findings from the conducted MANOVAs or MANCOVAs were followed up with ANOVAs and ANCOVAs with individual sub scale scores as dependent measures to ensure maximal sampling. Outliers were not deleted from any analysis due to small sample sizes. However, extreme outliers were checked for miss inputs by researchers. No outliers were deleted as a result. Due to the limitation of multiple testing issue and to reduce familywise error rate, post-hoc Bonferroni tests were undertaken if the main interaction was significant. This was to prevent chance findings from random sampling, to disentangle differences and to control for type I errors.

Psychosocial covariates to be added were assessed first and revealed the anxiety and depression factors were correlated $r = 0.54$, $p < 0.01$ in healthy controls and patients $r = 0.58$,

$p < 0.01$. However, covariates were not excluded from MANCOVAs since the exclusion criteria for correlating variables has been found to be $r > 0.80$ (Vatcheva, Lee, McCormick, & Rahbar, 2016). A separate MANOVA was conducted to test for homogeneity of regression slopes where MANCOVAs were conducted and interactions were modelled between the fixed factor, which was group and covariates added to the model. If no significant differences were found then it was concluded that subsequent MANCOVAs did not violate the assumption of homogeneity of regression slopes.

Caseness was assessed to explore if any mood variables related to behavioural outcome measures. Caseness refers to a clinical diagnosis of either anxiety or depression and is advantageous as it provides a quantification which can be used to clinically diagnose and understand the degree to which a disorder affects a patient (Westen, 2012). This allows caseness to be incorporated into categorical statistical analyses to measure relationships between variables which would otherwise not be possible. This method was used to investigate if caseness of anxiety and/or depression were associated with cognitive impairments compared to non-casness. Subscale sum scores $\geq 8$ were applied as a cut-off for caseness on the HADS scale that indicated borderline anxiety or depression and risk of developing clinical mood disorder. A regression was conducted with each outcome variable to assess if caseness had a relationship with any cognitive outcome measures. Caseness was coded as a dummy variable with categories coded as caseness and non-caseness of pure anxiety or depression. Then the dummy coding was applied where participants had a caseness score $\geq 8$ on both anxiety and depression and conducted a multiple regression of both caseness variables to determine their co-morbid effect on cognitive outcomes.

**Table 2.4:** Scaled Scores for patients and controls

| Measure | Healthy Controls Baseline (n = 29) | | Patients Baseline (n = 30) | | Difference | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **MMSE** | 29.03 | 1.05 | 28.40 | 1.69 | 0.63 | -0.64 |
| **WASI-II** | | | | | | |
| Verbal Comprehension | 124.83 | 16.16 | 115.40 | 19.86 | 9.43 | -3.70 |
| Perceptual Reasoning | 112.66 | 15.05 | 112.07 | 17.29 | 0.59 | -2.25 |
| FSIQ-4 | 120.90 | 14.55 | 113.83 | 16.68 | 7.06 | -2.14 |
| **D-KEFS** | | | | | | |
| Visual Scanning | 10.59 | 2.87 | 9.90 | 3.28 | 0.69 | -0.41 |
| Number Sequencing | 11.79 | 2.77 | 12.33 | 1.88 | -0.54 | 0.89 |
| Letter Sequencing | 11.55 | 3.13 | 12.47 | 1.61 | -0.91 | 1.52 |
| Number Letter Sequencing | 11.38 | 2.96 | 10.97 | 3.53 | 0.41 | -0.57 |
| **Verbal Fluency** | | | | | | |
| Letter Fluency | 13.83 | 2.94 | 11.30 | 3.71 | 2.53 | -0.77 |
| Category Fluency | 12.72 | 2.96 | 11.17 | 3.43 | 1.56 | -0.47 |
| Category Switching | 12.59 | 2.93 | 11.73 | 2.66 | 0.85 | 0.27 |
| Switching Accuracy | 12.38 | 2.72 | 12.00 | 2.48 | 0.38 | 0.24 |
| **Design Fluency** | | | | | | |
| Filled Dots | 12.52 | 2.75 | 11.10 | 2.64 | 1.42 | 0.10 |
| Empty Dots | 11.48 | 3.27 | 11.77 | 2.70 | -0.28 | 0.57 |
| Switching (Empty and Filled) | 11.52 | 2.50 | 11.80 | 3.52 | -0.28 | -1.02 |
| **Colour Word Interference Test** | | | | | | |
| Colour Naming | 10.86 | 1.87 | 10.69 | 2.31 | 0.17 | -0.44 |
| Word Reading | 11.21 | 2.55 | 11.75 | 2.05 | -0.54 | 0.51 |
| Inhibition | 12.69 | 1.85 | 10.82 | 3.48 | 1.86 | -1.63 |
| Inhibition Switching | 12.55 | 1.76 | 11.34 | 2.27 | 1.21 | -0.51 |
| **Tower Test** | | | | | | |
| Number of Moves | 149.90 | 82.78 | 119.53 | 34.17 | 30.36 | 48.61 |
| Total Time to complete (ms) | 504.21 | 164.93 | 517.55 | 169.98 | -13.34 | -5.05 |
| Achievement Scores | 11.41 | 2.85 | 12.07 | 2.79 | -0.65 | 0.06 |

| Measure | Healthy Controls Baseline (n = 29) | | Patients Baseline (n = 30) | | Difference | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| **BRIEF-A** | | | | | | |
| **Self-Report** | | | | | | |
| Inhibition | 51.17 | 6.79 | 51.87 | 8.70 | -0.69 | -1.90 |
| Shift | 52.17 | 9.75 | 52.83 | 10.39 | -0.66 | -0.64 |
| Emotional Control | 50.03 | 9.48 | 50.33 | 9.53 | -0.30 | -0.05 |
| Monitor | 49.38 | 8.02 | 50.83 | 10.86 | -1.45 | -2.83 |
| Initiate | 50.28 | 7.10 | 52.60 | 11.17 | -2.32 | -4.07 |
| Working Memory | 57.24 | 11.33 | 56.63 | 12.53 | 0.61 | -1.20 |
| Plan/Organise | 50.03 | 8.51 | 53.33 | 10.29 | -3.30 | -1.78 |
| Task Monitor | 53.69 | 8.94 | 58.37 | 13.41 | -4.68 | -4.47 |
| Organisation of Materials | 49.38 | 8.19 | 51.67 | 12.10 | -2.29 | -3.91 |
| BRI | 51.24 | 7.83 | 51.33 | 10.81 | -0.09 | -2.98 |
| MI | 51.86 | 7.00 | 55.97 | 13.42 | -4.10 | -6.41 |
| GEC | 51.31 | 6.62 | 54.20 | 11.30 | -2.89 | -4.68 |
| **CANTAB** | | | | | | |
| **Between Errors** | | | | | | |
| 4 boxes | 1.34 | 2.78 | 1.07 | 1.82 | 0.28 | 0.96 |
| 6 boxes | 8.07 | 8.57 | 7.07 | 6.90 | 1.00 | 1.67 |
| 8 boxes | 19.93 | 11.08 | 20.00 | 12.27 | -0.07 | -1.19 |
| **Double Errors** | | | | | | |
| 4 boxes | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 6 boxes | 0.14 | 0.35 | 0.27 | 0.94 | -0.13 | -0.59 |
| 8 boxes | 0.28 | 0.80 | 0.60 | 1.25 | -0.32 | -0.45 |
| **Strategy Score** | 33.10 | 7.56 | 32.60 | 5.44 | 0.50 | 2.12 |
| **Testosterone (pg/ml)** | 83.43 | 26.40 | 257.92 | 152.22 | -174.49 | -125.82** |
| **HADS** | | | | | | |
| Anxiety | 3.48 | 2.734 | 3.90 | 3.188 | -0.42 | -0.45 |
| Depression | 1.76 | 1.596 | 2.30 | 2.744 | -0.54 | -1.15 |

Abbreviations: SD, standard deviation; fsiq, full scale intelligence quotient; pg, picograms; ml, millilitre; ms, milliseconds; BRI, behavioural regulation index; MI metacognitive index; GEC, global executive composite; **p<0.01.

## 2.6 Results

Twenty nine healthy controls (mean age = 67.45 $\pm$ 6.96) and 30 patients (mean age = 66.47 $\pm$ 6.92) completed all neuropsychological and psychosocial assessments. No significant difference in age was found between patients and control ($t(57) = 0.54$, $p > 0.05$). Statistical analyses were conducted using SPSS 22.0 (IBM, 2013). Missing data was found in one healthy control participant on the D-KEFS colour word interference test. One patient's data was missing from the Brief-A Informant questionnaire and another from the D-KEFS colour word interference test. The estimation maximization procedure was used to estimate and replace all missing data which has been demonstrated as being an effective strategy for managing omitted data (Rubin, Witkiewitz, Andre, & Reilly, 2007).

### 2.6.1 Screening assessments

All healthy controls assessed on the MMSE passed the criteria for participation (table 2.1). MMSE scores did not significantly differ between patients (*mdn* = 29) and controls (*mdn* = 29) ($U = 351.00$, $z = -1.33$, $p > 0.05$). Levels of depression did not significantly differ between patients (*mdn* = 1) and controls (*mdn* = 1) ($U = 425.00$, $z = -1.55$, $p > 0.05$). Levels of anxiety did not significantly differ between patients and controls (table 2.2) ($t$ (57) = -0.54, $p > 0.05$). However, testosterone levels did significantly differ between patients (*mdn* = 212.585 pg/ml) and controls (*mdn* = 67.00 pg/ml) ($U = 53.00$, $z = -5.79$, $p < 0.01$). The assessment of the APoE4 gene was part of the hypothesis and rationale. However, data required to analyse this component were not available at the time of analysis due to the timeframe of the broader project.

Caseness of anxiety and depression showed that three healthy controls scored in the borderline zone for clinical anxiety. Patients' caseness of anxiety showed that five participants had borderline levels of anxiety. No relationship between anxiety caseness and cognitive variables were found in controls. However, patients did have a significant relationship between anxiety and double errors made in the eight box condition on the CANTAB SWM task ($F(1,28) = 14.41$, $p < 0.01$ where $R^2 = 0.34$ ($\beta$=1.92). This suggests that as caseness of anxiety increased in patients, number of double errors increased in the eight box condition. The patient group had two subjects presenting with caseness of depression. No significant relationship of depression casessness was found in controls or patients on any cognitive outcomes. Two patients had borderline caseness scores on both anxiety and depression. Both co-morbid mood factors did not have any relationship with cognitive outcomes.

### 2.6.2 Intellectual functioning

*WASI-II*

An independent samples t-test showed no significant WASI-II FSIQ-4 score differences between controls and patients ($t(57) = 0.74$, $p > 0.05$).

### 2.6.3 Executive functioning

*D-KEFS*

A MANOVA with all D-KEFS contrast scaled scores as dependent variables (table 2.2) showed no significant differences between groups ($F(5,53) = 1.18$, $p > 0.05$). Testosterone was added to the model with no significant effect ($F(5,52) = 1.17$, $p > 0.05$). Groups did not differ with the addition of testosterone ($F(5,52) = 2.03$, $p > 0.05$).

*BRIEF-A*

The brief executive function assessment self-report questionnaire (BRIEF-A) was subsequently analysed with MANOVAs. Fixed factors were entered as patients and controls. Inhibition, shift, emotional control, self-organisation, ability to initiate, working memory, monitor, behavioural regulation index, metacognition and global executive function scaled scores were entered as dependent variables (table 2.2). No significant differences were evident across dependent variables between patients and controls ($F(12,46) = 0.57$, $p > 0.05$). Testosterone was added as a covariate to the model but did not predict performance independent of group ($F(12,45) = 0.99$, $p > 0.05$). Furthermore, group performance differences were not accounted for by testosterone ($F(12,45) = 0.88$, $p > 0.05$).

Further exploratory analysis did not show any significant correlation between self-rated BRIEF-A questionnaire meta-cognitive Index scaled scores and D-KEFS contrast scaled scores. Correlations were not significant on the D-KEFS trail making test (r=-0.06, *p*=ns); verbal fluency test (r=0.05, *p*=ns), design fluency test (r=-0.10, *p*=ns), colour word interference test (r=0.08, *p*=ns) and tower test (r=-0.11, *p*=ns). This indicates that there were no correlations between meta-cognitive thought patterns and executive function.

### 2.6.4 Spatial reasoning

*Cantab*

No significant differences were found on the number of between errors made in the 4 box, 6 box and 8 box conditions across groups on the CANTAB SWM task ($F(3,55) = 0.18$, $p > 0.05$).

Further analyses showed no significant differences between number of between errors made when testosterone ($F(3,54) = 4.1$, $p > 0.05$) was added to the model. No significant difference was found between groups when controlling for testosterone ($F(3,54) = 1.11$, $p > 0.05$).

A MANCOVA was undertaken with the WAIS working memory index score added as a covariate to the number of between errors made. This allowed the isolation of the spatial reasoning portion of the SWM task whilst controlling for working memory. Findings showed a significant predictive relationship of the WAIS working memory index score on errors made on the SWM task ($F(3,53) = 8.96$, $p < 0.01$, $\eta^2 = 0.34$). Specific ANCOVAs showed that the WAIS accounted for variance on the number of total between errors made ($F(1,55) = 24.16$, $p < 0.01$, $\eta^2 = 0.31$), errors made in the 4 box condition ($F(1,55) = 12.24$, $p = 0.01$, $\eta^2 = 0.18$), errors made in the six box condition ($F(1,55) = 26.77$, $p < 0.01$, $\eta^2 = 0.33$) and errors made in the eight box condition ($F(1,55) = 15.17$, $p < 0.01$, $\eta^2 = 0.22$) while controlling for group. No effect of testosterone was found when it was added to the model when also controlling for WAIS WMI ($F(3,53) = 0.35$, $p > 0.05$). No between groups differences were found while controlling for WAIS WMI scores and testosterone levels ($F(3,53) = 0.48$, $p > 0.05$).

No significant differences were found on the number of double errors made in the 4 box, 6 box and 8 box conditions between groups on the CANTAB SWM task ($F(2,56) = 0.92$, $p > 0.05$). Testosterone was added to the number of double errors which showed that it did

predict the number of errors made ($F(2,55) = 10.46$, $p < 0.01$, $\eta^2 = 0.28$). Further ANCOVAs revealed testosterone had a significant effect on the number of errors made in the eight box condition ($F(1,53) = 13.25$, $p < 0.01$, $\eta^2 = 0.20$) and total number of errors made ($F(1,53) = 17.58$, $p < 0.01$, $\eta^2 = 0.25$) independent of group. The MANCOVA showed no differences between groups while controlling for testosterone ($F(2,55) = 1.39$, $p > 0.05$). Finally, no difference in strategy score was found in an independent samples t-test ($t$ (25) = 0.53, $p > 0.05$).

When testosterone and WAIS WMI were added to the number of double errors made a significant effect of testosterone was found ($F(2,54) = 10.60$, $p < 0.01$, $\eta^2 = 0.29$) on average independent of group. Further analysis showed the effects of testosterone were significant on total double errors ($F(1,55) = 20.25$, $p <0.01$, $\eta^2 = 0.01$) and number of errors in the eight box condition ($F(1,55) = 17.68$, $p < 0.01$, $\eta^2 = 0.24$). However, WAIS WMI scores did not predict the number of double errors made ($F(2,54) = 1.11$, $p > 0.05$) whilst controlling for group. No change was found between groups while controlling for WAIS WMI and testosterone ($F(2,54) = 1.63$, $p > 0.05$).

## 2.7 Discussion

No significant differences were found on the WASI FSIQ-4 and MMSE assessments indicating that intellectual functioning was similar between patients and controls which was in line with what was expected. Moreover, neuropsychological performance did not significantly differ between patients and controls on measures of the D-KEFS, BRIEF-A and CANTAB. This showed there were no differences in executive function and spatial reasoning, as was hypothesised. No significant psychosocial or mood differences were found, which was as projected. Testosterone levels did differ between groups which was not expected.

In the current study neuropsychological outcomes on the D-KEFS assessment showed no significant differences with and without covariates, as was expected. Between groups analysis of the CANTAB spatial working memory outcomes showed no significant differences on the number of between errors or double errors made between patients and controls which is in line with the hypothesis. However, testosterone levels across both groups did significantly predict the average number of errors made. This was prevalent in the double errors condition when controlling for working memory performance and testosterone which was a purer measure of spatial reasoning performance. This was in contrast to what was expected. Spatial reasoning ability measures have been reported as being highly sensitive to testosterone. One study assessing this relationship administered just a single dose (0.5mg) of testosterone to females and showed improvements on a 3D mental rotation task compared to those administered a placebo (Aleman, Bronk, Kessels, Koppeschaar, & van Honk, 2004). However, the study was limited because researchers did not measure both levels of testosterone and oestrogens. Therefore, endogenous oestrogens may have increased through the aromatization process to establish an equilibrium (Crosnoe, Grober, Ohl, & Kim, 2013).

This could have biased findings and all effects found may have been due to increasing oestrogen levels. Despite this shortcoming, other studies have found similar effects when testosterone was administered to females with higher spatial task loads and over a longitudinal time frame (Li, 2014; Postma et al., 2000).

Outcomes from the BRIEF-A measures showed no significant differences between groups. Low self-ratings of cognitive function were commonly found among participants (Pennequin, Sorel, & Mainguy, 2010). This may have had bearings on cognitive performance but in the present study, self meta-cognitive BRIEF-A scaled scores did not correlate with D-KEFS contrast scaled scores. This suggests that meta-cognitive perceptions of cognition did not have a bearing on cognitive performance. Nevertheless, this may not be the case for all executive function measures and clinically meaningful outcomes may have been missed. One study assessing participants on a number of neuropsychological measures, found significant correlations between the BRIEF-A self-reported cognitive impairments scale and executive functions (Rabin et al., 2006). Executive functions were assessed with the D-KEFS and Wechsler Memory Scale (WMS-III). Correlations were more pronounced in patients with mild cognitive impairment and those reporting cognitive complaints (Rabin et al., 2006). This suggests that self-reported cognitive impairments revealed clinically meaningful executive function deficits in patients compared to matched healthy controls. This may explain the lack of correlation found across groups between self-report measures and neuropsychological performance in the present study. This is because patients had cognition which matched to healthy controls that did not have any signs of MCI, thus there was no correlation between meta-cognition and executive function as illustrated by Rabin et al. (2006).

Anxiety and depression caseness revealed no relationship between neuropsychological performance and pure anxiety or depression caseness in healthy controls. However, caseness of anxiety did have a relationship between number of double errors made on the CANTAB eight box condition. Numerous researchers have shown an effect of anxiety on spatial reasoning (Shackman et al., 2006; Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013). This suggests that whereas testosterone may compensate for cognitive deficits, anxiety may lead to cognitive impairment even at baseline in patients that could not be compensated for by testosterone. However, this effect in patients was found on the more difficult items suggesting that easier spatial reasoning was intact despite caseness of anxiety.

Despite similar cognitive performance between patients and controls, some subtle significant outcomes were noted that require further interpretation. One unexpected result was that testosterone levels were significantly higher in patients compared to controls. While past patient samples in other assessments did not have higher than control levels of testosterone, the higher levels found in the current sample may not be unusual and has been shown in numerous patient cohorts (Klap, Schmid, & Loughlin, 2015). This could be because androgen receptor signalling is required for malignant cell proliferation and PC progression (Heinlein & Chang, 2004). Moreover, high serum testosterone levels have been proposed as a risk factor for increasing prostate specific antigen levels (PSA) levels. Although, there is a link between higher testosterone levels and poor cancer outcome, it may have inadvertently benefitted the sample in the study undertaken. This is because testosterone has been shown as being neuroprotective to cognition (Beauchet, 2006; Bialek, Zaremba, Borowicz, & Czuczwar, 2004), which may facilitate neuropsychological performance in patients with neurodegenerative disease or cognitive deficits to that of normal levels (Kurth et al., 2014). Furthermore, primary studies support this link showing that testosterone replacement therapy

can mitigate some of the deleterious effects of Alzheimer's disease. One study revealed improved verbal memory, spatial memory and constructional ability in Alzheimer's disease patients administered DHT (Cherrier et al., 2005). Moreover, patients with higher testosterone levels were less likely to develop Alzheimer's disease despite having gene expressions that increased Alzheimer's disease risk (Moffat et al., 2004). The extent to which patients were affected by risk of Alzheimer's was an aim of the study undertaken. Nevertheless, due to time constraints this information was not available in the current project. However, as part of future research, it may be proposed that APoE4 gene levels are measured in both groups to ascertain its effect on Alzheimer's development in PC patients and of interactions with testosterone. The patient cohort had higher than control levels of testosterone which may have offset the risk of Alzheimer's to allow for better cognition compared to past studies (Salminen et al., 2003). Thus, one possible mechanism for similar performance between patients and controls may be that the higher testosterone levels found in patients, facilitated compensation for cognitive deficits due to PC disease specific factors. This was evident as the sample assessed were not as severely affected on mood and cognitive measures as baseline patients assessed in past studies (Salminen et al., 2003; Salminen, Portin, Koskinen, Helenius, & Nurmi, 2004). However, during ADT the effects of this compensatory effect may be lost due to testosterone deprivation leading to cognitive decline during ADT. However, this requires careful consideration as it could affect ADT uptake, continuation and management of cognitive side-effects during therapy.

There are a number of shortcomings to the current study that must be acknowledged. Firstly, sample size was small, which limits the ability to detect smaller group differences (see Appendix 8 for power calculation). Hence, a larger sample size is required in the future; this will overcome the limitation of small groups in between subjects designs, which are limited

by between subject variance and so although differences were not identified here they may still be present but small in magnitude. A further confound that could be rectified by larger samples size is of the multiple testing issue that arose due to the number comparisons made between variables. This may have increased family wise error rates leading to false discoveries. Bonferroni corrections were employed to control for chance findings, however the corrected adjustment method has been known to be extremely conservative and comes at the price of increasing false negatives. Therefore, while no significant differences were apparent in the study undertaken, they may be present, but were not identified due to false acceptance of the null hypothesis implying there were no differences between groups.

The lack of relationship between mood and cognitive variables may reflect the nature of the measures used. Self-reported anxiety and depression questionnaires were employed to assess mood. However, informant questionnaires may have been beneficial on these measures to gauge another's perspective. This may lead to more accurate psychosocial understanding of PC patients. This could also give an insight into the patient's environmental factors such as family life which could affect psychosocial function. Therefore, informant questionnaires can add another dimension to the data which provides a network approach to psychosocial function from individuals related to or close to the patient (Edwards & Clarke, 2004). However, it was not the focus of the current study to assess informant reports. Formal assessment of mood may also allow better evaluation of a participant's mood levels through qualitative methods and diagnosis by a clinician. This may allow physicians to gauge information that is not revealed through self-report forms alone due to bias (O'Brien et al., 2001). Nevertheless, some researchers have shown that participants feel less embarrassment in reporting mood information on a questionnaire or computer than to a clinician (Kobak, Reynolds, & Greist, 1994). This implicates that both self-report and qualitative methods

should be administered to acquire a thorough understanding of psychosocial mood function in samples.

Another limitation includes that assessments of psychosocial function from the participant's perspective require a broader range of factors to be explored that were lacking in the current investigation. These are variables such as day to day functioning, quality of life, coping mechanisms or a specific questionnaire such as the prostate specific quality of life questionnaire (Stockler, Osoba, Corey, Goodwin, & Tannock, 1999). This may allow greater evaluation of patients' level of psychosocial function while awaiting upcoming treatment and the effect on cognition may be assessed from a wider range of outcomes. Whilst cognitive assessments undergo rigorous reliability and validity checks, they may lack the ability to assess real world cognition. Further research should take into account physical functioning, cognitive performance inside and in the real world environment for a thorough understanding of everyday functioning and quality of life through ecological validity assessments (Spooner & Pachana, 2006). This could be achieved by administering a variety of alternative assessments or measured through qualitative means such as interviews. This may provide researchers with a greater depth of information that is ecologically accurate, allowing a better understanding of factors that may lead to cognitive changes in PC patients before, during and after therapy.

In the current chapter, there were no behavioural differences between controls and patients however those data form an important baseline for longitudinal follow-up (refer to Chapter 4 & 5). Furthermore, there is strong evidence in the ageing literature that preservation of behavioural performance may be observed and yet there are significant alterations in the

neural architecture engaged during performance of those tasks. In other words, it is possible that neural differences may exists in advance of overt evidence of behavioural changes.

This is most evident in ageing populations. Some past studies have demonstrated normal spatial reasoning behavioural performance with altered brain activity to compensate for deficits. This is important because the study conducted showed an effect on spatial reasoning performance when testosterone was added as a covariate. Furthermore, spatial reasoning assessments are known to be sensitive to testosterone level fluctuations, therefore, deprivations of these levels may lead to spatial impairments or inabilities to compensate for deficits (Celec, Ostatníková, & Hodosy, 2015). One study assessed 25 older participants (mean age=70.9) and younger samples (mean age=22.4) on a mental rotation task under varying postural loads (sitting or standing balancing) (Van Impe et al., 2013). Brain imaging was undertaken with fMRI to identify neural associated mental rotation regions. Outcomes showed older participants had greater BOLD activation in frontal and parietal regions compared to the young cohort. Left lingual gyrus activation moreover modulated older participants' success on the task. However, task performance and reaction times did not differ between groups even with increasing complexity of the task in the scanner or with increasing postural loads. Another study used positron emission tomography (PET) to assess 10 young subjects (mean age=21.2) and 10 older participants (mean age=67.4) (Reuter-Lorenz et al., 2000). Researchers found no behavioural differences between groups but revealed mostly right lateralised activity in young participants while older participants showed activation in the anterior bilateral lobe. Additional frontal regions were active in older adults including bilateral dorsolateral prefrontal cortex.

A further study also showed this effect in normal healthy participants. Researchers employed a task in which participants were asked to locate the midpoint of either symmetrical or asymmetrical shapes (Wilkinson & Halligan, 2004). Significant differences in activation were

found in the anterior cingulate gyrus suggesting it had a facilitatiory effect on symmetry. Nevertheless, the presence or absence of symmetry did not affect speed or accuracy performance. Therefore, activation of the anterior cingulate gyrus and its behavioural correlate was not shown. Other studies validate this effect of behavioural and neural pattern divergence in cognitive domains including episodic memory (Suzuki et al., 2002), conflict resolution (Paulus, Hozack, Frank, & Brown, 2002), lexical decision making (Peng et al., 2003), guided movement (Handy, Grafton, Shroff, Ketay, & Gazzaniga, 2003) and spatial reasoning (Vingerhoets et al., 2001). Outcomes from previous studies suggest that cognitive compensation mechanisms are available in healthy ageing participants. This highlights the need for a control group in any further investigations when comparing controls to patients. This is because healthy ageing participants may have underlying neural networks that activate putative domain specific executive function and spatial reasoning regions that differ to young cohorts. Therefore, if activation in healthy ageing is not characterised when compared against older patients, any differences may be misconstrued as cognitive impairment in patients when in fact it is a normal function of ageing.

Diverging brain activation to behavioural performance on spatial reasoning tasks have also been found in patients with neurodegenerative conditions that may allow compensation for cognitive impairments. Parkinson's disease is characterised by deficits in motor control and planning due to cell death in the substantia nigra from dopamine deficiency (Marsden, 1982). This may lead to deficits on spatial reasoning tasks including the mental rotation task. A study which implemented the mental rotation task assessed 19 Parkinson's patients to compare performance and brain activation on the most and least affected hand of patients (Helmich, de Lange, Bloem, & Toni, 2007). Participants were tasked with identifying handedness of hands presented to them in the medial or lateral planes at various degrees of

rotation whilst undergoing fMRI. Outcomes showed that reaction times increased as a function of hand degree rotation from zero suggesting that patients did use spatial reasoning to mentally rotate hand photos. However, patients did have increased reaction times to hands in the lateral condition which was accompanied with a significant increase in activation of the right extrastriate area and occipito-parietal cortex when they were required to rotate the affected hand corresponding to their own affected hand. Regions with higher activation have been linked to visual processing and seem to have been recruited to minimize behavioural weaknesses. Moreover, increased psychocophysicological interaction (PPI) connectivity was found in the contralateral premotor cortex of right handed most affected participants compared to controls. This suggests that compensatory cerebral activity in unaffected neural areas should attempt to minimize behavioural performance on tasks of spatial reasoning in patients known to be affected with neural impairments. Although much research has been conducted in Parkinson's disease with motor tasks several studies support the theory of increasing activation in certain regions to support brain degeneration (Haslinger et al., 2001; Sabatini et al., 2000; Samuel et al., 1997).

Discrepancies in behavioural performance and neuroimaging findings have also been found in ageing patient populations when compared to normal ageing participants. One study measured cognition through positron emission tomography (PET) in a visual recall and semantic memory task in older patients with Alzheimer's Aβ deposition compared to age matched controls (Elman et al., 2014). Outcomes showed all groups had behavioural performance above chance levels and that there were no between group differences. All groups exhibited activation on the semantic recall and memory recall part of the task in the fronto-parietal network while deactivated areas were found in the default mode network. However, when only task correct items were compared between Alzheimer's patients and elderly controls, patients had greater activation in right lateral, superior parietal and occipital

cortical regions. Moreover, patients had relatively less deactivation in task related default mode areas compared to ageing controls. Finally, task activation regions were attenuated in patients with greater Aβ deposition compared to patients with low levels of Aβ deposition (Elman et al., 2014). This suggests that hyper-activation was beneficial in patients with Aβ deposition which increased neural plasticity to serve as an over-compensatory mechanism to support cognitive processes. These findings have been supported by other research groups showing that neural plasticity is a factor that can suppress disease impairments that are unobservable such as dopamine deficiency in patients with Parkinson's disease (Poletti, Emre, & Bonuccelli, 2011) and plaque build-up in Alzheimer's disease (Sumowski, Chiaravalloti, & DeLuca, 2009). Taken together, this suggests that subjects with compromised cognition may be able to compensate for impairments through recruitment of underlying neural circuits. This may facilitate similar performance of patients to controls as demonstrated behaviourally in the current study and requires further investigation of underlying neurology.

The underlying neural mechanisms in prostate cancer (PC) patients before or during ADT have rarely been examined. Studies conducted in healthy controls compared to ADT and non-ADT PC patients at baseline, have found similar task performance (Chao et al., 2012; Cherrier et al., 2010). Furthermore, structural MRI generally showed intact gray and white matter volumes using voxel-based morphometry at baseline (Chao et al., 2013). However, one study demonstrated that despite the lack of behavioural cognitive difference found between patients and controls over a duration of six months, patients had significantly decreased connectivity between the medial prefrontal cortex and regions required for cognitive control during ADT (Chao et al., 2012). This shows that there were underlying neural alterations that did not manifest in behavioural tasks. In the context of the current

study, it is important to establish whether similar patterns of group difference exist at baseline so that we can interpret longitudinal changes carefully and appropriately. Furthermore, the presence of group differences at baseline might help us to identify which men are most at risk of later cognitive decline associated with ADT. Functional imaging offers an advantage in being able to identify different aspects of brain pathology that may be subtle and cannot be detected by behavioural assessment alone. These outcomes offer valid and novel insights into the nature of cognition of patients and controls.

For the current study, this indicates that cognitive decline may not be detected through behavioural assessment alone in patients at the point of diagnosis. However, neuroimaging may highlight neural abnormalities in elderly PC patients. However, no neuroimaging studies have employed a comprehensive battery of cognitive assessments in PC patients before ADT, to evaluate executive function and spatial reasoning ability. These domains have been shown to decline in patients whilst undergoing ADT (Treanor et al., 2017). Therefore, the effects of neuroimaging deficits in patients before starting therapy must be investigated to mitigate any carry over effects to the actual therapy duration. This may help physicians devise a strategy or plan to restrain cognitive impairments during therapy.

The research suggests that ageing participants or those with cognitive impairments could compensate for their deficits. The exploration of these underlying mechanisms allows the differentiation between neural changes that are related to the ageing process or those that are disease specific. This will be explored in the next chapter. Moreover, potential neural changes found in patients could be exacerbated during therapy leading to detrimental effects on cognition. Therefore, characterisation of neural function in patients at the early stages may prevent unwanted effects of the therapy through careful strategic planning and management.

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# CHAPTER 3

Study 2: Neuroimaging comparison of neural function and underlying architecture between pre-treatment prostate cancer patients and healthy age-matched controls.

## 3.0 Introduction

Consistent with the behavioural literature relating to testosterone, cognition and aging, assessment of functional activation during paradigms that assess executive functions and spatial reasoning abilities is warranted, as these areas were shown to be affected in PC patients (McGinty et al., 2014; Treanor et al., 2017). Executive functions refers to a family of terms that encompass a wide number of cognitive sub domains such as inhibition, verbal ability, working memory, problem solving ability and mental plasticity (Alichniewicz et al., 2013; Allain, Nicoleau, Pinon, Etcharry-Bouyx, Barre, et al., 2005; Baddeley & Della Sala, 1996). These series of abilities are usually classified as a complex set of higher order skills that require top down processing to achieve goal oriented daily life activities such as independent function, ability to work and maintain social relations (Goel, Grafman, Tajik, Gana, & Danto, 1997; Grafman et al., 1996; Green, 1996).

Two key cognitive domains (executive function and spatial reasoning) were studied in the current thesis, using a functional imaging framework, owing to data suggesting there may be poor performance in PC patients both before and while undergoing ADT (McGinty et al., 2014; Salminen et al., 2003; Treanor et al., 2017). Inhibition, or inhibitory control, is an important aspect of executive function requiring higher order cognitive functioning to withhold an action, thought or response (Alichniewicz et al., 2013; May et al., 1999). It can also involve the self-control to interference (Diamond, 2013). Specific paradigms that employ inhibition include the stroop task (MacLeod, 1991), flanker task (Mullane, Corkum, Klein, & McLaughlin, 2009), anti-saccade task (Luna, 2009) and go/nogo task (Cragg & Nation,

2008). The task employed (stop signal task) has been found to assess motor response inhibition and shown to elicit brain activation in areas that have been associated with executive functions (Aron, 2007). Although spatial reasoning can also be measured in a variety of ways a prominent approach uses mental rotation of visually-presented objects (Potvin et al., 2013). Of note, the spatial reasoning task implemented (mental rotation task) incorporated a high spatial component but also required sub components of executive function such as short-term memory, working memory and mental flexibility (Pardo-Vazquez & Fernandez-Rey, 2012). The activation of areas associated with spatial ability from the task were important in the assessment of a spatial reasoning and executive function networks in patients. Furthermore, use of tasks with well-established patterns of activation in brain areas associated commonly with executive and spatial reasoning facilitates enabled the ability to not only investigate underlying neural architecture of behavioural responses in men with PC but also to assess the integrity of these networks using methods comprising resting state functional connectivity and arterial spin labelling. This allowed inferences to be made regarding age-related changes compared to changes in brain activity present before therapy in patients.

In this section the tasks employed in the current thesis are described. The stop signal task (SST) has a prevalent inhibition component to it that is considered a key component of executive function (Andrés, 2003; Aron, 2007; Stuphorn & Schall, 2006) and is suitable for assessments in a laboratory setting (Vince, 1948). The task employs the ability to supress inappropriate or no longer required responses (Verbruggen & Logan, 2009). Response inhibition impairments have been linked to disorders including attention deficit hyperactivity disorder (Nigg, 2001), obsessive compulsive disorder (Penades et al., 2007), and clinical disorders such as cancer (Chao et al., 2012). The paradigm typically involves a reaction time

response on go trials (no stop) and inhibition of responses to stop trials. The SST has been found to require intricate sensorimotor map representation, odd ball consideration, task switching, inhibition, and post error processing (Li, Huang, Constable, & Sinha, 2006). This is because the task requires retraction of an already initiated 'go' response thereby placing more emphasis on inhibition (Rubia et al., 2001). The task may transfer to everyday circumstances for example when an action that was on the edge of being implemented is suddenly changed in the evaluation of the condition. This requires quick motor inhibition in situations such as crossing a street or the decision whether to touch a hot object (Diamond, 2013).

The neural substrates of the SST have been explored in several studies, and provide strong evidence for the involvement of key brain regions in executing the task. For example, one study found that go trial presses elicited fMRI BOLD activation of the motor cortex, frontal and striatal cortex, whereas stopping (inhibitory trials) activated the right inferior frontal gyrus (rIFG) in 18 healthy subjects (mean age=29.2, SD=4.5) (Aron & Poldrack, 2006). Moreover, fast stop reaction times were shown to elicit greater activation. Another study showed similar regional activation in 14 healthy participants (mean age=29.4) (Chevrier, Noseworthy, & Schachar, 2007). Researchers found functional MRI BOLD activation in the right prefrontal and midline regions whereas inhibition activated the rIFG and basal ganglia. Furthermore, activation in the anterior cingulate was found during error detection. Further support of distinct regional involvement comes from a study demonstrating right fronto-parietal activations in 22 healthy participants on inhibitory trials (mean age=23.5) (Zandbelt, Bloemendaal, Neggers, Kahn, & Vink, 2013). These studies provide evidence that the SST elicits activation in consistent regions in healthy younger adults. Further brain regions have been implicated on the SST that have been associated with other behavioural aspects of the

SST. A study by Li et al. (2008) explored the behavioural correlates of post error slowing specifically in 40 slightly older healthy adults (age range=22-42). In this study behavioural adjustment was made during subsequent trials to adjust for errors made from previous trials and involves the slowing of reaction times. This reaction time latency allows better judgement on the task to increase accuracy and is behaviourally evident through increasing reaction times on inhibition trials (stop signal reaction time) and increasing go trial reaction times. However, it is a conflicted ability because the SST requires motor inhibition responses whilst also requiring slowing to increase accuracy. Researchers showed that neural correlates of this slowing effect were primarily in the right middle frontal gyrus and right inferior frontal gyrus which correlated with increasing stop signal reaction times and go reaction time as a function of error made (Li et al., 2008). Taken together the studies suggest a pattern of right prefrontal and parietal cortical activity during inhibition processing with midline regions being activated during go trials. Moreover, these areas correspond to typical areas associated with executive function (Takeuchi et al., 2013).

Similar tasks such as the GO/NOGO task have been found to elicit neural activation of overlapping regions analogues to the SST in the medial, mesial, and parietal and inferior frontal cortices (Rubia et al., 2001). One study found that both response switching from the go/nogo task and inhibition from the SST elicited BOLD fMRI activations in similar regions including bilateral IFG and presupplementary gyrus (Kenner et al., 2010). However, other studies have found that the go/nogo task has been associated with lateralisation to the left hemisphere DLPFC areas specialising in motor planning and response selection (Rubia et al., 2001). The SST has been correlated with right hemispheric laterization in the anterior cingulate cortex, inferior prefrontal, and parietal cortices which are known for specialisation of inhibition (Rubia et al., 2001). These areas  have predominantly been associated with

executive function, attention, and high working memory loads (Rubia et al., 2001). Lesion studies furthermore support right hemisphere dominance in which localised damage to the rIFG and MFG can lead to loss of inhibitory control (Drewe, 1975; Verfaellie & Heilman, 1987). Differences in activation may reflect dissimilarities in inhibitory requirements. This is because the go/nogo task typically requires an alternative response to be made while the SST necessitates inhibition of a response that was on the verge of being made (Verbruggen & Logan, 2009). This suggests the SST may be more able to assess functions related to everyday tasks whereas the go/nogo task assess a utility required in unusual cases (Diamond, 2013). This validates the SST as a suitable measure to assess inhibitory executive function, because it requires higher inhibitory loads when stop signals are presented compared to tasks such as the go/nogo task. Moreover, it activates cortical regions that have been implicated as being central to executive function such as the rIFG. The SST has not been found to be impaired by decreasing testosterone but literature does support that ageing participants have worse performance on the task compared to younger cohorts.

The mental rotation (MRot) task has been found to assess spatial reasoning ability. The task has been considered as measuring spatial reasoning as it necessitates object manipulation, monitoring, and updating of information as the task progresses (Potvin et al., 2013). It requires the mental rotation of shapes using executive function control and spatial orientation (Potvin et al., 2013). The assessment of spatial reasoning is of importance in patients undergoing ADT since testosterone levels are exhausted (Green et al., 2002; Green et al., 2004; Joly et al., 2006). Researchers have explored the effects of testosterone on cognition with findings leading to the conclusion that testosterone does affect cognition, and especially spatial reasoning. For example one study assessed the effects of normal endogenous serum testosterone levels and normal free testosterone levels on neuropsychological performance

(Moffat et al., 2002). Outcomes revealed a correlation between high visual memory, verbal memory, visuospatial function, and visuo motor scanning performance and high free testosterone levels. Higher mean free testosterone levels and longitudinal free testosterone levels were concomitantly associated with a decreased rate of visual memory decline (Moffat et al., 2002). Significantly impaired spatial reasoning performance was associated with visual memory decline in hypo-gonadal males (Moffat et al., 2002). The study therefore suggests a possible beneficial effect of free testosterone on cognition. A shortcoming was that participants classified as hypo-gonadal were on average eight years older than those participants with normal testosterone levels. Older participants have been known to have cognitive difficulties due to older age which may be a confounding factor (Lunenfield, 2003). Another study found that participants substituted with testosterone for six weeks had better spatial memory scores, and constructional ability scores compared to a placebo group (Cherrier et al., 2005). The above behavioural literature illustrates that much research from the past has found associations between testosterone levels and impaired spatial reasoning ability (Barrett-Connor, Goodman-Gruen, & Patay, 1999; Fonda et al., 2005; Moffat et al., 2002). The mental rotation (MRot) task has also been assessed in a longitudinal nine month neuroimaging (fMRI) study with ADT patients compared to matched healthy controls (Cherrier et al., 2010). Reductions in right parietal occipital lobes were apparent when ADT patients performed the MRot task compared to controls (Cherrier et al., 2010) leading to worse performance. This suggests ADT patients may suffer from spatial deficits during ADT treatment. Therefore, testosterone deprivation could lead to neuronal impairments which will be assessed in the current research.

Past animal studies add support of brain region activation and have found neural correlates of the task in the bilateral occipital parietal lobes and supramarginal gyri, which are thought to be associated with spatial map generation to mentally code locations of targets (Andersen &

Buneo, 2002). This is apparent through electrophysiological research of the superior parietal cortex in primates (Snyder, Grieve, Brotchie, & Andersen, 1998); individual cells in these areas are known to be responsible for eye-centred coordinates modulated by primate head or body position (Snyder et al., 1998). Moreover, monkeys with spatial neglect in one part of space often have lesions in the parietal cortex that inhibits spatial reasoning ability (Karnath, Ferber, & Himmelbach, 2001). The motor cortex has also been found to be activated during mental spatial manipulation but its activation is much debated throughout neuroimaging literature. This is because task activated regions must dissociate between regions activated due to motor planning and those activated due to the motor demands of the task (e.g. button response pressing).

Neural substrates associated with both SST and MRot have been speculated to be areas of abnormal activation in ADT patients due to ADT treatment. Functional MRI tasks can be used to measure underlying neural networks in PC patients before ADT in the presence of normal behavioural performance that would otherwise indicate normal brain function. This allows researchers to identify any affected brain regions before therapy that could accelerate adverse effects experienced by patients during treatment. This will allow physicians to implement strategies to manage the aetiology of side-effects to minimize cognitive impairments during therapy which could affect patient treatment uptake and continuation.

*Resting state connectivity*

Task-free functional connectivity is another tool with which to measure underlying neural changes that might occur in the absence of behavioural differences between groups. Resting state fMRI is measured by acquiring the BOLD signal at rest (Biswal, 2012). It can be used to measure correlations of neural patterns that occur between spatially separate regions (Biswal,

Van Kylen, & Hyde, 1997). Altered patterns of activity can thereby be identified that differ from standardised neural resting state networks (Damoiseaux et al., 2006; Fox & Raichle, 2007). These networks are distinct regions known to be activated simultaneously and are thought to collaborate together to form functional connections at rest (Lowe, Dzemidzic, Lurito, Mathews, & Phillips, 2000). The connections are important to assess as they are thought to overlap with brain regions that are active during behavioural performance (van den Heuvel & Hulshoff Pol, 2010). The measure thereby provides an indirect evaluation of white matter tract integrity that allows for the sharing of information between regions (van den Heuvel & Hulshoff Pol, 2010). Moreover, testosterone decline is also known to affect white matter tracts in patients with multiple sclerosis whereas testosterone substitution could alleviate inflammation and deterioration of white matter tracts (Kurth et al., 2014). Therefore, investigation of resting state networks was warranted due to the effect of androgens on white matter architecture and applies to PC patients deprived of the hormone.

The default mode network (DMN) is known to be one of the networks that is thought have a high level of activity compared to other identified networks and overlaps with functional regions that are active during the performance of task based assessments (Raichle & Snyder, 2007). Activity of the DMN was assessed in the current study as it has been associated with central processes including integration of emotional processing (Greicius, Krasnow, Reiss, & Menon, 2003); global monitoring (Gusnard, Raichle, & Raichle, 2001); divergent thinking (Heinonen et al., 2016) and mind drifting (Mason et al., 2007). These complex higher level process related to the DMN, indicate that it can provide a unique perspective in examining resting state connectivity that underpin behavioural cognitive concepts including executive function (Bullmore & Sporns, 2009; Garrity et al., 2007; Harrison, Yucel, Pujol, & Pantelis, 2007). Moreover, the DMN can be assessed in relation to other regions which are known to be underpinned by executive functions that were explored in the present study (Beaty,

Benedek, Barry Kaufman, & Silvia, 2015). The DMN has also been shown to be sensitive to testosterone levels and deprivations in androgen have been associated with Alzheimer's disease.

In support of this, some researchers suggest that testosterone levels could modulate functional connectivity in the DMN. One study found that testosterone administration in females transferring to transgender males had increased functional connectivity in the frontal cortex, medial temporal lobe and cerebellum compared to males transferring to the female gender (Mueller, Wierckx, Jackson, & T'Sjoen, 2016). Another study assessed the influence of a single dose of testosterone in healthy females (Schutter, Peper, Koppeschaar, Kahn, & van Honk, 2005). The administration showed greater resting state EEG connectivity between the left prefrontal and right parietal cortex. This network has been commonly identified as a network with low connectivity and linked to depression in females. Moreover, it is a known network important to executive function and consists of DMN areas. Therefore, the role of androgens in increasing connectivity may be beneficial to executive function whereas deprivation of androgens could lead to impairment. The same has been shown in males with high endogenous testosterone levels suggesting testosterone is a core hormonal modulator of resting state connectivity in the DMN and related executive function regions (Miskovic & Schmidt, 2009). These studies are supported by fMRI assessments of subcortical and cortical resting state connectivity analysis (van Wingen, Mattern, Verkes, Buitelaar, & Fernandez, 2010).

This is corroborated in age comparison research in which elderly participants have been shown to have decreased patterns of connectivity compared to younger cohorts (Damoiseaux et al., 2008). This suggests that circulating androgens have an effect on resting state connectivity areas linked to executive functioning. Nevertheless, other literature shows connectivity impairments between emotional processing nodes such as the amygdala and

default mode network and inferior frontal gyri and anterior cingulate cortex (Westlye, Kaufmann, Alnæs, Hullstein, & Bjørnebekk, 2017). These areas have also been found to have reduced resting state connectivity as a function of steroid administration (Westlye et al., 2017). Studies therefore infer that not only can resting state connectivity be modulated by testosterone levels but that declines in androgens are a natural part of ageing that can analogously affect resting state connectivity. This may affect inferences made on resting state network changes in patients that could be a function of normal ageing or due to disease specific or therapy factors. A control group was therefore warranted in the present exploration to characterise normal ageing resting state changes in relation to comparison to patients.

*Arterial spin labelling*

The BOLD signal is closely coupled to cerebral perfusion (Laurienti et al., 2003; Liu & Brown, 2007). Thus, changes in BOLD signal, whether induced by task-based paradigms or fluctuating at rest, may reflect fundamental alterations of underlying physiology rather than variation in functional architecture. Arterial spin labelling (ASL) is a relatively new technique that uses MRI to assess underlying cerebral blood flow changes that could occur in the absence of behavioural differences between groups. More specifically, measurement of functional MRI task-based or resting activation differences between or within groups may be affected by age-related changes or changes over time. This may be due to other relevant intermediary variables, such as cerebral perfusion, or blood flow (Chen, Rosas, & Salat, 2011; Leoni, Oliveira, Pontes-Neto, Santos, & Leite, 2017) rather than functional brain changes *per se.*

The link between ageing and cerebral perfusion has been explored in recent studies. Leoni and colleagues measured cerebrovascular reactivity, which was explored through the assessment of changes in cerebral blood flow (CBF) in response to carbon dioxide ($C0_2$) inhalation, leading to widening of blood vessels (vasodilatory effect). Healthy ageing participants were tested and compared to young participants (Leoni et al., 2017). Investigators found significantly reduced CBF in older (46 $\pm$ 9 mL/100g/min) compared to younger (57 $\pm$ 8 mL/100g/min) adults using pulsed arterial spin labelling (PASL). Moreover, reduced cerebrovascular reactivity was observed in elderly participants compared to young participants in gray matter (Leoni et al., 2017). This indicates that there are age related CBF impairments even in healthy older participants. Regional CBF decreases were found in the gray matter superior, middle and inferior frontal gyri, precentral and postcentral gyri, superior temporal gyrus, anterior cingulate gyrus, insula, putamen and supramarginal gyrus. However, the study was cross-sectional and variability as factors including gas breathing was not controlled systematically. Furthermore, the hemodynamic response measured had greater variability between subjects, which limited findings as these variables could not be controlled between subjects. A better option may have been to conduct a longitudinal study so that variables could be compared across time thereby limiting sources inter-subject variance.

Another more extensive study using PASL found reductions in CBF as a function of age in 23-88 year old participants (Chen et al., 2011). Groups were split into healthy young adults (mean age 30 $\pm$ 6.4), middle age adults (mean age 52.0 $\pm$ 5.9) and older adults (mean age 70.5 $\pm$ 10.4). Cerebral perfusion values were 52.6 $\pm$ 9.3, 52.0 $\pm$ 10.7, and 42.7 $\pm$ 8.8 ml/100gtissue/min respectively. Whilst no significant differences were found between young and middle aged adults, significant differences were evident in young and middle aged adults compared to older adults (Chen et al., 2011). Regional differences between groups were

found in the superior frontal, superior parietal, orbito-frontal, inferior-middle temporal gyri, insular, precuneous, supramarginal, lateral occipital and cingulate regions. However, subcortical perfusion was unchanged as a function of age (Chen et al., 2011). The study was cross-sectional but had a large sample size making it more generalizable to healthy ageing populations. This shows that there are differences in perfusion between middle aged participants and older participants suggesting that reductions in perfusion is a normal function of ageing. Moreover, in relation to the current study, decreases in CBF may be a function of normal ageing in PC patients before therapy that could lead to changes in the BOLD signal. Previous research shows that CBF impairments could lead to BOLD signal drop offs (Ances et al., 2009). This is important to the current study since outcomes were heavily dependent on fMRI outcomes. Therefore, if the BOLD signal is solely interpreted then researchers may be led to outcomes showing that patients had functional/cognitive deficits in cerebral function as a result of impending treatment or due to the therapy itself. In other words, given that cerebral blood flow is known to diminish with age, this could lead to lower BOLD signal activation independently of clinically-relevant disease related factors (Liu et al., 2012). This highlighted the need for a control group in the present study so that normal perfusion levels could be characterised and compared to patients to assess if any changes were due to cognitive functioning or perfusion differences. Therefore, ASL was employed to validate that cohort samples did not have perfusion abnormalities that affected fMRI activation results by leading to BOLD signal fluctuations.

In the context of the present study, and evidence that testosterone may influence brain connectivity at rest, it is important to establish whether testosterone levels influence CBF, particularly over longitudinal durations. Indeed, decline in testosterone levels associated with normal ageing may be one possible mechanism influencing changes in the CBF of normal

older samples. The association between cerebral perfusion and testosterone in ageing cohorts was explored in one study (Azad, Pitale, Barnes, & Friedman, 2003). Researchers assessed the effects of testosterone administration on cerebral perfusion using single photon emission tomography (SPECT) imaging. Seven hypogonadal subjects (age range=52-72) were administered with testosterone for three months. Results showed increased cerebral blood flow in the midbrain, anterior cingulate gyrus and superior frontal gyrus gray matter regions during treatment compared to baseline hypogonadal levels and was similar to that of controls. Moreover, testosterone administration led to better mood and quality of life (Azad et al., 2003). The study was limited in sample size but outcomes show that not only can imaging methods be used to detect the effects of hormonal changes on cerebral perfusion, but that testosterone does impact on cerebral perfusion as a function of normal ageing. This has implications for interpretation of either group differences in BOLD activation (i.e. patients and controls) or longitudinal analysis within patients. To date, no research has been reported that assesses the impact of androgens on cerebral blood flow using ASL. Thus, in the current study, a measure of ASL was used to examine whether changes in BOLD during task-based or resting fMRI reflected changes in cognition *per se* or simply altered cerebral perfusion.

### 3.1.1 Aim

The aim of this study was to characterise neural activation and functional connectivity in patients at baseline and controls. Moreover, the investigation aimed to assess whether cerebral perfusion accounted for any group differences.

### 3.1.2 Hypothesis

PC patients were not expected to have any changes in cerebral function or neural architecture before beginning androgen deprivation therapy. More specifically:

**a.** There were expected to be no in-scanner behavioural differences between healthy controls and patients which included:

- **i.** No difference in stop signal performance or reaction times between controls and patients.
- **ii.** No behavioural differences between controls and patients on the MRot task.

**b.** Furthermore, no significant neural activation differences between controls and patients on the stop signal or MRot tasks were expected including:

- **i.** That the SST would activate putative task regions from past literature. These areas being the right inferior frontal gyrus, middle frontal gyrus, anterior cingulate cortex and inferior parietal regions in both groups.
- **ii.** That there would be bilateral parietal activation when participants engage in the MRot task.

**c.** There would be no resting state connectivity differences between controls and patients when regions of interest relevant to executive function were selected and correlated with the default mode network.

**d.** No significant differences in cerebral perfusion were expected between controls and patients assessed through arterial spin labelling (ASL).

## 3.2 Methods

### 3.2.1 Design

A between subjects design was implemented to assess performance and neural activation in controls and patients on an event-related SST. MRot task performance and brain activation was measured with a block design interval model. Participants were scanned using functional magnetic resonance imaging (fMRI) before any cancer specific therapy commenced.

### 3.2.2 Participants

Participant details were as stated in chapter two.

## 3.3 Brain Imaging Tasks

**SST (SS) task parameters**

Participants were presented with a white fixation cross for 500ms followed by a yellow hash (#) symbol in the centre of the screen. Hash symbol durations were separated into go trials (no flicker) and stop trials (short flicker, medium flicker, and long flicker) presentation timings (figure 3.1). The hash symbol was always yellow in colour and presented on a black background. All stimuli durations were kept equal (700ms) and a jittered blank screen duration (ISI) was presented subsequently after each stimuli to make trial occurrence unpredictable. An algorithm was used to create ISI durations which were contingent upon the timing of the jitter at the beginning of each trial. The ISI was presented for 3000ms initially and either increased if a correct inhibition was made in the previous trial, or decreased by 50ms if a commission error was made in the previous trial where a button press is made on a stop trial. The ISI duration increased to a maximum of 3400ms and did not increase further even if correct inhibitions were made. The minimum ISI duration was 2600ms and did not decrease further even if further commission errors were made. Subjects were required to

respond on a keyboard to no flicker trials and to inhibit responses on stop trials. Go trials comprised 60 per cent of all trials and stop signals encompassed 40 per cent of trials [short flicker (15), medium flicker (5) and long flicker (20)]. Ordering of no flicker and flicker hash symbols were randomised (For durations of hash symbols see Figure 3.1). Apart from randomisation of stimuli each run was identical. The duration of each run was 5 minutes 14 seconds and experiments were split into two runs each consisting of 100 trials with 200 trials in total. The task was made using E-prime 2.0 (Schneider, 2002) which collected behavioural data consisting of keyboard responses (correct, inhibited, incorrect and reaction time).

*SST outcome measures*

Performance on the SST can be modelled as an independent horse race model which describes the processes between the probability of responding p(respond|signal) and the probability of inhibiting p(inhibit|signal) on stop signal trials (Logan & Cowan, 1984). The p(respond|signal) depends on three factors including (1) the stop signal delay (SSD), (2) go reaction time (go RT), and (3) stop signal reaction time (SSRT). When the SSD increases, the p(respond|signal) increases. This is because the stop process starts later and finishes later in comparison to the go process which has already begun thereby forcing a response. As subsequent SSDs are presented the p(respond|signal) decreases and go RT increases. This is because of the increased probability that the stop process finishes before the go process (Verbruggen & Logan, 2009). Furthermore, SSRT increases for subsequent SSDs because the probability that the stop process finishes after the go process increases.

*Stop signal reaction time calculation*

Stop signal reaction time (SSRT) was acquired with the most commonly used integration method. This method estimated the SSRT by subtracting the SSD from go RT distribution. The go RT distribution was acquired by rank ordering all go trial reaction times. The nth go RT was then selected and subtracted from the median SSD. This process was repeated for each participant and then averaged. For example in a session of 20 trials from which go RT can be measured, there are four stop trials (one correct stop and three incorrect stop). The probability of correctly stopping is 0.25, therefore, the probability of responding is 0.75. The stop signal is presented 350ms after the initial go onset of the go stimulus. Go RT are 300, 320, 340, 380, 400, 410, 420, 460, 500, 520, 540, 550, 590, 600, 700, 750ms.

*n* = number of go RTs (which can be measured) x probability of response on stop trials.

*n* therefore = 16 x 0.75 = 12

the $12^{th}$ reaction time is 550ms.

Therefore, the stop process finishing estimate is 550ms after the onset of the go stimulus. Subtraction of this from the SSD which is 350ms is 550-350 revealing an SSRT of 200ms (example from Eagle et al. (2008).

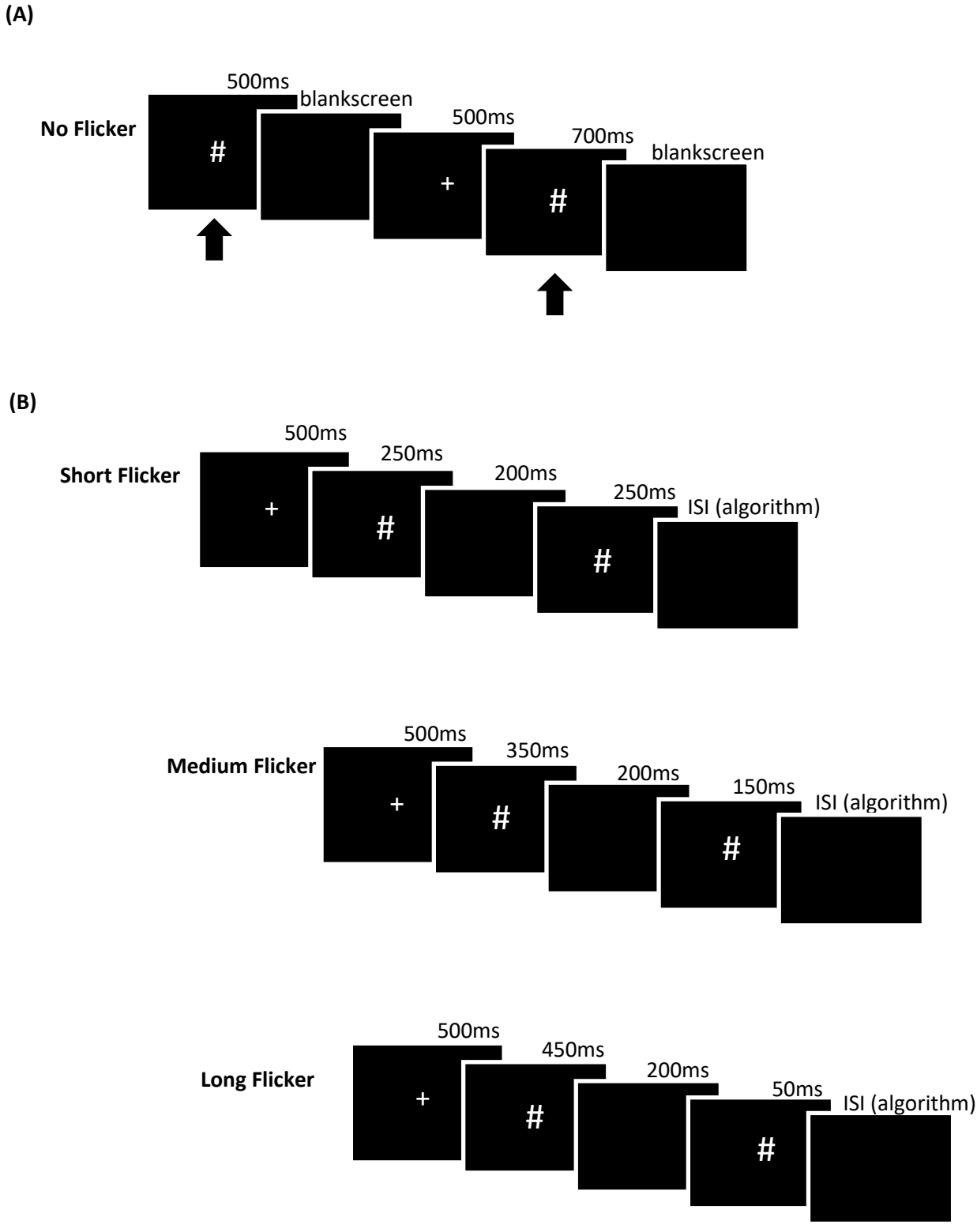


**Figure 3.1***: fMRI illustration of the SST. (A) No flicker timings in which participants must press the button on response pad. Arrows under hash symbol figures represent when participants must respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 2, blank screens labelled as ISI algorithm were varied in their duration and increased or decreased in duration depending on if the participant responded or inhibited correctly. Abbreviations: ms, milliseconds; ISI, interstimulus interval.*

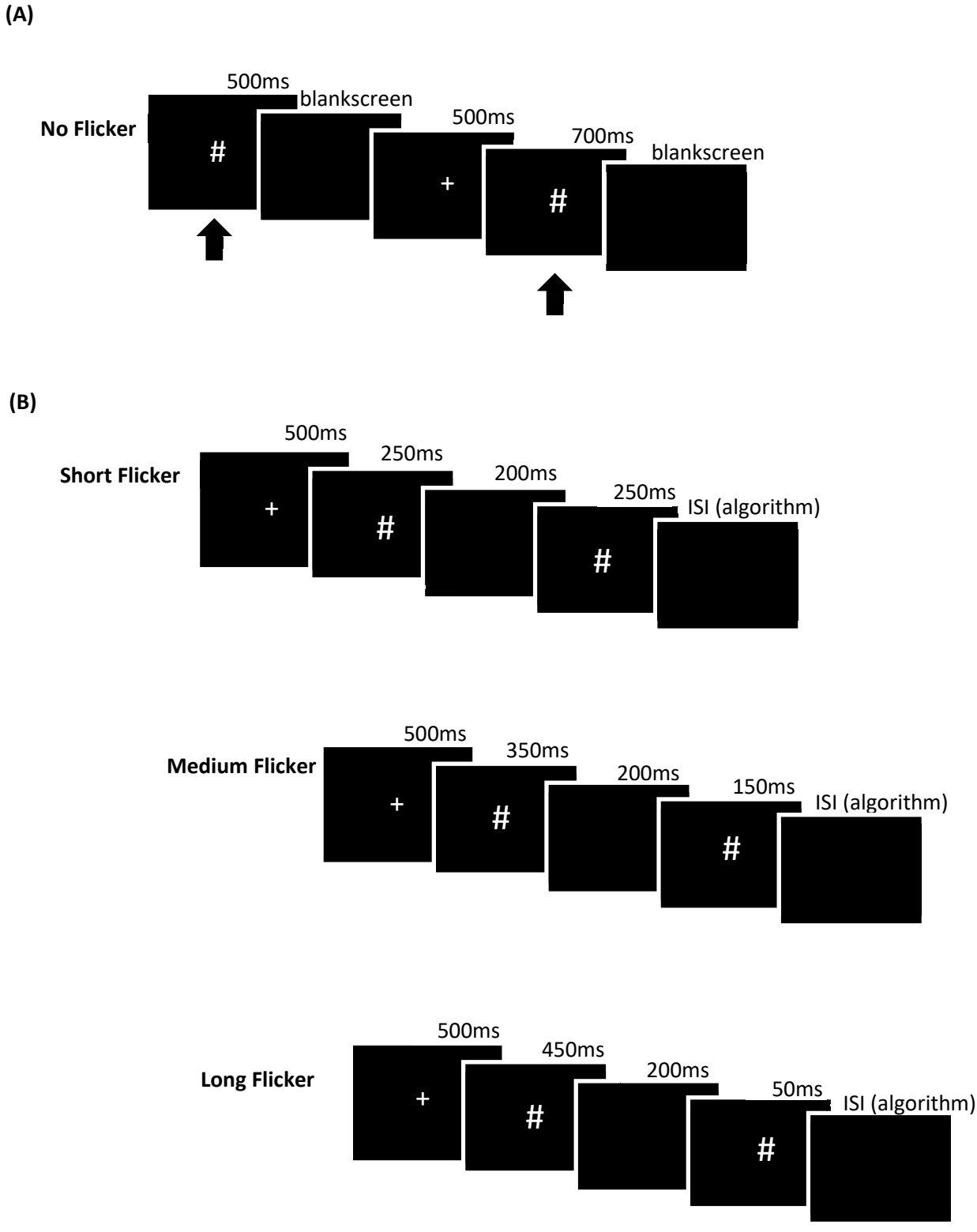


**Figure 3.1***: fMRI illustration of the SST. (A) No flicker timings in which participants must press the button on response pad. Arrows under hash symbol figures represent when participants must respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 2, blank screens labelled as ISI algorithm were varied in their duration and increased or decreased in duration depending on if the participant responded or inhibited correctly. Abbreviations: ms, milliseconds; ISI, interstimulus interval.*

**MRot task parameters**

The MRot task consisted of a 7.5 minute run with eight alternating 38 second blocks separated by 20 second rest periods. Blocks comprised of experimental and control conditions each repeated four times during the functional run. Eight shapes were presented per block. The task was adapted from Shepard and Metzler (1971) MRot task in which 3D shapes are displayed on a blank screen. In experimental blocks, one shape from each pair was rotated along its vertical axis at 60, 120, 180 or 300 degrees and contained identical shapes whilst the other half contained rotated mirrored shapes. Un-rotated identical or mirror shapes were presented in the control condition. Participants had to determine if shapes were identical or mirror images by pressing either their right index or middle finger respectively in both conditions. Slides were presented for 3750ms followed by a fixation point on a blank screen for 750ms.

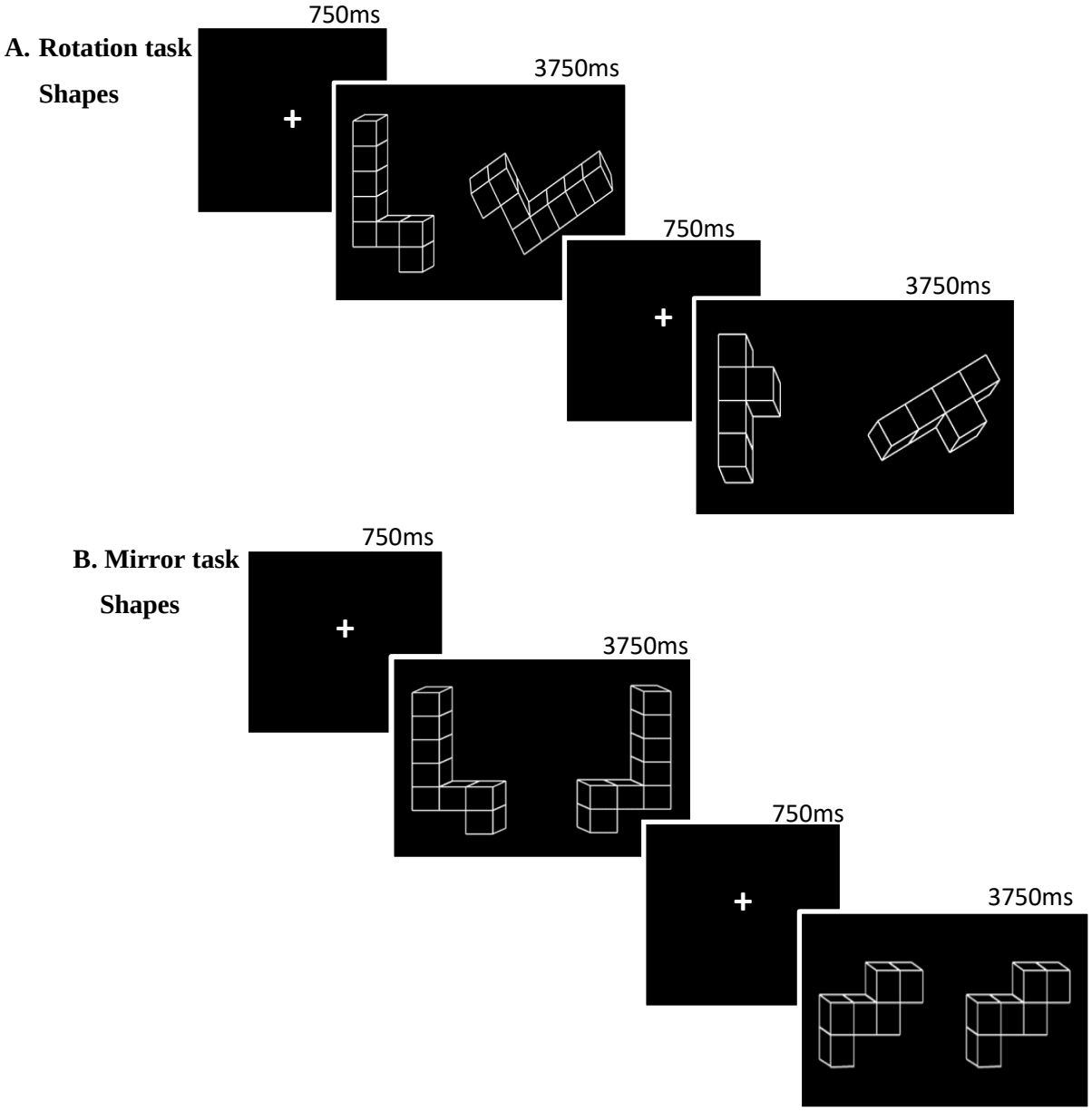


**Figure 3.2:** *Shows MRot task parameters and examples of presented shapes. A. Task rotated shape examples where subjects were required to make a response after mentally rotating shapes clockwise or anti-clockwise to judge if shapes were in the same orientation or mirror images of each other. B. same or mirror oriented shape pairs that require no rotation to distinguish if they were the same or different. Abbreviations: MRot, mental rotation task; ms, milliseconds.*

## 3.4 Imaging acquisition

All scans were acquired on a Philips 3.0T Achieve functional magnetic resonance imaging (fMRI) scanner with a 32 channel head coil receiver.

### 3.4.1 Structural scans

Sagittal $T_1$ structural images were obtained using a 3.0 T scanner (Philips). Blood oxygenation level dependent (BOLD) signals were acquired with a gradient echo planar imaging (FEEPI) sequence in the sagittal plane (thickness of 1mm), repetition time (TR) = 8.4s, echo time (TE) = 3.8ms, flip angle = 7.16°, field of view = 230 × 175 mm, matrix size = 288 x 288. Voxel dimensions were 1mm x 1mm x 1mm isotropic. 184 dynamics (images) were acquired for SSTs with forty contiguous axial slices (3mm thickness) per image. These were acquired with TR = 2.5s, TE = 3.80s, flip angle = 90°, field of view (FOV) = 240 × 240, matric size = 68 x 68. A tilted acquisition plane ($30^o$ to the rostral > caudal tilt) was used and adjusted individually to reduce signal dropout in frontal brain regions (orbitofrontal cortex) (Mennes et al., 2014).

### 3.4.2 Functional Scan Imaging Acquisition Parameters

Both SST and MRot functional scans were acquired with 3mm x 3mm x 3.5mm non-isotropic voxels. 184 dynamics (images) were acquired with 40 contiguous axial slices (3mm thickness) per image where the TR = 2.5s, TE = 3.80s, flip angle = 90°, field of view (FOV) = 240 × 240, matrix size = 68 x 68. A tilted acquisition plane ($30^o$ to the rostral > caudal tilt) was employed and adjusted individually to reduce signal dropout in frontal brain regions (orbitofrontal cortex) (Mennes et al., 2014).

### 3.4.3 Functional Resting state connectivity

Resting state scans were acquired with 30 contiguous axial slices (4mm thickness) using gradient echo planner imaging (FEEPI) with TR = 2.0s, TE = 3.5s, flip angle = 80°, field of view (FOV) = 80 × 80 matrix size. Voxel size were 3mm x 3mm x 4mm and 180 dynamics (images) were acquired. A tilted acquisition plane ($30^{o}$ to the rostral > caudal tilt) was used and adjusted individually to reduce signal dropout in frontal brain regions (orbitofrontal cortex) (Mennes et al., 2014). Scan duration was 6 minutes 13 seconds.

### 3.4.4 Arterial Spin Labelling (ASL)

Absolute cerebral blood flow was measured using pulsed arterial spin labelling (PASL) and was acquired with 15 axial slices (3.5mm thickness) at a distance of 3.5mm. A FAIR Double Acquisition back ground suppression (DABS) was used to acquire ASL data (Wesolowski, Gowland, & Francis, 2009) (TR = 8.0s, TE = 9.59s, flip angle = 90°, field of view (FOV) = 240 × 240$mm^2$ matrix size 68 x 66). Twenty-four dynamic images were acquired and voxel size were 3mm x 3mm x 7mm. A tilted acquisition plane ($30^{o}$ to the rostral > caudal tilt) was used and adjusted individually to reduce signal dropout in frontal brain regions (orbitofrontal cortex) (Mennes et al., 2014). The acquisition plane was also placed more toward the superior region of the brain so that maximum gray matter areas could be acquired in prefrontal and anterior regions. Scan duration was 6 minutes 66 seconds. Whole brain T1 sequences were acquired individually at 0.3, 0.6, 0.7, 1.1 and 1.3 seconds after bolus (endogenous water molecules in blood) were labelled (tagged by inversion). Control T1 sequences where no bolus was tagged were acquired eight seconds after tag images for later subtraction from T1 tag inversions. A calibration image was finally acquired with the same dimensions as aforementioned for PASL with only 6 dynamics. The calibration scan duration was 56

seconds and was acquired for an estimation of absolute cerebral blood flow in ml/100gtissue/min, which was applied to control-tag subtraction images in later analysis.

## 3.5 Statistical Analysis

### 3.5.1 FSL analysis

### 3.5.2 Preprocessing

Raw data files in ParRec from the Philips scanner were converted to Neuroinformatics Technology initiative (Niftii) format. Structural scans were reoriented using a reorient script in a Linux terminal. Structural T1s were skull stripped by employing a BET extraction tool in FSL 5.0.8 (Smith et al., 2004) BET brain extraction software (Smith, 2002). The point of interest on scans was corrected to determine the centre of the cortex for skull stripping. T1s were checked with FSL view to assess quality of skull extraction. Pre-processing steps included motion correction of images using FSL MCFLIRT. Regular slice timing correction was applied with a spatial smoothing full width by half maximum (FWHM) of 8mm. A high pass filter was applied to remove low frequency noise thereby allowing better signal to noise ratio (SNR). Functional images were co-registered to structural T1s and then co-registered images were registered to standard MNI standard space (Strother, 2006). Only voxels with significant ($p < 0.05$) functional activation were accepted as voxels with substantial signal change.

### 3.5.3 First Level analysis

Statistical analysis at the subject level on the SST was carried out at a single subject level first using the general linear model (GLM) in FSL. SST durations were separated into four conditions: go correct (gc), go incorrect (gin), stop correct (sc), and stop incorrect (sin) which were convolved with the GLM as regressors. The main contrast created to investigate inhibition compared to go activation was of go incorrect (gin) and stop correct (sc) trials compared to stop incorrect (sin) and go correct (gc) trials. As illustrated, gin and sc trials were paired together which was undertaken due to low trial power on gin trials. However,

this was further explored because although both trials types were related to inhibition, they used conceptually different mechanisms. This is because whereas, gin trials were associated with inhibition, they were errors made during go trials which may elicit activation in go related areas. Therefore, the pairing of trials may not be appropriate if this were the case since in the main contrast, the combination of gin and sc trials would not consist of solely inhibitory activation for comparison to go activation. Therefore, two exploratory contrasts of gin compared to gc trials and gc compared to gin trials were included to investigate if gin trials elicited inhibitory activation exclusively.

Similarly, sin trials were paired with gc trials to increase the power of go trials. Contrasts of sin compared to sc and sc compared to sin trials were created to investigate if sin trials were associated with primarily go trial activation. This is because sin errors were made during stop trials that may elicit inhibitory activity and not activation associated with go trials. Moreover, sin responses have been associated with post-error slowing adjustments. This is a mechanism by which subjects may incorporate a longer latency period from the start of a trial presentation to the response made after an error is detected so that better performance is achieved on subsequent trials (Li et al., 2008). However, this mechanism has also been associated with right dorso-lateral prefrontal cortex activation related to inhibition (Li et al., 2008). Therefore, it would not be appropriate to combine sin and gc trials in the main contrast if sin trials elicited inhibitory activity. The combination of contrasts also facilitated in balancing the main contrast so that it was not biased to the combined side with the majority of trials.

The SST was additionally decomposed into trials of long correct (lc), long incorrect (lin), medium correct (mc), medium incorrect (min), short correct (sc) and short incorrect (sin) trials and run contrasts. The main contrast was long flicker correct trials compared to go

correct trials as this allowed comparison of maximum inhibition activation compared to response (go trial) activation.

The MRot task was spilt into experimental (exp) and control (cont) task trial duration, which were convolved with the GLM. Experimental conditions were contrasted against the control condition. Contrasts were run with the general linear model in FSL and the canonical hemodynamic response function was used to model activation. Single subject analysis was performed with an uncorrected z-threshold ($P < 0.05$). Analysis of single subjects was combined using a higher level analysis and mixed Flame effects stats were implemented to correct for multiple comparisons with a z-threshold of 2.3. A cluster significance threshold of $P < 0.05$ was used to locate any voxel clusters surviving multiple comparisons.

First level analyses were entered into a group level analysis using FEAT's higher level statistical modelling approach (Smith et al., 2004; Woolrich et al., 2009). Patients and controls were compared using an independent samples t-test. Anxiety, depression and testosterone levels were added as covariates by first demeaning raw scores and then entering them as covariates for each participant. Demeaning was undertaken by taking the group mean for the covariate and subtracting it from each raw covariate score. Covariates were assessed at the individual level and with an F-test to explore the variance contributed by all covariates on group activation contrast maps. Contrast masking was used to contain variance from covariates (anxiety, depression and testosterone) to contrast activated regions of specific contrasts only.

Region of interest (ROI) analysis was done by masking specific regions taken from cluster activated areas above Z>2.3. Masks were binarised so that regions inside the mask were given a value of one and regions outside were given a value of zero using the fslmaths command. Featquery (http://www.FMRIb.ox.ac.uk/fsl/feat5/featquery.html) was used to analyse

ROIs in specific contrasts with output being mean percentage signal change over the functional run time course in the ROI.

### 3.5.4 Resting State seed based functional connectivity

Resting state analysis was undertaken by selecting predefined regions of interest cluster masks which were registered to pre-processed functional scans. Masks chosen were the default mode network (DMN) medial prefrontal cortex (mPFC) and right dorso-lateral prefrontal cortex (rDLPFC) as the seed region (Harvard-Oxford Cortical Structural Atlas). These areas were specifically chosen due to their putative involvement with executive functions (Koechlin & Summerfield, 2007; Seeley et al., 2007). All raw data went through preprocessing first where head motion in white matter and cerebrospinal fluid was filtered out. Region of interest of masks were then registered to each subject's functional resting state run. The BOLD data was further filtered between $0.001 < f < 0.01$.

A dorsolateral prefrontal cortex (DLPFC) mask was generated using a 5mm ROI around the peak group average voxel in the whole DLPFC (38 18 32). Correlations were then gauged between the seed region (DLPFC) and DMN mPFC. These were output into a separate file. Regressors were generated by finding the time course of the seed region of interest. The seed region time course was inputted into the first level single subject GLM so that a connectivity map could be generated with the time course of the seed region and DMN mPFC. Positive and negative contrasts were used to assess positive/negative correlation activations from the seed region and mPFC to other regions in the default mode network. Single subject level analyses were then combined into a higher level group analysis to assess combined group activation. Two analyses were performed in which the DMN mPFC and DLPFC were analysed and another analysis in which the DMN mPFC and rIFG were analysed. The rIFG was chosen due to its putative role in executive function and especially in the SST during

fMRI (Hughes, Johnston, Fulham, Budd, & Michie, 2013). The rIFG was masked by selecting the voxel presenting greatest activation (MNI coordinates: 50 30 -2) (Harvard-Oxford Cortical Structural Atlas) in the rIFG cluster area from the SST group activation map. A 5mm sphere was masked around these voxels for the resting state analysis. An independent sample t-test was conducted to assess the difference in resting state connectivity between patients and controls.

### 3.5.5 Arterial spin Labelling

Initial preprocessing steps were undertaken to establish there was normal cerebral perfusion estimates over time points that was obtained from a particular slice and voxel in each subject. This was accomplished by using a unique method where skull stripping was first undertaken of ASL data using the FSL BET extraction tool to create a mask of the brain (Smith et al., 2004). The BET extracted data were subsequently segmented into multiple T1 images, which revealed tag-control subtracted images. Next, T1 tag-control T1 images were averaged over repeated acquisitions. A slice was then selected in a gray matter superior portion of the brain that encompassed the prefrontal cortex as normal perfusion in this region was required for further analysis. Voxel co-ordinates were subsequently selected in the nominated slice making sure it was in a part of the brain where there was gray matter to measure cerebral perfusion in the slice and voxel across time points. This allowed the selection of a slice to estimate perfusion in just one voxel and image. This calibration image was also selected to calculate absolute perfusion in mL/100g/min units. Average perfusion was estimated by fitting the data from the slice and voxel selected to the kinetic curve over five time points (0.3, 0.5, 0.7, 0.9 and 1.1 ms) (Chappell et al., 2010). This pattern of perfusion has been shown to be exponential at first and then decreases rapidly as bolus exits the cortex. The curve was calculated at each time point by subtracting the tag image from the control image

and then averaging the result which is fitted on a voxel by voxel basis using Chapell's two compartmental model (Chappell et al., 2010). Further computations allowed gray, white and cerebrospinal fluid separation masks which were used to assess the kinetic curve over the five time points in each tissue type averaged over the whole brain. Moreover, a whole brain kinetic curve was explored and then just the top half of the brain perfusion was investigated. This procedure was undertaken in all subjects individually. If perfusion was severely abnormal due to motion effects or scanner software failure, then the scan of that subject was excluded from the analysis. The FSL package Bayesian inferencing toolkit (BASIL) (Chappell, Groves, Whitcher, & Woolrich, 2009) was used to analyse subject first level data where the tag and control images were subtracted to generate CBF images and registered to the BET extracted T1 structural image. The output revealed a CBF image tag-control averaged across the five inversion time durations. The CBF outputs were subsequently registered with the standard MNI template to standardise perfusion measures across subjects in gray matter only using the standardised brain.

In order to assess group level averaged cerebral whole brain and regional perfusion, higher level group combinations were undertaken using the fslmerge, fslmaths and fslstats commands to calculate average perfusion across groups. Regions of interest (ROIs) masks were generated of the rIFG and right and left supramarginal gyri and these were then binarised and superimposed onto whole brain perfusion images. Fslstats was then used to extract perfusion values in these ROIs. Comparisons were made between groups and ROIs by extracting average CBF on an individual participant basis. Data were then entered into SPSS and a one-way between subjects ANOVA was used to make comparisons.

## 3.6 Procedure

Participants were scanned at the Birmingham University Imaging Centre (BUIC) centre and screened before being scanned to confirm MRI safety protocols (Appendix 1). Participants were briefed about the nature of the study, their scan durations and the protocol that would be applied if any incidental findings were present.

Participants were fully de-metalled and placed in the supine position into the scanner by a qualified radiographer or trained scan operator. Padding was placed to the side and underneath the head to limit head movement. An emergency buzzer was given to participants in case of distress, so that they could alert the scanner operator mid-scan if they wanted to stop for any reason. A computer screen image projected from a projector was reflected to participants through a mirror placed on the head coil. Headphones were provided for communication to participants in the scanner room through the control room where either the radiographer or scan operator could communicate with the participant before individual scan runs. Ear plugs were provided for hearing protection.

### 3.6.1 Functional runs

Explanations of the SST and MRot tasks were administered before participants were scanned via a laptop computer. Participants completed 20 practice trial items from the SST and 15 items of the MRot task, which were dissimilar to the actual tasks presented to minimize practice effects. Practice trials were presented on a laptop computer in the waiting room or a separate quiet area. Participants were instructed to press their index finger once as quickly as possible when they saw the hash symbol appear after a fixation cross on the SST. However, if the hash symbol flickered, they were instructed to inhibit their response. They were also told not to wait until after the hash symbol completely disappeared off the screen and the blank

screen appeared to make a response. This instruction was required to avoid prediction responses and inhibition or post-trial responding.

Instructions for the MRot task included that participants should press their index finger as quickly as possible if shapes fit into each other after mentally rotating shape pairs. However, if shapes were a mirror image of each other and did not fit into each other then they should press their middle finger. Participants did the practice tasks on a desktop computer with screen and keyboard provided using E-prime 2.0 software, with analogous keyboard mapping to the scanner response pads.

Participants were instructed to lie as still as possible during the resting state scan and ASL scan. Finally, before entering the scanner, participants were told the layout of the scanner and a NATA technologies (NATAtech, 2006) response pad was provided inside the scanner. The ergonomic response pad was placed under the right hand of the participant in the scanner where they were able to press two buttons (index and middle) out of four available. Instructions were repeated to participants before each functional and structural scan through headphones. Total scan duration was 90 minutes.

## 3.7 Results

3.7.1 Behavioural Analysis

### 3.7.1.1 Stop Signal Task (SST)

In scanner behavioural performance was compared between healthy matched controls and patients with prostate cancer. Independent sample t-tests showed no significant differences between groups on overall go and stop signal accuracy ($t(57) = -1.04$, $p > 0.05$). No significant difference was found between groups on overall reaction time ($t(57) = 1.49$, $p > 0.05$). Moreover, no significant between groups difference was found on stop trial inhibition accuracy ($t(57) = -0.47$, $p > 0.05$) or stop signal reaction time ($t(57) = 0.27$, $p > 0.05$).

A further independent samples t-test was conducted with group as the fixed factor and stop signal flicker trials (short, medium and long) performance as dependent variables. The independent samples t-tests showed no significant differences on inhibition accuracy in the short flicker condition ($t(57) = -0.75$, $p > 0.05$) medium flicker condition ($t(57) = -1.47$, $p > 0.05$) or long flicker condition ($t(57) = 0.06$, $p > 0.05$) between patients and controls. Independent samples t-test of stop signal reaction time showed a significant difference between controls and patients in the short flicker condition ($t(36) = 2.05$, $p = 0.05$, $r = 0.32$) and medium flicker conditions ($t(44) = 2.38$, $p = 0.05$, $r = 0.34$) where patients had faster SSRTs compared to controls (Table 3.1). No significant SSRT between group differences were found in the long flicker ($t(57) = 0.79$, $p > 0.05$) condition. A MANCOVA showed no significant effects of anxiety ($F(2,21) = 0.80$, $p > 0.05$), depression ($F(2,21) = 2.20$, $p > 0.05$) or testosterone ($F(2,21) = 0.34$, $p > 0.05$) on stop signal flicker SSRT differences found between patients and controls.

**Table 3.1** SST Descriptive Statistics

| | **Healthy Controls (n=29)** | | **Patients (n=30)** | |
|---|---|---|---|---|
| | **Baseline** | | **Baseline** | |
| **Trial type** | **Mean** | **SD** | **Mean** | **SD** |
| **Go trials** | | | | |
| Accuracy (%) | 78.64 | 11.92 | 81.5 | 8.99 |
| Reaction time (ms) | 1317.34 | 311.55 | 1206.34 | 257.48 |
| **Stop trials** | | | | |
| Accuracy (% inhibited) | 65.82 | 22.42 | 68.46 | 20.47 |
| SSRT (ms) | 304.07 | 168.12 | 251.77 | 110.31 |
| **Short flickers** | | | | |
| Accuracy (% inhibited) | 79.54 | 26.41 | 84.44 | 23.72 |
| SSRT (ms) | 599.69 | 202.8 | 408.01 | 86.45* |
| **Medium flicker** | | | | |
| Accuracy (%inhibited) | 66.21 | 30.87 | 77 | 25.21 |
| SSRT (ms) | 564.6 | 183.38 | 540.33 | 261.65* |
| **Long flickers** | | | | |
| Accuracy (% inhibited) | 54.66 | 21.39 | 54.33 | 20.82 |
| SSRT (ms) | 615.95 | 147.57 | 557.62 | 114.6 |

Abbreviations: ms, milliseconds; SD, standard deviation; SSRT, stop-signal reaction time; SST, stop signal task; *p<0.05.

### 3.7.1.2 Mental Rotation (MRot) task

Independent samples t-tests were conducted and showed no significant difference in performance between patients and controls in the same oriented shape condition ($t(57) = -0.33$, $p > 0.05$). However, a significant difference was found between groups on mirror shape tasks ($t(57) = -2.19$, $p = 0.03$, $r = 0.28$) where patients performed better than controls (Table 3.2). No reaction time differences were found between groups in the same oriented shapes condition ($t(44.16) = -1.93$, $p > 0.05$) (equal variances were not assumed since Levene's test indicated unequal variances, ($F = 6.60$, $p = 0.01$). Therefore, degrees of freedom were adjusted from 57 to 44.16) or mirror shapes ($t(57) = -0.94$, $p > 0.05$). Finally, no significant differences were found between groups on performance ($t(57) = -1.86$, $p > 0.05$) or reaction time measures ($t(57) = -1.61$, $p > 0.05$) when trials were combined across same and mirror shapes.

Further ANOVAs were conducted to explore performance and reaction times on distinct shape orientations from zero. A one way ANOVA with group as the fixed factor and reaction times on the varying degrees of shape rotations (60, 120, 180 and 300 degrees) as four dependent variables was employed. The analysis showed no significant differences between groups in the 60 degree ($F(1,57) = 1.34$, $p > 0.05$), 120 degree ($F(1,57) = 0.86$, $p > 0.05$) and 180 degree ($F(1,57) = 0.005$, $p > 0.05$) conditions. However, a significant reaction time difference was found in the 300 degree condition ($F(1,57) = 6.70$, $p = 0.01$, $\eta^2 = 0.11$) where patients had slower reaction times compared to controls (Table 3.2 and figure 3.3). A similar ANOVA was performed with percentage correct in the varying degree conditions from zero as dependent variables. The analysis showed no significant performance differences in the 60 degree ($F(1,57) = 2.50$, $p > 0.05$), 120 degree ($F(1,57) = 3.15$, $p > 0.05$) or 180 degree ($F(1,57) = 0.01$, $p > 0.05$) conditions between groups. However, a significant difference was

found in the 300 degree condition ($F(1,57) = 5.03$, $p = 0.03$, $\eta^2 = 0.08$) where patients performed better than controls (Table 3.2 and figure 3.4).

Both mental rotation performance and reaction time in the 300 degree conditions were entered into a MANCOVA to assess if covariates mediated any of this difference. No effects of anxiety ($F(2,53) = 1.89$, $p > 0.05$), depression ($F(2,53) = 0.43$, $p > 0.05$) or testosterone ($F(2,53) = 1.35$, $p > 0.05$) were found to vary with differences in reaction time and performance. No significant reaction time or performance difference was found when controlling for all covariates ($F(2,53) = 0.86$, $p > 0.05$).

**Table 3.2** MR task Descriptive Statistics

| Trial type | Healthy Controls (n=29) | | Patients (n=30) | |
|---|---|---|---|---|
| | Baseline | | Baseline | |
| | Mean | SD | Mean | SD |
| **MR task performance** | | | | |
| **Same orientation shapes** | | | | |
| Accuracy (%) | 60.78 | 24.14 | 62.71 | 20.60 |
| Reaction Time (ms) | 2086.34 | 349.76 | 2229.72 | 199.53 |
| **Mirror orientation shapes** | | | | |
| Accuracy (%) | 46.55* | 28.33 | 61.04 | 22.18* |
| Reaction Time (ms) | 2138.55 | 287.86 | 2219.62 | 366.29 |
| **Total same and mirror orientation shapes** | | | | |
| Accuracy (%) | 53.67 | 18.30 | 61.88 | 15.64 |
| Reaction Time (ms) | 2112.44 | 285.65 | 2224.67 | 249.80 |
| **Divided degrees** | | | | |
| *60 degrees* | | | | |
| Accuracy (%) | 61.21 | 27.42 | 71.25 | 21.06 |
| RT (ms) | 1979.39 | 350.19 | 2105.53 | 464.89 |
| *120 degrees* | | | | |
| Accuracy (%) | 56.47 | 21.29 | 66.25 | 21.06 |
| RT (ms) | 2174.39 | 401.33 | 2261.48 | 316.01 |
| *180 degrees* | | | | |
| Accuracy (%) | 47.84 | 18.92 | 48.33 | 18.49 |
| RT (ms) | 2177.86 | 386.81 | 2170.52 | 391.34 |
| *300 degrees* | | | | |
| Accuracy (%) | 49.14 | 21.89 | 61.67 | 21.00* |
| RT (ms) | 2118.13 | 405.16 | 2361.16 | 293.43** |

Abbreviations: MR, mental rotation task; ms, milliseconds; RT, reaction time; SD, standard deviation; *p<0.05, **p<0.01.

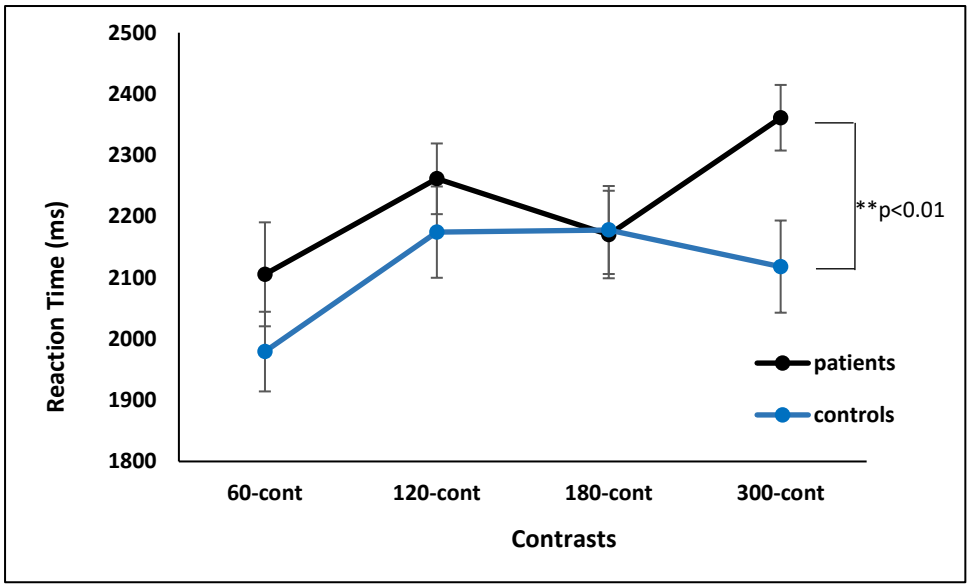


**Figure 3.3:** *MRot task average reaction time between patients and controls. Abbreviations: cont, control condition; MRot, mental rotation task; ms, milliseconds.*

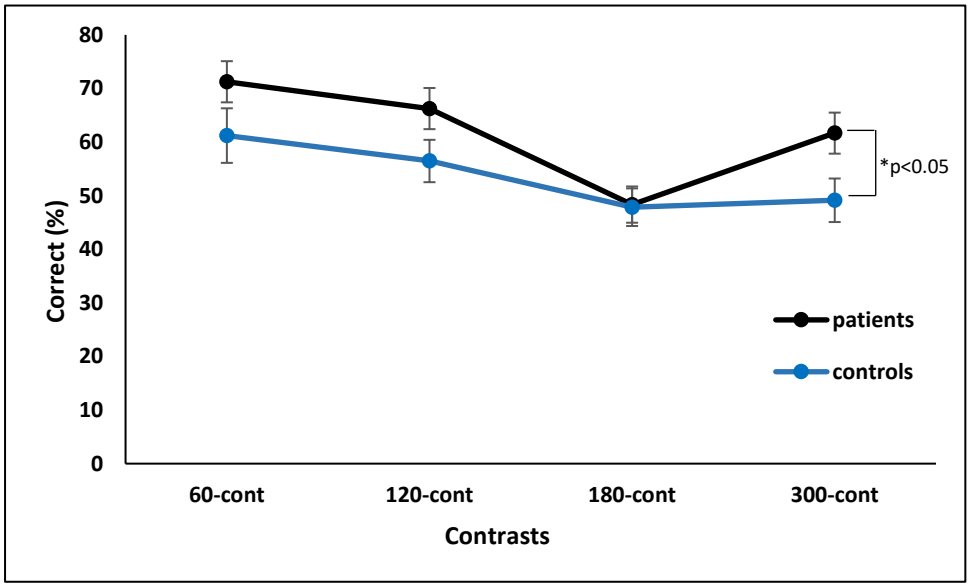


**Figure 3.4:** *MRot task average percentage correct between patients and controls. Abbreviations: cont, control condition; MRot, mental rotation task; *p<0.05; **p<0.01.*

### 3.7.2 Whole brain cerebral blood flow (Arterial Spin Labelling analysis)

Whole brain cerebral blood flow was explored first to validate that both groups did not have altered levels of cerebral perfusion. Whole brain CBF was classed as being abnormal if there were abnormalities such as hypo-perfusion (15-20 mL/100gtissue/min) or hyper perfusion in gray matter regions. These thresholds were implemented as they suggest there could be vascular abnormalities including arterial stenosis, occlusions or lesions etc. if such abnormalities are found (Pollock et al., 2009).

Analysis of baseline ASL data was compromised due to errors detected in the raw data of 22 cases (three patients, nineteen controls). Past research has shown that ASL is reliable over a longitudinal period and so in order to compare groups, data from follow up scans were used to estimate baseline perfusion. Researchers in one study assessed reliability in healthy older subjects (mean age = 75.5 $\pm$ 5.3) that underwent resting state ASL (Jiang et al., 2010). Participants were scanned using pulsed arterial spin labelling at baseline, three, six and twelve months. Outcomes showed no significant differences in perfusion between time points. Moreover, reliability of perfusion measured through intra-class correlations were moderate to good. Moderate ICC values were attributed to slight variances and changes in slice positioning and coregistration. The study is supported by further studies showing that ASL was reliable over a longitudinal period (Parkes, Rashid, Chard, & Tofts, 2004). However, reliability of ASL measured between baseline and periods in excess of one year were not reliable (Parkes et al., 2004). This may be due to the changing morphology and age associated change in brain architecture of ageing adults. Taken together, the studies suggest that ASL is reliable over a period of one year and that only slight variations can be expected due to slice positioning. Thus, ASL acquired at six months is expected to be similar to baseline scans in the current study. With this in mind, healthy control baseline scans that were still intact were

combined with the remaining scans of participants from follow-ups at six months. Three participants' scans were excluded from this analysis; two participants' scans were excluded due to excessive motion which created distortion and made the image unusable. The third participant's scan was not used due to withdrawal from the scanner during the ASL sequence. The final analysis was conducted with 26 control participants (mean age 67.69 $\pm$ 6.90). Whole brain average perfusion with this sample was 48.61 $\pm$ 51.88 ml/100gtissue/min. Group mean ASL perfusion subtraction analysis images are shown in Figures 3.5 and 3.6.

The prostate cancer ADT sample consisted of 24 patients (mean age 67.21 $\pm$ 6.35). Six patient scans were excluded due to scanner sequence failures and at the time of submission of this thesis, the follow up ASL data in these patients were not available for analysis in the manner in which corrupted baseline control data were incorporated. Whole brain average perfusion was 66.14 $\pm$ 70.01 ml/100gtissue/min.

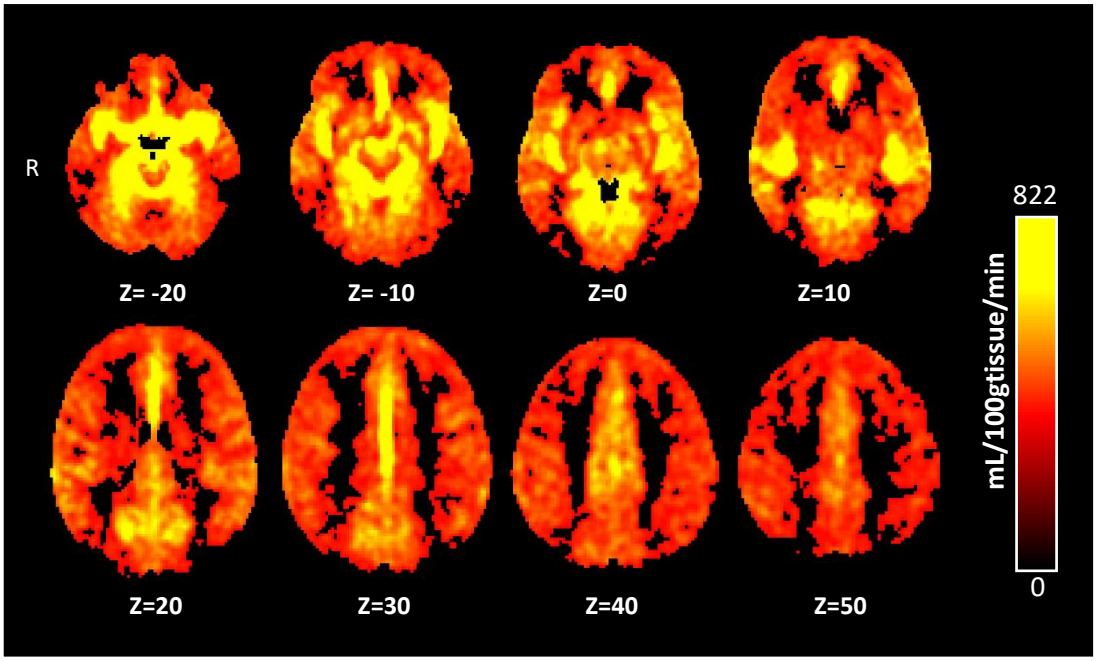


**Figure 3.5:** *Healthy Controls (n=26) ASL Group Analysis. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres.*

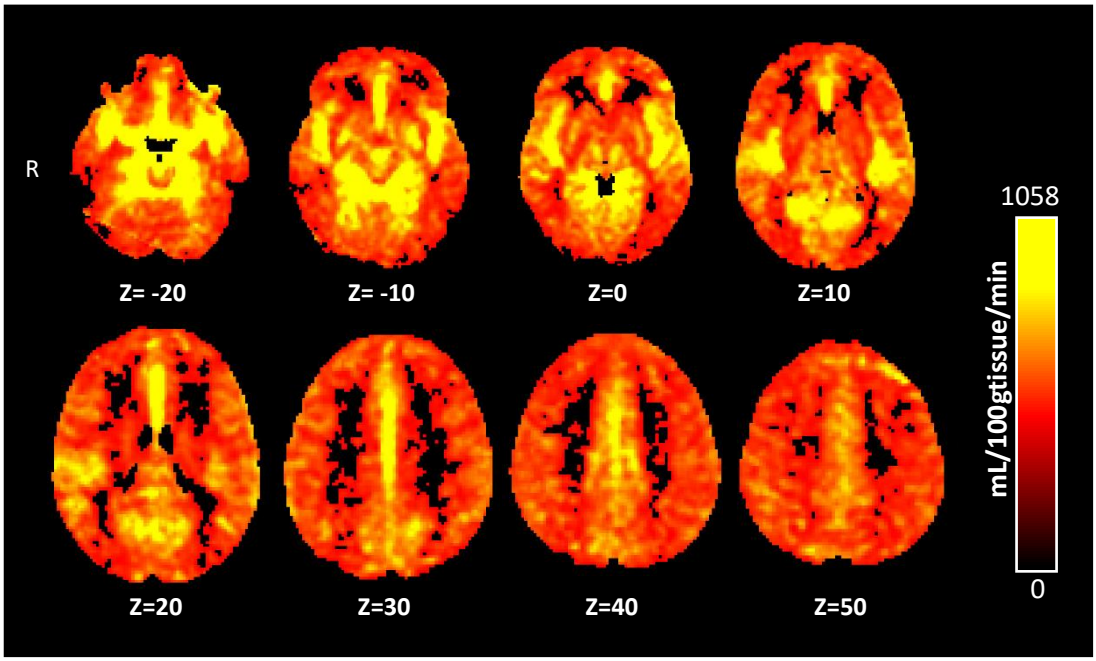


**Figure 3.6:** *PC patients ASL (n=24) Group Analysis. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres R, right.*

Analysis showed that whole brain perfusion was in the normal range (above 15-20 ml/100gtissue/min (Jann, Smith, Rios Piedra, Dapretto, & Wang, 2016; Pollock et al., 2009)). However, some areas did have hyper perfusion in both samples that was located in cerebrospinal fluid. This was normal and has been shown as being noise artefact (Duhamel, de Bazelaire, & Alsop, 2003). A one way independent Krushal Walis test was employed to compare group perfusion values. This test was used since it does not assume normality, is sensitive to outliers and can be used when samples are uneven. The analysis showed no significant whole brain CBF differences between the patient group (mean rank = 28) and controls (mean rank = 23.19) ($\chi^2(1) = 1.36$, $p > 0.05$). Thus, any differences between the group on functional MRI BOLD scans can be attributed to changes in neural function rather than perturbation of underlying perfusion.

## 3.7.3 Functional MRI analyses

### 3.7.3.1 Inhibitory Control (SST)

To determine whether brain function differed at baseline, task related activation was examined within each group (tables 3.3 and 3.4) and then a comparison between the groups was performed (Table 3.5). In healthy controls, activation during inhibitory control was apparent in putative inhibitory areas on the SST. These areas included the rIFG, rMFG and parietal regions. Left sided activation was also found in controls in the inferior frontal and middle frontal gyrus areas. The exploratory contrasts revealed activation in inhibitory areas on gin trials and go related areas on sin trials.

**Table 3.3**: SST group activation in healthy controls (n = 29)

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Contrast** | | | | | |
| **Go incorrect and stop correct trials minus stop incorrect and go correct trials** | | | | | |
| 2027 | 4.29 | -44 | 14 | 32 | L.Middle Frontal Gyrus |
| 1582 | 3.87 | 44 | -56 | 50 | R.Angular Gyrus |
| 1001 | 3.93 | -34 | -78 | 46 | L.Lateral Occipital Cortex |
| 724 | 3.36 | 44 | 26 | 16 | R.Inferior Frontal Gyrus |
| 705 | 4.49 | -44 | 52 | -8 | L.Frontal Pole |
| 210 | 3.5 | 54 | 30 | -2 | R.Inferior Frontal Gyrus |
| 191 | 3.17 | 28 | 6 | 48 | R.Middle Frontal Gyrus |
| **Exploratory Contrasts** | | | | | |
| **Stop incorrect and go correct trials minus go incorrect and stop correct trials** | | | | | |
| 268 | 3.32 | 8 | -68 | 2 | R.Lingual Gyrus |
| 111 | 3.31 | -8 | -46 | 62 | L.Postcentral Gyrus |
| **Go incorrect minus go correct trials** | | | | | |
| 375 | 3.48 | 62 | -48 | 28 | R.Angular Gyrus |
| **Go correct minus go incorrect trials** | | | | | |
| 701 | 4.09 | -42 | -16 | 16 | L.Postcentral Gyrus |
| **Stop incorrect minus stop correct trials** | | | | | |
| No Activation | | | | | |
| **Stop correct minus stop incorrect trials** | | | | | |
| 191 | 4.54 | 26 | 42 | 36 | R.Middle Frontal Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

Activation regions in patients were localised to the right occipital cortex only. Further exploratory group analysis carried out in the patient sample with a more lenient Z threshold of 2.0 did show activation of the rMFG (MNI: 24 32 42, Z = 3.33, cluster size = 721 voxels) and rIFG (MNI: 42 58 -2, Z = 3.31, cluster size = 809 voxels). Exploratory contrasts showed activation in go related trials on sin trials.

**Table 3.4:** SST Contrast Group Activations PC patients (n = 30)

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Contrast** | | | | | |
| **Go incorrect and stop correct trials minus stop incorrect and go correct trials** | | | | | |
| 633 | 3.94 | 48 | -70 | 24 | R.Lateral Occipital Cortex |
| **Stop incorrect and go correct trials minus go incorrect and stop correct trials** | | | | | |
| 1304 | 4.36 | -66 | -14 | 8 | L.Superior Temporal Gyrus |
| **Exploratory contrasts** | | | | | |
| **Go incorrect minus go correct trials** | | | | | |
| No Activation | | | | | |
| **Go correct minus go incorrect trials** | | | | | |
| No Activation | | | | | |
| **Stop incorrect minus stop correct trials** | | | | | |
| 450 | 3.82 | -64 | -18 | 38 | L.Postcentral Gyrus |
| **Stop correct minus stop incorrect trials** | | | | | |
| No Activation | | | | | |

Abbreviations: L, left; MNI, Montreal Neurological Institute; PC, prostate cancer; R, right; SST, stop signal task *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

A ROI analysis confirmed this ad hoc hypothesis (figure 3.7). A region of 210 voxels were masked in the contrast of go incorrect and stop correct minus stop incorrect and go correct where the rIFG (50 30 -2) was active in healthy controls. The mean BOLD signal was extracted from this region using featquery in controls and patients. An independent samples t test revealed no significant differences between the mean change in signal in the rIFG of healthy controls and patient BOLD levels ($t(57) = -1.11$, $p > 0.05$). This shows that there was BOLD signal change in patients at the group level in the rIFG, which is in line with healthy controls.

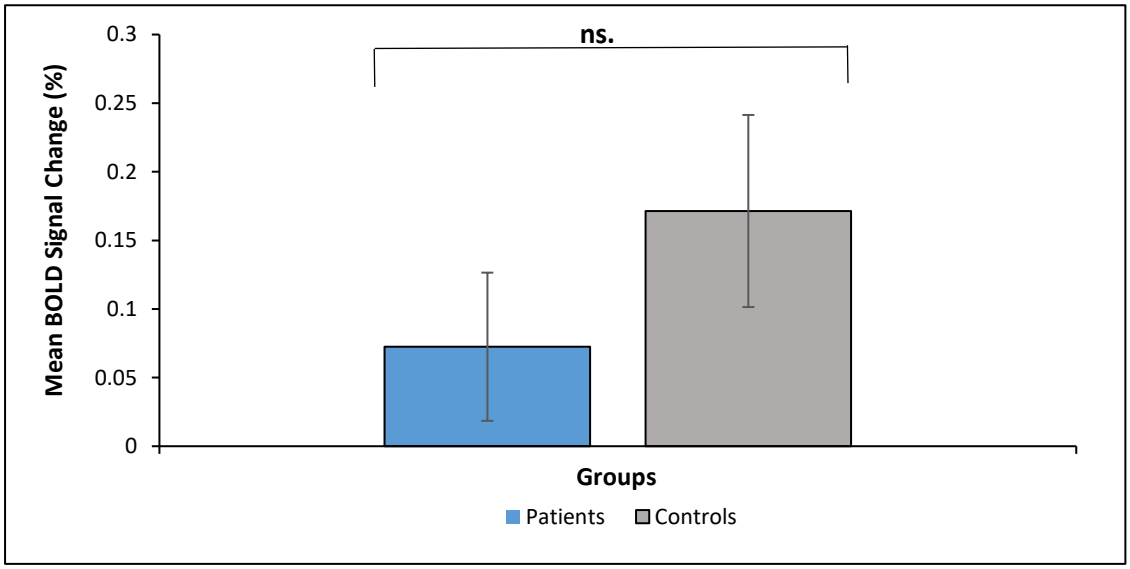


**Figure 3.7:** *Mean signal change on the SST in patients and controls in the rIFG. Abbreviations: ns, not significant.*

An independent samples t-test was used to assess whole brain differences between groups which did not show any significant activation differences between controls and patients suggesting that the rIFG was involved in both groups on the main contrast (Table 3.5). Furthermore, no significant activation differences were found between groups on the exploratory contrasts. All covariates were added to whole brain difference analysis to assess their impact on activation between patients and controls. Anxiety and depression covariates did not have any impact on activation between patients and controls in any contrasts. The pattern of activation shows differences in the limbic lobe and occipital lobe between controls and patients. However, activation maps showed that only the right amygdala was affected by testosterone in the go incorrect and stop correct minus stop incorrect and go correct contrast (Table 3.5). To further explore this finding, a one way ANCOVA was conducted with group (patients and controls) as the fixed factor, anxiety, depression and testosterone as covariates and mean signal change (%) of both groups in the amygdala as the dependent variable. Testosterone levels of patients and controls had a significant effect on signal change differences ($F(1,56) = 6.14$, $p = 0.02$) and accounted for 9% of variance in the model ($\eta^2 = 0.09$) in the amygdala. A significant difference was found in the amygdala while controlling for all covariates ($F(1,56) = 4.65$, $p = 0.04$, $\eta^2 = 0.07$). Estimated marginal means moreover showed BOLD signal increases in controls (mean = 0.12, standard error = 0.08) and decreases in patients (mean = -0.15, standard error = 0.08) when adjusting for testosterone. A linear regression was used to assess the relationship between testosterone and the amygdala signal change in patients. A significant model was found when testosterone was added ($F(1,25) = 4.59$, $p = 0.04$, $\beta=0.42$) where the dependent variable was mean signal change in the amygdala and independent variable was testosterone levels of patients. Mean signal was found to increase by 0.009% for every unit change in testosterone where the intercept was 0.08.

**Table 3.5:** SST Contrast Group Differences PC patients and Controls

| Contrast | Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|---|
| **Go incorrect and stop correct trials minus stop incorrect and go correct trials** | | | | | | |
| **Comparison** | | | | | | |
| **HC>PC** | 114 | 3.38 | 22 | -4 | -30 | R.Parahippocampal Gyrus |
| | 82 | 3.17 | 28 | -96 | 20 | R.Occipital Pole |
| **Comparison** | | | | | | |
| **PC>HC** | 83 | 3.04 | 20 | -80 | 0 | R.Occipital Fusiform Gyrus |
| **Covariate comparison** | | | | | | |
| **PC>HC while controlling for testosterone** | 89 | 3.15 | -30 | -6 | -20 | L.Amygdala |
| **Stop incorrect and go correct trials minus go incorrect and stop correct trials** | | | | | | |
| **Comparison** | | | | | | |
| **HC>PC** | 83 | 1.35 | 3.04 | 20 | -80 | R.Occipital Fusiform Gyrus |

Abbreviations: HC, healthy controls; L, left; MNI, Montreal Neurological Institute; PC, prostate cancer; R, right; SST, stop signal task *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

### 3.7.3.2 SST flicker activation

Typical areas were activated in controls and patients when stop signal trials were separated into components (Table 3.6 and 3.7). Long correct trials were the most difficult trials as evidenced from Table 3.1 and had the lowest performance and longest reaction times in both groups.

**Table 3.6:** SST Flicker Contrast Group Activations PC patients.

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Inhibition flicker contrasts** | | | | | |
| **Long correct trials minus go correct trials** | | | | | |
| 1693 | 4.43 | -34 | 20 | -4 | L.Insular Cortex |
| 272 | 3.79 | 44 | 22 | 30 | R.Middle Frontal Gyrus |
| **Medium correct trials minus go correct trials** | | | | | |
| 3241 | 5.07 | 58 | -46 | 16 | R.Angular Gyrus |
| 2550 | 4.37 | 44 | 18 | -10 | R.Frontal Orbital Cortex |
| 246 | 3.59 | -38 | -86 | 6 | L.Lateral Occipital Cortex |
| 226 | 4.03 | -42 | 32 | 24 | L.Middle Frontal Gyrus |
| 208 | 3.35 | 44 | -74 | -6 | R.Lateral Occipital Cortex |
| 204 | 3.29 | 48 | -30 | -14 | R.Inferior Temporal Gyrus |
| 133 | 3.63 | -2 | 48 | 32 | L.Superior Frontal Gyrus |
| **Short correct trials minus go correct trials** | | | | | |
| 7110 | 4.84 | 56 | -46 | 16 | R.Angular Gyrus |
| 2574 | 3.71 | 2 | -80 | 4 | R.Intracalcarine Cortex |
| 1227 | 3.84 | -58 | -58 | 28 | L.Angular Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; PC, prostate cancer; R, right; SST, stop signal task *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 3.7:** SST Flicker Contrast Group Activations Controls (n = 29)

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Inhibition flicker contrasts** | | | | | |
| **Long correct trials minus go correct trials** | | | | | |
| 8457 | 4.86 | 34 | 26 | -8 | R.Frontal Orbital Cortex |
| 8243 | 5.01 | 62 | -46 | 30 | R.Angular Gyrus |
| 3144 | 4.75 | -66 | -42 | 22 | L.Supramarginal Gyrus |
| 367 | 3.84 | -34 | 16 | -4 | L.Insular Cortex |
| 350 | 3.66 | -44 | 32 | 20 | L.Middle Frontal Gyrus |
| 314 | 3.67 | -44 | 54 | -6 | L.Frontal Pole |
| 226 | 3.42 | -58 | -4 | 26 | L.Precentral Gyrus |
| 108 | 3.91 | -24 | 16 | 50 | L.Superior Frontal Gyrus |
| **Medium correct trials minus go correct trials** | | | | | |
| 3539 | 4.33 | 32 | -60 | -16 | R.Temporal Occipital Fusiform Cortex |
| 1558 | 4 | 32 | 26 | -10 | R.Frontal Orbital Cortex |
| 1373 | 3.62 | 32 | 44 | 14 | R.Frontal Pole |
| 960 | 4.32 | -4 | 32 | 28 | L.Paracingulate Gyrus |
| 753 | 4.2 | -52 | -72 | -2 | L.Lateral Occipital Cortex |
| **Short correct trials minus go correct trials** | | | | | |
| 1899 | 4.11 | -18 | -80 | -12 | L.Occipital Fusiform Gyrus |
| 1838 | 4.57 | 54 | -36 | 32 | R.Supramarginal Gyrus |
| 1478 | 4.3 | 58 | 12 | 8 | R.Inferior Frontal Gyrus |
| 800 | 4.33 | -48 | -82 | 2 | L.Lateral Occipital Cortex |
| 750 | 4.07 | 48 | -62 | -10 | R.Lateral Occipital Cortex |
| 709 | 4.37 | -58 | -54 | 36 | L.Angular Gyrus |
| 463 | 4.18 | -4 | 32 | 28 | L.Paracingulate Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, $p < 0.05$)

Differences were found when independent samples t-tests were conducted (Table 3.8). The right supramarginal gyrus was more active in controls compared to patients on long correct trials. In contrast, the left inferior frontal gyrus showed greater activity in patients compared to controls.

**Table 3.8:** SST Flicker Contrast Group differences PC patients and Controls

| | | | MNI Coordinates | | | |
|---|---|---|---|---|---|---|
| | **Cluster (Voxels)** | **Z-MAX** | **X** | **Y** | **Z** | **Region** |
| **Inhibition Flicker contrast** | | | | | | |
| **Long correct trials minus go correct trials** | | | | | | |
| **Comparison HC>PC** | 204 | 2.95 | 54 | -32 | 30 | R.Supramarginal Gyrus |
| **Comparison PC>HC** | 153 | 3.45 | -56 | 20 | -6 | L.Inferior Frontal Gyrus |
| **Medium correct trials minus go correct trials** | | | | | | |
| **Comparison HC>PC** | | ns. | | | | |
| **Comparison PC>HC** | | ns. | | | | |
| **Short correct trials minus go correct trials** | | | | | | |
| **Comparison HC>PC** | | ns. | | | | |
| **Comparison PC>HC** | | ns. | | | | |

Abbreviations: HC, healthy controls; L, left; MNI, Montreal Neurological Institute; ns, non-significant; PC, prostate cancer; R, right; SST, stop signal task (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, $p < 0.05$)

Overlap images help to show differences in activation of patients and controls on the main contrast and activation areas on distinct flicker trials involved in inhibition. Differences in the left inferior frontal gyrus were found on the long correct minus go correct contrast (Table 3.8).

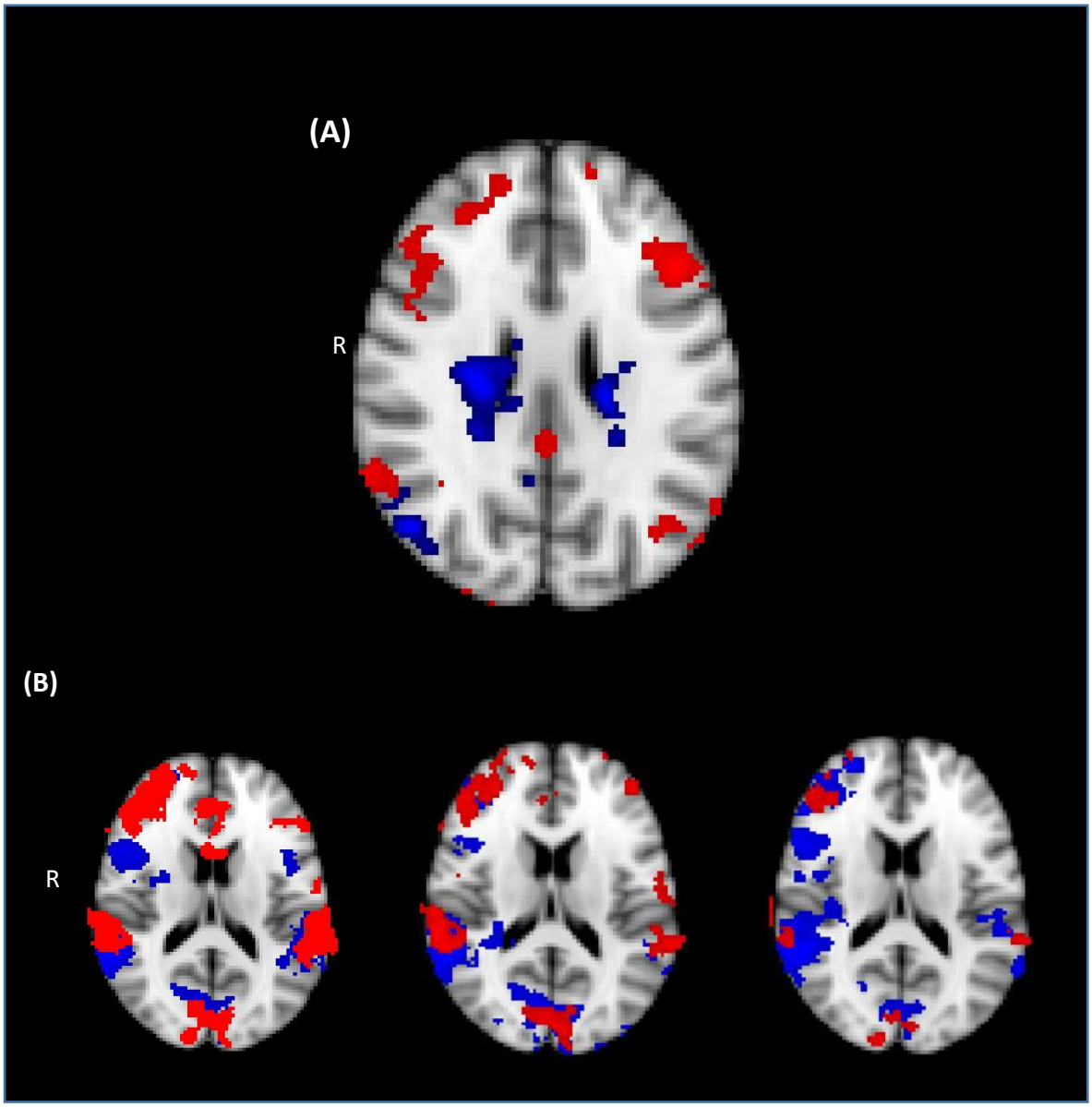


**Figure 3.8:** *SST overlap images of patients and controls where red activation colour maps indicate activation of controls and purple shows activation maps of patients. (A) Main contrast (go incorrect and stop correct trials minus stop incorrect and go correct trials) overlap activation maps for controls and patients. (B) lc-gc contrast. (C) mc-gc contrast. (D) sc-gc contrast. (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations: gc, go correct; lc, long correct; mc, medium correct; R, right; sc, short correct; SST, stop signal task.*

### 3.7.3.3 Spatial Reasoning (MRot task)

Patients and controls displayed neural activation in putative MRot task regions located in the parietal and occipital lobes (Table 3.9). However, patients also displayed activation in bilateral prefrontal middle frontal gyrus regions on the exp-cont contrast. Despite these outcomes suggesting that there may be some difference between groups, when a whole brain independent samples t-test was conducted, no significant differences were found on the main contrast of exp-cont.

**Table 3.9:** MRot tasks Contrast Group Activations Patients and Controls

**Group Level Patients**

| Contrast | Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|---|
| **exp-control** | 18160 | 5.22 | 20 | -72 | 50 | R.Lateral Occipital Cortex |
| | 529 | 5.32 | -26 | -8 | 44 | L.Precentral Gyrus |
| | 521 | 4.12 | -40 | 34 | 30 | L.Middle Frontal Gyrus |
| | 467 | 3.71 | 30 | 2 | 56 | R.Middle Frontal Gyrus |
| | 457 | 3.53 | 0 | 10 | 42 | Cingulate Gyrus |
| **cont-exp** | 467 | 3.64 | 12 | 42 | -6 | R.Paracingulate Gyrus |
| | 414 | 4.24 | -42 | 54 | -8 | L.Frontal Pole |

**Group Level Healthy Controls**

| Contrast | Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|---|
| **exp-control** | 30900 | 5.12 | -14 | -98 | 4 | L.Occipital Pole |
| | 849 | 3.67 | -34 | -12 | 50 | L.Precentral Gyrus |
| | 800 | 3.86 | 26 | 4 | 66 | R.Superior Frontal Gyrus |
| | 626 | 3.73 | 12 | 18 | 42 | R.Paracingulate Gyrus |
| | 564 | 3.57 | 32 | 24 | 6 | R.Insular Cortex |

Abbreviations: L, left; MNI, Montreal Neurological Institute; MRot, mental rotation task; R, right (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, $p < 0.05$)

#### 3.7.3.4 MRot task distinct rotation activation

Patients exhibited activation in bilateral occipital, precentral regions and left prefrontal regions when MRot task activations were split into distinct rotation components. Healthy controls similarly elicited neural activations in bilateral occipital, bilateral precentral, left prefrontal regions and slightly more right sided frontal sided regions (Tables 3.10 and 3.11). No significant differences emerged between patients and controls in an independent samples t-test.

**Table 3.10:** MRot task distinct rotations Contrast Group Activations PC patients (n = 30)

| Group Level PC patients | | | | | | |
|---|---|---|---|---|---|---|
| | | | MNI Coordinates | | | |
| **Contrast** | **Cluster (Voxels)** | **Z-MAX** | **X** | **Y** | **Z** | **Region** |
| **60 degree-control** | 7208 | 4.05 | 2 | -72 | 56 | R.Precuneous Cortex |
| | 2224 | 3.75 | -28 | -12 | 48 | L.Precentral Gyrus |
| | 402 | 3.48 | 0 | 8 | 46 | Paracingulate Gyrus |
| **120 degree-control** | 4551 | 4.62 | -24 | -64 | 52 | L.Lateral Occipital Cortex |
| | 1412 | 3.77 | 36 | -78 | 12 | R.Lateral Occipital Cortex |
| | 1124 | 4.06 | -34 | -84 | 0 | L.Lateral Occipital Cortex |
| | 1016 | 4.22 | 28 | -10 | 46 | R.Precentral Gyrus |
| | 787 | 3.92 | -26 | -2 | 42 | L.Middle Frontal Gyrus |
| **180 degree-control** | 11148 | 4.69 | -22 | -62 | 50 | L.Lateral Occipital Cortex |
| | 550 | 3.6 | -46 | -4 | 32 | L.Precentral Gyrus |
| | 438 | 4.07 | -26 | -8 | 48 | L.Precentral Gyrus |
| | 436 | 3.58 | 4 | 28 | 28 | R.Cingulate Gyrus |
| **300 degree-control** | 2113 | 4.5 | -16 | -70 | 56 | L.Lateral Occipital Cortex |
| | 749 | 4.15 | 50 | -32 | 36 | R.Supramarginal Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; MRot, mental rotation task; PC, prostate cancer; R, right *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, $p < 0.05$).*

**Table 3.11:** MRot task distinct rotations Contrast Group Activations
Healthy Controls (n = 29)

| Contrast | Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|---|
| **60 degree-control** | 3016 | 4.43 | -44 | -90 | -6 | L.Lateral Occipital Cortex |
| | 947 | 3.79 | 54 | -76 | -16 | R.Lateral Occipital Cortex |
| | 672 | 3.5 | 4 | -56 | 52 | R.Precuneous Cortex |
| **120 degree-control** | 3583 | 4.1 | -22 | -70 | 40 | L.Lateral Occipital Cortex |
| | 1051 | 3.44 | 20 | -78 | 24 | R.Cuneal Cortex |
| | 896 | 3.79 | -8 | -106 | -2 | L.Occipital Pole |
| **180 degree-control** | 7843 | 4.72 | -36 | -44 | 40 | L.Superior Parietal Lobule |
| | 3771 | 4.29 | 36 | 24 | -6 | R.Frontal Orbital Cortex |
| | 1099 | 3.75 | 44 | -82 | 18 | R.Lateral Occipital Cortex |
| | 685 | 4.3 | -46 | 8 | 20 | L.Inferior Frontal Gyrus |
| | 653 | 3.88 | -30 | -58 | -12 | L.Temporal Occipital Fusiform |
| | 454 | 3.53 | -32 | -4 | 50 | L.Precentral Gyrus |
| **300 degrees-control** | 4738 | 4.43 | 24 | -64 | 60 | R.Lateral Occipital Cortex |
| | 4176 | 4.42 | -46 | -80 | -4 | L.Lateral Occipital Cortex |

Abbreviations: L, left; MNI, Montreal Neurological Institute; MRot, mental rotation task; R, right *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

Activation in controls and patients on the main trial and on the various degree shape rotations from zero was overlaid to visually inspect group concordance. Figure 3.9 shows that there are regions in both groups that do not overlap. For example, controls had more widespread left supramarginal gyrus activation in the main contrast (Figure 3.9A) and 180 degree-control overlap group activation map showed more bilateral anterior and cingulate activation (Figure 3.8D). However, when examined using an independent samples t-test there were no statistically significant differences detected.

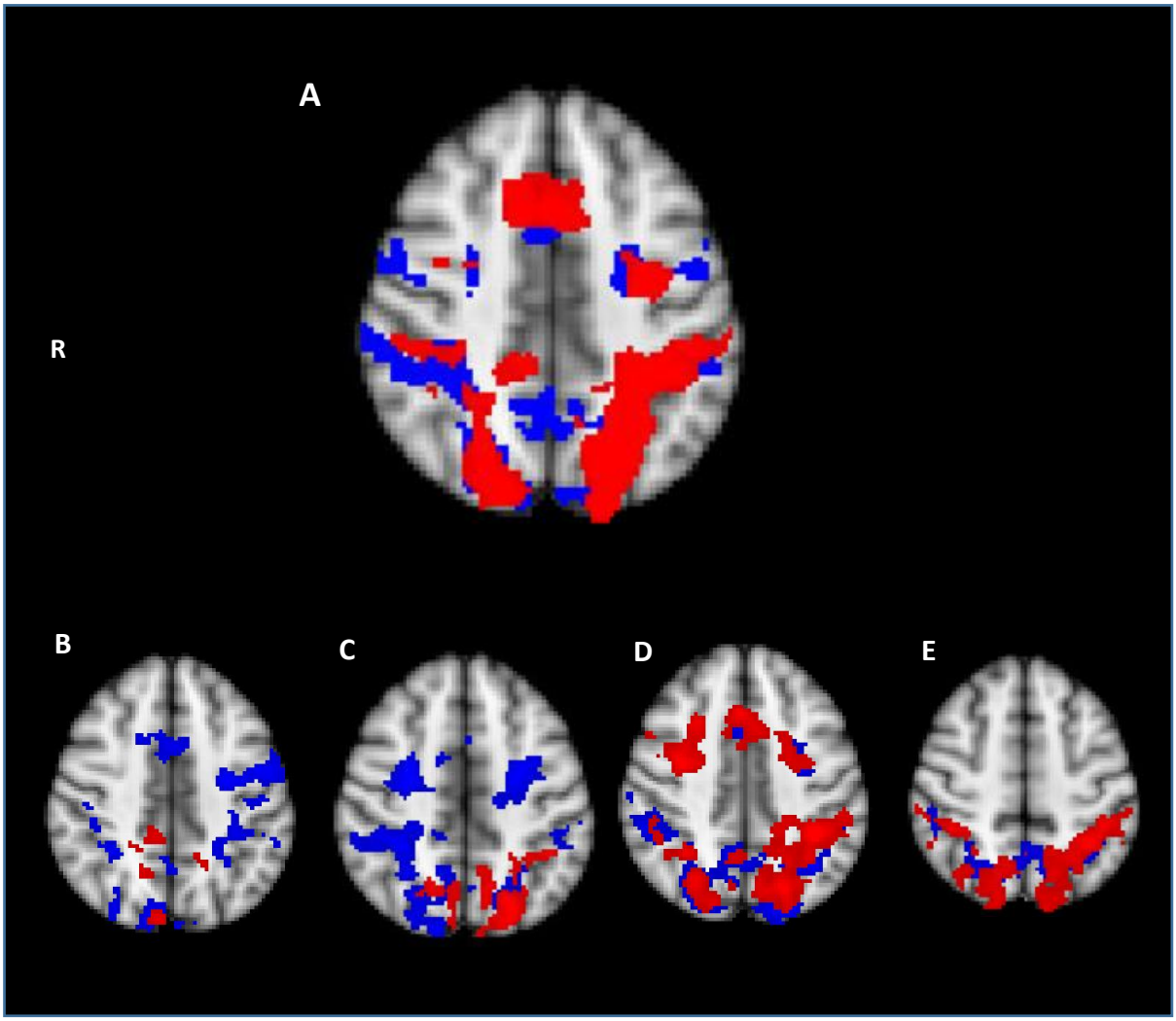


**Figure 3.9:** *MRot task overlap images of patients and controls where red activation maps show group activation in healthy controls and purple activation indicate group activation of patients. (A) Main group contrast activation maps for patients and controls (B) 60 degree contrast. (C) 120 degree condition. (D) 180 degree condition. (E) 300 degree condition (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations: MRot, mental rotation task; R, right.*

The analyses presented above suggests that there were generally no significant behavioural or neural differences between patients and controls on tasks of inhibition and spatial reasoning. However, underlying deficits in cognition may be more subtle and only detectable through advanced imaging methods such as resting state and regional arterial spin labelling analysis. This was explored subsequently to exclude the presence of any cerebral abnormalities in patients compared to controls at baseline. Any abnormalities could lead to group average BOLD signal drop offs which affect the interpretation of group differences analysis through bias. Moreover, any abnormalities found in patients could be exacerbated during further therapy from a clinical point of view.

## 3.8 Resting State Analysis

Resting state analysis was conducted by first acquiring the default mode network seeded from the medial prefrontal cortex (mPFC) mask. The network consisted of the posterior cingulate cortex (PCC) medial prefrontal cortex and bilateral inferior parietal lobes. A mask generated of the right dorsolateral prefrontal cortex (rDLPFC) was used to measure connectivity by seeding it to the DMN mPFC regions. Both these regions and networks are known to be imperative to fronto-parietal executive functions (Niendam et al., 2012; Spreng, 2012). Analysis of baseline healthy control scans showed positive correlations between the right frontal pole, right angular gyrus, left middle frontal gyrus (Z = 3.74 to 7.92) and the DMN mPFC. Negative connectivity with the DMN mPFC was found in the left precentral gyrus and left fronto-orbital cortex (Z = -4.14 to -5.44) indicated by negative BOLD (figure 3.11A). At baseline PC patients exhibited activation in the right frontal pole, right supramarginal gyrus and left frontal pole (Z = 4.71 to 7.53) with the DMN. Negative BOLD was found in the left lingual gyrus (Z = -4.8) (figure 3.10A).

Differences between groups were found in the right lingual gyrus where healthy controls had greater activation compared to patients (MNI: 6 -72 -12, Z = 4.18) with cluster size of 485 voxels. An ANCOVA was used to assess the magnitude of difference in the right lingual gyrus and whether testosterone modulated this difference (MNI: 54 -58 24, 747 voxels, Z max=4.71). The analysis revealed a BOLD signal change difference in the lingual gyrus between controls (0.025 $\pm$ 0.15) and patients (0.02 $\pm$ 0.19) ($F(1,56) = 4.01$, $p = 0.05$, $\eta^2 = 0.07$) that significantly co-varied with testosterone ($F(1,56) = 13.74$, $p < 0.01$, $\eta^2 = 0.20$). Therefore, testosterone differences alone facilitated 20% of variance in positive activation found. Estimated means confirmed this as signal change was higher in controls (mean=0.07, standard error = 0.03) and lower in patients (mean=-0.04, standard error = 0.03).

A small activation area in the rIFG was masked with the aim to investigate IFG connectivity with the DMN mPFC. The mask was selected and made from the activation acquired on the SST on the go incorrect and stop correct minus stop incorrect and go correct group activation contrast (MNI: 50 30 -2). A 5mm sphere was made around this co-ordinate and prepared for functional connectivity analysis. Healthy controls showed strong connectivity between the DMN and rIFG, right frontal orbital cortex and right paracingulate gyrus (Z = 4.31 to 6.1). Negative activation in healthy controls was found in the left precuneous cortex, left lateral occipital cortex, left superior frontal gyrus and left frontal pole (Z = -4.29 to -4.2) (figure 3.11B). PC patients at baseline showed strong connectivity in the right frontal pole and right temporal pole (Z = 5.94 to 7.74). Negative BOLD was found in the left superior occipital fusiform cortex and right parahippocampal gyrus (Z = -3.63 to 4.98) of PC patients (figure 3.10B).

Differences between groups were found where healthy controls had significantly greater activation in the left middle temporal gyrus (MNI: -70 -52 10, Z = 4.09, cluster size = 5811 voxels), left superior parietal lobule (MNI: -36 -58 66, Z = 3.8, cluster size = 730 voxels) and right insular cortex (MNI: 34 8 -4, Z = 3.74, cluster size = 660 voxels).

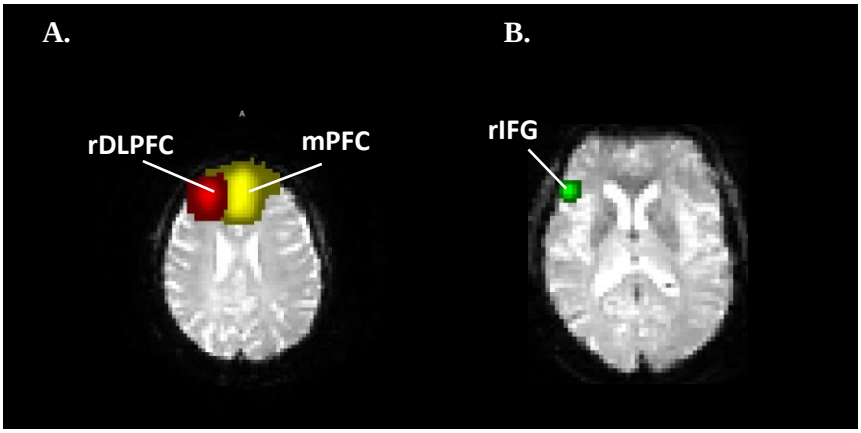


**Figure 3.10:** *Resting state analysis masks of seed regions and regions of interest. (A) Masks showing the mPFC which was seeded to create a functional connectivity map of the default mode network. The strength of connectivity from the DLPFC was measured to the DMN. (B) The strength of connectivity was similarly measured from the rIFG to the DMN. Abbreviations: A, anterior; DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; mPFC, medial prefrontal cortex; r, right.*

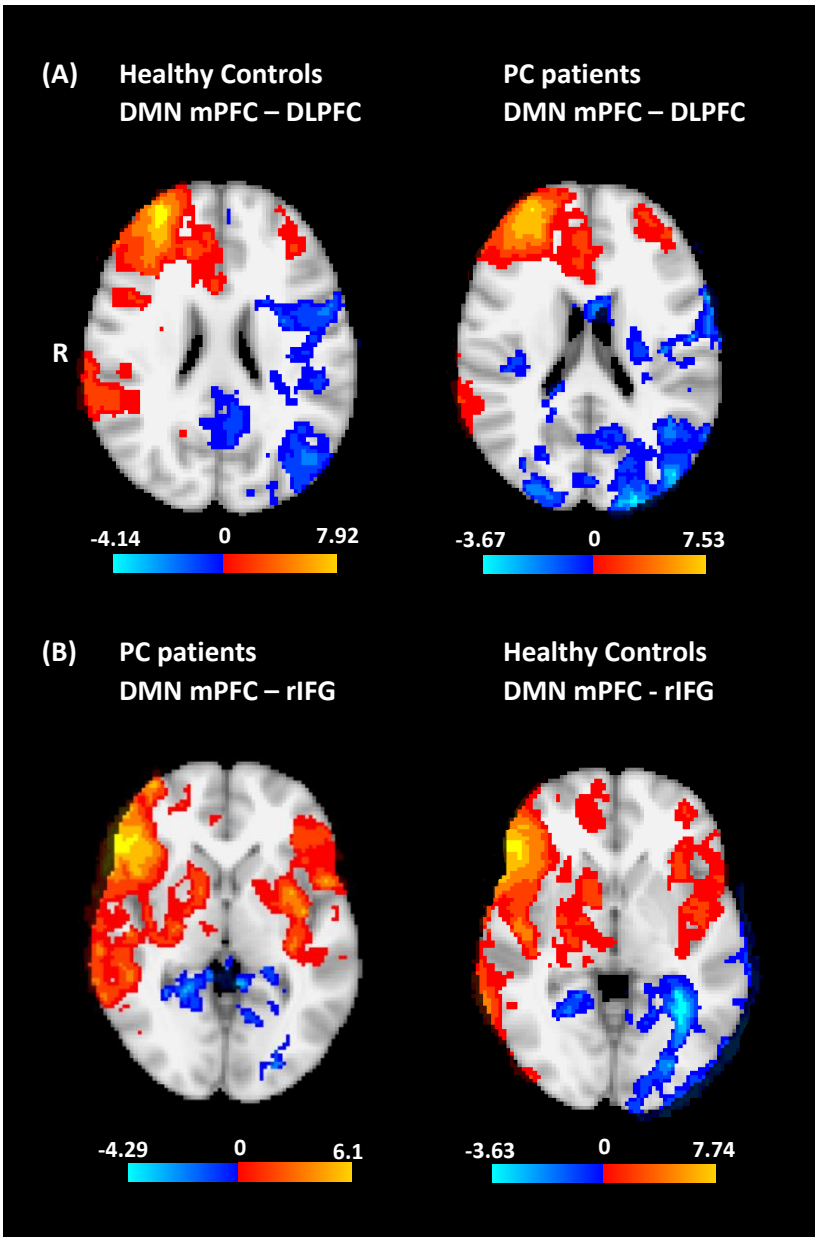


**Figure 3.11:** *Resting state analysis of patients and controls. (A) Patient and control resting state connectivity map with rDLPFC as region of interest to DMN. (B) Patient and control resting state connectivity map with rIFG as region of interest to DMN (Corrected for multiple comparisons, Z > +/-2.3; Cluster-wise threshold, p < 0.05). The colour bar represents negative BOLD in light blue to dark blue areas of brain maps and greater BOLD activation in red to yellow areas of brain maps. Abbreviations: DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; mPFC, medial prefrontal cortex; r, right.*

A post hoc analysis was conducted to explore if stop signal reaction times (SSRTs) correlated with resting state activity in the rIFG pars triangularis since this was where significant task-based activation was found. Previous research has shown that resting state BOLD signal changes negatively correlate with SSRT (Lee & Hsieh, 2017). The rIFG was selected from the FSL atlas which was masked and binarised. The dependent variable was mean signal change in the rIFG pars triangularis with predictor as SSRT. There was a linear trend of increasing SSRT with increasing BOLD signal change (figure 3.12). The mean signal increased by less than 0.001% for every millisecond increase in SSRT (where the intercept was 0.05). Nevertheless, stop signal reaction time during the SST did not significantly predict BOLD signal change at rest in the rIFG in healthy controls ($F(1,27) = 3.57$, $p > 0.05$) ($\beta$=0.34).

Stop signal reaction time likewise did not predict BOLD signal change in the rIFG of patients ($F(1,28) = 0.09$, $p > 0.05$) ($\beta = -0.06$). Mean signal decreased by less than 0.001% for every millisecond increase in SSRT (where the intercept was 0.15).

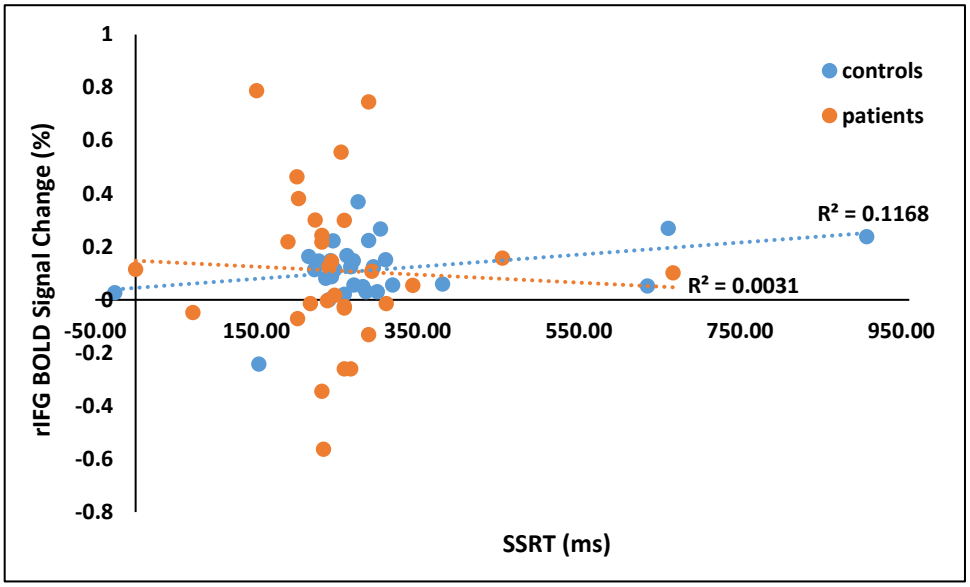


**Figure 3.12:** *Regression of mean BOLD signal change in the rIFG correlated with stop signal reaction time (SSRT) from the SST. Abbreviations: IFG, inferior frontal gyrus; ms, milliseconds; r, right; SST, stop signal task.*

## 3.9 Regional ASL perfusion

Figure 3.13 shows CBF values in regions of interest of controls and patients. Regions were selected based on activation areas of the stop signal and MRot task. Regional ASL analysis was conducted subsequently to ensure that putative task regions were first active before conducting the analysis. Perfusion assessments were employed in these regions to assess the integrity of BOLD signal activation found in patients and controls. Hypoperfusion has been known to lead to reductions in the BOLD signal which may lead to reduction in SST activation. The rIFG pars triangularis and pars opercularis were both masked and combined so that a regional estimate of the rIFG could be gauged. Bilateral supramarginal gyri were also masked to measure CBF in these regions that were related to the MRot task.

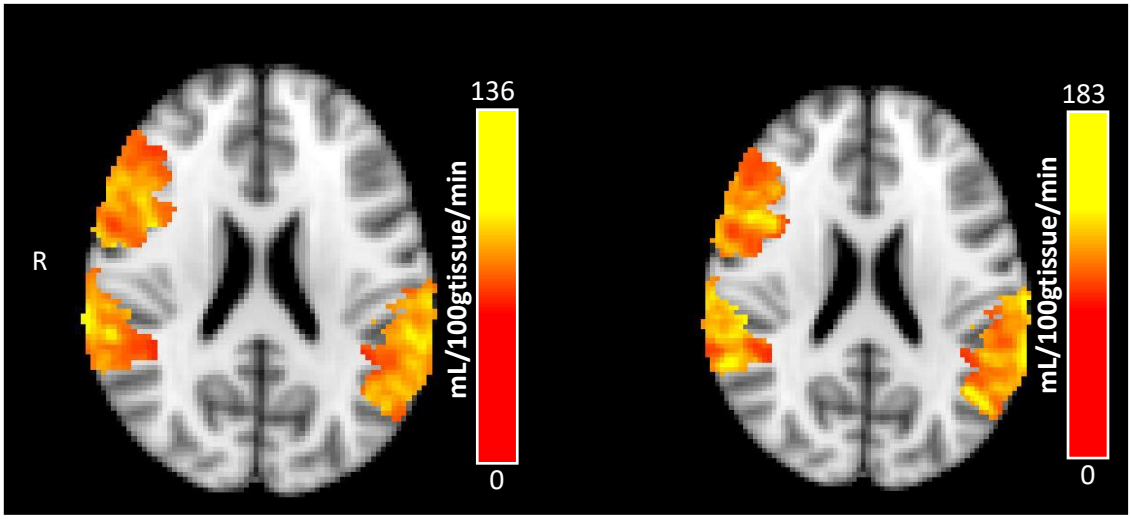


**Figure 3.13: (A)** *Healthy controls rIFG, right supramarginal and left supramarginal gyrus ASL group level perfusion maps.* **(B)** *PC patients Controls rIFG, right Supramarginal and left Supramarginal gyrus ASL Group Level perfusion maps. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the CBF map. Abbreviations: g, grams; min, minute; mL, millilitres.*

When the rIFG was isolated in healthy controls, average perfusion was 40.66 ± 13.45 ml/100gtissue/min (range 0-108.07 ml/100gtissue/min). The right and left supramarginal gyri were masked and superimposed on the whole brain perfusion map. The left supramarginal gyrus was estimated as having average perfusion of 39.45 ± 16.24 ml/100gtissue/min (range 0-137 ml/100gtissue/min). The right supramarginal gyrus was estimated as having average CBF of 45.97 ± 17.41 ml/100gtissue/min (range 0-119.57 ml/100gtissue/min).

The rIFG was similarly masked in patients and average CBF was calculated as 53.14 ± 70.01 ml/100gtissue/min (Range = 0 – 1058.96 ml/100gtissue/min). The right and left supramarginal gyri were masked with left supramarginal gyrus estimated as having average perfusion of 57.41 ± 21.05 ml/100gtissue/min (range 0-139.09 ml/100gtissue/min). The right supramarginal gyrus was estimated as have average CBF of 63.83 ± 23.39 ml/100gtissue/min (range 0-182.77 ml/100gtissue/min).

Past studies have shown increased cerebral perfusion in subjects with high endogenous testosterone levels (Ghisleni et al., 2015). Exploratory ad hoc analyses were conducted to assess if associations occurred between testosterone levels and whole brain or regional CBF. This warrants investigation since patients had significantly higher testosterone levels compared to controls and testosterone appears to covary with amygdala activation at baseline. This could have led to hyperperfusion that was modulated by testosterone, which could in turn mask patient impairment by effectively compensating for any functional deficits. Individual groups were compared first using Pearson's correlations. No significant correlations were found in controls between testosterone and average perfusion in the whole brain ($r = 0.04$, $p > 0.05$), rIFG ($r = -0.10$, $p > 0.05$), right supramarginal gyrus ($r = 0.01$, $p > 0.05$) and left supramarginal gyrus ($r = 0.009$, $p > 0.05$). Patients also showed no significant correlation between testosterone and average perfusion in the whole brain ($r = 0.05$, $p >$

0.05), rIFG ($r = 0.10$, $p > 0.05$), right supramarginal gyrus ($r = 0.13$, $p > 0.05$) and left supramarginal gyrus ($r = 0.05$, $p > 0.05$).

Both groups' combined perfusion estimates were analysed across a spread of values to assess if testosterone correlated with any regional perfusion estimates. No significant Pearson's level correlations were found between testosterone and average perfusion in the whole brain ($r = 0.18$, $p > 0.05$), rIFG ($r = 0.19$, $p > 0.05$), right supramarginal gyrus ($r = 0.24$, $p > 0.05$) and left supramarginal gyrus ($r = 0.20$, $p > 0.05$) across both groups. This indicates that no correlations were found between testosterone and whole brain or regional perfusion estimated averages in individual groups or across groups.

## 3.10 Discussion

The current experiment sought to examine performance and activation of relevant functional imaging measures in men prior to the onset of treatment for prostate cancer relative to healthy control participants. The purpose of the study was to establish a framework for subsequent longitudinal imaging data in both groups, taking into account the possibility that men who had recently received a diagnosis of cancer may show different patterns of activation because of the potential impact of mood disturbance on neural function. A number of important findings are reported. These a summarised first and then discussed in light of some unexpected results.

In line with expectations, overall performance of patients and controls on the tasks used inside the scanner did not differ. Reaction times on the SST and overall performance or reaction time on the MRot task were similar for patients and controls on the MRot task, as was expected. However, when mental rotation distinct rotations were divided, a difference was found on the reaction times between patients and controls in the 300 degree condition in which controls reacted quicker on the task. Nevertheless, patients had significantly better accuracy in judging mirror shapes compared to controls in the 300 degree condition. This was not in line with what was hypothesised but is consistent with differential engagement of a speed-accuracy trade off strategy. Reaction time and performance also increased as a function of shape orientation from zero showing that participants were attempting to mentally rotate shapes without assuming shape orientations. This was expected and in agreement with previous studies that have shown similar findings (Hertzog, Vernon, & Rypma, 1993; Lineweaver, Salmon, Bondi, & Corey-Bloom, 2005; Shepard & Metzler, 1971).

The neuroimaging data also supported the hypothesis that groups would not differ at baseline. This was evident in the majority of regions with some exceptions showing dissimilarities as a

function of testosterone and without testosterone. Analysis of specific contrasts showed that activation on go incorrect trials was distinct compared to go correct trials. This suggests that while the underlying mechanisms of go incorrect and stop trials differed, their underlying activation was in line with inhibitory function providing support for the combination of go incorrect and stop correct trials in the main contrast. Moreover, stop incorrect trials elicited activation in go related areas supporting the combination of stop incorrect and go correct trials. However, activated voxel clusters were not apparent in both cohorts at the group level. This may have been due to the threshold set for multiple comparisons to avoid false positives and therefore some clusters were not apparent at this level. Nevertheless, further analyses showed no significant differences on the exploratory contrasts between groups which indicated that similar areas were active in both groups. Therefore, the combination of trials was warranted and benefitted the main contrast to increase the power of trials. The main contrast of the SST showed activation in regions in line with past research in healthy controls on the SST these being the rIFG, rMFG and inferior parietal regions (Aron, 2007; Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Li et al., 2006). Although activation was not apparent in putative SST regions of PC patients in a group first level analysis, subsequent signal change analysis confirmed that there was activity in task-associated regions. Further support was provided by the lack of significant group differences between patients and controls when their activation was compared directly in task associated regions on the SST. Of note, testosterone had an effect on the right amygdala which was a unique finding from the study on the SST main contrast of go incorrect and stop correct trials minus stop incorrect and go correct trials. Moreover, the left amygdala was active in the contrast when controlling for testosterone which was unexpected and will be discussed in more detail below.

Differences on the SST were found in the left inferior frontal gyrus where patients had greater activation compared to controls on long correct trials only. This area may have been recruited

in patients on more difficult trials to compensate for executive function deficits. The left inferior frontal gyrus is a known mirror region area which can be recruited to facilitate executive function deficit (Kilner, Neal, Weiskopf, Friston, & Frith, 2009).

Differences were also apparent in the right amygdala on the main contrast where testosterone covaried with some differences. Post hoc, exploratory analysis of mean BOLD signal difference whilst covarying testosterone showed that testosterone did covary with activation in patients in the amygdala and may have aided to offset inhibition deficits in patients. Although the amygdala is not critical to inhibitory control, evidence from previous research involving the amygdala raises the possibility that alterations of activity in this area may serve to assist patients that had inhibition deficits. This could explain the lack of performance impairment found in patients whilst having atypical brain activation (Dillon & Pizzagalli, 2007). The amygdala is involved in fear conditioning and increased activation to stress responses. Researchers have found that the amygdala can modulate its responses to incorporate inhibition by briefly changing brain circuits involved in motor control (Sagaspe, Schwartz, & Vuilleumier, 2011; Stanton, Wirth, Waugh, & Schultheiss, 2009). The mechanism behind this change may be as a function of risk taking behaviour. One study implemented a typical SST, however, the risk taking component was hypothesised to be in the period where participants responded to go stimulus (Li, Chao, & Lee, 2009). It was supposed that long go trial responses reduced the risk of an incorrect inhibition since prolonged go latency allows better judgement on whether the stimulus presented is a go or stop trial. Therefore, shorter go trial reaction times were suspected to be associated with greater risk compared to longer reaction times. This was explored by separating contrasts of short go trial reaction times to long reaction times. Outcomes showed activation of the left inferior parietal lobes, posterior cingulate cortex, amygdala and prefrontal cortex during short

reaction time indicating greater risk taking behaviour. Amygdala activation was suspected to be a salient feature of risk taking and has been found in many previous studies (Nagai, Critchley, Featherstone, Trimble, & Dolan, 2004; Ohira et al., 2006). Risky decision making behaviour has also been correlated with testosterone and amygdala activation in animal studies of motor impulsivity and may explain the possible link between risk taking and amygdala activation (Batrinos, 2012; Cooper, Goings, Kim, & Wood, 2014; Goetz et al., 2014).

The amygdala has been known to have activation increases in response to high testosterone levels which in turn can increase emotional queues such as aggression, frustration etc. in males. One speculation may be that the nature of the SST increased these cues due to past failed inhibitions which allowed post-error corrections on subsequent trials. However, the exact level of testosterone required to elicit such emotional coupled psychological responses are not known which is a limit of this interpretation. Moreover, individual differences make it difficult to understand the interaction. This suggests that there could be some executive function component impairment in PC patients before treatment begins that was compensated for by higher testosterone levels. One possible neural mechanism may be better understood by a connective pathway between the amygdala and frontal connective network. A study assessing this connection measured resting state connectivity between healthy young and healthy older ageing adults (Cao et al., 2014). Older adults had a greater degree of connection between the right anterior cingulate cortex to the amygdala and superior temporal gyrus and IFG (Cao et al., 2014). This suggests that there may be a strong connective network between the amygdala and IFG that could be activated in response to inhibition activity and activation of the IFG. Moreover, due to a link between testosterone levels and risk taking behaviour, the network between the amygdala and IFG may be strengthened and activated to a greater

degree in the PC patients of the current study. Therefore, this connection between the amygdala in response to testosterone and IFG may be plausible during the SST given research showing connectivity between these networks at rest.

Amygdala activation was moreover found in patients when controlling for testosterone during inhibition on the SST which was an unexpected finding. This suggests that other disease specific factors may have elicited this activation. One possible explanation may be through the APoE4 allele that is known to be more prevalent in PC patients (Ifere et al., 2013). Animal model research has shown hyper neuronal synaptic activity in the amygdala of aged mice that have expressions of the APoE4 gene (Klein, Acheson, Mace, Sullivan, & Moore, 2014). Researchers speculate that the hyperactivity is likely due to reductions in the integrity of pyramidal cell neurons due to tau and beta amyloid formations. This makes neurons less likely to respond to gamma-aminobutyric acid (GABA) inhibitory signals resulting in over excitation (Nuriel et al., 2017). Thus, the above suggests that the APoE4 genotype has differential effects on parts of the brain where some areas display lower activity and others present with upregulated activity. This research warrants further investigation through single cell studies and human trials in relation to APoE4 (Hillman, 2014; Huneau, Benali, & Chabriat, 2015).

Further differences were also found in patients compared to controls in the supramarginal gyrus. This area is well known to be involved on the SST due to its engagement of fronto-parietal networks (Sharp et al., 2010).

Activation during mental rotation in patients and controls occurred in regions including bilateral parietal lobes and occipital regions, and this is in line with past research (Potvin et al., 2013; Zacks, 2008). No significant differences between groups were found on the MRot task as was expected and no effect of anxiety or depression was apparent between groups to

account for any group difference activation as was expected. No significant differences were found in executive function and spatial reasoning areas in whole brain resting state analysis or regional resting state analysis between patients and controls which was as hypothesised. Importantly, careful analysis of arterial spin labelling data, which measures cerebral perfusion, through either whole brain or regional analyses found no differences between patients and controls as was predicted.

The study demonstrates that older ageing PC patients and age matched healthy controls had similar neural activity that corresponded to behavioural performance before patients began any therapy. However some unforeseen outcomes were noted that require further clarification. An unexpected outcome was noted when task components were split on the MRot task and compared between groups. Differences were evident on the MRot task between groups on the 300 degree condition, where patients sacrificed speed for accuracy. This was reflected in better task performance by patients when judging whether shapes were mirror orientated compared to shapes orientated in the same plane. Further analysis showed no significant effects of anxiety, depression or testosterone on performance and reaction times in the MRot task. Past research has shown a correlation between greater testosterone levels and spatial reasoning ability and this may explain patient and control performance differences on mirror items (Lunenfield, 2003; Moffat et al., 2002). Given that patients had significantly higher testosterone levels, this might have resulted in slower reaction times but better accuracy. This effect was not expected but may be a function of age and effects of higher than normal testosterone levels defined by the control group, which could lead to prolonged reaction times. A study in support of this supposition found that reaction times on an executive function task increased up to mid-range endogenous testosterone levels in ageing healthy participants (Muller et al., 2005). However, testosterone levels beyond this

point resulted in increases of reaction time durations in the highest level testosterone groups. This outcome is supported and has been found in numerous studies of healthy ageing samples (Barrett-Connor et al., 1999; Moffat & Hampson, 1996). Thus, even if other disease-specific factors that can influence activation levels – such as mood disturbance - were present in patients, their higher testosterone levels act to compensate and protect performance. The outcome of longer reaction times in patients may have inadvertently allowed better accuracy. This may be a function of the MRot task design incorporated in the current study which had longer duration of shape presentations. Nevertheless, this trade-off was required to gain performance in line with past research in both groups to significantly activate underlying neural networks associated with spatial reasoning and could not be avoided.

The finding of better performance of patients in the mirror condition task is consistent with findings from past research. Previous literature has shown a correlation between greater testosterone levels and spatial reasoning ability which may explain patients' and controls performance difference on mirror items (Lunenfield, 2003; Moffat et al., 2002). Although no direct effect of testosterone was found on performance in the mirror 300 degree condition, literature shows that testosterone is converted to other hormonal substrates that could affect cognition but were not measured in the current study performance. These factors may have contributed to better performance in the 300 degree condition of patients compared to controls.

The underlying executive function networks of patients and controls were assessed to measure the strength of connectivity to SST specific regions and behavioural measures through resting state seed connectivity analysis. The exploration generally showed normal

patterns of fronto-parietal connectivity at rest. This was found when the dorsolateral prefrontal cortex and rIFG were used as seed connectivity regions with the default mode network medial prefrontal cortex in patients and controls. This supports the fronto-parietal network required for executive functions and is consistent with the idea that this network is especially engaged by the SST. One study found that spontaneous resting state activity was an effective indicator of inhibitory performance in middle aged and older aging participants (Lee & Hsieh, 2017). Researchers used resting state spontaneous brain activity measured by regional homogeneity and fractional amplitude of low-frequency fluctuations on blood oxygenation level dependent signals (Lee & Hsieh, 2017). Correlations showed that the bilateral inferior frontal gyri and default mode network negatively correlated with stop signal reaction time (Lee & Hsieh, 2017). This suggests that these regions were vital to inhibition functions. However, this is incongruent to what was found in the current study. Although non-significant, control SSRTs had a positive linear trending relationship with BOLD signal change whilst patients had a negative trending relationship. This could explain the lack of significant suprathreshold activity found at the group level in patients in the rIFG; it may be the case that there is a subtle functional deficit in the underlying resting state network, which in turn reduces task-related activation. This finding may suggest that there are very subtle functional changes in executive brain regions in men with prostate cancer prior to the onset of treatment. This has important implications for longitudinal research. Thus, an increased sample size would be helpful to confirm that this pattern is not simply a product of low power to detect activation in patient groups.

Another explanation may be that the lack of difference between samples was due to a compensatory effect in patients that was facilitated by testosterone. Indeed, one study showed that participants transferring gender from females to males had increased functional

connectivity in the DMN and right postcentral gyrus when administered testosterone compared to controls that were not undergoing gender reassignment treatment (Burke et al., 2018). Further support for the study comes from patients with Alzheimer's disease where resting state connectivity in the medial prefrontal cortex which is part of the DMN in one study was attenuated (Mevel, Chételat, Eustache, & Desgranges, 2011). This may have been due to white matter disruptions from amyloid beta plaque formations that are known to alter the pathway of axons between connective resting state regions (Mevel et al., 2011). However, these plaques and formations may be reduced through testosterone therapy as demonstrated by one group of researchers assessing older males and could apply to the patients in the current study whereby deficits were avoided through higher testosterone levels (Gouras et al., 2000).

The research above suggests that there may be testosterone mechanisms by which resting state is compensated for in the patient sample of the study undertaken and suggests this may have allowed PC patients to have a cognitive reserve. This was evaluated through resting state examinations in the current assessment since commonly identified neural networks at rest often overlap with functional areas. Negatively activated networks can thereby provide an indirect assessment of integrity in specific regions when they are activated (Zhao et al., 2016). Longitudinal investigations are required to assess the effects of deprivation of the testosterone reserve on resting state connectivity during and after ADT.

Given the known age-related changes in brain perfusion, it was important to measure and, where necessary, control for perfusion deficits in participants. In addition, any perfusion deficit differences between groups could explain either resting or task-related BOLD group differences. Hence, arterial spin labelling imaging in patients and controls was collected, and

showed whole brain and local perfusion was in the normal range (Pollock et al., 2009). However a shortcoming to data collection was that a significant proportion of scans had to be discarded in controls at baseline. Based on evidence that ASL is reliable in the short term and changes are not seen over a 12 month period, (Parkes et al., 2004), data from six-month scans in the affected control participants, which were all in the normal range, were pooled with other control baseline ASL data. This analysis should be considered exploratory but supports the idea that there were no group differences. Further data are required to confirm this. This was, however, outside the scope of the current thesis.

In conclusion, patients and controls had similar levels of function in the neural networks corresponding to executive function and spatial reasoning with some unforeseen exceptions (differences in RT and accuracy on the MRot task and small differences in brain activations). Variation in testosterone did account for some differences between groups. Those results that varied with testosterone showed that there were benefits to patients in having higher levels compared to controls that may have allowed for compensation of disease specific impairments. Nevertheless, other findings showed that higher levels were not beneficial and were posited to have impaired neural and cognitive function. Differences between patients and controls were suggested to be due to disease specific factors in patients that may be able to be recruited without testosterone manipulations. Another factor to consider was the efficacy of the control sample. This is explored in Chapter Four through a longitudinal study, which allowed an extensive investigation to confirm the control samples' appropriateness as a comparison cohort as a group without any cognitive or underlying neural impairments.

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# CHAPTER 4

Study 3: Neuropsychological performance and neuroimaging comparisons of neural function between baseline and follow-up.

## 4.1 Introduction

In the previous experimental chapters, data were presented on behavioural and imaging indices relevant to the measurement of change associated with depletion of testosterone in men with prostate cancer. The purpose of the current experiment was to conduct longitudinal assessment in participants to assess the integrity and reliability of cognitive performance across a period of six months. This was used to determine if any changes occurred in the healthy sample between baseline and follow-up and to determine if factors including testosterone contributed to any changes found. The within-subjects comparison allowed inferences to be made on the validity of the sample as being representative of healthy ageing controls over a longitudinal period that were compared to PC patients at baseline. Patients were not followed up because in the current timeframe of the study, insufficient numbers were available at follow-up for analysis. Nevertheless, it was important to confirm that the healthy sample did not show objective cognitive or brain function impairments over time. Previous assessments that compared ADT patients to healthy controls found differences between samples. As mentioned previously, however, the studies were limited because healthy controls were not matched for intelligence and education levels compared to patients (Green et al., 2002; Green et al., 2004). The current investigation thereby increased the robustness of outcomes found of patients in comparison to controls by assuring samples were matched and could be verified as participants without cognitive impairment (see chapters 2 & 3).

In the first part of the chapter, data are presented from participants who were evaluated through serial neuropsychological measures. Past research has determined that aging is

associated with executive function decline compared to young adults. One study assessing subjects on tasks of executive function updating, shifting and inhibition found a negative correlation between updating and ageing (Clarys, Bugaiska, Tapia, & Baudouin, 2009). Further investigations have shown similar patterns of executive function deficits in older ageing subjects (Alichniewicz et al., 2013; Mac Kay, 2016; May et al., 1999; Shao et al., 2014). Indeed, previous research indicates that executive functions deteriorate by -0.02 standard deviations on average per year after the third decade of life (Salthouse, 2010). In the context of treatment for prostate cancer, it is possible that this decline is accelerated beyond age-expected limits.

Spatial abilities that require the mental updating of spatial stimuli and mental manipulation are relevant to the current patient population and some studies suggest that these decline with age (Bo, Borza, & Seidler, 2009; Kumar & Priyadarshi, 2013; Schmiedek, Hildebrandt, Lovden, Lindenberger, & Wilhelm, 2009). However, a meta-analysis of 137 effect sizes found similar performance of normal aging participants to young subjects on tests of spatial ability including mental rotation and spatial perception (Techentin, Voyer, & Voyer, 2014). Nonetheless, speed of processing declined with age compared to accuracy alone (Techentin et al., 2014). This fits well with the notion of declining processing speed in elderly normal aging participants and was accompanied by large effect sizes (Park & Reuter-Lorenz, 2009). A shortcoming of the review however was that only cross sectional studies were included in the review, which limited the analysis of change over time that may have revealed further differences over time between groups.

Although previous research suggests that there are age-related differences in cognition, many studies tend to compare behavioural performance in the cognitive domains of young to adult participants. This approach does not account for ageing *per se*, which requires repeated measurement of relevant domains in an older cohort. Consequently, the present experiment

aimed to characterise an ageing healthy sample with a number of neuropsychological assessments while attempting to mitigate error confounds. Longitudinal change can be measured through statistical methods including omnibus tests or multivariate tests. However, these methods can hide or mask levels of cognitive decline due to the use of average performance. This may lead to under or over estimation of impairment (Jenkins et al., 2005). One effective method to measure significant statistical and clinical change while taking into account the scale of reliability between time points is the reliable change index (RCI) (Jacobson & Truax, 1991). The RCI is a technique to determine the proportion of participants that improve or decline in standardised performance score units over a longitudinal time frame and can account for practice effect (Zahra & Hedge, 2010). The RCI is particularly useful in small sample size studies that have less power but where the average is not representative of a normal distribution of scores (Zahra & Hedge, 2010; Zahra, Hedge, Pesola, & Burr, 2016).

Similar to the absence of behavioural group differences at baseline, there may not be behavioural change over time on objective measures, however group differences in neural activity can be elicited over time. Thus, in addition to a comprehensive behavioural examination, the current chapter also investigated the possibility of underlying neurological changes that may occur in the absence of any cognitive behavioural differences. Several studies have explored cerebral neural mechanisms in older adults with outcomes showing that ageing was associated with underlying neural changes that were not overtly visible through neuropsychological assessments (Celec et al., 2015; Van Impe et al., 2013; Wilkinson & Halligan, 2004). These subtle differences may occur even in normal healthy ageing due to numerous gross differences in the cerebral structure of older adults compared to younger

adults with developing brain structures. These data are first reviewed before attention turns to potential functional compensation by brain regions when early structural changes occur.

A range of different techniques that examine properties of brain tissues support the idea that there are age-related structural changes that might underpin a decline in brain functions with age. For example, post-mortem examination of brain tissues demonstrated that ageing is associated with reducing brain weight, reductions in brain volume, dilated ventricles (ventriculomegly) and enlargement of sulci (Skullerud, 1985).(Skullerud, 1985). Further, microscopic studies show loss of neuronal bodies in the neocortex, hippocampus and cerebellum (Ellis, 1920; Nairn, Bedi, Mayhew, & Campbell, 1989). These neurons have been found to shrink and often have structural abnormalities (Terman & Brunk, 1998). Sparser cerebral vasculature has also been demonstrated in older adults (Riddle, Sonntag, & Lichtenwalner, 2003) which has been associated with declines in synaptic density and loss of dendritic spines (Bertoni-Freddari et al., 2002; Morrison & Hof, 1997). Other post-mortem studies show accumulative mitochondrial damage, incapable DNA repair ability and failure of glia cells to remove neurons with impaired DNA in older participants (Brunk & Terman, 2002; Rutten, Korr, Steinbusch, & Schmitz, 2003). Volumetric MRI studies have revealed that prefrontal cortices are most affected compared to other brain regions. Moreover, prefrontal cortex volume has been shown to have relatively strong, negative correlations with age ($r= -0.56$) in the majority of literature (Raz & Rodrigue, 2006; Raz, Williamson, Gunning-Dixon, Head, & Acker, 2000). Conversely the weakest correlation between age and brain volume occurs in the occipital cortical region with age (Raz, Rodrigue, Kennedy, & Acker, 2007).

Regional changes may give some insight into the nature of functional changes that occur with ageing. Longitudinal volumetric investigations conducted on the cerebral ventricles can give an indication of cerebral structure since white matter and nuclei tracts fall around them. This

indicates that measurement of these regions can provide an indirect summary measure of the entire central nervous system. Studies measuring ventricular size have generally found that they increase in size by an average of 2.9% per year (Raz et al., 2004). Five studies of older participants (mean age 70) revealed a yearly ventricular enlargement of 4.25% (Hu & Li, 2012; Mueller et al., 1998; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003; Sullivan, Pfefferbaum, Adalsteinsson, Swan, & Carmelli, 2002; Tang, Whitman, Lopez, & Baloh, 2001) whereas younger subjects (below 40 years age) only exhibited a 0.43% increase in expansion (Cahn et al., 2002; DeLisi et al., 1997; Ho et al., 2003; Saijo et al., 2001). This suggests the rate of expansion accelerates with age.

An important topic of research globally focuses on early structural changes in the medial temporal regions including the hippocampus, particularly given their putative roles in Alzheimer's disease (AD). Hippocampal volume has been shown as a good predictor of AD in ageing samples (Laakso et al., 2000). Moreover, entorhinal volume may be a better prospective predictor for detecting transition from average ageing to AD (Gosche, Mortimer, Smith, Markesbery, & Snowdon, 2002). Within subjects longitudinal studies reveal that shrinkage of these regions among healthy adults is twice that of younger participants (Liu et al., 2003). Therefore, exploration of cognitive performance in healthy ageing samples is warranted to identify and exclude participants with dementia or Alzheimer's like symptoms.

Morphometric changes in global or regional brain structure could be due to numerous co-morbid factors. These include disease such as diabetes and hypertension or environmental influences such as lifestyle. Chronic elevation of blood pressure in particular affects over 55% of Americans and can alter the cognitive effects of ageing (den Heijer et al., 2005). Past literature has shown that subjects with medically controlled hypertension may be at less risk of cognitive decline compared to undiagnosed hypertensive subjects (Dufouil, Alperovitch, Ducros, & Tzourio, 2003). Research also points to abnormal white matter and reduced gray

matter volume in ageing subjects who are chronic hypertensives (Raz, Rodrigue, & Acker, 2003; Raz, Rodrigue, Kennedy, et al., 2003). This has been known to lead to vascular diseases affecting regional cerebral blood flow which can cause white matter hyper intensities and gray matter changes (Raz et al., 2007). Thus, it is important to collect information of relevant medical conditions so that these can be factored in to analyses of group by time outcomes.

Although the overall depiction indicates cognitive decline with ageing, vast individual differences exist. Many older subjects are able to outperform young participants on some cognitive tasks and others perform equally well compared to younger cohorts (Craik & Jennings, 1992). The reasons for this variability is of great interest. One hypothesis suggests that older adults are able to compensate for cognitive inabilities. Functional imaging studies provide a neural network view of cognition that changes with age. Aging has been shown to localise neuronal activity to specific regions rather than delocalise co-ordinated activity between brain regions in some cohorts (Andrews-Hanna et al., 2007). This suggests loss of global integrative function which has been associated poor regional co-ordination and worse cognitive performance (Andrews-Hanna et al., 2007). In contrast, some regional networks such as the prefrontal cortex have been shown to become less localised and connected with other regions as a function of ageing (Cabeza, 2002; Park & Reuter-Lorenz, 2009). However, younger participants have been found to utilise contained and distinct prefrontal regions to perform the same higher level executive tasks making the region function more proficiently in younger cohorts (Cabeza, 2002; Park & Reuter-Lorenz, 2009). However, researchers have also found that older adults with more delocalised activity outperform older adults whose activity is localised on higher level executive function tasks (Cabeza, 2002; Cabeza, Anderson, Locantore, & McIntosh, 2002). This suggests that regional associations in the

elderly may be a compensatory response to recruit additional brain areas during highly demanding cognitive activities in which the mechanism differs compared to younger adults. This may be because isolated regions may lose function with age that then begin to connect with other areas to increase cognitive efficiency.

Another hypothesis proposed that the mirror neuron systems may allow compensation in ageing adults and presents itself as over activation in neural networks, particularly in prefrontal regions. Over activation in older participants has typically been found in mirror or opposite regions to where putative activation has been found in young adults in foregoing research (Reuter-Lorenz & Cappell, 2008). The interpretation of over activation has been much debated since its nature and neural correlates are ambiguous (Reuter-Lorenz & Lustig, 2005). Many studies report over activation with age equivalent performance. This suggests that the surplus activity has an advantageous purpose as a compensatory mechanism without which poor performance would result (Reuter-Lorenz & Cappell, 2008). Moreover, the compensation hypothesis suggests that over activation should lead to higher performance among older adults even whilst being performance matched at a group level with those who are younger. Although significant correlations are lacking, positive performance correlations have been found supporting the compensatory hypothesis of age related over activations (Cabeza et al., 2004; Reuter-Lorenz & Lustig, 2005). Transcranial magnetic stimulation (TMS) studies have been applied in elderly participants in activation sites. One study found impaired performance in elderly participants when TMS was applied to one hemisphere in the prefrontal cortex on recognition memory tasks. This forced unilateral involvement on participants showing that bilateral activation may be required in seniors to successfully complete the task compared to younger cohorts (Rossi et al., 2004). Taken together, these data suggest that even though structural brain changes occur with ageing and these relate to a decline in cognitive performance, at the early stages of decline when performance differences

may not be evident on standard testing, regional functional brain compensation may occur. Accordingly functional MRI methodologies are relevant to the question of whether there are ADT-induced cognitive change in men with prostate cancer.

The potential utility of fMRI measures must of course take into consideration the potential effect of normal testosterone decline on the brain in a healthy ageing cohort. Research suggests that these hormones have neuroprotective features in the brain and are able to cross the blood brain barrier (Pike, Carroll, Rosario, & Barron, 2009). Testosterone levels have been found to decline in healthy samples even in short time frames of six-months. One longitudinal six month assessment of healthy participants ranging from 30-90 years age found downward trends of testosterone over the period (Harman, Metter, Tobin, Pearson, & Blackman, 2001). Furthermore, numerous longitudinal investigations have demonstrated relationships between declining testosterone levels during ageing and cognitive impairments which has also been supported by neuronal and cerebral perfusion investigations (Moffat et al., 2002; Moffat et al., 2004). The study conducted therefore aimed to characterise neural function in healthy elderly ageing participants and to determine factors including testosterone that influenced cognition over a longitudinal duration. Moreover, the study sought to assess the reliability of activation across the six month period as this would then enable future assessment of the impact of testosterone depletion using ADT in PC.

In light of the literature above in ageing healthy participants, cognitive performance and its neural correlates were characterised over a longitudinal study duration to assess reliability of behavioural performance between time points. Furthermore, the study design allowed the assessment of the underlying integrity of neural correlates of neuropsychological function. Due to the longitudinal nature of the study, variables such as mood and testosterone that may

have affected cognition over the time period could be factored into the analysis. This facilitated in identifying if the control sample in the assessment of PC patients at baseline were suitable as matched controls of normal ageing and in a longitudinal assessment of patients and controls.

### 4.1.1 Aim

The aim of this study was to characterise the cognition of the healthy control sample across a longitudinal duration using behavioural measures.

Another aim was to assess the reliability and stability of the neural correlates of executive function and spatial reasoning domains over a period from baseline to six months in healthy ageing controls.

### 4.1.2 Behavioural Cognition Hypothesis

The hypothesis for the study were as follows:

**Main hypotheses:**

**a.** Healthy elderly participants will not have any significant performance differences between baseline and follow-up on the Delis Kaplan Executive function test (D-KEFS) (Trail Making test, Verbal Fluency test, Colour Word interference and Tower test), self- report Behavioural Rating Inventory (BRIEF-A) and Cambridge Neuropsychological Test Automated Battery (CANTAB) spatial working memory (SWM) task.

**b.** There will be no significant differences in levels of serum testosterone in healthy participants between baseline and follow-up.

**c.** Change in testosterone will not contribute to any variance in neuropsychological performance change between time points.

### 4.1.2 Neuroimaging Hypotheses

**a.** There will be no behavioural differences on the stop signal task (SST) or mental rotation (MR) tasks between baseline and six months.

**b.** There will be no neural activation differences in healthy participants from baseline to six months on either the stop signal task or mental rotation task.

**c.** There will be no significant correlation differences between baseline and six months on resting state seed functional connectivity analysis between seed regions and regions of interest.

**d.** There will be no significant differences in cerebral blood flow in healthy subjects at baseline compared to six months assessed by arterial spin labelling (ASL).

## 4.2 Methods

### 4.2.1 Design

A longitudinal within subjects design was used to investigate cognitive performance at baseline and six months in healthy participants. Neural activation was assessed through MRI scans acquired to analyse neural differences in functional task related activation, and differences between time points on resting state connectivity and arterial spin labelling.

### 4.2.2 Participants

Twenty nine healthy male participants (mean age = 67.81 $\pm$ 6.92) were initially recruited to participate in the study. Three participants were lost to follow-up due to loss of interest or other reasons (domestic, household circumstance changes etc.). Therefore, the final healthy elderly sample included 26 healthy participants assessed at baseline (mean age = 68.54 $\pm$ 6.10). Ethical approval was acquired from the University of Birmingham ethics committee for the assessment of neuropsychological measures. Healthy older ageing subjects were recruited from various organisational institutions which contained facilities catering towards the needs of healthy elderly populations. This included establishments such as gym facilities, elderly care homes, sports facilities (Birmingham bowls, golf establishments) and religious organisations (churches, temples, mosques etc.). Some participants were additionally recruited from the University of Birmingham elderly participant's database.

### 4.2.3 Inclusion and Exclusion criteria

As described in chapter 2 section 2.2.1 table 2.1 - community based control group

### 4.2.4 Ethical Approval

As described in chapter 2 section 2.2.2

### 4.2.5 Saliva Sample Collection

As described in chapter 2 section 2.2.3

## 4.3 Behavioural Outcome Measures

Participants were administered the D-KEFS, BRIEF-A self-report scale, WAIS-IV, CANTAB and HADS as described in chapter 2 section 2.3 at baseline and six month follow-up.

## 4.4 Brain Imaging in Scanner Task Measures

The SST and MRot tasks were as described in chapter 3 section 3.3.

## 4.5 Imaging Acquisitions

Structural T1, T2 functional scans and resting state and arterial perfusion scans were acquired as described in chapter 3 section 3.4.

## 4.6 Neuropsychological Outcomes Statistical Analysis

The main purpose of the analysis was to assess cognitive change over time and if any change was accounted for by testosterone or psychosocial factors. A p value of either 0.05 or less was accepted as statistically significant (two-tailed). Paired samples t-tests were conducted to assess the level of psychosocial (anxiety and depression) change and testosterone level change that occurred from baseline to follow-up. Further multivariate techniques including within subjects MANOVAs and ANOVAs were employed to assess main effects and interactions between outcome measures and time. The repeated measures factor was time (baseline and six months) and primary outcome variables of each cognitive measure employed were the dependent variables in each analysis. Covariates were added to the models to assess their effect on change between time points (see Appendix 11 for more detail). One noteworthy influence was that the comparison of many variables by the analysis

methods implemented, may have led to issues with multiple testing due to the number of comparisons made between variables. A reliable change index was calculated to measure change between time points and chi-square test was employed to measure the proportions of the sample that had a change in performance between time points in relation to testosterone (Appendix 8).

**Table 4.1: Scaled Scores for Neuropsychological baseline, six month follow-up and change over time**

| Measure | Baseline (n=26) | | 6-month (n=26) | | Difference | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| **D-KEFS** | | | | | | |
| **Trail Making Test** | | | | | | |
| Visual Scanning | 10.62 | 2.90 | 11.31 | 2.09 | 0.69 | 2.85 |
| Number Sequencing | 11.85 | 2.62 | 12.81 | 1.79 | 0.96 | 2.52 |
| Letter Sequencing | 11.81 | 3.01 | 12.04 | 1.95 | 0.23 | 3.24 |
| Number Letter Sequencing | 11.46 | 2.89 | 12.15 | 2.31 | 0.69 | 3.43 |
| **Verbal Fluency Test** | | | | | | |
| Letter Fluency | 14.00 | 3.06 | 13.73 | 2.97 | -0.27 | 1.64 |
| Category Fluency | 13.04 | 2.88 | 13.46 | 2.69 | 0.42 | 2.23 |
| Category Switching | 12.92 | -2.74 | 12.23 | 3.60 | -0.69 | 5.06 |
| Switching Accuracy | 12.68 | 2.67 | 12.31 | 3.65 | 0.12 | 5.33 |
| **Design Fluency** | | | | | | |
| Filled Dots | 11.73 | 2.82 | 12.23 | 3.05 | 0.50 | 3.30 |
| Empty Dots | 11.62 | 2.74 | 11.65 | 2.91 | 0.03 | 2.99 |
| Switching (Empty and Filled) | 12.58 | 3.44 | 12.58 | 2.90 | 0.00 | 3.01 |
| **Colour Word Interference Test** | | | | | | |
| Colour Naming | 11.12 | 1.56 | 11.27 | 2.60 | 0.15 | 2.29 |
| Word Reading | 11.35 | 2.68 | 11.81 | 2.04 | 0.46 | 2.40 |
| Inhibition | 13.00 | 1.56 | 12.96 | 1.54 | -0.04 | 1.54 |
| Inhibition Switching | 12.92 | 1.38 | 13.23 | 1.11 | 0.31 | 1.38 |
| **Tower Test** | | | | | | |
| Number of Moves | 155.04 | 84.88 | 147.46 | 49.47 | 7.58 | 76.15 |
| Total Time to complete (ms) | 494.88 | 166.61 | 505.96 | 164.13 | 49.15 | 205.33 |
| Achievement Scores | 9.15 | 3.18 | 12.50 | 2.20 | 3.35 | 3.76 |

Abbreviations: ms, milliseconds; SD, standard deviation.

| Measure | Baseline (n=26) | | 6-month (n=26) | | Difference | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| **BRIEF-A** | | | | | | |
| **Self-Report** | | | | | | |
| Inhibition | 50.42 | 6.65 | 50.04 | 7.25 | -0.38 | 5.49 |
| Shift | 50.46 | 8.68 | 51.08 | 9.50 | 0.62 | 7.19 |
| Emotional Control | 48.85 | 9.12 | 49.73 | 8.38 | 0.88 | 8.81 |
| Monitor | 49.90 | 8.44 | 48.19 | 7.69 | 0.69 | 15.98 |
| Initiate | 50.08 | 7.47 | 49.50 | 6.56 | -0.58 | 6.87 |
| Working Memory | 56.76 | 11.79 | 47.31 | 5.40 | -9.46 | 10.08 |
| Plan/Organise | 49.54 | 8.62 | 50.34 | 9.80 | 0.81 | 7.13 |
| Task Monitor | 52.69 | 8.82 | 52.23 | 8.94 | -0.46 | 7.06 |
| Organisation of Materials | 49.96 | 8.12 | 50.23 | 7.80 | 0.27 | 6.38 |
| BRI | 50.04 | 7.30 | 40.42 | 6.84 | -9.62 | 5.61 |
| MI | 51.50 | 7.22 | 55.42 | 9.03 | 3.92 | 7.19 |
| GEC | 50.84 | 6.37 | 95.73 | 15.18 | 46.85 | 20.49 |
| **CANTAB** | | | | | | |
| **Between Errors** | | | | | | |
| 4 boxes | 0.58 | 1.33 | 0.72 | 1.46 | 0.14 | 1.04 |
| 6 boxes | 6.12 | 6.53 | 6.04 | 6.22 | 0.07 | 6.64 |
| 8 boxes | 17.85 | 9.41 | 18.32 | 13.00 | 0.47 | 10.75 |
| **Double Errors** | | | | | | |
| 4 boxes | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 boxes | 0.15 | 0.37 | 0.12 | 0.32 | -0.03 | 0.45 |
| 8 boxes | 0.27 | 0.83 | 0.84 | 1.57 | 0.57 | 1.88 |
| **Strategy Score** | 31.88 | 6.99 | 31.31 | 6.40 | 0.57 | 0.59 |
| **HADS** | | | | | | |
| Anxiety | 2.88 | 2.14 | 3.00 | 2.91 | 0.12 | 2.07 |
| Depression | 1.50 | 1.39 | 2.00 | 1.94 | 0.50 | 1.70 |

Abbreviations: BRI, behavioural regulation index; GEC, global executive composite; MI metacognitive index; SD, standard deviation.

## 4.7 Neuroimaging Statistical Analysis

### 4.7.1 FSL analysis

FSL analysis was as described in chapter 3 section 3.5.1 with healthy participants assessed at both baseline and six months on the SST and MRot task.

Additionally, region of interest (ROI) analysis was undertaken. The ROI method allowed the assessment of small clusters of voxels in task related regions thereby reducing the amount of error due to multiple comparisons of voxels that could be prone to type-I errors and chance findings during whole brain analysis between baseline and follow-up (Logan, Geliazkova, & Rowe, 2008). Within subjects ANOVAs were employed to assess regional BOLD signal change between time points. Significant interactions were followed up with paired sample t-tests that were corrected using the Bonferroni method. Likewise, paired sample t-tests were conducted in intact ASL scans to assess the stability of whole brain and regional perfusion between baseline and follow-up. While ROI analysis may reduce chances of errors, outcomes from the analysis should still be interpreted with caution due to issues of multiple testing since many factors were compared increasing the chances of type-I errors. This may lead to increases of chance findings where the null hypothesis was rejected for the alternate hypothesis to increase familywise errors. Consequently, signal change differences between time points may be susceptible to error.

### 4.7.2 SPM Analysis

SPM analysis was undertaken to investigate intra-class correlations (ICC) in voxel regions of interest. The ICC method is an analysis technique used to measure test-retest reliability of the spatial distribution of BOLD fMRI voxel activation across separate sessions (Caceres et al., 2009) (Appendix 12). MATLAB version 7.9.0.529 (R2009b) was used with SPM12 (Welcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK (SPM)) to allow the conversion of raw data files to NIFTII format. Pre-processing steps and group level analysis were implemented with SPM12 as detailed in Appendix 12. Contrasts made for both the MRot and SST were analogous to those generated using FSL (chapter 3 section 3.5.1). Maximally activated voxel regions were then masked and extracted using the SPM integrated Marsbar software (Matthew, Jean-Luc, Romain, & Jean-Baptiste, 2002) from the baseline group activation contrast maps and inputted into an ICC toolbox designed by Caceres et al. (2009) to measure activation consistency from baseline to follow-up (Appendix 12).

## 4.8 Procedure

*Behavioural*

Healthy participants underwent behavioural assessments as described in chapter 2 section 2.4. Subjects were administered identical behavioural assessments at baseline and six month follow-up.

*Neuroimaging*

Procedure was as described in chapter 3 section 3.6 and analogous at baseline and follow-up in healthy participants. Functional runs were administered as described in chapter 3 section 3.6.1 in healthy participants at baseline and follow-up

## 4.9 Behavioural Cognitive Performance results

Twenty six participants completed all phases of the study from baseline to six months. Time between baseline assessment and six month follow-up ranged from 122 days to 223 (mean = 77.46 $\pm$ 17.37) days. Participants' mean age at baseline was 68.54 $\pm$ 6.10 ranging from 56 to 80 years old. At six months participants' mean age was 69.27 $\pm$ 6.07 ranging from 56 to 81. Statistical analyses were undertaken with SPSS 22.0. Missing data was found for one participant at baseline and follow-up. The participant was excluded from the colour-word interference test due to colour blindness. To avoid the exclusion of the participant in statistical terms, an expectation maximization procedure was used to analyse missing values. This has been shown as an effective method for managing missing data (Rubin et al., 2007).

### 4.9.1 Screening assessments

All healthy elderly participants included, passed the criteria for participation. Subjects scored an average 27 $\pm$ 0.92 on the MMSE which was above the cut-off criteria of 24. On average, participants had higher free testosterone levels at the six month follow-up (mean = 91.63pg/ml, *SE* = 5.47) compared to baseline (mean = 82.84pg/ml, *SE* = 5.39). This difference was not significant ($t(25) = -1.20$, $p > 0.05$). Participants had marginally lower anxiety scores as measured by the HADs scale at baseline ($M = 2.88$, $SE = 0.42$) compared to six months ($M = 3.00$, $SE = 0.57$). This difference was not significant ($t(25) = -2.85$, $p > 0.05$). Furthermore, participants had marginally lower depression scores at baseline (mean = 1.50, *SE* = 0.27) compared to follow-up (mean = 2.00, *SE* = 0.38). This was not a significant difference ($t(25) = -1.50$, $p > 0.05$).

### 4.9.2 Intellectual Functioning

All participants performed in the normal range for their particular age groups on subtests of the WASI-II as evidenced from the Full scale intelligence quotient 4 scores (FSIQ-4) (Mean = 123.62 $\pm$ 12.33, range 88-141). Moreover, perceptual reasoning (mean = 115.23 $\pm$ 13.23) and verbal comprehension index scores (127.12 $\pm$ 15.18) were in the normal range. Subtests encompassing FSIQ-4 included the block design task, matrix reasoning, vocabulary task and similarities task.

### 4.9.3 Executive functioning

*D-KEFS*

Overall, behavioural performance was equal between both time points within healthy participants. An omnibus MANOVA with all D-KEFS contrast scaled scores included, showed no significant differences in healthy controls between time points ($F(5,46) = 0.85$, $p > 0.05$). All one-way follow up ANOVAs showed non-significant interactions between each test and time variable. A MANCOVA was conducted to assess the influence of testosterone level with findings showing no significant effect of the hormone ($F(5,45) = 2.01$, $p > 0.05$). Furthermore, no significant difference in D-KEFS contrast scaled scores was found between time when controlling for testosterone ($F(5,45) = 0.833$, $p > 0.05$).

*BRIEF-A*

The brief executive function assessment (BRIEF-A) self-report scores were analysed with MANOVAs. The first conducted MANOVA assessed differences in healthy controls between baseline and six months. Inhibition, shift, emotional control, self-organisation, ability to initiate, working memory, monitor, behavioural regulation index, metacognition and global executive function scaled scores were entered as dependent variables. Fixed factors were entered as patients and controls. No significant differences were found across dependent variables between baseline and follow-up ($F(6, 45) = 0.19$, $p > 0.05$). Furthermore, testosterone did not predict scores on average between time points on the BRIEF-A ($F(6, 44) = 1.50$, $p > 0.05$). No differences were found between time points while controlling for testosterone level ($F(6, 44) = 0.19$, $p > 0.05$).

Further exploratory analysis showed no significant correlations between the self-rated BRIEF-A questionnaire meta-cognitive Index scaled score and D-KEFS contrast scaled scores. Correlations were not significant on the D-KEFS trail making test ($r = 0.27$, $p > 0.05$),

verbal fluency test (r = 0.10, $p > 0.05$), design fluency test (r = -0.01, $p > 0.05$), colour word interference test (r = 0.17, $p > 0.05$) and tower test (r = -0.23, $p > 0.05$).

### 4.9.4 Spatial reasoning

CANTAB SWM *between errors*

A MANOVA was conducted to assess spatial reasoning performance. Number of between errors made on the CANTAB SWM was the dependent variable and assessed between time points in a within subjects' analysis. Findings showed no significant differences between the number of between errors made between time points on the CANTAB SWM task in the 4 box, 6 box and 8 box conditions ($F(3,47) = 0.07$, $p > 0.05$). Further analyses showed no significant contribution of testosterone to performance on average errors made between baseline and follow up ($F(3,46) = 0.30$, $p > 0.05$).

A MANCOVA was undertaken by adding the WAIS working memory index scores (WMI) as a covariate to the number of between errors made on the CANTAB SWM task. This allowed the isolation of the spatial reasoning domain of the SWM task while controlling for working memory. Findings showed a significant predictive effect of the WAIS working memory index scores on between errors made on the SWM task as was expected ($F(2,46) = 4.24$, $p < 0.01$, $\eta^2 = 0.22$). Specific ANCOVAs showed that the WAIS_WMI predicted scores in the 4 box condition ($F(1,48) = 6.81$, $p = 0.01$, $\eta^2 = 0.12$) and 8 box condition ($F(1,48) = 5.26$, $p = 0.03$, $\eta^2 = 0.10$). Spatial reasoning scores did not significantly differ between time points while controlling for WAIS performance ($F(2,46) = 0.08$, $p > 0.05$). No effect of testosterone was found when it was concurrently added with WAIS WMI scores on average spatial reasoning scores between time points ($F(3,45) = 0.71$, $p > 0.05$). Moreover, no

significant difference was found between time points whilst controlling for WAIS WMI scores and testosterone levels ($F(3,45) = 0.08$, $p > 0.05$).

CANTAB SWM *double errors*

A subsequent MANOVA was conducted with double errors as the dependent variable to assess the number of errors made between time points by healthy subjects. This showed no significant differences on the number of double errors made in the 4, 6 and 8 box conditions between time points in healthy subjects ($F(2,48) = 1.30$, $p > 0.05$). When testosterone was added to the model, it had no effect on double errors made on average across baseline and follow-up ($F(3,46) = 1.78$, $p > 0.05$). Furthermore, testosterone did not covary with the number of double errors made between time ($F(3,46) = 1.03$, $p > 0.05$).

A further MANCOVA was implemented with the WAIS scores as a covariate on the number of double errors made by healthy participants on the CANTAB SWM task. The analysis showed WAIS scores did not predict the number of double errors made independent of time ($F(3,46) = 0.03$, $p > 0.05$). Moreover, no predictive effect of the WAIS was found on double errors made between time points ($F(3,46) = 0.95$, $p > 0.05$) When testosterone was added with the WAIS to the number of double errors made, it had no predictive effect on number of errors made on average across baseline and six months ($F(3,45) = 1.52$, $p > 0.05$). No significant difference was found between time points while controlling for testosterone level and WAIS WMI scores ($F(3,45) = 1.01$, $p > 0.05$). No difference in strategy score was either found in a paired sample t-test ($t(25) = 0.53$, $p > 0.05$).

### 4.9.5 Reliable Change index Outcomes

Table 4.2 shows the proportion of reliable change that occurred between baseline and follow-up. A paired sample t-test was conducted to assess if any difference in neuropsychological performance was evident between baseline and six months follow up in healthy participants. No significant difference was found ($t(36) = 0.23$, $p > 0.05$) suggesting that performance was similar between baseline (mean = 1.41 $\pm$ 0.90) and at six months (mean = 1.35 $\pm$ 1.09) (Table 4.1).

A chi-square test (3x3) was undertaken to assess the effect of the proportions of participants that had testosterone increases, decreases or same levels on cognitive improvement, decline or same performance over time (table 4.2). The independent variable was testosterone with three categorical levels these being reliable testosterone level increases, decreases or levels that stayed the same between baseline and six months. The outcome variables were the proportion of participants that either had cognitive performance RCI improvements, declines or stayed the same over baseline to six months (table 4.2).

Results showed a significant relationship between testosterone level change across time and performance on the CANTAB eight box double errors condition between time points ($X^2$ (2, $N = 26$) = 7.93, $p = 0.02$). More specifically, the relationship that predicted the most variance in the outcome showed that the proportion of participants with reliable testosterone declines across time also had reliably worse performance on the CANTAB eight box double errors condition (std. residual = 2.6). Another significant relationship was found between testosterone level change across time and the BRIEF-A self-report self-monitor scale ($X^2$ (2, $N = 26$) = 12.67, $p = 0.01$). More specifically, the relationship that predicted the most variance in the outcome showed that the proportion of participants with reliable testosterone

increases across time had lower scores on the BRIEF-A self-reported self-monitor scale (std. residual = 3.3).

**Table 4.2: Change from baseline to six months with proportion improved and declined. Calculated using RCI method.**

| Measure | Mean difference | Min difference | Max difference | RCI Threshold Improved N | % | Declined N | % |
|---|---|---|---|---|---|---|---|
| **D-KEFS Trail making Test** | | | | | | | |
| Visual Scanning | 0.69 | -6.00 | 7.00 | 1 | 3.85 | 2 | 7.69 |
| Number Sequencing | 0.96 | -3.00 | 9.00 | 0 | 0.00 | 1 | 3.85 |
| Letter Sequencing | 0.23 | -8.00 | 11.00 | 1 | 3.85 | 2 | 7.69 |
| Number Letter Sequencing | 0.69 | -10.00 | 10.00 | 1 | 3.85 | 2 | 7.69 |
| Total | | | | 3 | 2.88 | 7 | 6.73 |
| **Verbal Fluency Test** | | | | | | | |
| Letter Fluency | -0.27 | -4.00 | 3.00 | 2 | 7.69 | 1 | 3.85 |
| Category Fluency | 0.42 | -4.00 | 5.00 | 1 | 3.85 | 1 | 3.85 |
| Category Switching | -0.62 | -8.00 | 5.00 | 2 | 7.69 | 2 | 7.69 |
| Switching Accuracy | -0.31 | -7.00 | 7.00 | 2 | 7.69 | 2 | 7.69 |
| Total | | | | 7 | 6.73 | 6 | 5.77 |
| **Design Fluency** | | | | | | | |
| Filled Dots | 0.50 | -8.00 | 7.00 | 1 | 3.85 | 2 | 7.69 |
| Empty Dots | 0.04 | -4.00 | 8.00 | 2 | 7.69 | 0 | 0.00 |
| Switching (Empty and Filled) | 0.00 | -4.00 | 8.00 | 1 | 3.85 | 0 | 0.00 |
| Total | | | | 4 | 3.85 | 2 | 1.92 |
| **Colour Word Interference Test** | | | | | | | |
| Colour Naming | 0.15 | -9.00 | 2.00 | 0 | 0.00 | 2 | 7.69 |
| Word Reading | 0.46 | -3.00 | 11.00 | 0 | 0.00 | 1 | 3.85 |
| Inhibition | -0.04 | -4.00 | 2.00 | 3 | 11.54 | 0 | 0.00 |
| Inhibition Switching | 0.31 | -3.00 | 2.00 | 1 | 3.85 | 0 | 0.00 |
| Total | | | | 4 | 3.85 | 3 | 2.88 |
| **Tower Test** | | | | | | | |
| Number of Moves | -7.58 | -207.00 | 117.00 | 0 | 0.00 | 3 | 11.54 |
| Time to complete | -0.08 | -5.00 | 1.00 | 1 | 3.85 | 0 | 0.00 |
| Achievement Scores | 0.73 | -3.00 | 5.00 | 1 | 3.85 | 1 | 3.85 |
| Total | | | | 2 | 2.56 | 4 | 5.13 |
| Total D-KEFS change | | | | 13 | 2.78 | 16 | 3.42 |

| Measure | Mean difference | Min difference | Max difference | RCI Threshold Improved N | % | Declined N | % |
|---|---|---|---|---|---|---|---|
| **BRIEF-A** | | | | | | | |
| **Self-Report** | | | | | | | |
| Inhibition | -0.38 | -10.00 | 11.00 | 1 | 3.85 | 1 | 3.85 |
| Shift | 0.62 | -10.00 | 19.00 | 0 | 0.00 | 2 | 7.69 |
| Emotional Control | 0.88 | -18.00 | 15.00 | 2 | 7.69 | 0 | 0.00 |
| Monitor | -0.96 | -19.00 | 14.00 | 1 | 3.85 | 1 | 3.85** |
| Initiate | -0.58 | -17.00 | 15.00 | 1 | 3.85 | 2 | 7.69 |
| Working Memory | -2.19 | -27.00 | 15.00 | 2 | 7.69 | 0 | 0.00 |
| Plan/Organise | 0.81 | -11.00 | 22.00 | 1 | 3.85 | 1 | 3.85 |
| Task Monitor | -0.46 | -14.00 | 11.00 | 1 | 3.85 | 0 | 0.00 |
| Organisation of Materials | 0.27 | -14.00 | 10.00 | 3 | 11.54 | 0 | 0.00 |
| BRI | -0.23 | -13.00 | 13.00 | 1 | 3.85 | 2 | 7.69 |
| MI | -0.04 | -12.00 | 21.00 | 1 | 3.85 | 1 | 3.85 |
| GEC | 0.38 | -9.00 | 17.00 | 2 | 7.69 | 1 | 3.85 |
| Total Self Report | | | | 16 | 5.13 | 11 | 3.53 |
| **CANTAB** | | | | | | | |
| **Four boxes** | | | | | | | |
| Between Errors | 0.12 | -2.00 | 3.00 | 3 | 11.54 | 1 | 3.85 |
| Double Errors | -0.31 | -14.00 | 15.00 | 2 | 7.69 | 1 | 3.85 |
| Total | | | | 5 | 9.62 | 2 | 3.85 |
| **Six Boxes** | | | | | | | |
| Between Errors | 0.58 | -19.00 | 21.00 | 3 | 11.54 | 4 | 15.38 |
| Double Errors | -0.04 | -1.00 | 1.00 | 3 | 11.54 | 2 | 7.69 |
| Total | | | | 6 | 11.54 | 6 | 11.54 |
| **Eight Boxes** | | | | | | | |
| Between Errors | -0.04 | -1.00 | 1.00 | 2 | 7.69 | 3 | 11.54 |
| Double Errors | 0.58 | -3.00 | 5.00 | 2 | 7.69 | 4 | 15.38* |
| Total | | | | 4 | 7.69 | 7 | 13.46 |
| Strategy | -0.58 | -12.00 | 14.00 | 1 | 3.85 | 2 | 7.69 |
| Total CANTAB change | | | | 15 | 9.62 | 15 | 9.62 |
| **Total reliable change all neuropsychological assessments** | | | | **28** | **4.49** | **31** | **4.97** |
| Testosterone | 8.79 | -59.01 | 77.85 | 2 | 7.96 | 3 | 11.54 |

Abbreviations: BRI, behavioural regulation index; GEC, global executive composite; MI metacognitive index; RCI, reliable change index; *p<0.05; **p<0.01.

### 4.10.1 Brain imaging results

### 4.10.1 Behavioural in Scanner Task Analysis

Tasks administered during fMRI were first behaviourally evaluated to assess longitudinal performance between time (baseline and six months).

Inhibitory Control *(SST)*

Table 4.3 shows SS task performance on go and stop trials between baseline and six month follow-up. Paired sample t-tests revealed a significant difference of inhibitory performance in which participants performed better at follow-up compared to baseline on stop trials ($t(25) = -2.51$, $p = 0.02$). No significant differences were found on other measures of go accuracy, go reaction time and stop signal reaction time. This suggests there were some behavioural differences of participants from baseline to six months.

A 2 x 3 ANOVA was performed where one variable was time with 2 factors and another variable was flicker trial accuracy. Flicker trials were separated into short, medium and long trials. Simple effects revealed a significant difference of time ($F(1,25) = 7.16$, $p = 0.01$, $\eta^2 = 0.22$). Furthermore, performance across flickers independent of time were significantly different ($F(2,25) = 7.16$, $p < 0.01$, $\eta^2 = 0.63$). Nevertheless, a flicker by time interaction did not reveal any significant differences ($F(2,24) = 0.407$, $p > 0.05$). This shows that significant differences were found between short, medium and long flicker trials. Specifically, better performance was found on short flicker trials compared to medium flicker trials and worst on long flicker trials compared to both short and medium flicker trials. However, performance across time did not significantly differ across the distinct flicker trials. A similar ANOVA was undertaken for flicker stop signal reaction times (SSRT). No significant differences were found in this ANOVA measuring average flicker SSRTs between time points. However, a

significant difference was found in SSRTs across flicker trials independent of time ($F(2,7) = 11.03$, $p = 0.007$, $\eta^2 = 0.76$). No interaction between time and flicker duration was observed ($F(2,16) = 1.36$, $p > 0.05$) showing that whilst SSRT between flicker durations did differ, no difference was evident between time.

**Table 4.3 SST Descriptive Statistics**

| Trial type | Healthy subjects (n = 26) Baseline | | Healthy subjects (n = 26) Six months | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| **Go + stop trials** | | | | |
| Accuracy (%) | 85.35 | 13.15 | 85.38 | 14.99 |
| Reaction time (ms) | 712.83 | 178.30 | 714.09 | 154.25 |
| **Stop trials** | | | | |
| Accuracy (% inhibited) | 64.38 | 23.04 | 75.87 | 16.92 |
| SSRT (ms) | 283.81 | 219.4 | 257.54 | 99.4 |
| **Short flickers** | | | | |
| Accuracy (% inhibited) | 77.56 | 26.89 | 89.62 | 12.09 |
| SSRT (ms) | 470.5 | 122.97 | 446.85 | 90.67 |
| **Medium flicker** | | | | |
| Accuracy (%inhibited) | 65.38 | 31.53 | 80.77 | 21.89 |
| SSRT (ms) | 524.36 | 164.89 | 456.84 | 44.83 |
| **Long flickers** | | | | |
| Accuracy (% inhibited) | 54.04 | 21.73 | 64.33 | 21.92 |
| SSRT (ms) | 531.51 | 118.43 | 526.83 | 46.12 |

Abbreviations: ms, milliseconds; SD, standard deviation; SSRT, stop-signal reaction time; SST, stop signal task.

Spatial Reasoning *(MRot task)*

A paired sample t-test showed a significant performance difference between same and mirror orientation trials on average across baseline and six months ($t(25)= -2.059$, $p = 0.05$). However, reaction times did not significantly differ between time points on average in the same and mirror oriented shape conditions (Table 4.4).

Further analysis of distinct rotations in a two way repeated measures ANOVA where time and rotation orientations were independent variables showed a significant simple effect of time independent of rotation orientations ($F(1,25) = 4.24$, $p = 0.05$, $\eta^2 = 0.15$). A significant difference was also found on performance across varying degrees of rotation ($F(3,75) = 7.87$, $p < 0.01$, $\eta^2 = 0.24$). More precisely, performance was better on average in the 60 degree condition compared to the other conditions between baseline and follow-up. Moreover, participants' performance was worse in the 180 degree condition compared to all other conditions. Therefore, difficulty increased as a function of shape orientation up to 180 degrees but did not decrease further on higher than 180 degree orientation shapes. No significant interaction was apparent ($F(3,75) = 1.45$, $p > 0.05$) suggesting that performance differences between time and variation in shape rotation from zero did not differ (figure 4.1). An analogues repeated measures ANOVA was conducted to gauge reaction time differences on rotated shape pairs. No significant effect of time was found ($F(1,25) = 0.21$, $p > 0.05$) suggesting that reaction times were similar at baseline and six months devoid of varying shape rotations. However, reaction times on average were significantly different depending on the rotated shape pair presented independent of time points ($F(3,75) = 3.42$, $p = 0.02$, $\eta^2 = 0.12$). This reflects findings from performance conducted above since slowest reaction times were evident on the 180 degree condition indicating the difficulty of this shape orientation (figure 4.2). No interaction effect was apparent ($F(3,75) = 0.10$, $p > 0.05$) indicating that the

mean difference between time points on varying shape orientations from zero, did not significantly differ.

**Table 4.4 MRot task descriptive statistics averages**

| Trial type | Healthy participants (n = 26) | | Healthy participants (n = 26) | |
|---|---|---|---|---|
| | Baseline | | Six months | |
| | Mean | SD | Mean | SD |
| **MRot task performance** | | | | |
| **Same orientation shapes** | | | | |
| Accuracy (%) | 60.81 | 24.91 | 67.54 | 193.84 |
| Reaction Time (ms) | 2102.04 | 324.17 | 2175.27 | 368.31 |
| **Mirror orientation shapes** | | | | |
| Accuracy (%) | 46.63 | 29.33 | 55.29 | 24.73 |
| Reaction Time (ms) | 2153.40 | 291.79 | 2160.11 | 393.27 |
| **Total same and mirror orientation shapes** | | | | |
| Accuracy (%) | 53.72 | 18.56 | 61.41 | 15.66 |
| Reaction Time (ms) | 2127.72 | 285.27 | 2167.69 | 352.85 |
| **Divided degrees** | | | | |
| *60 degrees* | | | | |
| Accuracy (%) | 60.58 | 27.09 | 68.75 | 23.52 |
| RT (ms) | 2026.54 | 313.01 | 2039.74 | 488.75 |
| *120 degrees* | | | | |
| Accuracy (%) | 55.29 | 20.97 | 66.83 | 22.34 |
| RT (ms) | 2167.22 | 423.85 | 2186.38 | 475.96 |
| *180 degrees* | | | | |
| Accuracy (%) | 49.52 | 18.87 | 50.00 | 15.41 |
| RT (ms) | 2181.81 | 407.14 | 2229.25 | 445.54 |
| *300 degrees* | | | | |
| Accuracy (%) | 49.52 | 23.04 | 60.10 | 23.46 |
| RT (ms) | 2135.30 | 379.47 | 2215.37 | 458.76 |

Abbreviations: MRot, mental rotation task; ms, milliseconds; RT, reaction time; SD, standard deviation.

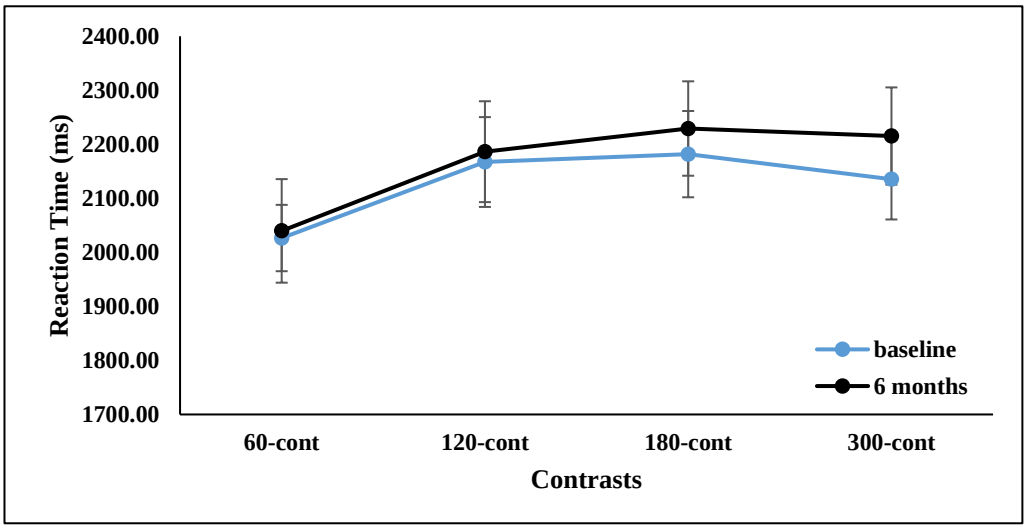


**Figure 4.1:** *MRot task average reaction time at baseline and six months as a function of shape orientation from zero. Abbreviations: cont, control condition; MRot, mental rotation task; ms, milliseconds.*

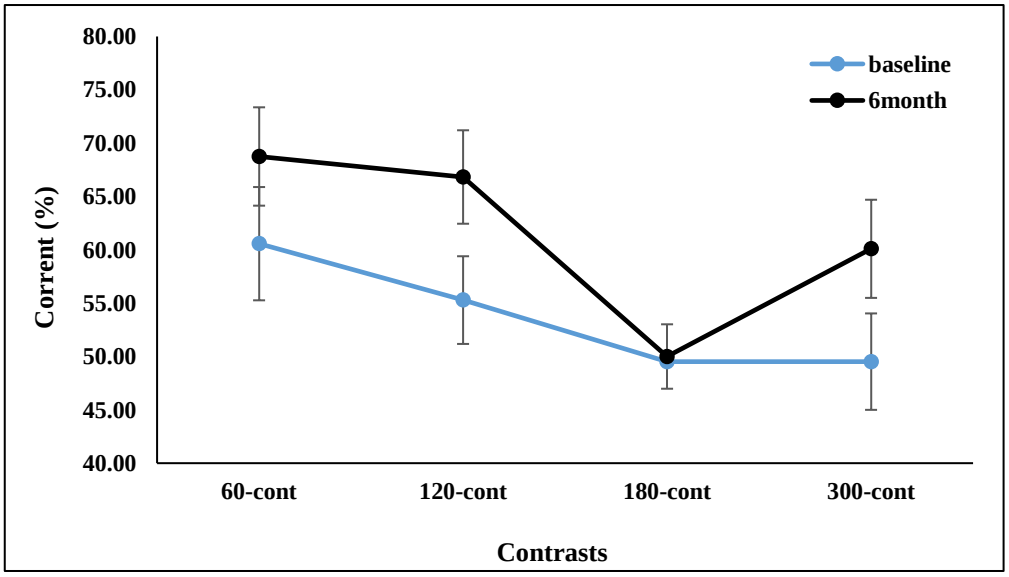


**Figure 4.2:** *MRot task average percentage correct at baseline and follow-up as a function of shape orientation from zero. Abbreviations: cont, control condition; MRot, mental rotation task.*

### 4.10.2 Whole brain cerebral blood flow (Arterial Spin Labelling analysis)

Whole brain cerebral blood flow was explored between longitudinal time points initially to confirm that healthy elderly participants did not have altered levels of cerebral perfusion over time. This was assessed to exclude the presence of perfusion abnormalities that may affect further conducted fMRI BOLD activation outcomes. Eight Participants' CBF scans were available from the baseline data (mean age 69.38 $\pm$ 3.46) and the same participants' scans were used for a longitudinal analysis at six months (mean age 70 $\pm$ 3.39).

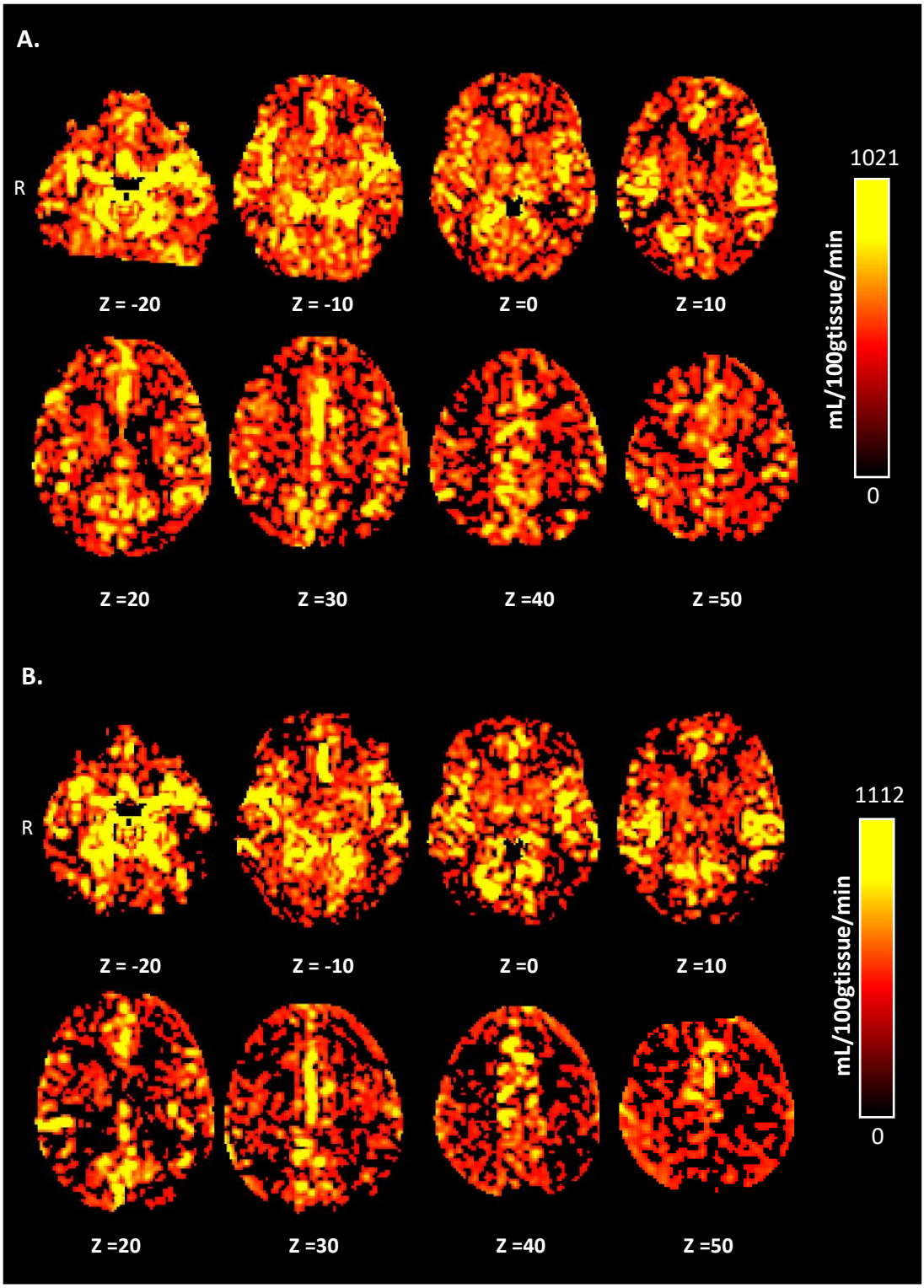


**Figure 4.3:** *Whole brain ASL maps of healthy subjects at: (A) Baseline (n = 8) (B) Six Months (n = 8). The colour bar represents least perfusion in black to red areas of CBF maps and greatest perfusion in yellow areas of CBF maps. Abbreviations: g, grams; min, minute; mL, millilitres, R, right.*

The analysis of ASL at a whole brain level showed no significant differences between estimated baseline CBF (54.20 $\pm$ 11.60 ml/100gtissue/min) (range 0-1021.30 ml/100gtissue/min) and six month CBF (50.09 $\pm$ 8.75 ml/100gtissue/min) (range 0-1112.05 ml/100gtissue/min) in a paired sample t-test ($t(7) = 1.28$, $p > 0.05$). This showed that whole brain CBF was above the level considered abnormal and that CBF did not significantly differ between baseline and six months.

## 4.11 Functional MRI analysis

### 4.11.1 Inhibitory Control (SST)

Tables 4.5 and 4.6 show activation whole brain maps elicited in the twenty-six healthy ageing participants while performing the SST at baseline and follow-up (figure 4.4). To explore the activation patterns associated with inhibitory control, a main contrast of inhibition was generated (go incorrect and stop correct trials compared to stop incorrect and go correct trials) and first assessed within each group. Other contrasts were also generated to assess activation on go trials that reflected motor responses (stop incorrect and go correct trials minus go incorrect and stop correct trials). Activation was found in putative SST areas at baseline in areas including the rIFG (pars triangularis and opercularis), rMFG and right inferior parietal lobe. Additional areas were also apparent including the left IFG and left MFG. Six month activation showed similar patterns of activity. At the group level, exploratory contrasts showed activation in inhibitory areas on go incorrect trials at baseline and six months and go associated activity on stop incorrect trials at six months.

A two sample paired t-test was performed on the main contrast of interest (go incorrect and stop correct compared to stop incorrect and go correct trials) to assess whole brain differences between time points. No significant whole brain activation differences were found between time baseline and follow-up. Furthermore, no significant activation differences were found between baseline and follow-up on the exploratory contrasts.

**Table 4.5:** SST Contrast Activation areas Healthy participants Baseline (n = 26).

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Go incorrect and stop correct trials minus stop incorrect and go correct trials** | | | | | |
| 2027 | 4.29 | -44 | 14 | 32 | L.Middle Frontal/Inferior Frontal Pars operularis |
| 1582 | 3.87 | 44 | -56 | 50 | R.Inferior Parietal Lobe |
| 1001 | 3.93 | -34 | -78 | 46 | L.Lateral Occipital |
| 724 | 3.36 | 44 | 26 | 16 | R.Inferior Frontal Gyrus Pars Triangularis |
| 705 | 4.49 | -44 | 52 | -8 | L.Frontal Pole |
| 449 | 3.47 | 26 | -96 | 18 | R.Lateral Occipital |
| 260 | 3.43 | 2 | 56 | -14 | Frontal Pole/Medial Frontal |
| 210 | 3.5 | 54 | 30 | -2 | R.Inferior Frontal Gyrus Pars Triangularis |
| 191 | 3.17 | 28 | 6 | 48 | R.Middle Frontal Gyrus |
| 151 | 3.41 | -6 | -74 | 46 | L.Precuneus |
| 144 | 3.43 | -28 | -96 | 0 | L.Occipital Pole |
| 131 | 3.14 | -2 | -42 | 32 | Posterior Cingulate Gyrus |
| 123 | 3.68 | -40 | -16 | -34 | L.Temporal Fusiform |
| 104 | 2.97 | 16 | 10 | -24 | R.Frontal Orbital |
| 98 | 3.66 | -38 | -66 | 20 | L.Lateral Occipital |
| 87 | 3.29 | 38 | 44 | 18 | R.Frontal Pole |
| **Stop incorrect and go correct trials minus go incorrect and stop correct trials** | | | | | |
| 361 | 3.5 | -54 | -14 | 12 | L. Secondary Somatosensory |
| 268 | 3.32 | 8 | -68 | 2 | R.lingual Gyrus |
| 111 | 3.31 | -8 | -46 | 62 | L. GM Primary Motor |
| **Exploratory contrasts** | | | | | |
| **Go incorrect minus go correct trials** | | | | | |
| 248 | 3.68 | 60 | 46 | 30 | R.Angular Gyrus |
| **Go correct minus go incorrect trials** | | | | | |
| No Activation | | | | | |
| **Stop incorrect minus stop correct trials** | | | | | |
| No Activation | | | | | |
| **Stop correct minus stop incorrect trials** | | | | | |
| 1894 | 4.79 | 44 | -38 | 46 | R.Supramarginal Gyrus |
| 982 | 4.42 | 24 | 34 | 40 | R.Middle Frontal Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task.
*(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.6:** SST Contrast Activation areas Healthy participants six months (n = 26).

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Go incorrect and stop correct trials minus stop incorrect and go correct trials** | | | | | |
| 3212 | 4.85 | -34 | -68 | 34 | L.Lateral Occipital Superior |
| 1760 | 5.03 | 46 | -66 | 32 | R.Lateral Occipital Superior |
| 1522 | 4.57 | -50 | 14 | 14 | L.Inferior Frontal Gyrus |
| 538 | 4.58 | 4 | -38 | 32 | Posterior Cingulate Gyrus |
| 392 | 4.45 | -58 | -62 | -14 | L. Inferior Temporal Gyrus |
| 335 | 4.09 | 46 | 30 | 28 | R.Middle/Inferior Frontal Gyrus Pars Triangularis |
| 308 | 4.29 | 16 | 16 | -20 | R.Fontal Orbital |
| 235 | 4.17 | 24 | 36 | 30 | R.Frontal Pole |
| 226 | 3.84 | -16 | -54 | 10 | L.Precuneus |
| 220 | 4.29 | 20 | 64 | -4 | R.Frontal Pole |
| 177 | 3.63 | 54 | -62 | 12 | R.Lateral Occipital Inferior |
| 75 | 4.11 | 16 | 42 | -16 | R.Frontal Pole |
| 66 | 3.92 | -6 | 56 | -10 | L.Frontal Pole |
| 64 | 3.59 | 36 | -26 | 42 | R.Postcentral Gyrus |
| 52 | 3.69 | 18 | 32 | -6 | R.White Matter |
| 50 | 3.72 | 42 | -80 | -8 | R.Lateral Occipital Inferior |
| **Stop incorrect and go correct trials minus go incorrect and stop correct trials** | | | | | |
| 26132 | 5.03 | 46 | -66 | 32 | R.Lateral Occipital Superior |
| 4008 | 4.29 | 20 | 64 | -4 | R.Frontal Pole |
| 1455 | 4.29 | 16 | 16 | -20 | R.Orbito-Frontal |
| 305 | 3.4 | -28 | 6 | -10 | L.White Matter |
| 174 | 3.69 | 22 | -12 | -28 | R.Parahippocampal Gyrus |
| 142 | 3.1 | -12 | 52 | 30 | L.Frontal Pole |
| 141 | 3.52 | -10 | -24 | 50 | L.Precentral Gyrus |
| 111 | 3.42 | -14 | 2 | 12 | L.White Matter |
| **Exploratory contrasts** | | | | | |
| **Go incorrect minus go correct trials** | | | | | |
| 914 | 3.66 | 0 | 56 | -14 | R.Medial Frontal Cortex |
| **Go correct minus go incorrect trials** | | | | | |
| No activation | | | | | |
| **Stop incorrect minus stop correct trials** | | | | | |
| 174 | 3.69 | -56 | -28 | 34 | L.Supramarginal Gyrus |
| **Stop correct minus stop incorrect trials** | | | | | |
| 384 | 5.49 | -40 | -74 | 42 | L.Lateral Occipital Cortex |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task.
*(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

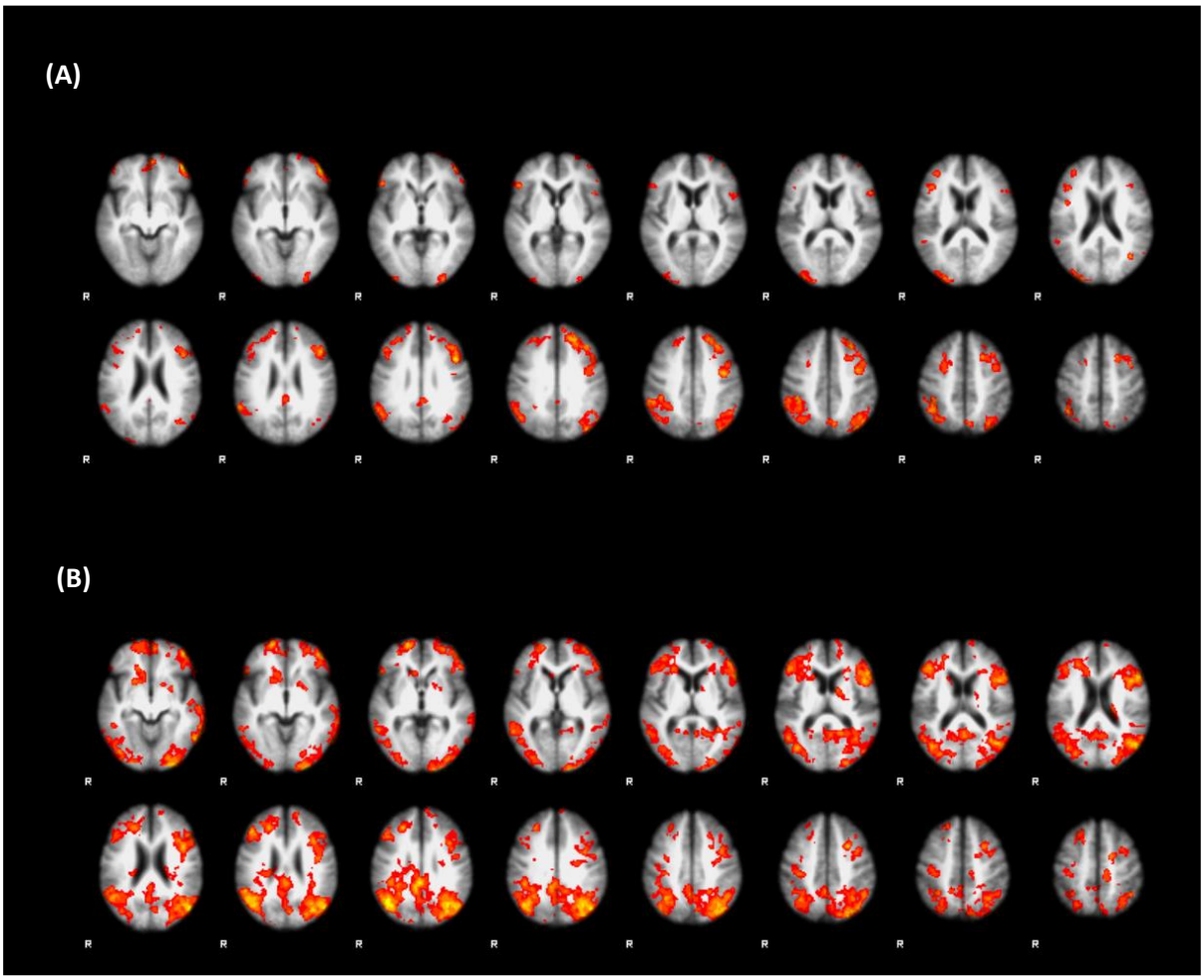


**Figure 4.4:** *SST whole brain group activation. A. baseline group activation. B. Six month group activation. Abbreviations: R, right; SST, stop signal task.*

#### 4.11.1.2 Inhibition Stop signal flicker activation.

Tables 4.7 and 4.8 show activation areas on the SST in healthy participants at baseline and follow-up. To explore the activation patterns associated with inhibitory control, durations from long correct, medium correct and short flicker trials were separated and compared in contrasts to overall go trials. These contrasts were assessed for activation at single time points (baseline and six months). left middle frontal gyrus, left supramarginal gyrus and left precentral gyrus regions were activation on long correct compared to go correct trials. Most putative inhibition regions on the medium correct compared to go correct trials activated were in the rIFG pars opercularis, left middle frontal gyrus, right middle frontal gyrus and left supramarginal gyrus. The short correct trials compared to go correct trials showed activation in the parietal cortex mostly consisting of areas including the right supramarginal gyrus and left lingual gyrus.

Two sample paired t-tests were performed on all contrasts to assess whole brain differences between baseline and six months. No significant differences emerged between baseline and follow-up on the contrasts of long correct compared to go correct trials; medium correct trials compared to go correct trials and short correct trials compared to go correct trials.

**Table 4.7:** SST Flicker Contrast Activations in Healthy Participants at Baseline

| Baseline healthy participants (n = 26) | | | | | |
|---|---|---|---|---|---|
| **Cluster** | | **MNI Coordinates** | | | |
| **(Voxels)** | **Z-MAX** | **X** | **Y** | **Z** | **Region** |
| **Long correct trials minus go correct trials** | | | | | |
| 6748 | 4.71 | 54 | 12 | 2 | R.Inferior Frontal Gyrus Pars Opercularis |
| 4282 | 4.58 | 62 | -46 | 32 | R.Supramarginal Gyrus |
| 3456 | 4.09 | 8 | -80 | 10 | R.Intracalcarine |
| 1576 | 4.81 | -68 | -40 | 20 | L.Supramarginal Gyrus |
| 241 | 3.15 | -50 | 30 | 18 | L.Inferior Frontal Gyrus Pars Triangularis |
| 228 | 3.55 | -40 | 54 | 0 | L.Frontal Pole |
| 199 | 3.34 | -32 | 18 | -4 | L.Insular |
| 186 | 3.53 | -54 | -6 | 26 | L.Precentral Gyrus Premotor |
| **Medium correct trials minus go correct trials** | | | | | |
| 3539 | 4.33 | 32 | -60 | -16 | R.Temporal Fusiform Gyrus |
| 2022 | 4.35 | 62 | -34 | 20 | R.Planum Temporal |
| 1558 | 4 | 32 | 26 | -10 | R.Frontal Orbital |
| 1373 | 3.62 | 32 | 44 | 14 | R.Frontal Pole |
| 1005 | 4.16 | -56 | -32 | 10 | L.Planum Temporal |
| 960 | 4.32 | -4 | 32 | 28 | Parascingulate Gyrus |
| 753 | 4.2 | -52 | -72 | -2 | L.Lateral Occipital Inferior |
| 331 | 3.48 | -44 | 46 | 16 | L.Frontal Pole |
| 236 | 3.31 | -34 | 14 | -2 | L.Insular |
| 142 | 3.01 | 54 | -24 | -12 | R.Middle Temporal Gyrus |
| **Short correct trials minus go correct trials** | | | | | |
| 1899 | 4.11 | -18 | -80 | -12 | L.Occipital Fusiform Gyrus |
| 1838 | 4.57 | 54 | -36 | 32 | R.Supramarginal Gyrus |
| 1478 | 4.3 | 58 | 12 | 8 | R.Inferior Frontal Gyrus |
| 800 | 4.33 | -48 | -82 | 2 | L.Lateral Occipital Inferior |
| 750 | 4.07 | 48 | -62 | -10 | R.Lateral Occipital Inferior |
| 709 | 4.37 | -58 | -54 | 36 | L.Angular Gyrus |
| 463 | 4.18 | -4 | 32 | 28 | R.Parascingulate Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task.
*(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.8:** SST Flicker Contrast Activations in Healthy Participants Six Month Follow-up

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **Long correct trials minus go correct trials** | | | | | |
| 4879 | 5 | 60 | -42 | 32 | R.Supramarginal Gyrus |
| 4244 | 4.78 | 30 | 26 | -10 | R.Fronto-Orbital |
| 1569 | 4.36 | -46 | -56 | 42 | L.Angular Gyrus |
| 1296 | 4.24 | -38 | 10 | -2 | L.Insular |
| 1153 | 4.3 | -60 | -52 | 0 | L.Middle Temporal Gyrus |
| 825 | 4.35 | -6 | 32 | 18 | Anterior Cingulate Gyrus |
| 767 | 3.77 | 0 | -102 | 6 | Occipital Pole |
| 399 | 4.09 | -44 | 8 | 36 | L.Middle Frontal Gyrus |
| **Medium correct trials minus go correct trials** | | | | | |
| 2394 | 4.28 | -52 | -60 | 24 | L.Angular Gyrus |
| 2072 | 3.85 | 64 | -40 | 6 | R.Middle Temporal Gyrus |
| 1807 | 3.67 | -12 | 54 | 28 | L.Frontal Pole |
| 1218 | 4.45 | 48 | -56 | 42 | R.Angualar Gyrus |
| 1094 | 3.6 | -26 | 4 | 34 | L.White Matter |
| 847 | 3.92 | 0 | 32 | 20 | Anterior Cingulate Gyrus |
| 666 | 3.46 | -2 | -86 | -8 | Lingual Gyrus Middle |
| 577 | 3.75 | 30 | 20 | -22 | R.Frontal Orbital |
| 552 | 3.92 | -56 | 6 | 2 | L.Precentral Gyrus |
| **Short correct trials minus go correct trials** | | | | | |
| 1551 | 4.56 | 52 | -54 | 40 | R.Angular Gyrus |
| 1358 | 4.35 | 50 | -70 | -14 | R.Lateral Occipital Inferior |
| 806 | 4.32 | -48 | -58 | 44 | R.Angular Gyrus |
| 791 | 4.54 | 30 | 28 | -12 | R.Frontal Orbital |
| 650 | 3.65 | 40 | 44 | 14 | R.Frontal Pole |
| 549 | 3.95 | 40 | 24 | 40 | R.Middle Frontal Gyrus |
| 421 | 3.5 | -44 | -70 | -4 | L.Lateral Occipital Inferior |
| 378 | 4.01 | -44 | 8 | 36 | L.Middle Frontal Gyrus |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right; SST, stop signal task.
*(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

#### 4.11.1.3 Region of interest analysis with main contrast and distinct flicker contrasts.

Region of interest analyses was undertaken to assess regional consistency of mean BOLD signal change (%) between time points on the main inhibition contrast (table 4.9) and flicker contrasts (table 4.10). The rIFG, rMFG, left inferior temporal gyrus (LITG) and left middle frontal gyrus (LMFG) were masked using FSL atlas and the FSL maths command to binary masks (Harvard-Oxford Cortical Structural Atlas). A repeated measures ANOVA was conducted with time (baseline and six months) and percent signal change in regions on the main contrast and flicker contrasts separately. Testosterone was added as a covariate to assess its effect on signal change between time points in ROIs as it was the main factor of interest in the conducted study. The effect of testosterone was controlled for on all main effects. Its relation to interaction was reported only.

*Main Contrast (go incorrect and stop correct trials compared to stop incorrect and go correct trials).*

The ROI analysis on the inhibition contrast showed no significant main effect of averaged signal change across all ROIs between time points ($F(1, 24) = 0.35$, $p > 0.05$). A significant main effect was shown between the signal changes of ROIs regardless of time. Mauchly's test of sphericity was violated ($X_2(2) = 9.32$, $p < 0.01$), therefore, degrees of freedom were adjusted using Greenhouse Geisser estimates of sphericity ($\varepsilon$=0.75). This revealed a significant ANOVA ($F(1.50, 36.00) = 7.07$, $p < 0.01$, $\eta^2 = 0.38$). No significant effect was found on the interaction term of signal change in ROIs between time points ($F(2, 48) = 0.89$, $p > 0.05$). Variance in testosterone level change approached significance on the interaction term between baseline and six months ($F(2, 48) = 2.63$, $p > 0.05$) (Figure 4.5).

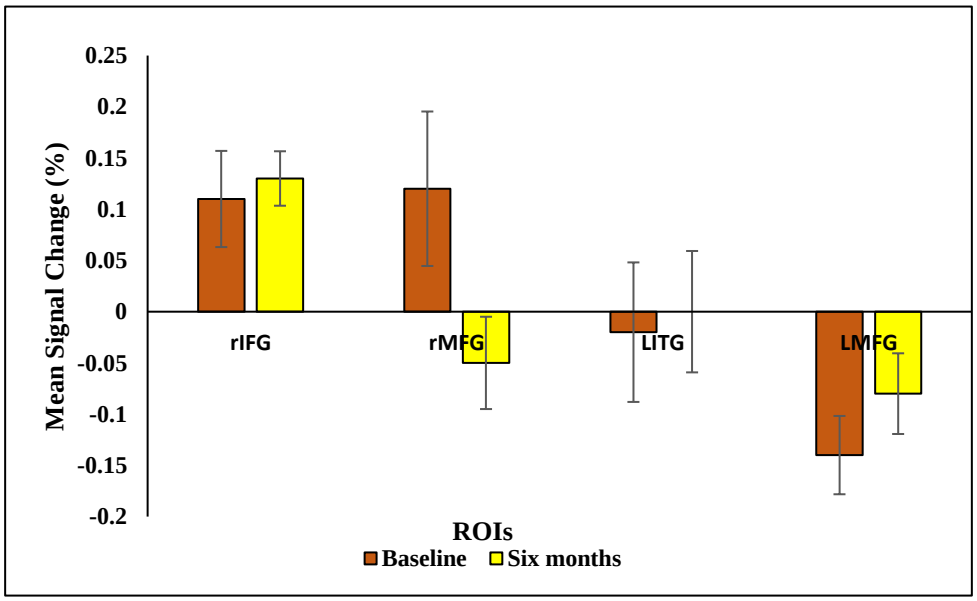


**Figure 4.5:** *Mean signal change on main SST contrast (go incorrect and stop correct compared to stop incorrect and go incorrect). Abbreviations: IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; L, left; MFG, middle frontal gyrus; r, right; SST, stop signal task.*

Flicker Signal Change

*Contrast long correct compared to go correct trials.*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 0.20$, $p > 0.05$). A main effect was shown between the signal changes of ROIs when controlling for time. Mauchly's test of sphericity was violated ($X_2(5) = 36.56$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.51$) ($F(1{,}55, 37.10) = 6.65$, $p < 0.01$, $\eta^2 = 0.22$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(5) = 30.64$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.57$) ($F(1.71, 40.95) = 0.58$, $p > 0.05$). Testosterone did not covary with signal change in ROIs between time points ($F(1.71, 40.95) = 0.52$, $p > 0.05$).

*Contrast medium correct compared to go correct trials.*

No significant main effect of signal change averaged across all ROIs was found between time points ($F(1, 24) = 0.10$, $p > 0.05$). No main effect was shown of signal change between ROIs when controlling for time. Mauchly's test of sphericity was violated ($X_2(5) = 45.45$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.50$) ($F(1,49, 35.67) = 1.73$, $p > 0.05$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(5) = 33.51$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.56$) ($F(1.67, 39.98) = 1.16$, $p > 0.05$). Change in testosterone between time did not covary with change in regional BOLD signal between time points ($F(1.67, 39.98) = 0.25$, $p > 0.05$).

*Contrast short correct compared to go correct trials.*

The ROI analysis showed no significant main effect of signal change averaged across all ROIs between time points ($F(1, 24) = 0.03$, $p > 0.05$). No main effect was found when signal change of ROIs were compared while controlling for time. Mauchly's test of sphericity was violated ($X_2(5) = 35.19$, $p < 0.01$), therefore, degrees of freedom were altered using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.52$). This revealed a sign non-significant ANOVA ($F(1.55, 37.25) = 2.24$, $p > 0.05$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(5) = 28.99$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.57$) ($F(1.70, 40.98) = 0.16$, $p > 0.05$). Difference in testosterone between baseline and six months did not covary with any

differences in the interaction between time points and mean regional signal change ($F(1.70, 40.98) = 0.41$, $p > 0.05$).

**Table 4.9**: ROI Signal Mean Signal Change Descriptives SST

| | **Healthy subjects (n = 26) Baseline** | | **Healthy subjects (n = 26) Six months** | |
|---|---|---|---|---|
| **Region** | **Signal change (%)** | **SD** | **Signal change (%)** | **SD** |
| Contrast go incorrect and stop correct compared to go incorrect and go correct trials | | | | |
| rIFG | 0.11 | 0.24 | 0.13 | 0.14 |
| rMFG | 0.12 | 0.38 | -0.05 | 0.23 |
| LITG | -0.02 | 0.35 | 0.00 | 0.30 |
| LMFG | -0.14 | 0.19 | -0.08 | 0.20 |

Abbreviations: IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; L, left; MFG, middle frontal gyrus; R, right; ROI, region of interest; SD, standard deviation; SST, stop signal task *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.10**: Mean Signal Change Descriptive Stop Signal Flickers

| Region | Healthy subjects (n = 26) Baseline | | Healthy subjects (n = 26) Six months | |
|---|---|---|---|---|
| | **Signal change (%)** | **SD** | **Signal change (%)** | **SD** |
| **Contrast long correct compared to go correct trials** | | | | |
| rIFG | 0.08 | 0.18 | 0.10 | 0.20 |
| rMFG | 0.01 | 0.42 | 0.12 | 0.45 |
| LITG | 0.24 | 0.39 | 0.25 | 0.26 |
| LMFG | 0.14 | 0.24 | 0.13 | 0.20 |
| **Contrast medium correct compared to go correct trials** | | | | |
| rIFG | 0.05 | 0.16 | 0.05 | 0.20 |
| rMFG | 0.09 | 0.21 | 0.08 | 0.22 |
| LITG | 0.14 | 0.38 | 0.14 | 0.30 |
| LMFG | 0.00 | 0.34 | 0.13 | 0.44 |
| **Contrast short correct compared to go correct trials** | | | | |
| rIFG | 0.06 | 0.14 | 0.05 | 0.19 |
| rMFG | 0.10 | 0.17 | 0.08 | 0.21 |
| LITG | 0.15 | 0.24 | 0.14 | 0.27 |
| LMFG | 0.04 | 0.31 | 0.08 | 0.45 |

Abbreviations: IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; L, left; MFG, middle frontal gyrus; R, right; SD, standard deviation. *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

Overlapping of distinct flicker contrasts trials in figure 4.6 show areas contributing to activation on the separate flicker contrasts. This provides a better view that can be visually inspected. Activation seemed to be apparent in areas including the rIFG and RMFG. Furthermore, parietal areas were activated supporting a fronto-parietal network that is in line with an executive function network at baseline and follow-up. Additional areas of activity were present in the left IFG and left MFG. The lc-gc and mc-gc contrasts showed most activation.

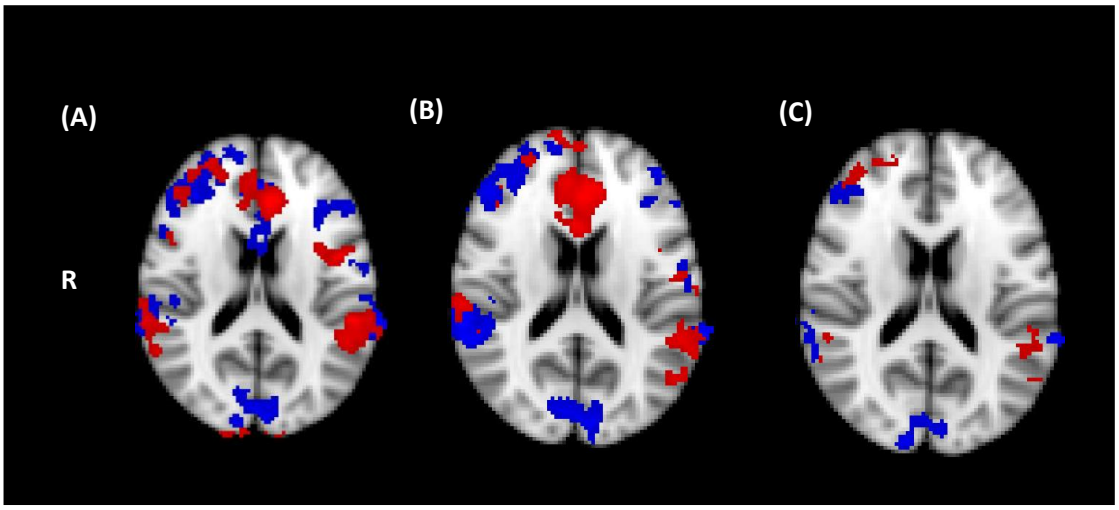


**Figure 4.6:** *SST overlap images of healthy participants at baseline and six-month follow-up where red activation colour maps indicate activation of participant group activation at baseline and purple activation group maps at six months. (A) Group activation maps on the contrast lc-gc. (B) Group activation maps on the contrast mc-gc. (C) Group activation maps on the contrast sc-gc (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations*: *lc, long correct; mc, medium correct; R, right; sc, short correct; SST, stop signal task.*

#### 4.11.1.4 Inhibition Intra-class Correlation Analysis

Intra-class correlation (ICC) analysis illustrates reliability across specific regions (Table 4.11). This was calculated by comparing baseline group activation in SPM with a paired sample t-test to follow-up group activation (go incorrect and stop correct activation compared to stop incorrect and go correct activation). Median intra-voxel reliability showed the most reliability on the main contrast and subsequently less reliability across regions as task difficulty decreased. This indicates that reliably greater activation was required in putative stop signal areas as task difficulty increased.

**Table 4.11:** Intra-class Correlations SST (uncorrected, p<0.001)

| ROI | MNI coordinates | | | t-max | main contrast | | $ICCv_{med}$ (STE) | | Average Flickers |
|---|---|---|---|---|---|---|---|---|---|
| Contrast | X | Y | Z | | gin+sc-sin+gc | lc-gc | mc-gc | sc-gc | ICC(STE) |
| R.Supra-Marginal Gyrus | 54 | -34 | 35 | 7.25 | 0.57 (0.08) | 0.32 (0.12) | 0.01 (0.10) | 0.07 (0.08) | 0.13 (0.09) |
| ACC | 3 | 35 | 23 | 7.21 | 0.39 (0.13) | -0.01 (0.11) | 0.11 (0.09) | 0.18 (0.08) | 0.09 (0.06) |
| R.IFG Pars Opercularis | 54 | 14 | -1 | 6.84 | 0.43 (0.10) | 0.29 (0.09) | 0.04 (0.12) | 0.09 (0.06) | 0.14 (0.08) |
| L.IFG Pars Opercularis | -45 | 11 | 20 | 6.83 | 0.43 (0.15) | 0.15 (0.07) | -0.10 (0.10) | 0.12 (0.15) | 0.08 (0.08) |
| R.MFG | 39 | 38 | 26 | 6.70 | 0.41 (0.09) | 0.30 (0.09) | 0.04 (0.12) | 0.09 (0.07) | 0.14 (0.08) |
| **Average Contrasts ICC (STE)** | | | | | 0.45 (0.032) | 0.21 (0.06) | 0.02 (0.03) | 0.11 (0.02) | |

Abbreviations represent: ACC, Anterior Cingulate Gyrus; gin+sc-sin+gc, go incorrect and stop correct compared to stop incorrect and go correct; IFG, Inferior Frontal Gyrus; lc-gc, long correct trials compared to go correct trials; mc-gc, medium correct trials compared to go correct trials; MFG, Middle Frontal Gyrus; MNI, Montreal Neurological Institute; sc-gc, short correct trials compared to go correct trials; STE, standard error; SST, stop signal task.

### 4.11.2 Spatial Reasoning (MRot task)

Tables 4.12 and 4.13 show activation regions of the MRot task at baseline and six months. Activation maps at baseline and follow-up are shown in figure 4.7. Activation was apparent in the parietal, precentral (premotor), precuneous, and occipital lobes at baseline and six months in healthy participants. A two samples paired t-test was run to assess any whole brain differences on the main contrast of rotations compared to control task (exp-cont) between baseline and six months. This revealed no significant activation differences between time points.

**Table 4.12:** MRot task Contrast Activations Separated by Healthy Participants assessed at Baseline (n = 26).

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **contrast exp-cont** | | | | | |
| 30900 | 5.12 | -14 | -98 | 4 | L.Occipital pole |
| 849 | 3.67 | -34 | -12 | 50 | L.Precentral Gyrus |
| 800 | 3.86 | 26 | 4 | 66 | R.Superior Frontal Gyrus |
| 626 | 3.73 | 12 | 18 | 42 | Paracingulate Gyrus |
| 564 | 3.57 | 32 | 24 | 6 | R.Insular Cortex |
| **contrast cont-exp** | | | | | |
| No activation | | | | | |

Abbreviations: cont, control condition; exp, experiment condition; L, left; MRot, mental rotation task; MNI, Montreal Neurological Institute; R, right *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.13:** MRot task Contrast Activations Separated by Healthy Participants assessed at six months (n = 26).

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **contrast exp-cont** | | | | | |
| 19379 | 5.02 | -18 | -80 | 50 | L.Lateral Occipital Cortex Superior |
| 295 | 3.39 | 56 | -32 | 46 | R.Supramarginal Gyrus |
| 212 | 3.49 | 60 | -2 | 18 | R.Precentral Gyrus |
| 181 | 3.21 | -16 | -24 | 4 | L.Thalamus |
| **contrast cont-exp** | | | | | |
| 609 | 3.41 | 46 | -58 | 32 | R.angular Gyrus |
| 598 | 3.95 | 12 | 56 | 32 | R.Frontal pole |
| 543 | 3.71 | -52 | -66 | 42 | L.Lateral Occipital Superior |

Abbreviations: cont, control condition; exp, experiment condition; L, left; MRot, mental rotation task; MNI, Montreal Neurological Institute; R, right *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

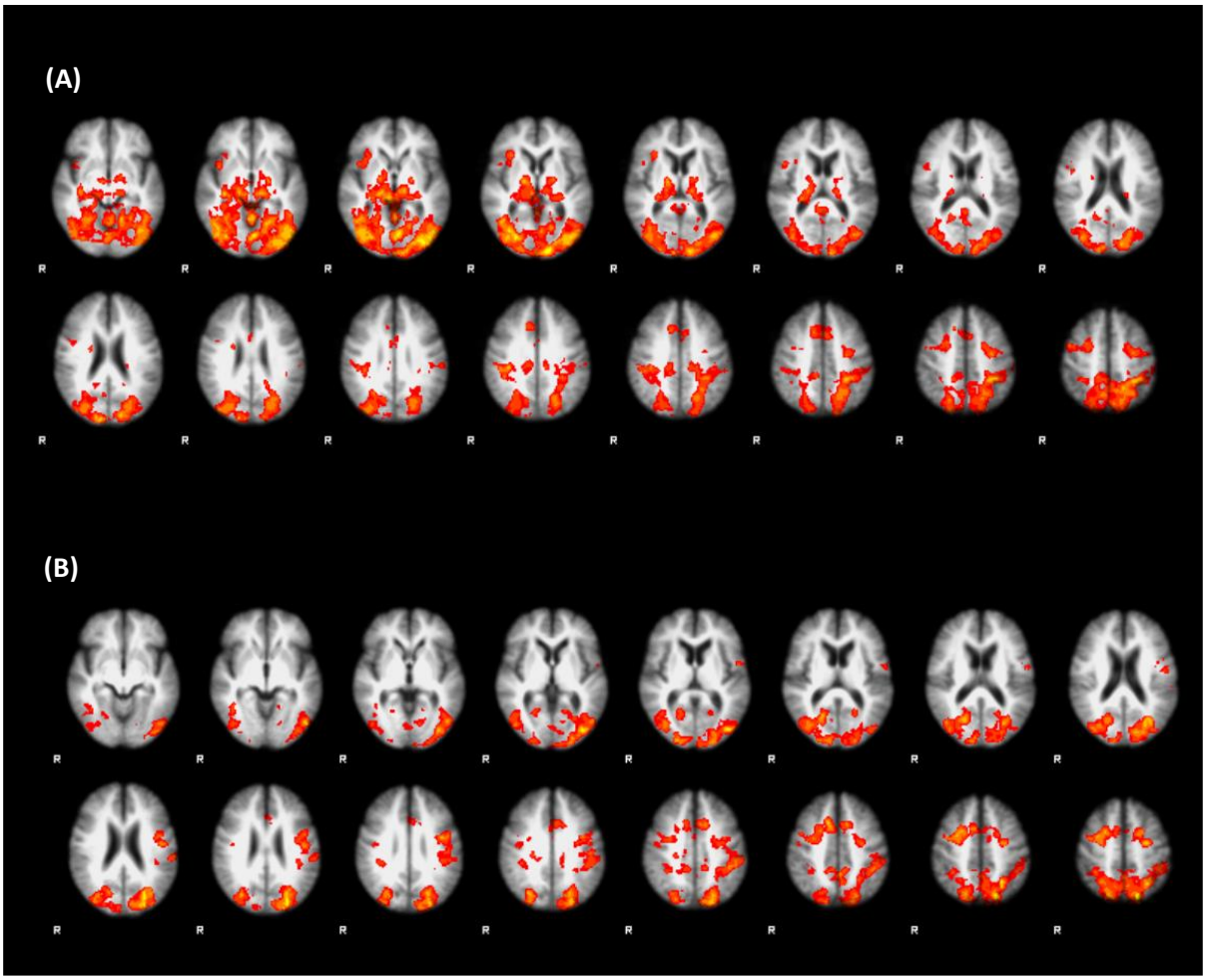


**Figure 4.7:** *MRot task whole brain activation. (A) Baseline MRot activation. (B) six month MRot activation. Abbreviations: MRot, mental rotation task; R, right.*

### 4.11.2.1 Spatial reasoning distinct rotation activation

Tables 4.14 and 4.15 shows activation during distinct rotation parts of the task when shape pairs were required to be rotated at baseline and follow-up. No significant whole brain differences were found between baseline and follow-up activations assessed with a two samples paired t-test.

**Table 4.14:** MRot task Contrast Activations in healthy participants at baseline (n = 26)

| Cluster (Voxels) | Z-MAX | MNI Coordinates | | | Region |
|---|---|---|---|---|---|
| | | X | Y | Z | |
| **contrast 60 degree-control** | | | | | |
| 3016 | 4.43 | -44 | -90 | -6 | L.Occipital Cortex Inferior |
| 947 | 3.79 | 54 | -76 | -16 | R.Occipital Cortex Inferior |
| 672 | 3.5 | 4 | -56 | 52 | R.Precuneus Cortex |
| **contrast 120 degree-control** | | | | | |
| 1454 | 3.53 | -22 | -70 | 40 | L.Lateral Occipital Cortex Superior |
| 606 | 3.16 | 32 | -84 | 30 | R.Lateral Occipital Cortex Superior |
| 543 | 3.61 | -44 | -86 | -6 | L.Lateral Occipital Cortex Inferior |
| **contrast 180-control** | | | | | |
| 7843 | 4.72 | -36 | -44 | 40 | L.Supramarginal Gyrus |
| 3771 | 4.29 | 36 | 24 | -6 | R.Frontal orbital Cortex |
| 1099 | 3.75 | 44 | -82 | 18 | R.Lateral Occipital Cortex Superior |
| 685 | 4.3 | -46 | 8 | 20 | L.Inferior Frontal Gyrus |
| 653 | 3.88 | -30 | -58 | -12 | L.Temporal Occipital fusiform Cortex |
| 454 | 3.53 | -32 | -4 | 50 | L.Precentral Gyrus premotor Cortex |
| **contrast 300-control** | | | | | |
| 4738 | 4.43 | 24 | -64 | 60 | R.Lateral Occipital Cortex Superior |
| 4176 | 4.42 | -46 | -80 | -4 | L.Later Occipital Cortex Inferior |

Abbreviations: L, left; MRot, mental rotation task; MNI, Montreal Neurological Institute; R, right *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.15:** MRot task Contrast Activations at six months in healthy participants (n = 26).

| Cluster (Voxels) | Z-MAX | MNI Coordinates X | Y | Z | Region |
|---|---|---|---|---|---|
| **contrast 60 degree-control** | | | | | |
| No activation | | | | | |
| **contrast 120 degree-control** | | | | | |
| No activation | | | | | |
| **contrast 180-control** | | | | | |
| 15847 | 4.75 | -24 | -68 | 36 | L.Lateral Occipital Superior |
| 3296 | 4.58 | 2 | 18 | 36 | Anterior Cingulate Gyrus |
| 523 | 3.35 | 56 | -32 | 46 | R.Supramarginal Gyrus |
| **contrast 300-control** | | | | | |
| 9430 | 4.39 | -20 | -76 | 48 | L.Lateral Occipital Superior |
| 2709 | 4.32 | 28 | 0 | 50 | R.Precentral Gyrus |
| 575 | 3.66 | 60 | -2 | 8 | R.Central Opericular |

Abbreviations: L, left; MRot, mental rotation task; MNI, Montreal Neurological Institute; R, right
*(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

#### 4.11.2.2 Region of interest analysis with main contrast and distinct rotation contrasts.

Subsequently, ROI analysis was undertaken to assess regional consistency of mean BOLD signal change (%) between time points on the main contrast to compare mean signal change during rotation compared to the control condition in specific regions. Regions were extracted by first masking the regions of interest from putative MRot task regions. The left precentral gyrus, right superior frontal gyrus and right supramarginal gyrus were masked using FSL atlas and the FSL maths commands (Harvard-Oxford Cortical Structural Atlas). A repeated measures ANOVA was conducted with time (baseline and six months) and percent signal change in regions on the main exp-cont contrast (Table 4.16) and contrasts in the separated rotations (Table 4.17). Testosterone was added as a covariate to assess its effect on signal change between time points in ROIs.

*Contrast Exp-cont*

The ROI analysis on the exp-cont contrast showed no significant main effect of averaged signal change across all ROIs between time points ($F(1, 24) = 0.63$, $p > 0.05$). A main effect was found between the signal changes of ROIs regardless of time ($F(2, 48) = 3.33$, $p = 0.05$, $\eta^2 = 0.12$). No significant effect was found on the interaction term of signal change in ROIs between time points ($F(2, 48) = 2.19$, $p > 0.05$). Testosterone change between time points did not account for any difference in this interaction ($F(2, 48) = 0.21$, $p > 0.05$).

*Contrast 60-cont*

The ROI analysis showed a significant main effect of averaged signal change across all ROIs between time points ($F(1, 24) = 8.56$, $p < 0.01$, $\eta^2 = 0.25$). A significant main effect was found between signal change in ROIs regardless of time. Mauchly's test of sphericity was violated ($X_2(2) = 20.68$, $p < 0.01$), therefore, degrees of freedom were adjusted using Greenhouse Geisser estimates of sphericity ($\varepsilon$=0.63) ($F(1.27, 31.70) = 10.81$, $p < 0.01$, $\eta^2 = 0.30$). A significant interaction was found of signal change in ROIs between time points. Mauchly's test of sphericity was violated ($X_2(2) = 15.29$, $p < 0.01$), therefore, degrees of freedom were adjusted using Greenhouse Geisser estimates of sphericity ($\varepsilon$=0.68) ($F(1.36, 33.99) = 14.70$, $p < 0.01$, $\eta^2 = 0.37$). A paired sample t-test showed a significant signal change difference in the left precentral gyrus between baseline and six months ($t(25) = 4.70$, $p < 0.01$) (Figure 4.8). Moreover, testosterone had a significant effect on the interaction term ($F(1.46, 35.04) = 9.96$, $p < 0.01$, $\eta^2 = 0.29$) demonstrating that it accounted for a significant proportion of variance in signal change in the left precentral gyrus.

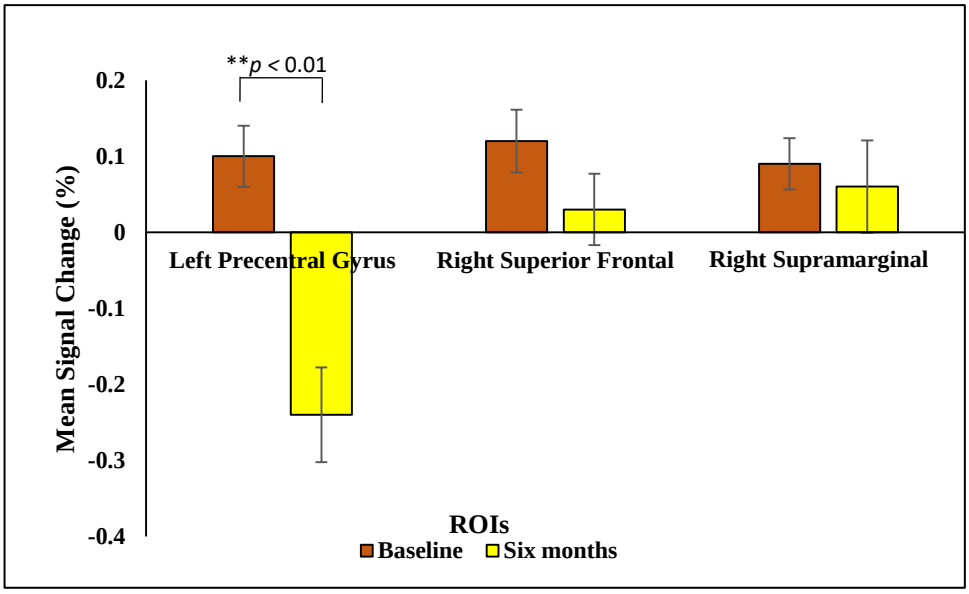


**Figure 4.8:** *MRot mean signal change on 60-cont contrast showing a significant difference in the left precentral gyrus. Abbreviations: MRot, mental rotation task; ROIs, regions of interest.*

*Contrast 120-cont*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 1.25$, $p > 0.05$). A main effect was shown between the signal changes of ROIs when controlling for time ($F(2, 48) = 3.54$, $p = 0.04$, $\eta^2 = 0.13$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(2) = 7.96$, $p = 0.02$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.77$) ($F(1.54, 37.14) = 0.89$, $p > 0.05$). Change in testosterone did not covary with any differences between signal change between regions and time ($F(1.54, 37.14) = 3.39$, $p > 0.05$).

*Contrast 180-cont*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 1.55$, $p > 0.05$). No main effect was shown between the signal changes of ROIs when controlling for time. Mauchly's test of sphericity was violated ($X_2(2) = 10.32$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of

sphericity (ε=0.73) ($F(1.47, 35.25) = 1.25$, $p > 0.05$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(2) = 13.53$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity (ε=0.69) ($F(1.38, 33.23) = 1.11$, $p > 0.05$).

*Contrast 300-cont*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 0.01$, $p > 0.05$). A significant main effect was shown between the signal changes of ROIs when controlling for time. Mauchly's test of sphericity was violated ($X_2(2) = 9.32$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity (ε=0.76) ($F(1.50, 36.00) = 6.66$, $p < 0.01$, $\eta^2 = 0.22$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months ($F(2, 48) = 0.89$, $p > 0.05$). Testosterone was not shown to covary with change in signal between baseline and six months ($F(2, 48) = 2.63$, $p > 0.05$)

**Table 4.16:** Mean Signal Change Descriptives MRot task

| | **Healthy subjects (n = 26) Baseline** | | **Healthy subjects (n = 26) Six months** | |
|---|---|---|---|---|
| **Region** | **Signal change (%)** | **SD** | **Signal change (%)** | **SD** |
| **Contrast Exp-Cont** | | | | |
| Left Precentral Gyrus | 0.10 | 0.20 | 0.08 | 0.13 |
| Right Superior Frontal | -0.11 | 0.18 | -0.05 | 0.11 |
| Right Supramarginal | 0.03 | 0.19 | 0.04 | 0.14 |

Abbreviations: cont, control condition; exp, experiment condition; Montreal Neurological Institute; SD, standard deviation *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

**Table 4.17:** Mean Signal Change Descriptives Distinct MRot rotations

| | **Healthy subjects (n = 26)** | | **Healthy subjects (n = 26)** | |
|---|---|---|---|---|
| | **Baseline** | | **Six months** | |
| **Region** | **Signal change (%)** | **SD** | **Signal change (%)** | **SD** |
| Contrast 60-cont | | | | |
| Left Precentral Gyrus | 0.10 | 0.20 | -0.24 | 0.32 |
| Right Superior Frontal | 0.12 | 0.21 | 0.03 | 0.24 |
| Right Superamarginal | 0.09 | 0.17 | 0.06 | 0.31 |
| Contrast 120-cont | | | | |
| Left Precentral Gyrus | 0.08 | 0.20 | 0.13 | 0.15 |
| Right Superior Frontal | 0.11 | 0.17 | 0.19 | 0.23 |
| Right Superamarginal | 0.16 | 0.17 | 0.14 | 0.29 |
| Contrast 180-cont | | | | |
| Left Precentral Gyrus | 0.09 | 0.16 | 0.17 | 0.18 |
| Right Superior Frontal | 0.08 | 0.16 | 0.14 | 0.18 |
| Right Superamarginal | 0.13 | 0.26 | 0.15 | 0.17 |
| Contrast 300-cont | | | | |
| Left Precentral Gyrus | -0.02 | 0.23 | 0.01 | 0.22 |
| Right Superior Frontal | 0.01 | 0.23 | 0.02 | 0.21 |
| Right Superamarginal | 0.07 | 0.29 | 0.06 | 0.24 |

Abbreviations: cont, control condition; Montreal Neurological Institute; SD, standard deviation. *(Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05).*

Overlap images assist in demonstrating activation visually in healthy participants at baseline and follow-up (figure 4.9). No activation was apparent in contrasts requiring least rotation at six months, however, activation was present at baseline (60-cont and 120-cont). The 180-cont contrast showed most activation in putative MRot areas including the bilateral supramarginal gyri, precentral gyri and parietal lobe areas.

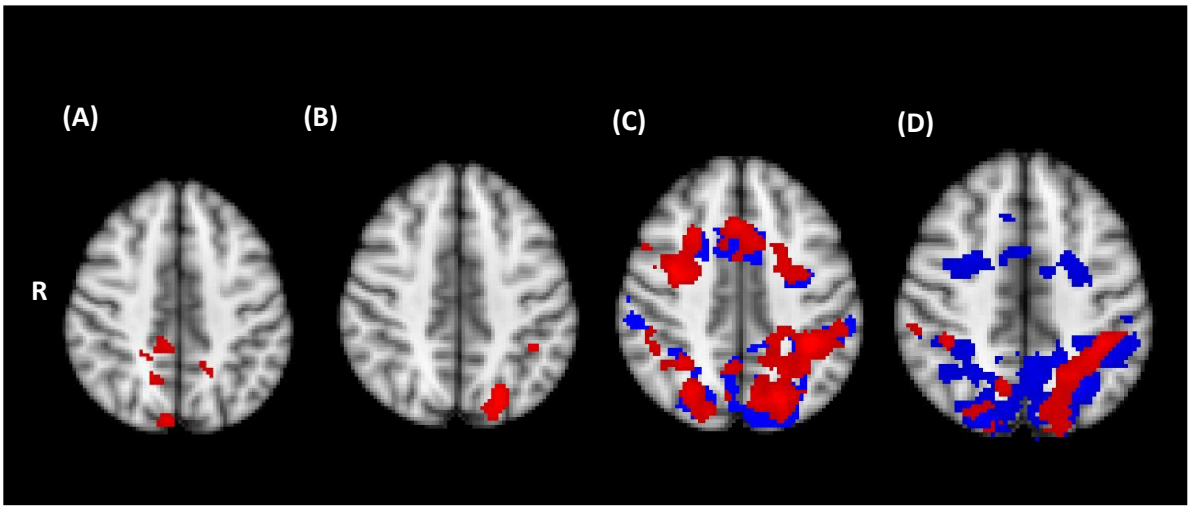


**Figure 4.9:** *MRot overlap images of healthy participants at baseline and six-month follow-up where red activation colour maps indicate group activation at baseline and blue activation group maps show activation at six months. (A) Group activation maps on the contrast 60-cont. (B) Group activation maps on the contrast 120-cont. (C) Group activation maps on the contrast 180-cont. (D) Group activation maps on the contrast 180-cont. (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations: cont, control condition; MRot, mental rotation task; R, right.*

#### 4.11.2.3 Spatial reasoning Intra-class Correlation Analysis

Intra-class correlation (ICC) analysis shows reliability across specific regions. Regions were selected from a one sample t-test of exp-cont contrast at $p < 0.01$ from baseline group activation in SPM**.** Intra-voxel reliability was found to be highest in the main contrast (exp-cont).

**Table 4.18:** Intra-class Correlations MRot task

| Contrasts ROI | MNI coordinates X | Y | Z | t-max | $ICC_v$ med (STE) exp-cont | 120-cont | 180-cont | 300-cont | 60-cont | Average ROI ICC (STE) |
|---|---|---|---|---|---|---|---|---|---|---|
| L.Precentral | -45 | -4 | 23 | 5.18 | 0.30 (0.14) | -0.08 (0.16) | 0.10 (0.11) | 0.22 (0.17) | -0.04 (0.18) | 0.05 (0.07) |
| L.Precuneus | -18 | -76 | -35 | 4.88 | 0.32 (0.13) | 0.08 (0.09) | 0.32 (0.16) | 0.20 (0.10) | -0.02 (0.10) | 0.15 (0.07) |
| L.Superior Parietal | -24 | -58 | 47 | 3.37 | 0.31 (0.14) | 0.08 (0.09) | 0.30 (0.15) | 0.20 (0.11) | -0.02 (0.10) | 0.14 (0.07) |
| L.Supra-marginal Gyrus | -48 | -55 | 26 | 4.08 | 0.31 (0.12) | 0.05 (0.20) | 0.17 (0.11) | 0.03 (0.17) | 0.15 (0.29) | 0.10 (0.04) |
| R.IPL | 45 | -28 | 29 | 3.57 | 0.17 (0.13) | 0.14 (0.16) | 0.20 (0.17) | 0.18 (0.14) | 0.13 (0.12) | 0.16 (0.16) |
| L.IFG Pars Triangularis | -48 | 17 | 5 | 3.35 | 0.06 (0.22) | 0.21 (0.25) | 0.21 (0.16) | -0.02 (0.16) | 0.05 (0.13) | 0.11 (0.06) |
| **Average Contrasts $ICC_v$ (STE)** | | | | | 0.25 (0.15) | 0.08 (0.03) | 0.22 (0.03) | 0.14 (0.04) | 0.04 (0.03) | |

Abbreviations represent: cont, control condition; exp, experiment condition; $ICC_v$ med, intra-voxel median; IFG, Inferior Frontal Gyrus; IPL, Inferior Parietal Lobe; STE, standard error; MNI, Montreal Neurological Institute; ROI, region of interest.

## 4.12 Resting State Analysis

Resting state analysis was conducted by first acquiring the default mode network seeded from the medial prefrontal cortex (mPFC) functional connectivity mask. The network consisted of the posterior cingulate cortex (PCC) medial prefrontal cortex and bilateral inferior parietal lobes at baseline and analogous regions were active at six months. The mask generated of the right dorsolateral prefrontal cortex (rDLPFC) (Harvard-Oxford Cortical Structural Atlas) was used to measure connectivity to the DMN mPFC at baseline and six months. Both these regions and networks are known to be imperative to fronto-parietal executive functions (Niendam et al., 2012; Spreng, 2012). Analysis of baseline scans showed positive correlations in the right hemisphere frontal pole, right supramarginal gyrus and left middle frontal gyrus (Z =3.48 to 7.43) with the DMN mPFC. Negative connectivity with the DMN mPFC was found in the right primary motor cortex and left frontal orbital cortex (Z = -3.78 to -5.26) indicated by negative BOLD. Six month follow-up illustrated connectivity of the right frontal pole, right supramarginal gyrus and left frontal pole (Z =2.3 to 7.19) with the DMN. The left inferior temporal gyrus was negatively correlated with the DMN (Z =-5.38).

Further analysis was conducted to assess the integrity of resting state connectivity underlying executive functions associated with the SST. With this in mind, a small activation area in the rIFG was masked (Harvard-Oxford Cortical Structural Atlas) with the aim to investigate its connectivity with the DMN mPFC. The mask was selected and made from go incorrect and stop correct compared to stop incorrect and go correct group activation contrast (MNI: 50 30 -2). A 5mm sphere was made around this point and prepared for functional connectivity analysis. Baseline analysis revealed connectivity of the rMFG gyrus, right angular gyrus, left precentral gyrus and precuneous cortex (Z = 2.47 to 7.25) with the DMN mPFC. Negative activation was found in the left occipital fusiform gyrus and left superior parietal lobe (Z = -4.3 to -4.56). Six month follow-up analysis showed strong connectivity in the rIFG gyrus pars

Triangularis, paracingulate gyrus and left fronto-orbital cortex (Z = 4.42 to 7.18). Negative BOLD was found in the left postcentral gyrus (Z = -5.03). A paired sample t-test was used to find any whole brain resting connectivity differences however, no significant differences were found.

## 4.13 Regional ASL Perfusion

Region of interest CBF estimates were compared between baseline and six months longitudinally in the eight subjects' intact data from baseline and six months (refer to mean age and standard deviations in section 4.10.2). Regional ASL analysis was conducted subsequently to ensure that putative task regions were active before conducting the analysis. Regions were selected based on activation areas of the SST and MRot task in healthy participants. Perfusion assessments were employed in these regions to assess the integrity of BOLD signal activation. Hypoperfusion has been known to lead to reductions in the BOLD signal which may lead to less task activation. The rIFG pars triangularis and pars opercularis were both masked and combined so that a regional estimate of the rIFG could be gauged (Harvard-Oxford Cortical Structural Atlas). Bilateral supramarginal gyri were also masked to measure CBF in these regions that were associated with the MRot task (Harvard-Oxford Cortical Structural Atlas). Baseline activation was analysed in chapter three showing that perfusion was above the range that suggests abnormal perfusion in the specified ROIs (section 3.9).

Estimated mean CBF at baseline in the rIFG was 40.43 $\pm$ 10.80 ml/100gtissue/min (range 0-381.44 ml/100gtissue/min) which did not significantly differ from six month estimates (37.86 $\pm$ 10.11 ml/100gtissue/min) (range 0-354.53 ml/100gtissue/min) ($t(7) = 0.57$, $p > 0.05$). The right and left supramarginal gyri were also masked and superimposed on the whole brain perfusion map (Harvard-Oxford Cortical Structural Atlas). The left supramarginal gyrus was estimated as having a baseline mean perfusion of 37.08 $\pm$ 11.90 ml/100gtissue/min (range 0-

595.55 ml/100gtissue/min) that did not significantly differ from six month CBF (37.79 ± 8.38 ml/100gtissue/min) (range 0-492.30 ml/100gtissue/min) ($t(7) = -0.13$, $p > 0.05$). Baseline average CBF was estimated at 47.12 ± 13.38 ml/100gtissue/min (range 0-840.78 ml/100gtissue/min) in the right supramarginal gyrus which did not significantly differ to six month CBF estimates (45.69 ± 14.26 ml/100gtissue/min) (range 0-515.77 ml/100gtissue/min) ($t(7) = 0.29$, $p > 0.05$). These estimates did not significantly change between time points and were above the range suggested as being abnormal (above 15-20 ml/100gtissue/min) (Jann et al., 2016; Pollock et al., 2009). Moreover, CBF values acquired in the sample of this study were in line and similar to those found in ageing adults of a comparable age and in similar regions (Leoni et al., 2017; Liu et al., 2012).

Thus, normal CBF was apparent across time points in healthy ageing participants. Therefore abnormal perfusion was unlikely to have affected the BOLD signal activation; normal CBF should lead to unimpaired BOLD activation thereby allowing any longitudinal BOLD activation changes to be interpreted in light of changes in higher order function rather than underlying neurophysiology.

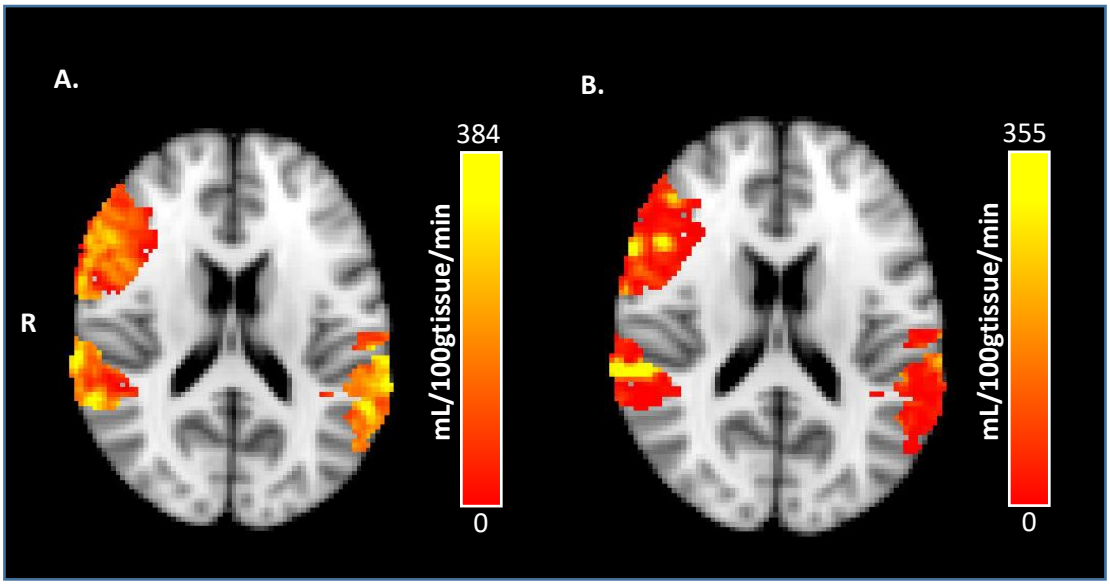


**Figure 4.10:** *rIFG gyrus and right and left supramarginal gyri masked ASL perfusion of: (A) baseline (n = 8) (B) six months healthy participants (n = 8). The colour bar represents least perfusion in red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres; R, right.*

## 4.14 Discussion

The main aim of this study was to examine the stability of executive function and spatial reasoning performance in healthy older participants over a period from baseline to six months and to determine factors that may affect cognition over this time frame. Moreover, neuroimaging assessments were employed to investigate the neural underpinnings of executive function and spatial reasoning and to assess factors that may affect neural activation over the longitudinal period. Findings from the present study revealed little difference in testosterone levels between time in accordance with the hypothesis aforementioned and past research in healthy controls (Boxer, Kenny, Dowsett, & Taxel, 2005; Lu, Masterman, Mulnard, & et al., 2006; Young, Baker, Liu, & Seeman, 1993). Healthy elderly participants were assessed with the MMSE and WASI-II which are known to be robust measures in detecting memory impairments and intellectual impairments among adults (Gontkovsky, 2016; O'Bryant et al., 2008). Findings showed all participants scored above the cut-off ranges on the assessments and were in the normal range according to specific age groups. This was in line with what was predicted and confirms that the sample of healthy controls were from the general population without intellectual difficulties or any apparent neurodegenerative conditions.

Cognitive performance on the D-KEFS and CANTAB SWM tasks did not vary significantly in healthy participants between time points in accordance with the hypothesis. Furthermore, performance was consistent with analogous executive function and spatial reasoning ability tasks in elderly healthy controls (Albrecht, Masters, Ames, Foster, & The, 2016; Johnson, Storandt, Morris, & Galvin, 2009; Vanderploeg, Curtiss, & Belanger, 2005). Multivariate models employed to analyse distinct D-KEFS and CANTAB performance with group by time comparisons, revealed no significant differences from baseline to six months on any tasks. Moreover, these findings remained stable between time points while controlling for

testosterone. Outcomes from the BRIEF-A measures showed no significant differences between longitudinal time points. Low self-ratings of cognitive function have been reported in past research (Pennequin et al., 2010). This may have bearings on cognitive performance but the current study showed no significant correlation between dependent BRIEF-A measures and D-KEFS contrast scaled scores. This suggests that self-rated meta-cognition was accurate compared to participants' neuropsychological performance which is consistent with past research (Rabin et al., 2006). The RCI change did show some relationship between proportion of testosterone change and proportion of cognitive change that are explored in more depth in subsequent paragraphs. Finally, psychosocial function also remained stable between time periods evidenced by HADS anxiety and depression scores in norm ranges (table 4.1). This is in line with the current hypothesis and previous research (Cella, Dymond, & Cooper, 2010; Hek et al., 2013; Wetherell et al., 2001).

Neuroimaging outcomes showed no neural activation differences between baseline and follow-up on the SST. No significant activation differences were present on the contrast of interest on the MRot task (exp-cont). This is in line with what was predicted since healthy participants' cerebral function were not expected to change over six months. Moreover, stop signal and MRot task activation were in putative inhibitory and spatial reasoning areas which was consistent with past research. This was also as hypothesised and further validates the sample of ageing participants had normal cerebral function. No significant differences were found in comparisons of resting state connectivity between the seed network and region of interests between time periods. Additionally, no significant CBF differences were found in elderly subjects between baseline and six months. This was as hypothesised and suggests cerebral perfusion and resting state connectivity did not change or deteriorate between time points. However, these outcomes should be interpreted with caution as comparisons between time were only assessed in eight participants. Psychosocial levels did not change in

participants between baseline and six months as expected and did not account for activation in fMRI task regions. Testosterone levels did not significantly differ between baseline and follow-up as anticipated.

The above behavioural cognitive findings illustrate that no significant differences were found in healthy older ageing participants between time points. However, while group level statistical comparisons were made, they do not address the question of identifying the extent to which individual subjects can be expected to reliably improve or decline on neuropsychological measures over time. The current study used the reliable change index (RCI) to measure change in participants over a time so that assessment of longitudinal change could control for practice effects (Barr, 2002). The effectiveness of the RCI method was explored in one study (Frerichs & Tuokko, 2005). Researchers assessed six reliability methods and demonstrated that the RCI was regarded as the most useful clinical diagnostic utility to detect reliable change in healthy older participants (Frerichs & Tuokko, 2005). Furthermore, when coupled with a threshold of $\pm$ 1.645 (90% CI) the RCI method gave a better indication of true change which addresses the potential influence of practice effects (Hensel, Angermeyer, & Riedel-Heller, 2007). Previous research incorporating the RCI has commonly found stable or better performance amongst healthy subjects in longitudinal duration studies compared to patient populations (Alves et al., 2014; Lampe, Sitskoorn, & Heeren, 2004; Stordal et al., 2005; Taylor, Barker, Heavey, & McHale, 2015). However, these studies employed samples that primarily comprised of young healthy cohorts with stable performance across time. Research comparing aging participants to young samples have commonly found subtle but significant differences in performance using cross sectional and longitudinal methodologies (Adolfsdottir, Wollschlaeger, Wehling, & Lundervold, 2017; Cabral Soares et al., 2015). One study explored healthy aging in subjects between the ages of

18-80 over 2.5 years (Salthouse, 2010). Researchers found aging was associated with significant negative changes across domains of spatial reasoning, visualisation, perceptual speed and vocabulary (Salthouse, 2010). This may suggest that the current investigation was limited in its duration and that any significant changes detected through the RCI method may be apparent over periods longer than six months. Nevertheless, findings from the current study did show some unexpected change including a relationship between the proportion of testosterone change over time and BRIEF-A self-report monitor scale. The BRIEF-A self-monitor scale measures the ability of a subject to be aware of a participant's own behaviour on others. It thereby captures how the subjects own behaviour compares with expectations of standard behaviours (Gioia et al., 2000). Further interpretation of this effect may include that high testosterone levels lead to the reduced conscious ability to perceive social cues or corrective behavioural patterns. Researchers in one study gave 16 female participants either testosterone or a placebo in a within subjects design (van Honk & J.L.G. Schutter, 2007). Participants were administered tasks in which a morphed facial expression was presented to them. Subjects were instructed to consciously recognise and index facial expression as either expressing threat i.e. disgust, fear or anger or non-threat i.e. surprise, sad, happy. When participants were administered testosterone, they had a significant reduction in recognising faces expressing treat. Moreover, participants were less aware of faces expressing anger compared to any other expressions. This suggests that higher testosterone levels impaired the ability of participants to consciously detect socially corrective facial signals of anger. This may have further implications for predisposing individuals with higher testosterone to assertive dominance behaviour that could lead to reduced awareness of self and surrounding standardised behaviour patterns. This may be an aspect of executive function that is affected as a function of higher testosterone levels and may explain the relationship on the self-monitor scale and testosterone relationship of the current undertaking.

Greater testosterone levels at follow-up in the conducted exploration were also associated with reliably better performance on the CANTAB spatial working memory eight box condition. This may be expected in light of studies showing that the domain of spatial reasoning is highly sensitive to testosterone manipulation (Moffat et al., 2002; Moffat et al., 2004). The effect of testosterone may have been associated with the eight box condition as it required a higher level of spatial reasoning ability thus being higher in sensitivity to testosterone level. This suggests that the task is suitable in the assessment of patients undergoing ADT since their levels are depleted. Therefore, the CANTAB SWM can differentiate testosterone-depleted performance from normal ageing related performance in a longitudinal assessment.

While neuropsychological findings generally revealed little difference between time points in line with the hypothesis, neuroimaging analyses revealed some interesting scanner task specific effects. Stop trial accuracy was significantly better at six months compared to baseline. This may reflect some practice effects in participants as they performed better at follow-up. However, this effect could also be due to environmental factors such as anxiety or stress during the participants' first visit for the experiment to an MRI scanning facility. Self-reported anxiety measurements were obtained but may not truly identify stress factors just prior to scanning. Thus, the second visit to the facility may have reduced anxiety levels to allow better performance on the task.

Other outcomes of separate flicker behavioural outcomes showed no significant interactions. However, stop signal reaction time did change as a function of flicker trial difficulty which is analogous to past research (Li et al., 2006). Longer SSRT latency was found on long flicker trials since they required high levels of executive function and inhibition rather than prediction to correctly inhibit. This was demonstrated in pilot study two (appendix 4: study 2) and three in which long flicker duration induced long SSRTs and was aimed for. Medium

flicker trials induced long latency SSRTs however, they were of low power rendering the outcome prone to error. Short flicker trials had the quickest SSRTs and best trial accuracy. This is analogous to past research showing that shortening of the SSD should result in better inhibition performance (Li et al., 2006; Li et al., 2008; Verbruggen & Logan, 2009). Spatial reasoning performance in participants showed that they performed better when they had to judge shapes in the same orientation compared to mirror orientations. This is a common finding as mirror trials increase the difficulty of the trial (Shepard & Metzler, 1971; Wan, Chen, Wu, & Qian, 2011). Moreover, reaction time increased as a function of shape orientation from zero which is analogues to findings from past research (Hertzog et al., 1993; Lineweaver et al., 2005; Shepard & Metzler, 1971). This suggests the healthy sample was mentally rotating shapes and were not guessing responses. If they were estimating responses then reaction time may be equal for all trials. The 300 degree condition did not elicit worse performance or longer reactions time which may be due to rotation of shapes in the anti-clockwise direction. This may have allowed participants to rotate shapes in the 300 degree condition quicker with better accuracy but may have required additional mental rotation related cerebral resources to change the direction of shape rotation.

Neuroimaging revealed putative SST activation concentrated in the rIFG gyrus, middle frontal gyrus and bilateral parietal lobes supporting a fronto-parietal network of activity and consistent with previous research (Hughes et al., 2013; Zhang & Li, 2012). Exploratory analysis of specific contrasts showed that activation on go incorrect trials was distinct compared to go correct trials and in line with inhibitory function. Stop incorrect trials also exhibited activation in go correct related areas which provided support for the combination of trials in the main contrast. However, activated voxel clusters were not apparent at both time points at the group level. This may have been due to the threshold set for multiple comparisons to avoid false positives and therefore some clusters were not apparent at this

level. Nevertheless, further analyses showed no significant differences on the additional contrasts between time points which indicates that similar areas were active at baseline and follow-up in the controls. Therefore, the combination of trials was warranted and benefitted the main contrast to increase the power of trials.

Region of interest analysis enabled the analysis of specific task associated regions activated at baseline and follow-up. This permitted the analysis of regional activation consistency without the restrictions imposed by whole brain analysis. The ROI analyses on contrasts of the stop signal task showed consistent activation in putative SST inhibitory areas including in the rIFG and rMFG. However, other regions including the LITG and LMFG were investigated as possible compensatory mechanism areas in older ageing samples as they were activated at either baseline or follow-up in whole brain activation maps. All regions had consistent BOLD signal change whether it was positive or negative BOLD between baseline and follow-up on the main inhibition contrast and across separate flicker trials. The main inhibition contrast was approaching significance in covarying with testosterone change between baseline and follow-up. This showed a linear trend indicating that as testosterone levels increased across time, differences between regional BOLD signals changed, although not significantly. Left hemispheric regions did not seem to contribute as a compensation mechanism as they had predominately negative signal change. However, when contrasts were split into flicker trials they did show some contribution. However, through averaging of the signal across all trials, they may have resulted in negative signal change. In addition, owing to the large number of analyses conducted to thoroughly explore the parameters of the task, this result should be interpreted cautiously and evaluated in independent samples.

The MRot task heavily relied on the parietal regions and precentral premotor regions as seen from the contrast of exp-cont. Region of interest analysis showed consistent activation across baseline and follow-up in putative regions on the main contrast and when contrast ROI

analysis were separated for each distinct rotation contrast (i.e. 120-cont, 180-cont and 300-cont contrasts). However, a significant interaction was found between regional signal change and time in the 60-cont contrast. More specifically, the left precentral gyrus had greatest change between time points. This covaried with change in testosterone indicating that as testosterone levels increased from baseline to follow-up, signal change in the left precentral gyrus decreased from baseline to six-months. Although testosterone change was associated with precentral gyrus change, it only accounted for 12% of variance. One explanation for signal change in the precentral gyrus (premotor cortex) between time points is that the MRot task has been found to elicit activation in the precentral gyrus that has been associated with mental imagery of self-hand movements during mental shape rotations (motor imagery) (Vingerhoets, de Lange, Vandemaele, Deblaere, & Achten, 2002). In the current study, motor imagery may have been a strategy implemented by participants in a novel task setting at baseline. However, at six months, participants may have recruited parietal brain regions due to practice effects or due to a different strategy employed that is compensated by testosterone. This may be more relevant to the 60-cont contrast as the rotation from zero on this sub component of the task required minimal rotation resulting in less precentral gyrus involvement at six months due to some practice effects. This is supported by BOLD signal change showing precentral gyrus involvement in the 120, 180 and 300 degree sub components of the task at six months (table 4.17). Thus the 60-cont sub component of the task may be more susceptible to testosterone change allowing differentiation of performance in PC patients undergoing ADT compared to performance as a result of normal ageing in especially the left precentral gyrus.

Further findings showed reliable activation in regions identified from baseline at an intra-voxel level in the main contrast (exp-cont). Reliability across conditions was slightly weaker but regional activation was highest when high degrees of shape rotation were required

indicating that these regions were consistently activated when difficulty increased to mentally rotate shapes (table 4.18). A limitation of the intra-class correlation method may be that there were activation differences when analyses were conducted in SPM compared to FSL. For example, the ACC was active in SPM baseline group analysis but was not active using the FSL analysis approach. This may be attributed to a higher sensitivity of SPM in the pre-processing steps of pipeline analysis (Morgan, Dawant, Li, & Pickens, 2007). Nevertheless, SPM has also been associated with simple noise models and FSL which underestimates spatial smoothness (Eklund, Nichols, Andersson, & Knutsson, 2015). Further research should attempt to compare outcomes from both software's analysis methods to isolate differences in analysis methods.

Subtle underlying brain architecture was investigated using resting state analysis and ASL. Outcomes consistently showed activation in the fronto-parietal network when the right dorso-lateral prefrontal cortex and rIFG gyrus were selected regions of interests. Moreover, negative deactivations were found in the opposite hemispheres to those that were activated such as the inferior parietal and superior parietal regions as was expected and has been related to stop signal performance in other studies (Lee & Hsieh, 2017). Arterial spin labelling was in the normal range and above the level classified as hypo-perfusion at baseline and six months (Pollock et al., 2009). Furthermore, no change was found in the healthy sample between time-points showing the suitability of the sample for longitudinal assessment during further investigations. A shortcoming to this analysis was of limited subjects assessed. This may limit the power of the analysis leading to increased type II errors where the null hypothesis was falsely retained.

Limitations of the study conducted include that there was a probability that the null hypothesis was retained in favour of a significant difference (Faul, Erdfelder, Lang, & Buchner, 2007). This is because a small sample size was employed that could leading to type

II errors. However, the longitudinal nature of the study allowed the elimination of between subject variability which added power to findings reducing the chances of type II errors. A further shortcoming included that there was a high probability of type I errors in both the behavioural assessments and neuroimaging assessments. This was due to the number of analyses conducted between variables and between voxels in whole brain analyses, raising the issue of multiple comparisons that increase familywise error rates. Thus there was a chance that small findings were as a result of chance. Therefore, these small comparisons that were exploratory should be interpreted with caution. Future study methodologies should implement larger samples sizes to decrease the chance of type-I and type-II errors. Another shortcoming was that the investigation only had a follow-up duration of only six months. Previous research has found subtle yet significant cognitive deterioration in healthy older adults with longitudinal follow-ups of three years. Nevertheless, past studies have implemented a limited cognitive battery therefore in depth insights into cognition could not be gauged. Moreover, norms and averages of neuropsychological assessments are likely to change over very long periods. This could invalidate outcomes of studies with very long follow up periods. A final shortcoming was that spatial reasoning ability was neuropsychologically evaluated with a task comprising both a spatial condition and a working memory condition. The variability in working memory was controlled for to gauge purely spatial reasoning. However, the removal of working memory through statistical methods may not truly remove all components of the domain from spatial ability. Nevertheless, neuroimaging did show and support that spatial reasoning ability was intact in healthy participants across the longitudinal duration suggesting that neural underpinnings were reflective of behavioural performance that demonstrated that spatial reasoning did not decline over the six months period.

In conclusion, healthy participants assessed had normal and reliable executive function over a longitudinal time course that was supported by neural underpinnings indicating that the performance in the domain was intact in healthy ageing older participants. Spatial reasoning was also found to be stable over the time period with neuronal activation in putative regions confirming that cognitive function in the domain was reliable in the healthy sample. Testosterone did have an effect on reliable performance across time suggesting that it could either enhance or impair cognition at later time points. Nevertheless, the effects were subtle and did not indicate that the whole domain was affected but rather that sub components of the spatial domain were affected.

The study confirms that the sample compared to patients were normal ageing healthy older ageing controls that did not confound the assessment of patients due to behavioural, biological or cerebral abnormalities. Moreover, it allows researchers to confirm that comparisons between patients and controls were robust. This may impact on patients informed choices of treatment, continuation of treatment and management of cognition in PC patients before therapy is administered.

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# CHAPTER 5

## 5.1 General Discussion

The current study sought to measure cognitive change associated with the reduction of testosterone levels (i.e. androgen deprivation therapy; ADT) used to treat men with prostate cancer (PC). It adopted an innovative approach combining clinically-relevant tasks to assess cognition and mood, together with sensitive functional neuroimaging measures. The thesis presented data from a cohort of men with prostate cancer prior to treatment onset and age-matched controls, all assessed twice over a six month period. Longitudinal data in prostate cancer patients were not available for analysis, however, the thesis provides a comprehensive framework for ongoing research, as well as novel insights from available data.

The treatment of PC has taken many forms including surgical methods (prostatectomy and orchiectomy), brachytherapy and cryotherapy that have been shown to be effective in reducing the risk of metastatic spread (Bill-Axelson et al., 2014; Gomella, 2009). However, these forms of treatment are associated with many side-effects such as erectile dysfunction, gynecomastia, mood impairment and impotence (Louda et al., 2012). Management of these adverse effects requires life-long treatment to recover from and may require additional testosterone replacement therapy. Androgen Deprivation therapy is a chemical castration method that has been shown to be as effective as other forms of treatment in preventing tumorigenesis and increasing survival rates (Heyns et al., 2003; Raina et al., 2007). Nevertheless, it has also been associated with side-effects similar to those of the previously mentioned surgical and radiation methods. However, an advantage of ADT is that these adverse effects can be reversed post ADT because testosterone levels endogenously return to baseline levels. Other treatments cannot offer this advantage due to complete prostate or

testes ablation and removal. Nonetheless, ADT has also been associated with deleterious effects on cognition and this has been reported to cause distress in patients (Sountoulides & Rountos, 2013). Although, many inconsistencies have been reported in the literature of the domains affected in patients during ADT, systematic reviews and meta-systematic reviews have found executive function and spatial reasoning abilities to be affected in ADT samples across multiple studies (McGinty et al., 2014; Treanor et al., 2017). However, as outlined in the current thesis, limitations of past assessments in the assessment of cognition in ADT samples meant that clear guidance for treating doctors and men with PC was not available. Therefore, the current investigation was employed to characterise cognition in PC patients undergoing ADT using an extensive battery of neuropsychological assessments and neuroimaging techniques at baseline compared to healthy controls. This was undertaken because past researchers have found that patients had cognitive impairments at baseline compared to healthy samples, therefore the pre-treatment period requires exploration (Salminen et al., 2003). The study undertaken was therefore conducted with the aim of providing physicians with the necessary information to facilitate the management of patients before they began ADT to prevent adverse effects from developing in the domains of executive function and spatial reasoning during therapy. This may allow patients to make informed choices on treatment uptake and continuation.

A critical shortcoming of past research included that some studies did not incorporate matched control samples. This was evidenced, for example, in studies where controls had higher intelligence scores and higher levels of education compared to patients (Green et al., 2002; Green et al., 2004). Therefore, any differences between groups were biased in favour of healthy controls, which would erroneously ascribe impairments to patients; this has implications for evidence-based practice. Specifically, men may elect to avoid what is a successful treatment for advanced PC due to concerns about the impact on their thinking

skills, even though the data currently supporting this outcome are not optimal. Thus, the present study therefore attempted to overcome several limitations by including assessment of potential confounds (for example, mood and testosterone levels) and matching groups. In addition, it aimed to characterise neural and cognitive function in the healthy control sample to validate that they did not have or develop any underlying cognitive deficits over a longitudinal period of six months. If any changes occurred in controls then it is important that these are accounted for in a longitudinal study of patients. Thus, in the current thesis, analyses and data collection focussed on identifying covariates including testosterone and mood factors that accounted for a significant proportion of variance on any differences. Moreover, it allowed inferences to be made on the usefulness of the sample compared to patients. The current PhD project was part of a broader study in which PC patients were compared over a longitudinal time period of 12 months before beginning ADT, during therapy and after therapy. Therefore, patient assessment was limited to investigating key background principles and development of neuroimaging paradigms rather than assessing patient outcomes owing to time constraints.

The hypotheses of the study were largely supported. At baseline, testing of patients and controls using a comprehensive battery of executive function and spatial reasoning neuropsychological and neuroimaging methods was used to test the hypothesis that there would be no significant difference in cognition and neurological function between patients and controls' before patients began therapy. This hypothesis was largely met with differences between patients and controls in testosterone levels and spatial function. The hypothesis was tested to determine if any cognitive impairments were present in PC patients before ADT treatment. If any deficits were found then this may provide physicians and therapists with the necessary information to develop a strategy to tackle any cognitive impairments which can

stem from the cancer diagnosis period to treatment period. Moreover, as testosterone has been shown to be a covarying factor between cognition and was higher in the patient sample, it may have been a contributing compensatory factor that resulted in null effects between patients and controls. Thus, ADT could exacerbate cognitive deficits in the treatment period due to its deprivation effect on androgens. Although a power analysis was conducted in the current undertaking, further mediating analyses are required to assess this relationship in larger sample sizes to account for null hypothesis testing effects.

The second key analysis of the current thesis involved longitudinal change in measures used. The global hypothesis was that ageing healthy controls had stable cognition over a longitudinal period of six months with unchanging neural function over the time period. This was to validate that the sample was representative of ageing healthy male participants that had stable performance over six months. If the hypothesis was not met, then outcomes from when patients were compared to controls may be confounded. This is because controls' performance and neural function was compared to patients to mitigate cognitive impairments that occur as a function of healthy ageing. The exploration confirmed that age matched healthy controls had no significant differences in cognitive performance in the domains of spatial reasoning and executive function between baseline and six months. Moreover, no underlying neural deficits were found that confounded neuropsychological and in scanner cognitive outcomes. This demonstrated that the control sample was matched compared to patients for age and intelligence without any underlying cognitive or neural impairments. Furthermore, it indicates that the control sample was a suitable comparator group to patients in the analyses that will arise in a future longitudinal comparison.

## 5.2 Implications for the study and treatment of prostate cancer with testosterone depletion

At a simplistic level, it is perhaps unsurprising that patients' and controls' performance did not differ before therapy commenced, although a range of factors might have led to a different outcome. For example, the men with PC had recently had a life-altering diagnosis which may impact negatively on mood. There is a known association with mood and cognition, and so it was important to consider whether the patient cohort could have presented with cognitive deficits prior to treatment for reasons other than testosterone depletion. The current study, however, found that mood was similar between patients and healthy controls before therapy indicating that mood levels were in the normal range. In addition, the similarity in cognitive performance between groups reflects the relatively stable neuropsychological profile in both groups on initial testing. Nevertheless, this finding warrants further consideration, given that men with PC had slightly higher testosterone levels at baseline.

Higher than normal testosterone levels could facilitate or support cognitive processes in the presence of cognitive impairment. This is of interest to the current study because an unexpected finding was that patients had significantly higher testosterone levels compared to controls at baseline assessment. Studies in patients with Alzheimer's disease and multiple sclerosis (Cherrier et al., 2005; Gold et al., 2008) provide support for the notion that higher testosterone levels may offer a protective effect in the presence of early cognitive decline. Patients administered testosterone in these studies had better cognition despite having underlying neurological conditions. The study by Cherrier et al. (2005) showed that administration of testosterone increased peak testosterone levels in patients with Alzheimer's disease and mild cognitive impairments. The increases corresponded with better performance in skills and domains including spatial memory, verbal memory and problem solving ability

compared to a patient cohort administered a placebo (Cherrier et al., 2005). This suggests that the increased testosterone was beneficial as a neuroprotective agent and may be a contributing factor that allowed patients to compensate for lower cognitive ability that may occur as a result of disease specific factors. Studies have also shown that low testosterone levels may be a vulnerability factor for major depressive disorder (McIntyre et al., 2006). Whereas administration of testosterone in patients resistant to serotonin reuptake inhibitors (SSRI) has been found to improve cognition in males suffering from major depression (McHenry, Carrier, Hull, & Kabbaj, 2014; Seidman & Rabkin, 1998; Seidman & Walsh, 1999). Testosterone administration has been found to improve anxiety symptoms in males (Cooper & Ritchie, 2000; McHenry et al., 2014). Psychosocial factors are known to facilitate cognition and underlying neural activity (Baxter et al., 1989; Chen et al., 2014; Kalanthroff, Cohen, & Henik, 2013). This suggests that while testosterone is a contributing factor of adenocarcinoma growth, it may be protective to cognition which hides underlying and unnoticeable psychosocial impairments that affect cognition. No psychosocial impairments were found in patients at the group level or found to have a relationship with cognitive outcomes suggesting that higher testosterone levels may have compensated for any impairments. Nevertheless, caseness analysis showed that PC patients that had borderline anxiety disorder had impaired spatial reasoning function on the more difficult subtest of the CANTAB SWM. This could affect patient daily life and has implications for the strategies implemented by physicians and specialists to target cognition in the early stages to avoid cognitive impairments. These approaches should therefore be effective when testosterone is depressed in patients during therapy or when mood factors may be elevated, possibly due to impending treatment.

Risks have been associated with testosterone administration in PC patients undergoing ADT including increases in PSA, development of cancer into metastatic growth and recurrence of

PC (Fowler & Whitmore, 1981; Huggins & Hodges, 2002). Physicians should therefore be aware of strategies or alternatives that could be implemented to tackle side effects especially those that involve biochemical administration methods. This is because previous human and animal assessments have been conducted with testosterone that is aromatizable to oestrogen. Therefore, oestrogen may partially have an effect of cognitive function rather than testosterone alone. Some studies suggest that androgen receptor production may be regulated by androgens (Lu, McKenna, Cologer-Clifford, Nau, & Simon, 1998) or oestrogens (McAbee & Doncarlos, 1999) in the central nervous system. Studies in which males are compared to females often show that men have better spatial reasoning compared to females which is attributed to the difference in testosterone. However, women have been found to outperform men in cognitive domains including short-term memory, verbal ability, perceptual speed and accuracy (Hampson, 2002). It is unknown whether these differences are due to gender differences or hormonal dissimilarities. One study assessed cognition in transgender subjects before undergoing oestrogen replacement therapy and after therapy (Miles, Green, & Hines, 2006). Participants were concurrently administered cyproterone acetate to depress testosterone levels while undergoing oestrogen therapy. Outcomes showed no cognitive differences after hormone treatment in domains of spatial reasoning, verbal fluency, verbal memory, visual memory and working memory compared to baseline levels before treatment. This shows that while testosterone was depleted, oestrogen administration may have substituted for deficits normally caused by testosterone deprivation. Similarly, the effect of oestrogen therapy may be suitable in PC patients when testosterone is deprived to allow cognition to remain intact. Moreover, this may allow patients to retain cognitive function to those of levels before ADT administration.

## 5.3 The role of neuroimaging approaches in studying cognitive decline associated with testosterone

Neuroimaging was employed in the study to assess the integrity of PC patient and healthy participant cerebral neuro-architecture through functional imaging methodologies. Neural correlates were investigated because past research has shown that underlying neurological structures may be affected despite participants having normal behavioural performance (Chao et al., 2013; Chao et al., 2012). To explore if this was the case, the present study utilised functional magnetic resonance imaging (fMRI) to assess brain activations and neural architecture differences with techniques including in scanner task activations and resting state fMRI seed connectivity. This allowed interrogation of differences in activation in underlying cerebral networks between groups pertaining to the domains of executive function and spatial reasoning. Cerebral perfusion was measured in the current study with a novel arterial spin labelling (ASL) technique. This allowed inferences to be made on underlying BOLD task and resting state activations through ASL at rest (Krainik et al., 2013; Wang et al., 2017). This is because the BOLD signal is an indirect measure of neuronal activity that is influenced by cerebral perfusion into cerebral tissue. In the context of studies of ageing or longitudinal studies, it is important to discount the effect of perfusion changes on BOLD differences over time. Although this is important for all such research, it is particularly relevant when the results of imaging studies have the potential to impact on clinical care. It would be erroneous to suggest that ADT is associated with cognitive changes through fMRI studies if the underlying basis for alterations in BOLD signals was instead differences in perfusion. Whilst changes in perfusion might not be favourable, the impact on clinical decision making is different given the suggestion that men treated for PC with ADT describe their experience of cognitive changes as distressing (Green et al., 2004). Therefore, the use of ASL here was able

to provide a unique perspective that supports accurate interpretation of BOLD task activations.

Treatment of potential confounding factors in the analysis of functional imaging data extends to other variables, including clinical factors such as testosterone. In the current study, testosterone was covaried with all neural measures to assess its association in patients and controls. Outcomes in patients and controls showed a difference in activation on the stop signal task in which patients had more activation in the left inferior frontal gyrus. This may be speculated to have been recruited through mirror neural circuitry to assist in inhibition performance. A possible mechanism by which this compensation may have occurred could be from the contribution of additional androgens to pyramidal cell mirror neurons in the left IFG (Kilner et al., 2009). The IFG has a putative abundance of pyramidal neurons (Jacot-Descombes et al., 2012) and has been shown to contain androgens in multiple layers of the gyrus (Kritzer, 2004). Dendritic density of these neurons has been linked to androgens because the hormone may facilitate dendritic spine formation by preventing ischemia and cell apoptosis (Yaffe, Grady, Pressman, & Cummings, 1998). This allows for a greater potential for the left IFG to mirror activity from the right IFG through facilitation of androgens in the patient sample of the current study. Testosterone was also shown to co-vary for activation in the amygdala on the stop signal task in PC patients. This suggests there may have been some effect of testosterone in facilitating compensatory activity in patients to produce inhibitory performance similar to that of controls. This shows the role of testosterone on executive function performance where the higher testosterone levels found in patients may have allowed them to produce similar performance to that of controls. Consequently, this finding has important implications; when testosterone is deprived during ADT, this compensatory recruitment may no longer be available and this may, in turn, lead to worse executive function performance as has been reported in past studies. Resting state and ASL analysis did

not uncover any facilitation of higher testosterone levels suggesting that BOLD activation during functional paradigms were valid and were not biased through testosterone modulations. Additionally, resting state executive function networks were not impaired in patients indicating that functional activation was not affected by underlying neural architecture impairment such as deteriorating white matter tracts that facilitate communication between cerebral regions.

In addition to baseline comparisons, the analysis of neuroimaging data extended to healthy control networks longitudinally. Although there were some difficulties with the ASL analyses, this initial evidence suggests that there is stable neural activity and perfusion across time points. The lack of impact of testosterone, which showed no differences between time points, suggests that these paradigms will be sensitive to the effects of ADT. This is the first comprehensive study to examine domain-relevant neural activity in this manner and suggests that brain imaging differences between patients and controls will be useful in future research. The investigations conducted showed that testosterone could influence neural activity in patients before ADT to allow for normal cognition similar to that of healthy participants while PC disease specific factors are present. This could explain the range of deficits found in patients during therapy. Longitudinal assessment of healthy controls moreover supports this outcome by demonstrating that testosterone was unaffected and that this did not lead to any differences between time points. Reliability measures further support that there were no activation differences between time-points which were not influenced by testosterone. This implies that the control group was a healthy ageing cohort that was and will be useful in the comparison of patients cross-sectionally and longitudinally.

Although the above demonstrates intact cognition in patients and controls, further research is required to assess if androgens are converted to testosterone or oestrogen through the aromatization process after entering the blood brain barrier. The use of non-aromatizable testosterone may be a viable option to investigate this in future assessments as it is not converted to estrogen (Pospisilova et al., 2012). Additionally, although fMRI is a useful tool, it suffers from temporal limitations whereby slice acquisitions are acquired after the neuronal event. This is because acquisitions of images by fMRI rely on the neurovascular coupling whereby the HRF that occurs to deliver oxygen for glucose metabolism transpires after a delay of the neuronal event (Hillman, 2014). Thus, post processing is required after data collection to calibrate for this difference so that an estimate is gauged of the actual time of event (Hillman, 2014). This may hamper the accuracy of temporal associations between brain regions in relation to mediating variables. Therefore, other modalities such as electroencephalography (EEG) should be considered in conjunction with fMRI to help bridge this gap. This may provide for a better approach with sufficient spatial resolution through fMRI and superior temporal resolution from EEG to concurrently measure activation that corresponds to neuronal activity (Huneau et al., 2015).

## 5.4 Limitations of the current study

The current thesis was conducted in the context of a larger longitudinal study, which was supported by the work presented here. Three key limitations arose during the course of the study: 1) lack of sufficient data at six-month follow up in men with PC, 2) sample size of the groups, and 3) corruption of some ASL data which limited planned analyses. In addition, multiple comparisons were made in the study that could limit outcomes from a behavioural and neuroimaging perspective which will first be addressed before moving on to discuss the limitations identified above. However, the current research and the imaging community incorporates control for this in software used for analysis. Moreover, only corrected threshold outcomes were reported in the current study. There is no accepted control for behavioural data. Bonferroni is considered too stringent, for example. Thus, whilst researchers in the present study were aware of these issues, findings were not stringently controlled for in this preliminary study. This was partly because the data required exploration so that any variables that might be relevant could be identified for future analyses when a fuller data set is available.

As aforementioned a shortcoming of the study was that patients and controls were compared cross-sectionally. While this was advantageous in gaining data before ADT, it lacked the capacity to generate and make inferences of factors that caused cognitive decline before and during therapy. The cross-sectional methodology additionally confined the current study outcomes as sample sizes were small which limited power. Since participants were assessed at a single time point, power requirements were not met which may lead to type II errors where the null hypothesis is falsely accepted. Implications of this are that cognitive and neural deficits before therapy may be missed due to small sample size allowing only the largest effects to be detected whereas smaller effects may be missed (Tsang, Colley, & Lynd, 2009). Caseness analysis was performed with mood measures to assess the relationship of

mood with cognition. However, no other variable could be compared such as testosterone due to the sample size. The comparison of healthy controls at longitudinal time points benefitted from the study design since between subjects variability was avoided due to within subject comparisons. However, calculation of the reliability change index (RCI) allowed measurement of proportions of participants that increased or decreased in performance over six months. This was advantageous as the RCI has been found to be sensitive when there are small sizes (Zahra et al., 2016). Moreover, the relationship between change in cognitive performance and change in testosterone could be assessed using statistical methodologies to provide links between factors that mediated this reliability over time in executive function and spatial reasoning.

Another power constraint of the present investigation utilising neuroimaging was that only eight healthy controls' arterial spin labelling (ASL) scans could be compared to patients. This increased chances of errors that may have biased findings. An attempt was made to include six month scans from missing controls' which were combined with the eight baseline intact participants' ASL data. This allowed a hypothetical healthy control group at baseline to be compared to PC patients. The basis of the concept came from past research showing that cerebral perfusion acquired with ASL was stable in healthy ageing older participants over a period of one year (Parkes et al., 2004). Moreover, only eight healthy controls' data could be compared between time points. Therefore, findings should be interpreted with caution due to a lack of power in the paired sample analysis.

A fundamental restriction of ASL is that the signal obtained from ASL is highly sensitive to noise and is dominated by thermal and physiological artefacts. Low SNR has been attributed to occur due to the relatively low ratio of blood (2%) to tissue ratio in the cerebral cortex and rapid decay rate of labelled bolus during transit time. This makes it difficult to employ ASL but can be improved by using background suppression techniques which implement multiple

inversion techniques to acquire a higher SNR (Ghariq, Chappell, Schmid, Teeuwisse, & van Osch, 2014). However, due to the above limitations of ASL, outcomes and reliability estimates from the currently conducted study may have been affected (Liu & Brown, 2007).

### 5.5 Future Directions

The optimal way in which to understand the cause of cognitive decline in ADT patients would be to recommend a longitudinal study. Patients should be assessed longitudinally before baseline close to the diagnosis phase to the time approaching therapy, during therapy and post ADT. This may allow researchers to observe changes present before therapy and their aetiology from the diagnosis phase to impending therapy, during therapy and after therapy. Physicians may thereby be able to make better judgements of whether ADT is the factor causing cognitive impairment or other co morbid factors (Caruana, Roman, Hernández-Sánchez, & Solli, 2015). Moreover, they can devise treatment methodologies to prevent adverse effects before therapy and during ADT to allow patients a better chance at continuing treatment. An analogous methodology was implemented by Cherrier et al. (2009) to reduce practice effects before baseline. However, researchers could utilise this methodology with a comprehensive battery of neuropsychological assessments and neuroimaging measures to identify factors affecting cognition before ADT commencement. Power of longitudinal assessments have been found to be higher due to within subject comparisons which allow smaller differences to be detected between time periods and groups (Caruana et al., 2015). Although the current study was designed to achieve this, it was not feasible in the timeframe of candidature to complete this. Ongoing data collection will provide an opportunity to expand and extend the current novel framework and provide evidence-based data for clinical decision making. A strength of the present study was that age

and intelligence of the healthy sample was controlled for which improved upon some past research (Green et al., 2002; Green et al., 2004). However, there are other factors that could be assessed in future research. These includes for example the exploration of genetic markers such as APoE4 and IGF that were collected in the present undertaking but not processed due to the time span of the project. These variables are important in the assessment of cognition in PC patients as they have been shown to be more preventative in this sample compared to normal ageing samples (Ballentine Carter, 2012). A shortcoming which was not listed but may benefit from future research is that while behavioural and neuroimaging approaches incorporated in the current assessment have their individual strengths, their complementary value in comparison to each other was not utilised. A combined and comparative analysis methodology is recommended during further research especially in longitudinal studies, as it could provide a more complete understanding of cognition and underlying pathways in relation to the aetiology of any deficits found.

As outlined in the previous section, corruption of neuroimaging data impeded data analysis. In future, additional participants should be recruited to address and account for scanner data corruption or participant attrition rates during longitudinal follow-up. This was a shortcoming in the assessment of healthy controls in the current study with ASL which should be rectified by additional recruitment. A further limitation may be addressed by the inclusion of a comparison group of PC patients that are not undergoing ADT. This group may be more appropriate as it allows experimenters the ability to match groups more closely on factors including body fat index, physical functioning, medication regimes and genetic factors (Alibhai et al., 2010; Clay et al., 2007). This may elucidate the effect of solely ADT without the presence of confounding or comorbid factors.

Recent advances in MRI acquisition of perfusion include the simultaneous combined acquisition of PCASL and the BOLD signal. This is advantageous because unlike PASL alone, which was implemented in the study conducted, it has better SNR. A recent technique to combine both ASL and BOLD techniques used a pseudo continuous ASL (PCASL) sequence that could be implemented by future researchers to increase SNR (Fernandez-Seara, Rodgers, Englund, & Wehrli, 2016). A dual acquisition ASL sequence was employed with background suppression and 3D gradient and spin echo (GRASE) readout while BOLD was acquired with 2D multi slice echo planar imaging (EPI). This was carried out during a visual stimulation (motor) task in 10 healthy participants (mean age=23.3, SD=4.8). The dual acquisition (DA) sequence was compared to a PCASL dual echo EPI (DE-EPI) ASL acquisition sequence during the same task. A further experiment was implemented in five healthy participants to assess the potential of the DA sequence to measure maximum (M) mapping which is the maximum possible cerebral metabolic rate of oxygen ($CMRO_2$). This was measured with a gas breathing challenge (hypercapnia and $CO_2$ gas delivery) and acquired using DA. Results showed that the dual ASL and BOLD acquisition had a temporal SNR (tSNR) that was three time higher in the ASL sequence compared to the DE-EPI sequence. Furthermore, the BOLD tSNR obtained in the DA sequence was comparable to that of the BOLD signal obtained during the DE-EPI sequence but was slightly lower than the DE-EPI sequence. Nevertheless, this was not significant and was attributed to longer TRs during the DA sequence. The DA sequence M-mapping gas breathing acquisition during the motor task showed BOLD signal changes in bilateral motor cortices and gray to white matter contrasts analogous to past research (Fernandez-Seara et al., 2016). This suggests the DA sequence was better at detecting ASL and BOLD simultaneously allowing better calibrated images with higher tSNR compared to past techniques employing DE-EPI. Moreover, the DA

sequence allows better estimations of M-mapping during functional sequences due to better ASL tSNR acquired. One limitation was that long TRs during the DA sequence reduced the BOLD signal. Moreover, sample sizes were small but the study does validate that the DA sequence can improve tSNR in future assessments during functional MRI paradigms compared to techniques implemented in the past.

The current study has important implications for care of men who choose to have ADT and require rehabilitation after diagnosis, before therapy and during therapy. Significant effort has been directed towards developing strategies that ameliorate the negative cognitive effects of ADT. Some approaches that have been implemented in past research include exercise, improving family cohesion and dietary care. Exercise has been shown to be effective in managing the cognitive effects of ADT and could improve patient quality of life. One study assessed quality of life and general health in 29 patients (mean age=69.5, SD=7.3) undergoing ADT that were assigned to an exercise regimen program for 12 weeks (Galvão, Taaffe, Spry, Joseph, & Newton, 2010). Patients reported better quality of life, decreased fatigue and had reductions in levels of C-reactive protein which is a marker for cancer and inflammation compared to 28 ADT patients that were not administered exercise therapy (mean age=70, SD=7.3). The brief period of exercise demonstrated its benefits on biological factors through a non-medicated medium. A limitation in view of the current study was that exercise therapy was commenced while participants were two months into ADT. This is promising as benefits of exercise were still evident, however, the effectiveness of exercise has not been explored before treatment. Nevertheless, a further group of researchers showed the effectiveness of a three month exercise program in ADT patients (mean age=69.6, SD=6.5) when the program was initiated earlier into ADT treatment (10 days) (Cormie et al., 2015). Findings revealed that patients had lower levels of fat, less total cholesterol, higher muscular strength and reported better sexual function, less fatigue, better mental health and

decreased psychosocial distress compared to ADT patients that were not administered an exercise program (Cormie et al., 2015). This supports that exercise was beneficial in improving physical and mental aspects of life in patients during the initial period of treatment. Moreover, the exercise intervention was implemented at a period when adverse effects of ADT such as the initial flare effect is known to be fatal and can also be distressing and uncomfortable for patients (Sountoulides & Rountos, 2013). Next steps should be to assess exercise in PC patients before undergoing ADT in relation to cognition during therapy. This has not been explored but past research shows favourable effects of exercise in healthy ageing participants that could be applied to patients (Colcombe & Kramer, 2003; Erickson et al., 2011; Hamer & Chida, 2008).

Another approach to tackle adverse effects before ADT may include working with the patient in the context of their family. Researchers have found that communication within the family of patients with cancer may affect patient recovery and treatment coping ability. Stressful events such as the diagnosis of cancer can trigger clinical levels of depression not only in patients but also in family relatives and partners. One study assessed the relatives (mean age=44.17, SD=18.87) of 48 patients (mean age=54.60, SD=15.87) diagnosed with colorectal, breast or prostate cancer (Edwards & Clarke, 2004). Patients and relatives were administered measures of anxiety, depression, illness concerns, physical functioning and family functioning. Outcomes showed that 12.8% of patients and relatives had clinical levels of depression. Moreover, 20.8% of relatives were shown to have risks of developing clinically diagnosable depression. Multi-level modelling revealed a relationship between communication within the family and psychosocial dysfunction, with unclear communication being associated with higher anxiety and depression levels. Furthermore, there was a significant interaction between patient with prostate cancer levels of anxiety and family communication. Intraclass correlations also found that 21 percent of variance from depression

and 15 percent of variance from anxiety came from family membership and support (Edwards & Clarke, 2004). This indicates that the capability of the family to act openly to express feelings directly correlated with lower levels of depression and anxiety. A limitation of the study was that it was cross sectional and did not measure whether family functioning affected depression and anxiety levels during and after the main treatment period. This is important because while many patients assessed in the study conducted did not have a clinical diagnosis of anxiety or depression at baseline, these factors may develop during treatment or after treatment especially in those patients with borderline mood disorders before impending therapy. Therefore, interventions to increase family cohesiveness could be implemented but require longitudinal follow-up to assess their efficacy during and after therapy in controlling mood impairments.

In conclusion the current study conducted used neuropsychological and neuroimaging measures in the domains of executive function and spatial reasoning to assess PC patients before they underwent ADT. Findings showed mostly comparable levels of cognition and underlying neural architecture compared to healthy controls in these domains. However, some differences were evident in patients that may have been facilitated by higher testosterone levels to compensate for disease specific cognitive impairments and deficits in cerebral function even before ADT. This has implications on strategies that are devised and implemented by physicians to resolve side effects prior to therapy so that behavioural cognitive impairments are not intensified during ADT. Moreover, outcomes have consequences on patient therapy uptake and maintenance. The second part of this research assessed healthy controls longitudinally. Controls did not have any signs of cognitive or neural impairment in the domains of executive function and spatial reasoning across a longitudinal period. This demonstrated that the healthy control group was a valid comparison sample to patients. Furthermore, the study of healthy controls showed that the cohort had

reliable cognitive performance and activation across time points. This suggests that controls could be implemented in a longitudinal study in which patients are compared to controls. This forms the basis of future research to uncover and treat adverse effects associated with ADT in PC patients.

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# Appendices:

## Appendix 1: BUIC Screening form

# MRI SAFETY SCREENING QUESTIONNAIRE

**EVERYONE must fill out this form BEFORE entering the MRI suite**. The MRI suite has a very powerful magnetic field that may be hazardous to those with metallic, electronic, magnetic or mechanical implants or devices. All information will be kept strictly confidential.

| | | | |
|---|---|---|---|
| **Name:** | | **Date of Birth:** | |
| **Email:** | | **Tel No:** | |

## Section A – To be completed by EVERYONE entering the MRI suite

| Please indicate if you have any of the following: | YES | NO | If yes please explain |
|---|---|---|---|
| Cardiac Pacemaker, pacing wires or defibrillator | | | |
| Aneurysm clip (metal clips put around blood vessels during surgery) | | | |
| Electrical Stimulator for nerves, bone or brain | | | |
| Ear or Eye implants (e.g. cochlear implants) | | | |
| Implanted insulin, drug or infusion pump | | | |
| Stent, catheter, coil or filter in any blood vessel | | | |
| Orthopaedic hardware (e.g. artificial joints, metal plates, | | | |
| Any other type of prosthesis or implant | | | |
| Gun pellets, shrapnel, bullets or metal fragments | | | |
| Any surgery or an operation | | | |

## Section B – Complete ONLY if you are being scanned or intend to go inside the scanner room

| Please answer the following questions carefully | YES | NO | Staff Notes |
|---|---|---|---|
| Have you had an MRI scan before? | | | |
| Are you claustrophobic? | | | |
| Have you ever been a welder, machinist, grinder or worked with | | | |
| Do you suffer from any medical condition that may be relevant | | | |
| Do you have any tattoos or body piercings (other than | | | |
| Do you wear dentures, a dental plate or a brace (not | | | |
| Do you have any transdermal skin patches (e.g. nicotine | | | |
| (*Females only*) Are you or could you be pregnant? | | | |
| Please state your weight (kg) | | | |
| Other information (e.g. spectacle prescription) | | | |

## Please tick the boxes before being scanned or going inside the scanner room

I confirm that the above information is accurate to the best of my knowledge.

I will remove all metal including mobile phones, keys, watches, coins, credit cards, body piercings, jewellery, false teeth, hearing aids etc. before entering the scanner room. (Lockers available in waiting room.) ☐

I acknowledge that BUIC has taken reasonable precautions to screen for potential difficulties and is not liable for any event that might result from incorrect answers to the above. ☐

| Signed: | | Date: | |
|---|---|---|---|

Form verified by (*Authorised Personnel only*):

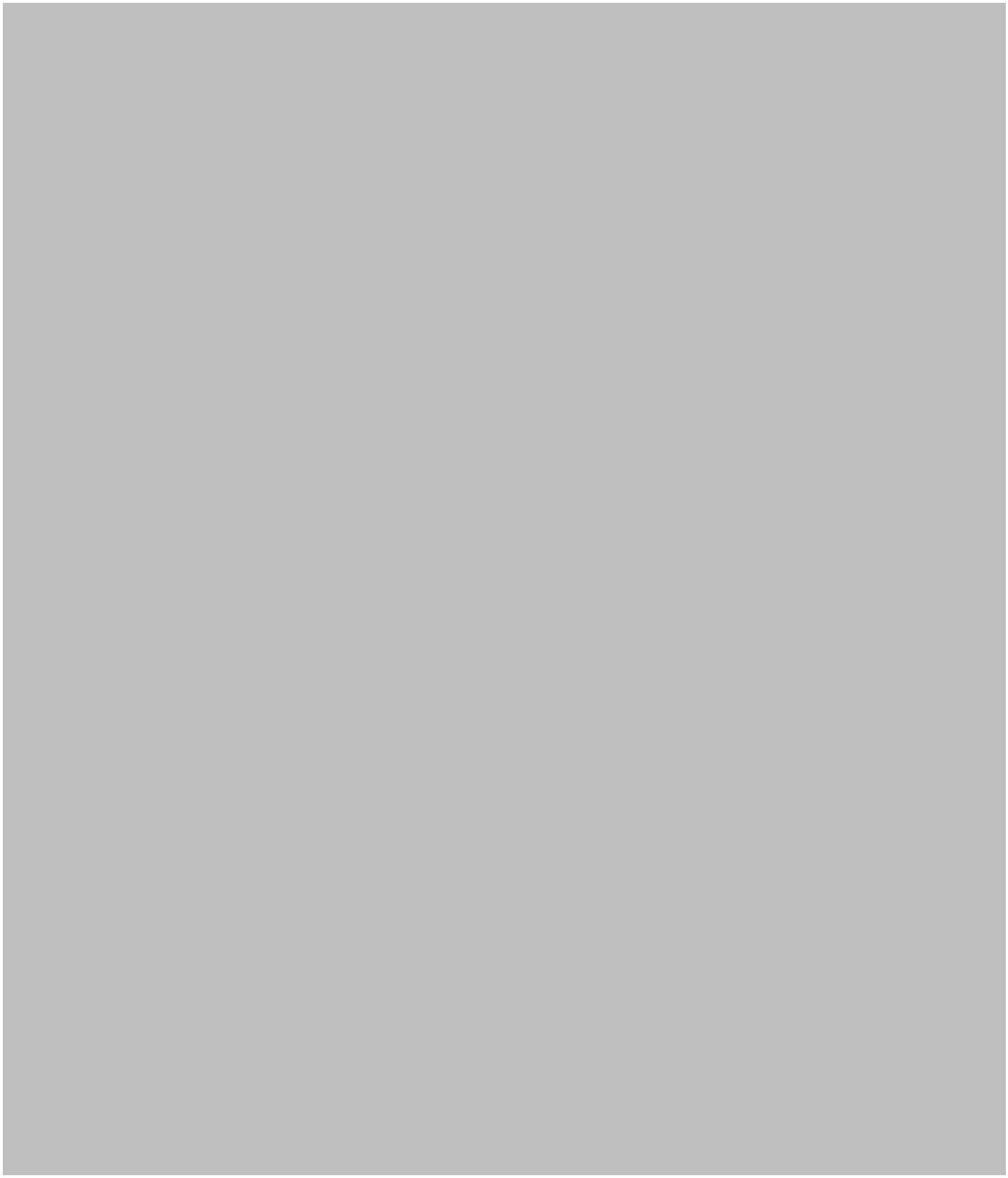
| Print Name: | | Signed: | | Date: | |
|---|---|---|---|---|---|

Staff Note: This form is only valid for six months from date of initial screening v4.0 July 2010

## Appendix 2: Ethical Approvals

**Patients assessment approval**

From: Susan Cottam
Subject: Application to use Programme of Work ERN_11-0429AP26
Date: 05 December 2015 5:22:03 PM GMT
To: Amanda Wood, Elizabeth Grunfeld Cc: Paras Joshee



## Appendix 3 Neuropsychology measures

### Mini-Mental State Exam - II (MMSE-II) – Standard Version (Folstein et al., 1975).

The test is known to purely assess multiple cognitive domains and thereby allows the detection of surface abnormalities in cognitive domains in a quick and easy manner. The 2nd edition was incorporated into the assessment which has been enhanced by past researchers to improve its clinical utility and standardization. The blue form version was employed since it was administered once only as a screening tool, therefore learning effects were minimised. The MMSE-II was implemented as a screening test in the current study to exclude participants with dementia or Alzheimer's like symptoms. One study assessing validity and reliability showed that the MMSE –standard version had high internal consistency, test-retest reliability, interrater reliability and good concurrent validity for detection of mild cognitive impairment (MCI) and Alzheimer's disease (AD) (Baek, Kim, Park, & Kim, 2016). However, researchers found decreased sensitivity of the test to detect MCI among a normal aging population (Baek et al., 2016). Nevertheless, it was sensitive in detecting Alzheimer's in the normal aging population (Baek et al., 2016). Moreover, a number of studies among various samples and languages show satisfactory and respectable reliability and validity among aging populations (Awan et al., 2015; Boban et al., 2012; Keskinoglu et al., 2009). This was moreover found in the clinical differentiation of mild cognitive impairment, dementia or Alzheimer's disease (Awan et al., 2015; Boban et al., 2012; Keskinoglu et al., 2009). The cut-off score for the MMSE was kept at 24 and any participants scoring below this range were excluded from the study. The cut-off was selected based on the MMSE manual showing that scores of less than 24 were considered as abnormal and indicative of participants having dementia **(Folstein, Folstein, & McHugh, 1975).**

### Wechsler Abbreviated Scale of Intelligence (WASI-II) (Wechsler, 2011).

Participants were assessed on the block design test; vocabulary test, matrix reasoning test and similarities sub-test. Each sub-test is unique and encompasses verbal comprehension and perceptual reasoning abilities to calculate a full-scale IQ score. The test was used to rapidly, efficiently and thoroughly measure IQ. Intelligence scores were obtained to screen and exclude participants with scores significantly below standardised norms as low scores. This is because low IQ scores indicate that performance on further measures may be compromised which could inflate sampling error. The WASI full scale intelligence quotient 4 (FSIQ-4) was the primary measure used in the current study and has a mean of 100 with SD of 15. Any participants with FSIQ-4 scores lower than 85 were excluded from further analyses as this indicated borderline of below average intelligence levels.

Sub-tests

*Block design test*: Evaluated the combination of visual stimuli, fluid intelligence, organisation and visuo-motor speed. Different start points were provided for adults. Participants were given an initial model with examples provided by the examiner on the first two items. They were subsequently instructed to make the design themselves on further items. Each item had a time limit and participants were awarded bonus points for successful re-construction of designs the quicker they were made. The subtest was discontinued after two repeated failures.

*Vocabulary*: Examined word definitions, word knowledge, crystalized intelligence and language development. Participants were asked to define a word presented to them. The manual outlined scoring criteria for the assessments ranging from 0 to 2 points per word definition. The test was discontinued after three repeated scores of 0.

*Matrix Reasoning*: Assessed concepts of visual intelligence, fluid intelligence, spatial reasoning and spatial organisation. Subjects were required to choose one item from five which fit within a square matrix to complete a pattern. Each item received a score of 0 or 1. The test was discontinued after three consecutive errors.

*Similarities*: Measured the ability of verbal theory formation, crystallised intelligence, abstract cognition and categorical thinking. Participants were asked to verbally describe a common feature between two words or concepts presented orally by the examiner. Participants were given points of either zero, one or two based on criteria in the WASI manual. The sub-test was discontinued after three consecutive scores of zero.

*Reverse rules and prompts*: The test was employed to thoroughly assess intelligence. Moreover, the WASI has a set of reverse rules on each subtest. Reverse rule items were only administered if participants made an error on the first two items of each sub-test, in which case the examiner must administer items in reverse until two consecutive correct scores were obtained. Reverse items were generally easier than actual items on sub-tests and facilitated in reinforcing the criteria of the task. If two consecutive correct scores were not obtained in reverse, then the test was discontinued as this indicated that participants were not likely able to perform remaining items on the task. Prompts were given only on the vocabulary and similarities subtest on either zero or one point items and were indicated in the WASI manual sub-test sections.

Reliability has been on the WASI-II with internal consistency coefficients per age group calculated through transformation of fishers-Z scores (Wechsler, 2011). Reliability coefficients obtained for adult samples between the ages of 55-84 were generally high and ranged from 0.87 - 0.94 for the block design task, 0.90 – 0.95 for the vocabulary task, 0.90-0.91 for the matrix reasoning task and 0.90-0.93 for the similarities sub-test. Composites of these sub-tests show reliabilities ranging from

0.95-0.96 on the verbal comprehension index (VCI), 0.93-0.95 on the percentile rising index (PRI) composite score, 0.96-0.97 on the full scale IQ – 4 (FSIQ-4) and 0.94-0.95 on the full scale IQ – 2 (FSIQ-2) composite score. Test retest reliability has been measured by testing a group of participants twice between 12 and 88 days. Outcomes reveal adequate test–retest reliability coefficients in adults between the ages of 55-90. Coefficients range from 0.83 – 0.96 across subtests and composite calculated scores. Inter-scorer agreements were also high and ranged between 0.98-0.99 and were calculated using intra-class correlation coefficients (Wechsler, 2011).

Validity has been assessed through content scores (content validity) and construct validity (Wechsler, 2011). Content validity was found to be strong when internal structures of the test were examined. Confirmatory factor analysis showed that subtests relating to PRI and VCI measured separate constructs of verbal comprehension and perceptual reasoning abilities. Confirmatory factor analysis also demonstrated that there were latent variables which highly correlated with g-factor intelligence or general intelligence. This is advantageous because the test shows validity in being able to measure intelligence. However, caution must be applied for interpretation of scores outside of the FSIQ. Intercorrelations of sub-test scores were generally modest and ranged between 0.40-0.70 in adult samples. This suggests that some aspects of cognition overlapped on sub-tests and that performance on one measure informed performance on other measures. Correlations with analogues intelligence measures (WISC-IV/WAIS-IV) have been found to be acceptable and in the range of 0.71-0.92.

**Delis Kaplan Executive Function System (D-KEFS) test (Delis et al., 2001a).**

The D-KEFS is a set of standardised assessments used to measure higher level cognitive functions referred to as executive functions. This draws on a myriad of cognitive skills such as attention, language, memory etc. to produce higher order levels of abstract thought and creativity. The D-KEFS is comprised of nine subtests which can be administered as either stand-alone assessments or as part of a whole assessment depending on the needs of the examiner or researcher. The test is known for measuring mild brain damage in especially frontal lobe areas. The test is able to empirically measure executive functions and fundamental skills required to achieve higher order abilities. This is because the scale of difficulty on each subtest is incremental and assesses basic cognitive abilities initially including for example language, attention, memory etc. and then moves onto items of higher difficulty, allowing the isolation of any deficits present on earlier task items. Furthermore, the test has high ceiling and floor benchmarks. This allows all relevant scores to be obtained regardless of an individual's ability on each test.

<u>Characteristics of sub tests included</u>

*D-KEFS Trail making test:* Consisted of a visual scanning task and four dot connection tasks. The primary task was a letter and number switching task. This allowed the assessment of flexibility of switching on a spatial motor task. The other sub components permitted examination of the components required to complete the primary task including visual scanning, number sequencing, letter sequencing and motor speed.

*D-KEFS verbal fluency test:* Measured the ability to generate words from overlearned concepts in a phonemic manner whilst also requiring switching and set-shifting between category fluency elements.

*D-KEFS Design Fluency test*: Assessed non-verbal ability and required that subjects draw as many different designs in 60 seconds as possible. The first condition was basic requiring simple design generation. Condition two required inhibition whilst also measuring design generation fluency. The final condition assessed fluency and shifting or switching ability which gauged visual attention, motor speed, perception and design construction skills. Executive functions recruited on the task included visual construction generation, creativity and inhibition.

*D-KEFS Colour word interference test*: Evaluated inhibition of overlearned concepts of words read and colours named. Inhibition was necessary on condition three which was the primary test and required conflicting responses of naming discordant ink colours to the words that were written. Furthermore, if participants were successful, the last condition required switching between inhibition and flexibility on read words. The task was analogous to the design by stroop (Stroop, 1935) with the added element of cognitive flexibility on the final task.

*D-KEFS Tower test:* Several executive functions including problem solving, spatial planning, rule learning, inhibition of impulsive responses, and rule maintenance were assessed from the task. Visual attention and visual-spatial skills were also measured on the task.

Scoring of all tasks were standardised so that raw scores were converted to age group scaled scores that had a mean of 10 and standard deviation of three. Scores on some tasks were also combined and contrasted to reflect cognitive function on a particular domain. Primary contrast scaled scores were used in the current experiment providing global scores for typifying overall performance on a task domain. Moreover, the contrast scaled score was calculated as primary assessment score controlled for performance on basic performance. Scaled scores provided classification of contrast scores in age normative ranges.

Validity of the D-KEFS has been cited in the D-KEFS manual (Delis, Kaplan, & Kramer, 2001b). Accuracy and error measurement intercorrelations of scaled scores have generally shown they are positive indicating that good performance on one variable is associated with good performance on the other variable. Correlations measured between primary scaled scores show low positive relationships

between tests. This suggests that the tests are related to each other but that they measure a unique aspect of executive function with only slight overlap.

Reliability measurements of D-KEFS subtests indicate mid to good reliability estimates. The technical manual differentiates standard error of measurements expressed as standard deviation units and indicates the amount of measurement error observed in an individual's test score (Delis et al., 2001b). An inverse relationship has been found between the reliability coefficient and standard error of measurement illustrating that as reliability increases, measurement error decreases. Test-retest reliabilities are grouped by all ages since sample sizes were small when assessed per age group. This creates high variability in coefficients. Test-retest coefficients by subtest are as follows: Coefficients for the trailing making test were shown to be in the range of 0.38 - 0.77 with the primary measure being the time to completion. Verbal fluency coefficients range from 0.36 - 0.80. Design fluency scores range from 0.32 - 0.58. Colour word interference coefficients range from 0.62 – 0.76. Coefficients for the tower test were 0.44. Overall coefficient values indicate mid to good reliability on sub-tests with higher reliability on initial assessments with less reliability on primary measures.

**Wechsler Adult Intelligence scale (WAIS-IV) (Wechsler, 2009).**

Subtests of the WAIS sub-tests selected were associated with working memory.

**Sub tests included:**

Digit span task: The task is composed of three assessments. One in which digits were said and immediately recalled by participants. The next task required the researcher to say digits which were then recalled backwards by subjects. The final task required participant to recall digits in sequential order from lowest to highest after researchers said them. This allowed isolation of simple memory recall and more complex working memory associated domain performance that required frontal brain regions.

Arithmetic: Items on the test ranged from requiring simple mental arithmetic to more complex arithmetic that necessitated attention, memory and manipulation of already mentally held information.

The WAIS has high internal consistency and when assessed over a twelve week time period, test retest reliability ranges from 0.70 to 0.90 in participants aged 60 – 80. Inter scorer coefficients have been reported as being 0.90 which is considered very high, indicating that scorers generally agreed on scoring criteria.

Content validity has been assessed through confirmatory factor analysis revealing moderate relationships between the digit span task and arithmetic task in measuring working memory constructs. Relationships of tasks to working memory were revealed as being slightly weaker in those aged 70-90 compared to those aged 16-69 in which higher correlation relationships were shown.

Intercorrelations of the WMI were 0.60 for the digit span and arithmetic tests suggesting that performance on one test informs performance on the other sub-test. Moreover, the working memory has been found to correlate with the nine sub-tests of the D-KEFS moderately (0.37-0.63). This is expected since executive function has been theorised to be part of the working memory system (Baddeley & Della Sala, 1996).

The WAIS working memory index composite score has a resultant mean score of 100 with SD of 15

**Cambridge Neurological test Automated Battery (CANTAB) (Cambridge Cognition, 2006)**

The Spatial Working memory task (SWM) sub-test was employed to assess spatial reasoning skills by assessing the ability to retain spatial information for later manipulation in working memory. The task was also used to measure heuristic planning and strategy and has been known to be sensitive to measures of executive functions.

The task requires a process of elimination strategy where participants were asked to find a blue token in a number of boxes and then place the token into an empty column on the right hand side of the screen. The token was never in a box that already contained a token. There were as many tokens to find as there were boxes on the screen which increased with trial successions. Location of tokens, number of boxes and colour of boxes were changed on each trial to avoid generalised search strategies.

The version used to assess participants consisted of a practice phase of four three box sets and then six assessments of two each of four boxes, six boxes and eight boxes (3x3 boxes practice) + (2x4) + (2x6) + (2x8).

Outcomes from the task were number of errors made which could be divided into two sub outcome measures.

Between errors: This was the number of times a box was revisited after a token was already found. Therefore, a lower number indicated less errors made.

Double errors: These were the times categorised when participants revisited a box with a token already found and the time when a box was revisited that was empty during the same search to find a token.

Total errors: this was the number of total combined errors made therefore lower scores indicated better performance.

Another outcome measure implemented was strategy. Authors have recommended an efficient search strategy for completing the task (Owen, Downes, Sahakian, Polkey, & Robbins, 1990). Based on this

method the most proficient method has been suggested to start at a specific box then once a blue token has been found, to return to the starting box and continue with a new search from there. An estimate of strategy was acquired when the participant began a new search strategy with a different box. This was only however measured in the six and eight box condition (Cambridge Cognition, 2006).

Validity of the CANTAB has been evidenced in studies of correlations between subtests of executive functioning and spatial reasoning (Kim, An, Kwon, & Shin, 2014; Torgersen, Flaatten, Engelsen, & Gramstad, 2012). Validity of the test has also been investigated in healthy participants showing moderate to high correlations with traditional cognitive assessments of WAIS, Controlled Oral Word Association Test, Animal Naming test, Trail Making Tests A and B, Stroop test and Green Story Recall test (Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013). The CANTAB is advantageous due its ease in computerised administration and logging of outcome measures can be programmed through the software. This saves time and reduced experimenter errors.

The resultant score on the number of errors made in the between errors condition and number of double errors made in the four, six and eight box conditions were converted to z-scores. The normal distribution falls between +1.96 and -1.96 SD of the mean.

## Psychosocial measures

### Behaviour Rating Inventory of Executive Function – Adult version (BRIEF-A) Self report (Gioia et al., 2000).

The behaviour rating inventory is a meta-cognitive psychosocial questionnaire assessing clinical cognitive constructs relating to executive function. Executive function components assessed from the BRIEF-A included inhibition, attentional shift, emotional control, self-monitoring, initiation of tasks, working memory, planning and organisation, task monitor and organisation of materials. Cognitive domains were then grouped into sub-indexes comprising of the sub-tests aforementioned. The behavioural regulation index (BRI) was the first index acquired which represented the ability to regulate control of emotional responses. It was composed of inhibition, shift, emotional control and self-monitoring scale. The next index was the metacognitive index (MI) which represented problem solving ability, planning, organisation and working memory. Finally, a global executive composite (GEC) was acquired as a summary score measuring all scales. This is a summary score that represented all clinical scales. Furthermore, inconsistency scores and negativity scores have been implemented into the questionnaire to measure negative emotions associated with participants' meta-cognition and inconsistency throughout the questionnaire. This was measured to assistant in assessing if the questionnaire was filled in, in haste or in a systematic pattern. Inconsistency and negativity scores can also be used as a means to measure validity of questionnaires. Outcome measures were raw scores that were converted to T-scores and have a mean of 50 and SD of 10.

Three measures of reliability have been made on the BRIEF-A. The BRIEF-A manual shows that internal consistency of the BRIEF-A is moderate to high with alpha coefficients of 0.80-0.94 (Gioia et al., 2000). The GEC index has been shown to have coefficients of between 0.93-0.96 in mixed clinical/healthy samples. This reflects that items were measuring the same underlying construct of meta-cognitive executive function abilities. Test-retest reliability correlations of self-report measures were found to range between 0.82-0.94 on clinical scales and 0.93-0.96 on the GEC over an average of 4 weeks (Gioia et al., 2000).

Content validity has been measured through agreement by experienced neuropsychologists on BRIEF-A constructs. Average agreement across items ranged from 35%-98%. Low agreement was evidenced due to the removal of the task completion scale which was moved to another domain as it fit better statistically. Construct and discriminant validity has been assessed through correlations with other similar measures of meta-cognition and psychosocial function. Moderate correlations have were found with measures of anxiety and depression which may be explained by previous literature reporting depressive-executive dysfunction syndrome and anxiety induced executive function impairments. Exploratory factor analysis has been implemented by researchers to assess validity based on the internal structure of the BRIEF-A. A two factor model was revealed as being most suitable with each factor consisting meta-cognition and emotional control. Meta-cognition was comprised of sub domains of planning and organisation, initiation of tasks, working memory and task monitor. The second factor comprised of inhibition, shifting and self-monitoring. Factors were strongly correlated with each other. These variables remained constant in young and older adult cohorts (50-89 years) and in males and females (Gioia et al., 2000).

**Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983)**

The HADS is a self-rating instrument that was used to detect the presence of depression and anxiety present mentally or somatically (Zigmond & Snaith, 1983). It focuses on non-physical symptoms so that it can be used to diagnose depression in subjects with significant physical ill-health such as cancer or specific diseases. Validity has been assessed with large cohort samples using exploratory factor analysis. Researchers have shown that a two factor model best explains variance on the scale with factors being depression and anxiety accounting for 57% of variance. Moreover, high test-retest intra-class correlations have been reported with values of 0.944 (Michopoulos et al., 2008). Items on the HADS were scored from 0-3 with a maximum potential score of 21 on anxiety and depression measures. Cut-off scores were defined as being 0-7 = normal; 8-10 = borderline and 11-21 = abnormal on either anxiety or depression parts of the questionnaire. The range of scores obtained in the current sample was from 0-13 on the anxiety part of the questionnaire and 0-9 on the depression part of the questionnaire.

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**Appendix 4: Participant Information Sheet**

# Participant Information Sheet

**Study title: Modelling risk and resilience factors associated with cognitive changes following testosterone depletion in older men.**

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you.

**One of our team will contact you at a later date to go through the information sheet with you and answer any questions you have.**

Thank you for taking the time to read the information sheet. The sheet is 9 pages long. Please make sure you have read all 9 pages.

If you require any help with the information sheet please call

Please read this information sheet carefully.

**What is an information sheet?**

The information sheet explains to you clearly and openly all the steps and procedures of the study. The information is to help you to decide whether or not you would like to take part in the research.

**What is this research study about?**

This study aims to understand whether undergoing androgen deprivation therapy affects cognition in patients with prostate cancer. We are interested in men who **have** prostate cancer compared to healthy participants who do not have such a condition and who are not undergoing androgen deprivation therapy. We wish to assess this from both a behavioural perspective, brain imaging perspective and from a quality of life perspective.

Currently, we know very little about the cognitive effects of androgen deprivation therapy in prostate cancer patients. However, researchers who have looked at cognitive effects have found inconsistent findings which are contradictory and limited. Only three studies in the past have examined androgen deprivation therapy and cognitive side effects using brain imaging. This study will use a number of cognitive tests and brain imaging tests together in order to find a consistent pattern of findings between one time point and a follow up study of six and 18 months. This will in turn benefit future prostate cancer patients to retain as much cognitive functioning as possible and to have a higher quality of life.

**Why have I been invited to take part?**

- You have been invited to take part because you are a healthy male who does not have prostate cancer or any other serious medical or neurological condition.
- You are male and are between the ages of 50 - 80.
- You have not received androgen deprivation therapy in the past.
- You do not have any signs of epilepsy, history of stroke, or neurodegenerative conditions.
- You speak and write English.

A total of 100 men will take part in this study. Fifty will be patients just about to have androgen deprivation therapy and another 50 healthy men will not have had prostate cancer or are not undergoing androgen deprivation therapy.

**Can everyone take part in this study?**

If you are in one of the above groups, you can take part in this study.

When the researcher telephones you, they will ensure that you are able to take part in the project. They will explain the study in more detail and you will be able to ask ***any*** questions.

**Do I have to take part in this study?**

No. Participation in this research project is **voluntary**. It is up to you to decide if you would like to take part in this study.

If you do decide to take part in this study, we will ask you to sign a consent form.

If you agree to take part in the study, but then change your mind, you can leave the study without giving any reason. You can withdraw yourself from the project at any time without any explanation.

**What will happen to me if I decide to take part?**

If you decide to take part, an appointment will be arranged at a time most convenient for you. Appointments will take place at the University of Birmingham.

In your appointment at the University of Birmingham, a researcher will go through this information sheet with you again. You will have the opportunity to ask any questions you may have. If you decide to take part, you will be asked to sign a consent form.

If you are happy with the procedure and have signed the consent form, you will be asked to complete a number of tasks in a screening phase. These should take no longer than 50 minutes. If you score below average on any of these tests, then a letter will be sent to your general practitioner (GP) and you may be excluded from the study. You will be given medical supervision from the GP and will not have to continue in the study. If you are eligible to continue further in the study then you will have a blood and saliva swab test. This will be taken in order to measure your testosterone levels and for detection of certain genetic strains. The genes include: Apolipoprotein E4 (ApoE4), catechol-O-methyltransferase (COMT), dopamine active transporter (DAT1), brain derived

neutrophic factor (BDNF), monoamine oxidase (MAO-A), ER-alpha (estrogen receptor-alpha), ER-beta, AR (androgen receptor), Aromatase gene (CYP19), *GNB3* single-nucleotide polymorphism rs1047776. Information on yours or other individuals' genetic information will not be revealed to you during or after the study has been completed. Blood samples will be processed at the University of Birmingham. Saliva samples will be transported to Coventry University within 7 days for processing.

You will also be given a number of questionnaires about your general mood and quality of life. If you have any problems completing the questionnaires, a researcher will be able to help you. On average the questionnaires should take one hour to complete.

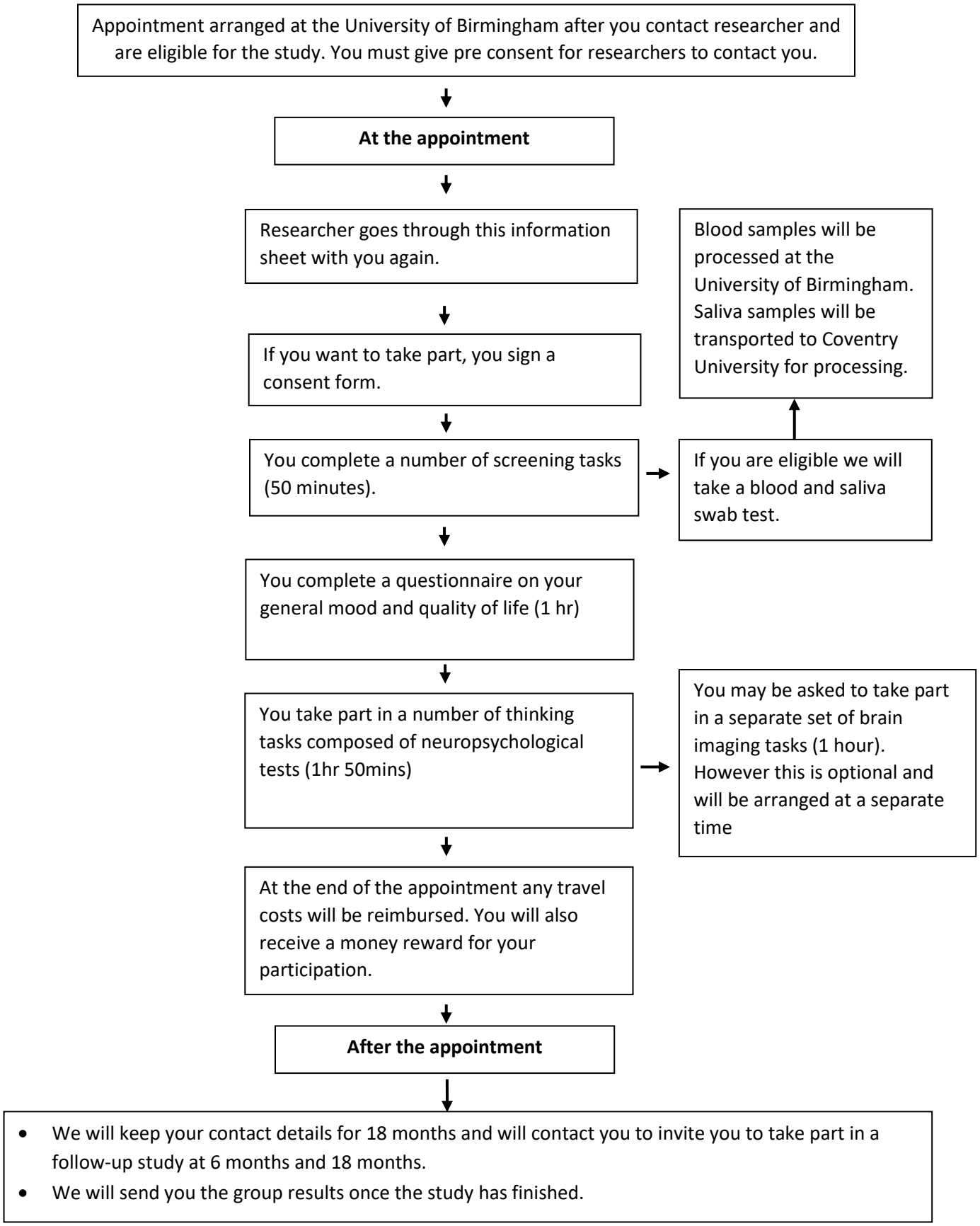
You will then be asked to complete a number of paper based thinking skill tasks (cognitive tasks) (2 hours 10 minutes). Regular breaks between tasks will be taken to ensure you do not get tired. Altogether the appointment should last about **4.5 hours**. If you become tired during the appointment, we will be happy for you to return on another day when it is convenient for you. You may be asked to complete tasks in a non-invasive brain imaging machine called a functional magnetic resonance imaging scanner. This will take approximately 1 hour. However, this is optional and a separate session and appointment time will be arranged for this. If you do decide to participate then you will be rewarded for your participation as detailed below.

Your answers are entirely confidential and will not be disclosed to anyone outside the research team, but your GP may be forwarded your scores for your wellbeing and optimal treatment.

At the end of each visit you will receive £30 for your participation

**Please see diagram 1 on the next page which shows the study procedure.**

## Diagram




**Will you access my medical records?**

We would like to access your medical records in order to obtain information on your health status and previous medical history.

We will access your medical records ***only*** if you provide additional consent to this. If you are happy for us to access your medical records, you will be asked to indicate this on the consent form. If you do not want us to access your medical records, you do ***not*** have to provide additional consent to this.

***You can take part in the rest of the study even if you do not want us to access your medical records.***

If you agree for the researchers to access your medical records, only the following researchers will have access to the identifiable data:

Dr Amanda Wood (study supervisor), Dr Elizabeth Grunfeld (study supervisor) and research assistants suitably qualified.

Other study investigators and staff will have access only to de-identified data files (that is, your name will not be associated with the medical data).

**What happens next?**

Once the study has been completed we will send you a summary of the group results.

**Will you contact me again?**

The researchers will keep your contact details and will contact you in the following 18 months to ask if you would like to take part in the follow-up studies. We will ask you to give further consent to participate in future studies. The follow up study will consist of the same tests which you took part in, in the first study.

Agreeing to participate in this study does ***not*** mean you will have to take part in the follow up study. However, it does mean that we will contact you again and ask you if you would like to take part in the follow up study.

**Will I get rewarded for my time?**

You will receive £30 for your participation after each visit

# BIRMINGHAM UNIVERSITY IMAGING CENTRE

www.buic.bham.ac.uk

## GENERAL MRI INFORMATION SHEET FOR RESEARCH PARTICIPANTS

**Dear Research Participant**

Thank you for volunteering to undergo a research magnetic resonance imaging (MRI) brain scan at the Birmingham University Imaging Centre (BUIC).

Your participation at this research centre will help benefit understanding in the areas of knowledge about the central nervous system, in neuroscience and medicine. This information sheet broadly describes the MRI procedure, and answers some common questions. If you have any further questions, please do not hesitate to contact your researcher.

You can find out more information about what we do from our web site www.buic.bham.ac.uk

**What is MRI?**

MRI is a relatively recently developed technique which combines the use of magnetic fields and radio-waves to image the body. MRI does not use *any* ionizing radiation or X-rays and there are *no* known side-effects or cumulative risks.

For your safety you will be asked to fill out an MRI Safety Screening Questionnaire, and to remove all metallic items before you enter the magnet room. FMRI (functional MRI) uses similar methods to conventional clinical MRI to obtain 'functional brain images'. The technique relies on indirectly identifying small changes in blood flow/oxygenation in different parts of the brain.

**The MRI Scanner**

As you can see from the picture opposite, the scanner is a large cylinder which has a tube (bore) running through the middle, open at both ends.

You will enter the scanner tube on a moveable bed, laid down on your back, head-first, with your lower legs remaining outside the magnet's bore.

**Before You Arrive**

Individuals who are concerned about feeling claustrophobic during the scan may wish to discuss this with the researcher before their arrival. However, if you are nervous, please do not worry as we will do everything we can to ensure that you are comfortable and relaxed during the scan. You will hold a call button at all times which you can press to attract our attention and an intercom system will allow you talk to the scanner operator and the researcher. If you wish, a friend or relative is welcome to accompany you to the Centre. There is a reception area close to the scanner room for them to wait during the scanning procedure.

**When You Arrive at the Imaging Centre**

Prior to your scan you will be asked to fill out an MRI Safety Screening Questionnaire. This is necessary to ensure that it is safe for you to undergo an MRI scan. Certain participants cannot be scanned and should not enter the magnet area, for example people who have a heart pacemaker surgically implanted.

You will be given a full explanation of the scan or study by the scanner operator/researcher and there will be an opportunity for you to ask any questions that you may have. When you are ready, and agreeable to participate, you will be asked to sign a form obtaining your ethical consent to undergo the research study. In this form you will see that you may withdraw from the study at any time without having to give a reason. Whether you choose to participate or not will not make any difference to any normal medical care you are currently receiving.

Next you will be asked to remove all metallic objects from your person - before you enter the magnet room. Lockers are available for any valuables if required.

**Starting the Scanning Procedure**

Once you are positioned within the scanner, comfortable, and have no further questions, the researcher and scan operator will go into the adjoining room (where the control equipment is located) and speak to you from there via a 2-way headphone / microphone communication system. There is a large window between these rooms and a camera so that you can be monitored at all times.

We will be able to see and talk to you whilst you are within the magnet throughout the entire study. You will be given a call button which you may hold throughout the scan. You may talk to us via the intercom system within the magnet and you may press the call button at any time to attract our attention. You may stop the scan, come out of the magnet or leave at any time without having to give any reason.

**During the Scan**

During the scanning you will hear various tapping and bleeping noises (some may be quite noisy, so earplugs will be supplied). This is the normal sound of the magnet taking pictures. Different types of scan sequences make different noises, so please don't be alarmed. You may also notice some mild vibration of the scanner bed. This too is normal. We will talk to you after each scanning sequence to make sure you are still comfortable, and you may talk to us at any time by pressing the call button in your hand.

***Please note that during the scanning period itself it is absolutely vital that you keep your head completely still.***

Some of the time, the scanner will be silent but please try to keep as still as possible during these periods as well. The length of the study will vary according to your particular study type, so please check with your researcher for exact times.

**Results of the Research**

Please note that this centre is not a clinical diagnostic department, but is wholly research orientated. Consequently, we are unable to give individual assessment or medical opinion on any clinical condition

you may be experiencing (which is the responsibility of your doctor). However, your participation is invaluable and will form part of the growing knowledge and future progress of understanding the brain.

Your pictures will be analysed over several weeks/months, usually as part of a group of anonymous volunteer scans. Research papers are published by researchers at this centre in national and international academic journals of excellence, most of which are able to be purchased, or are sometimes freely available. More information can be found at our web site or from your researcher.

**Unexpected findings on your scan**

As you are aware, the images obtained of your brain are for specific research purposes only and are generally not suitable for diagnostic opinions. They do not form any part of your official medical records. However, although the pictures are not diagnostic scans, in the unlikely event that any unusual findings are noted incidentally by the scan operator, further advice will be sought and you will be contacted at a later date to discuss any follow-up.

**After the Scan**

Once you have collected your belongings and spoken to the researcher/scan operator, you may leave. There are no known after-effects from being scanned in an MRI scanner.

UNIVERSITY OF BIRMINGHAM

**Birmingham University**

**Imaging Centre**

**www.buic.bham.ac.uk**

BUIC Birmingham University Imaging Centre

## PARTICIPANT INFORMATION SHEET

**Title of project:**

**Part 1**

**Introduction to the research and invitation to take part:**
You are being invited to take part in a research study. It is important that you understand why the research is being done and what it will involve before you decide whether or not to take part. Please read the following information carefully, and please discuss this with others if you wish. Feel free to ask us if there is anything that is not clear or if you would like more information.

**What is the purpose of the study?**
The study will be examining the relations between brain structure and brain function using magnetic resonance imaging.

**What kinds of stimuli will be presented?**
The stimuli will be visual images (pictures, words), sounds, tactile input or smells. Any stimuli that might potentially be distressing will be shown to you beforehand to enable you to judge if you feel distress. You will be able to withdraw from the study at any time (below).

**Do I have to take part?**
No. It is up to you whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. Withdrawing from the study will not affect you in any way (e.g., your future medical treatment).

**What will happen to me if I take part?**
You will undergo an MRI scan and you may also be asked to carry out a task while in the scanner. As you carry out the task we will measure changes related to brain activity which will inform us about how brain areas operate while a task is being undertaken. The scanning session will last about 45 min, during which time you will be asked to lie still.

**What is magnetic resonance imaging?**
Magnetic resonance imaging involves changes the gradient of a magnetic field to produce shifts in the alignment of atoms in the body of the person being scanned. The changes in alignment can be used to measure the structure and function of the tissues. When the brain is scanned we can derive information about both brain structure and function. The procedure is non-invasive and carries no known harm outside of safety issues for operating in a high magnetic field (e.g., if you have a cardiac pacemaker). For this reason you will be asked

to go through a safety questionnaire with a scan operator prior to being allowed to proceed into the scanning environment.

**What are the possible benefits of taking part?**

By learning more about how the brain works, by using MRI, we will be able to develop better ways of diagnosing changes in brain function, and we will learn about how to improve brain function to optimize performance.

**What happens at the end of the research study?**

The results will be written for scientific publication. In addition we will report them in a newsletter that we will distribute to all participants and to hospitals. All data will be reported anonymously.

**Using your data in other research** - we would ask you in the *consent form* if you are willing to share the data we collected from you with other researchers. If you agree we would upload your anonymized data on a server and make it available for research use. There are two levels of data sharing: sharing only with researchers associated with the University of Birmingham; or sharing it as an open access resource. In the latter case we would make the data available on the web as an open access resource. The latter adhere with current government and international policies on scientific data. ***You can choose to allow sharing your data in any or neither forms.*** In case of brain imaging data, you should be aware that there are software that can generate the skeleton shape of the face based on MR imaging data, hence by having access to your data one can potentially reconstruct your face. Furthermore, similar to fingerprint brain are individually unique, thus in the future technology may become available enabling to recognize an individual from the brain structure. If you are happy to share your data we would request you to fill up a short questionnaire about your general health and demographic. The data will never contain any personal details about you (name, address, etc.).

**What if there is a problem?**

It is possible that lying in the scanner might cause some back or neck pain, and it is possible to feel a burning sensation. If you experience any discomfort you can press the emergency buzzer and you will be brought out of the scanner immediately. Any complaint about the way the study has been conducted or any possible harm you may have suffered will be addressed. The detailed information on this is given in Part 2.

**Will my taking part in the study be kept confidential?**

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

***Contact Details* - BUIC Management:**

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

**Part 2**

**What will happen if I do not want to carry on with the research study?**

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. However, note that if you have agreed to share your data with other researchers (with UoB or as open access), you can only withdraw it on the day it was collected.

**What if there is a problem?**

The University of Birmingham has an insurance policy in place which provides cover for claims arising from negligent harm.

If you have any problems with the conduct of the study then you can contact various people:

(i) phone the Research Governance Committee of the University of Birmingham, who have considered this project, on [redacted] who will arrange for your concerns to be investigated.

(ii) Dr Amanda Wood, Ms Nina Salman or Ms Denise Clissett at the School of Psychology, University of Birmingham [redacted].

**Unexpected findings on your scan**

As you are aware, the images obtained of your brain are for specific research purposes only and are generally not suitable for diagnostic opinions. They do not form any part of your official medical records. However, although the pictures are not diagnostic scans, in the unlikely event that any unusual findings are noted incidentally by the scan operator, further advice will be sought and you will be contacted at a later date to discuss any follow-up.

**Will my taking part in this study be kept confidential?**

Our procedures for handling, processing, storing and destroying your data are all compliant with the Data Protection Act 1998. All information that is collected about you during the course of the research will be kept strictly confidential.

**Who is organising and funding the research?**

The research is organised by the University of Birmingham.

**Who has reviewed the study?**

This study was given a favourable ethical opinion by the Research Ethics Committee, University of Birmingham.

You will be given a copy of the information sheet and a signed consent

form to keep. Thank you for considering taking part and taking the time

to read this sheet.

**What are the possible disadvantages and advantages of taking part?**

There may be a chance that you may not feel well after your blood or saliva swab test. If so then you **MUST** immediately inform a nurse, doctor or staff personal on duty.

There is a possibility in this study that the tasks we complete with you may show that you have one or more difficulties in different areas of cognition or behaviour (for example the screening phase of the test could suggest a neurodegenerative disease such as dementia).

If difficulties are found, we will let your GP know and you may be able to get the help you need. This could be seen as an advantage of this study.

You may find it difficult to come to terms with such results. This may be seen as a disadvantage of this study.

You may also find the tasks tiring. We will make sure we take regular breaks. If you are unhappy and do not want to do the tasks, we will not continue the study. In this case you will not be given a cash reward but will be reimbursed for travelling costs.

The information we get from this study may not benefit you directly. However, it will help to ensure that men who need to undergo androgen deprivation therapy in the future are able to preserve as much cognitive function as possible.

It is important that you weigh up both the advantages and disadvantages of taking part in this study. Please talk to a researcher if you have any questions or want to discuss this further.

**What will happen if you find that I have difficulties?**

If we do find that you have some difficulties with the screening tasks we will inform your GP in a letter. They may wish to discuss these results with you so that appropriate support can be identified for you.

**What will happen if I don't want to carry on with the study?**

You can leave the study at any moment without giving a reason.

Any information collected which can be used to identify you (such as name, address) will be destroyed. Only information from you which has been made anonymous will be analysed. Anonymous means that no one will be able to identify you from that information.

**What will happen if I lose my mental capacity?**

Mental capacity means that you are unable to make informed decisions. Sometimes when people have mental health difficulties, their ability to make decisions can worsen during periods of ill health. This might affect your ability to provide us with informed consent.

If this happens, the researchers will withdraw you from the study and any data collected will be withdrawn from the study.

**What if I am already taking part in another study?**

If you are taking part in another study you can still choose to take part in this project. However, you need to make sure that taking part in multiple studies will not interfere negatively with your work/home life. Taking part in a study requires time and effort. If you are already taking part in another study and feel you do not have enough time to take part in this study, you don't have to.

**What if there is a problem?**

If you have a concern about any part of this study, you should speak to the researchers who will do their best to answer your questions

If you continue to have concerns then you can contact the ethics committee of X on Y.

You can also contact the Patient Advice and Liason Service ("PALS"). The nearest office to our premises is PALS University Hospital Birmingham NHS Foundation Trust,

The Birmingham and Solihull Mental Health NHS Trust PAL service offers 24 hour service, 7 days per week. They can be contacted on FreePhone: , Phone: (24 hours, 7 days per week)
They are located at

PALS also has an online resource (http://www.pals.nhs.uk/) that offers details of their service as well as the location of other offices that might be more convenient to you.

**Will my taking part in this study be kept confidential?**

Any data collected from you will be kept strictly confidential.

You will be assigned a unique code. Results from the questionnaires and tasks will be stored next to your code in a different place to where your contact details (name, address, telephone

number) and consent forms are stored. No information which can be used to identify you will leave the University of Birmingham premises.

All hard copies of documents will be stored in locked filing cabinets in the lab of Dr Amanda Wood, which will be locked when the researchers involved in this study are not there. All other information will be stored on password-protected computers. Information which is stored on computers will have your name and contact details removed so that the information cannot be traced directly to you.

Only researchers from this research team at the University of Birmingham will have access to your information. They will store your information after the study has ended and will contact you in up to 18 months to ask if you would like to participate in follow-up studies.

You can ask to see the information stored about you if you wish to do so. If you believe the information stored is incorrect, the researchers will correct it.

If you wish to leave the study, information which may be used to identify you will be destroyed.

All verbal information you disclose will also be kept confidential.

Confidentiality will only be broken if the researcher has serious concerns for your safety or if they have serious concern that you or others may be harmed.

**Will my doctor (GP) know that I am taking part in this study?**

Your family doctor will be informed that you are taking part in this study. They will be sent a brief letter saying that you are taking part in this study. Your doctor will also be sent a copy of your report if you have scored under average in the screening phase of the experiment. The report will say how you performed on the tasks.

**What will happen to the results of this study?**

Results from your participation will be analysed together with results from all of the other participants in this study. We may publish these results in scientific journals. Results that are published will typically be group results. We will **not** publish individual results with identifying information. This means it will be impossible for anyone to identify who participated in this study from the publications.

We will also send you a clear summary of group results and the publications if you would like to receive these.

**Who is organising the research?**

The research is sponsored by the University of Birmingham. The following researchers are involved in this study:

Dr Amanda Wood – Chief Investigator
Dr Elizabeth Grunfeld – Co - Supervisor

Mr Paras Joshee – Doctoral Researcher

**Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your interests. This study has been reviewed and given favorable opinion by ..... Research Ethics Committee.

**Who can I talk to about this study?**

You may want to talk about the project with:

- your family
- your friends
- your doctor
- the researchers (contact details listed below)

Please feel free to contact **Dr Elizabeth Grunfeld** or **Dr Amanda Wood** if you have any questions:

**Appendix 5: Phone Screening Form.**

# fMRI study phone screening sheet

**Screening date:_____ Appointment:_____**

**Screened by:_____ Arriving Via:_____**

**Meeting @ where?_____ time?_____**

Name_____ ID: _____ Phone number_____

Sex_____ Date of birth_____ Email_____

Handedness R/L

Sinistral family members Y/N If YES, then who (are they blood relatives):

OK to speak? ☐ Had chance to read participant information sheet? ☐

**Medical details: Any current or past history of being diagnosed with the following:**

| Diagnosis? | Yes/No | When? Where? How? What? Why? Who? Medication? |
|---|---|---|
| Cancer | YES / NO | What cancer? Treatment? |
| Any difficulties walking or climbing stairs? | YES / NO | |
| Heart disease, incl angina | YES / NO | |
| *Diabetes | YES / NO | |
| Implanted insulin, drug or infusion pump | YES / NO | |

| | | |
|---|---|---|
| Hypertension | YES / NO | |
| *Head injury or concussion | YES / NO | |
| *Stroke | YES / NO | |
| *Meningitis / encephalitis | YES / NO | |
| *Epilepsy / seizures | YES / NO | |
| Asthma | YES / NO | |
| Depression / anxiety / other psychiatric | YES / NO | |
| Speech or language problems | YES / NO | |
| Any implanted metal | YES / NO | |
| Stent, catheter, coil, filter in any blood vessels | YES / NO | |
| Aneurysm clips (metal clip put around blood vessels) | YES / NO | |
| Body piercing(s) | YES / NO | |

| | | |
|---|---|---|
| Cardiac pacemaker or defibrillator | YES / NO | |
| Cochlear, otologic, or ear implant | YES / NO | |
| Hearing aid | YES / NO | |
| Eye implants | YES / NO | |
| Tattoos or permanent makeup | YES / NO | |
| Artificial limb or prosthesis | YES / NO | |
| Broken bones | YES / NO | How? Bump head? Hospital? What happened after? Painkillers? Dizzy? Insert any pins? |
| Bone/joint pin, screw, nail, wire, plate | YES / NO | |
| Surgical staples | YES / NO | |
| Electrical stimulator for nerves, bone or brain | YES / NO | |

| | | |
|---|---|---|
| Any implant held in place by a magnet (e.g., dental) | YES / NO | Permanently fixed? |
| Coloured contact lenses | YES / NO | |
| Welder, machinist, grinder or worked with metal without eye protection? | YES / NO | |
| Any metal fragments (e.g., shrapnel), gun pellets, bullet | YES / NO | |
| Any surgery or operation of any kind | YES / NO | Insert any pins? |
| MRI before | YES / NO | At UoB? |
| Claustrophobia | YES / NO | |
| Transdermal skin patches (nicotine patch) | YES / NO | |
| Smoker? | | |

Alcohol and other (illicit) drug history (if yes, then details of frequency, amount):

**Current medications (details of name, purpose):**

**Mention unexpected findings procedure……**

**Are you happy for us to contact your GP? Y/N**

**Once completed would you like to be emailed a lay summary? Y/N**

**Appendix 6: Consent Form**


# Consent form

THE UNIVERSITY OF BIRMINGHAM

School of Psychology
The University of Birmingham
Edgbaston
Birmingham B15 2TT
United Kingdom

Study name: **The Effects of Androgen Deprivation Therapy on Cognition**

Participant identification number:

Name of researcher spoken to:

*Please write your inititals in the box*

1. **I confirm that I have read and understand the information sheet dated..... (version.....) for the above study.** ☐

2. **I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.** ☐

3. **I understand that my participation is voluntary and I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.** ☐

4. **I agree to have a blood test.** ☐

5. **I agree to my GP being informed of my participation in the study** ☐

6. **I agree to be contacted at a later date up to six months from now.** ☐

☐

7. **I understand that I will be withdrawn from the study if my ability to provide consent changes. Data already collected from me will be kept.**

8. **I agree to take part in the above study** ☐

☐

9. **Consent to access medical records** *(please tick <u>one</u> of the boxes)*

<u>I agree</u> for the researchers to access **my** medical records. ☐

If you agree above, then please acknowledge the following by ticking the small box:

I understand that relevant sections of my care record and data collected during the study may be looked at by responsible individuals from The University of Birmingham or from regulatory authorities, where it is relevant to my taking part in this research

I acknowledge the above ☐

<u>I do not agree</u> for the researchers to access **my** medical records. ☐

| Name of Participant Signature | Date | |
|---|---|---|
| Name of person taking consent | Date | Signature |

Please contact **Dr Elizabeth Grunfeld** or **Dr Amanda Wood** if you have any questions:

## Appendix 7: Neuropsychological measures – duration breakdown

Study measures

| Timing of measure | | Measure | Domain | Time taken (mins) |
|---|---|---|---|---|
| | | | | |
| **Screening** | | | | Total – 45 mins |
| screening | Mini Mental State Exam (MMSE)<br>Wechsler Abbreviated Intelligence Scale II (WASI-II)<br>fMRI screening<br>Consent form. | | Cognitive impairment (dementia), premorbid intelligence, verbal intelligence. | 45 |
| Demographic information | | | | |
| screening | Age, marital status, ethnicity, occupation, living arrangements, education level | | | 2 |
| Clinical information (gained beforehand in PC patients). | | | | |
| From notes | Diagnosis (inc date), tumour grade, treatments received (with dates), ADT treatment (inc start date/end date/frequency), co morbidities, family history (dementia, Alzheimer's) | | | 2 |
| **Neuropsychological battery** | | | | Total – 1 hr 30 mins |
| | Delis Kaplan Executive Function System (DKEFS)<br><br>max duration allocated per DKEFS subtest:<br><br>- Trail making task parts A & B (11 mins)<br>- Verbal fluency test (5 mins)<br>- Design fluency test (5 mins)<br>- Colour word interference test (6 mins)<br>- Tower of Hanoi test (19 mins) | | Executive function/working memory | 46 |
| | CANTAB spatial working memory task (SWM) | | Spatial reasoning | 15 |
| | Behaviour Rating Inventory of Executive Function Adult Version (Brief A) | | Executive function/working memory | 20 |
| **Psychosocial battery** | | | | Total = 10mins |
| | Hospital anxiety and depression scale (HADS) | | Mood | 10 |
| | | | | Total Time = 2 hrs 45 mins |

## Appendix 8: Power Analysis

### Power Analysis

Power analysis was conducted using G*power software (Faul et al., 2007). Power required was calculated by main outcomes for each test. In the current study, MANOVAS were implemented based on main outcomes from each behavioural assessment. Number of participants required was based on effect size. The $f^2(v)$ effect sizes were implemented and used to calculate power. This $f^2(v)$ effect size is a measure of the difference between groups that is conveyed on a general dimensionless scale. The $F^2$ has been categorised by Cohen (1988) as being $f^2 \geq 0.02$, $f^2 \geq 0.15$ and $f^2 \geq 0.35$ and signifies small medium and large effect sizes respectively.

Effect sizes employed to calculate sample size were inputted as medium and large which for MANOVAS had been shown to be based on $f^2(V)$ equal to 0.15 and 0.35 respectively. These effect sizes were selected as they were practical. A 0.80 power β was employed which shows a 4:1 ratio trade-off between β (type 2 error) and alpha risk (type one error). The alpha error probability was 0.05. This is the probability of making a type one error where a false positive is made and the null hypothesis is rejected. The, sample size calculated to avoid the above errors are presented in the table below:

| Effect size $f^2(V)$ | Assessment | Power | Number of Groups | Critical F | Alpha error prob | Outcomes | Sample size required |
|---|---|---|---|---|---|---|---|
| 0.15 | D-KEFS | 0.80 | 2.00 | 2.32 | 0.05 | 5 | 92.00 |
| 0.15 | BRIEF-A | 0.80 | 2.00 | 1.84 | 0.05 | 12 | 128.00 |
| 0.15 | CANTAB SWM | 0.80 | 2.00 | 2.03 | 0.05 | 8 | 110.00 |
| 0.35 | D-KEFS | 0.80 | 2.00 | 2.46 | 0.05 | 5 | 44.00 |
| 0.35 | BRIEF-A | 0.80 | 2.00 | 1.96 | 0.05 | 12 | 62.00 |
| 0.35 | CANTAB SWM | 0.80 | 2.00 | 2.03 | 0.05 | 8 | 52.00 |

Main outcomes were entered per neuropsychological assessment and analysis conducted. The main outcomes for the D-KEFS assessment reflected the five primary contrast scaled scores on the trail making test, verbal fluency test, design fluency test and tower test. The BRIEF-A assessment had twelve main outcomes which were Inhibition, self-shift, self-emotional control, ability to initiate, working memory, monitor, planning and organisation, behavioural regulation index, organisation of materials, behavioural rating index, metacognition index and global executive function scores. The

CANTAB main outcomes reflected conditions in the between errors four box, six box, eight box and total errors. Double error conditions added were errors in the four box, six box, eight box and total errors.

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## Appendix 9: Imaging Methods

### Functional Magnetic Resonance Imaging Approaches.

Functional magnetic resonance imaging (fMRI) was a key component in the current study. It was implemented because fMRI has significantly increased researchers' ability to understand the localisation of brain activity. Due to the availability of fMRI scanners as well as the functional aspects of fMRI to measure cognition, research has grown in popularity. Furthermore, the non-invasiveness of MRI allows researchers to scan subjects a number of times and for longer durations thus increasing signal to noise ratio.

The above techniques comes with a variety of ways to analyse data that each have positive and negative characteristics. This is because each tool can measure only partial but not all neural computations that occur during an imaging run. However, firstly to understand fMRI the causal relationship between the blood oxygen level dependent (BOLD) signal and neural activity must be examined. The BOLD signal does not correlate perfectly with action potentials but it measures a mix of continuous membrane and action potentials. Recent evidence suggests that the design of fMRI experiments may yield perfect causal relations between the BOLD signal and neural activity. However, this is not the case and the BOLD signal can only explain neural activation partially. While this is complicated to in understanding the BOLD signal, it offers the opportunity to understand neural information beyond action potentials and includes a variety of signals which are important in neural computations.

**The BOLD signal**

Magnetic resonance imaging measures the effect of diploes in a magnetic field on electromagnetic radio frequency waves (Logothetis & Pfeuffer, 2004). Magnetic resonance (MR) signal arises from hydrogen nuclei in water (Haacke, Lai, Yablonskiy, & Lin, 1995). These nuclei are the only dipoles present in sufficient density to sustain any signal at high spatial resolutions (Haacke et al., 1995). The energy transition of these diploes between energy states leads to an MR signal. Transitions occur on the basis of nearby tissue or physiological activity. This is represented as MR image measurements. When human tissue is present in the longitudinal axis (B0) field of the MR scanner, hydrogen nuclei are in a low energy state referred to as the resting state (Logothetis & Wandell, 2004). The presence of a high tesla magnet (1.5T to 7T) causes nuclei to align either parallel or anti-parallel to the main magnetic field (van der Zwaag et al., 2009). Magnetic resonance measurement begins when the researcher introduces a radio frequency (rf) pulse into tissue. The pulse forces nuclei from their resting state equilibrium into a higher energy state. This excitation however is only effective at the larmor resonance frequency (Logothetis & Wandell, 2004). This frequency is proportional to the magnetic field strength and gyromagnetic constant of hydrogen nucleus. This is the ratio of its magnetic dipole moment, to its angular momentum measured in radian per second per tesla (**rad**$\cdot s^{-1} \cdot T^{-1}$). The RF pulse elicits the specific amount of energy required to transfer nuclei into a higher energy state and realign in the magnetic field. Magnetic field pulses and rf excitations can be applied in a variety of timing and amplitude parameters. Variations in these parameters allow investigation of other various properties of the brain including structure, perfusion imaging, diffusion weighted imaging and functional neural activity. Information of tissue in the surrounding area is evaluated from the rate at which hydrogen nuclei return to a low energy state.

Exponential decay processes such as $T_1$ and $T_2$ describe the relaxation of nuclei back to a low energy state (Jarek, Flesher, & Shin, 1997). The T1 constant measures longitudinal relaxation in the direction of the main B0 magnetic field. The T2 constant measures transverse relaxation of the dipole in the x-y planes perpendicular to the B0 field. Transverse relaxation referred to as spin-spin interactions or spin dephasing allow transitions of energy to the local

field at neighbouring nuclei. These interactions cause exponential signal decay and are referred to as T2 (Michaeli et al., 2002). However, when decay occurs in tissue, transverse relaxation is additionally rapid due to field inhomogeneity's and this is referred to as T2* (Chavhan, Babyn, Thomas, Shroff, & Haacke, 2009). Inhomogeneity's depend on physiological states and blood supply which is dependent on neural activity for glucose metabolism. Therefore, T2* is an indirect measure of neural activity (Detre & Wang, 2002).

**BOLD Contrast**

The BOLD contrast mechanism is the mechanism relating neural activity to the T2* measure. The T2* parameter is dependent on neutral activity and changes in relative concentration of oxygenated and deoxygenated blood. Oxygenated haemoglobin (hb) is not paramagnetic however, deoxygenated haemoglobin (dhb) does have this property which influences the T2 parameter. The T2 value increases quadratically with field strength and the effect of T2* increases exponentially in the presence of dhb (Ogawa, Lee, Kay, & Tank, 1990). Researchers found that contrast changes with variations in blood oxygen demand (Ogawa, Lee, Nayak, & Glynn, 1990). Contrast increases were therefore attributed to paramagnetic dhb from red blood cells (Ogawa, Lee, Nayak, et al., 1990). Increases in dhb may be proposed to decrease BOLD signal. However, enhancements in signal typical of fMRI experiments during neural activity have been proposed to be due to overcompensation of blood cerebral blood flow (cbf) for decreases in oxygen (Fox & Raichle, 1986). A shortcoming to this description is the disparity between supply and consumption remains unclear. This is because glucose consumption during neural activity matches demand but over supply of oxygen does not (Logothetis & Wandell, 2004). Few theories postulate that cerebral vasculature delivers fixed amounts of glucose to specific regions. Therefore, if more than one system requires aerobic or anaerobic processes that require glucose, then oxygen is likewise delivered in surplus (Magistretti & Pellerin, 1999). Further speculation suggests that inefficient delivery mechanisms create inefficient diffusion. Therefore, an oversupply is required to fulfil demand through passive diffusion at high flow rates (Buxton & Frank, 1997). This glycolysis process is favourably upregulated over oxygenation during brain activation and the ratio of oxygen to glucose falls (Dienel, 2012). Therefore, excess oxygen delivery is termed as aerobic glycolysis whilst glycolysis during hypoxia is termed anaerobic glycolysis (Mergenthaler, Lindauer, Dienel, & Meisel, 2013).

These explanation can be tested by comparing theory with the time course of BOLD. The hemodynamic response function (HRF) is the amplitude and time course of the BOLD response to a brief stimulus (Logothetis & Wandell, 2004). Sensory and motor control responses are quick and can end in a few milliseconds. However, the BOLD response described through the HRF can occur up to a few seconds after stimulus response. This typically rises to a plateau of 6-9 seconds after stimulus onset and then returns to baseline with an undershoot sometimes in post stimulus intervals (Logothetis & Wandell, 2004). Predictions can be made from HRF responses to model into linear time invariant systems in which long duration responses are predicted from short duration events (Robson, Dorosz, & Gore, 1998). The neuro-metabolic connection between source and demand through cellular and neurovascular coupling as a function of the BOLD response have been investigated though deoxyglucose autoradigraphic techniques. This technique allows the ability to spatially gauge regional cerebral activation and glucose depletion in animals (Sokoloff, 1977). Further research has also examined local blood flow and oxygen consumption in humans using radiotracer techniques such as positron emission tomography (PET) which measures the indirect effects of neural activity on cerebral blood volume. Although this coupling between regional blood flow and neural activity exists, the cellular mechanisms and sites dominating these processes remains inconclusive.

Investigators have suggested that vascular density rather than number of neurons correlate highly with synapses (O'Kusky & Colonnier, 1982; Schuz & Palm, 1989). Researchers showed that cortical vascular networks can be divided into four layers which overlap with brodman layer cytoarchitecture. The first level has the lowest vascularization and consists of layers oriented parallel to neural fibers within the lower molecular layer. Layers have increasing amounts of vascularity from layers two to four with increasing numbers of perisynaptic elements rather than neuronal somata (Duvernoy, Delon, & Vannson, 1981). Subsequently, primary sensory areas are characterised by higher capillary density (Duvernoy et al., 1981).

Cerebral metabolic rate (CMR) can be used to measure neural activity since glucose is metabolised through aerobic or anaerobic glycolysis to adenosine triphosphate (ATP) by neurons for energy (Mergenthaler et al., 2013). The function of specific neurons may reflect their shape, electrical activity and size (Mergenthaler et al., 2013). This raises questions of how cerebral flow is coupled to energy depletion by neurons and which specific cellular mechanisms govern these processes. Astrocytes, which are a specific type of glial cell have

the functional characteristics to make them ideal gateways between neutrophil and intraparenchymal capillaries. Furthermore, they are connected with neurons and brain vasculature. Researchers propose that for each synapse, two $Na^+$ ions from an astrocyte uptake one glutamate molecule and one glucose molecule enters the same astrocyte. This glycolysis process of glucose thereby releases two ATP molecules and two lactate molecules which can be manipulated by neurons to produce 18 ATP molecules. This occurs in neuronal sites through oxidation with the introduction of phosphate molecules (phosphorylation) (Sibson et al., 1998). Nuclear magnetic resonance (NMR) spectroscopy studies reveal that the consumption of the excitatory neurotransmitter glutamate, is equivalent to the rate at which glutamate is changed to glutamine (Sibson et al., 1998). This conversion occurs in astrocytes and conversion energy is delivered through glycolysis (Schousboe, Scafidi, Bak, Waagepetersen, & McKenna, 2014). Astrocytes are further abundant in glucose transporters which are driven by $Na^+$ gradients and therefore a tight coupling exists between Glucose and $Na^+$ uptake. Studies illustrate that up to 80-90% of energy demands are from glutamatergic neurons in rodents (Sibson et al., 1998) and humans (Pan et al., 2000).

Investigators have endeavoured to isolate neural events that trigger vascular cerebral blood flow and consume the most energy. Researchers assumptions are based on the number of vesicles released per synapse during action potentials and post-synapse receptor activation with vesicle release. The metabolic consequences of activating single receptors, ion fluctuations and neurotransmitter recycling are modelled to calculate energy expenditure (Logothetis & Wandell, 2004). Post-synaptic events have been attributed to consume the largest amount on energy through glutamate in humans (70%) (Harris, Jolivet, & Attwell, 2012). Some conflict remains on dissociations found between neural signalling to initiate CBF verses energy consumption driving CBF. This is because neurochemicals such as gamma-aminobutyric acid (GABA) or glutamate have been known to control blood flow in areas such as the cerebellum in purkinje cells and interneurons (Caesar, Offenhauser, & Lauritzen, 2008). This is supported in a study demonstrating that GABA is required to elicit CBF in the cerebellum of rats (Thomsen, Offenhauser, & Lauritzen, 2004). Researchers tested their hypothesis through GABA blockade and increased Purkinje cell spiking activity. This led to an increase in energy consumption but did not change blood flow. Outcomes indicate that CBF changes required to support signals such as BOLD, are not due to neuron spiking activity, but that other neurochemicals could trigger increases in CBF (Thomsen et al., 2004). Findings propose that presynaptic activity and neuronal recovery initiate energy

production. This leads to the conclusion that CBF mechanisms are controlled by energy production prediction rather than those that measure depletion of energy.

The estimation of neural signals from the BOLD response is complex. The coupling between neural activity and the vascular response (hemodynamic response) is essential in defining amplitude and spatial resolution of the BOLD signal. Hemodynamic response efficiency is likely to be low in regions of sparse vascularization. However, areas such as white matter may have low vascularization density with increases in neural activity and energy metabolism. This is because white matter requires energy to restore ionic gradients which are distributed by the spread of action potentials at the Ranvier nodes (Harris & Attwell, 2012). The BOLD response has rarely been reported in white matter despite known energy consumption. Moreover, if any activity is found, then researchers may disregard findings altogether. Other shortcomings of the hemodynamic efficiency theory could be from parenchymal vascularity. This is because these arteries have constriction points which regulate cortical arterial flow. These changes in arterial diameter could determine hemodynamic efficiency and whether a region exhibits BOLD signal (Duvernoy et al., 1981; Harrison, Harel, Panesar, & Mount, 2002; Reina-De La Torre, Rodriguez-Baeza, & Sahuquillo-Barris, 1998). Further experiments conducted through simultaneous recordings of intrinsic signal of parenchymal flow with electrodes reveal that regional activation flow in one area often leads to reduced flow to the adjacent cortex. This can enhance local spatial resolution favourably when in fact activation does not match the tissue region from which signal are generated (Harrison et al., 2002). Taken together, the issues above suggest that caution should be applied in the interpretation of the BOLD signal in localized brain regions. This is because no reliable quantitative relationship can be made between the HRF amplitude and neural activity. This may still be the case when the BOLD response is controlled for by manipulating local blood flow into surrounding tissue (Hyder, Renken, Kennan, & Rothman, 2000; Kida, Kennan, Rothman, Behar, & Hyder, 2000).

### BOLD signal to Noise Ratio

The signal to noise ratio (SNR) is a measure of the desired signal to the level of background noise. This is the comparison of the signal acquired from the image to the background noise from the image. A quantitative description of SNR is the mean signal intensity in a region of interest (ROI) and the standard deviation of signal intensity measured in a region from which

no tissue is obtained (i.e. outside the ROI when the ROI is not present). Multiple variables can be manipulated by researchers such as Tesla strength and slice thickness which can assist in increasing SNR of images as these parameters influence background noise. However, scanner hardware can affect SNR and a linear trend has been found with SNR and field strength (Redpath, 1998). This is due to the increases in bulk magnetization of protons with higher field strengths in the $B_0$ field allowing magnetization over additional protons. This leads to increased spatial resolution as more protons emit signal relative to low field strengths (van der Kolk, Hendrikse, Zwanenburg, Visser, & Luijten, 2013).

Other sources of noise during fMRI can stem from respiration, cardiac pulses, motion or from task performance. Therefore, these sources of noise must be captured to compare with noise from outside of the ROI. Since artefact detection during fMRI is over a duration, imaging noise in individual images may not be suitable. However, mean temporal SNR (tSNR) can determine SNR of an fMRI time series (Triantafyllou et al., 2005). Improvements in contrast to noise ratio (CNR) BOLD in relation to SNR have been found with higher scanner field strengths (Wald, 2012). Furthermore, no other parameters were found to affect BOLD CNR signal excluding tSNR. This is important since CNR is based on contrast image quality rather than raw signal. Time series SNR can be calculated as the signal divided by the noise in standard deviations of the time series. The CNR heavily relies on the tSNR because it is the proportion signal intensity change between the activated and non-activated state, divided by the noise standard deviation of the time series total (Wald, 2012). This is important because it provides a measure of the slightest activations that can be identified and determines the statistical significance of strong and weak activation in a population.

In some ways CNR of a time series is preferred for structural MRI sequences as it allows the comparison of gray matter to white matter (Hyde, Biswal, & Jesmanowicz, 2001). This is because contrast refers to the properties to which the signal is sensitive. Therefore, areas with short $T_1$ values will be brighter such as white matter and dark areas indicating long $T_1$ weighted values. In summary, SNR is used to assess quality of single images, tSNR is used to assess the quality of and fMRI time series. Contrast to noise ratio can be used to evaluate the statistical significance of experimentally elicited signal variabilities in gray and white matter. These signals are then captures by other MR coils into K-space and eventually into images. Images are transferred from a spatial number in K-space using fourier transformations This is

a mathematical concept used to separate the signal obtained, into the frequency components that initially made the signal (Twieg, 1983).

**BOLD Signal in Ageing**

The BOLD signal is acquired through an indirect measure of cerebral perfusion which is largely dependent on neurovascular components and glucose metabolism. Widespread research has shown that the vascular system undergoes numerous changes as a function of age (Farkas & Luiten, 2001). Changes during ageing can be silent or can lead to more serious vascular compromise such as cerebral ischaemia or stroke. Atherosclerotic changes and compromises encompass the most prevalent forms of change during ageing. Variations with increasing age as an independent risk factor (Knox, Yates, Chen, & Klara, 1980) include vessel wall thickening (Furuta, Ishii, Nishihara, & Horie, 1991), smooth muscle cell necrosis (Masawa et al., 1994) and basement membrane thickening (Nagasawa et al., 1979). These factors reduce flexibility, elasticity and compliancy of affected vessels including capillaries, arterioles and cerebral arteries (Kalaria, 1996). Changes in arterial structure with age can eventually occlude vessels leading to either compromised or redistributed of blood flow. This will affect the BOLD signal as the signal relies on perfusion through this architecture which if narrowed or blockaded, may lead to signal drops and decreased SNR (tSNR). Moreover, decreases in resting cerebral blood flow have been found in cortical and subcortical brain regions with advancing age (Krejza et al., 1999).

Other sources of vascular change in ageing may a vascular reactivity component. This is a known reactivity of vasculature to chemical modulators such as upsurges in carbon dioxide concentrations resulting in arterial dilation (Tamaki, Nakai, Yokota, & Ogata, 1995). Decreased responsiveness has been found in animal models and in healthy ageing humans (Silvestrini et al., 2000; Tamaki et al., 1995). The BOLD signal in normal ageing has been measured using spatial and temporal features of the HRF. In theory if the HRF in young and old subjects is the same, then there should be no age related change in neural activation patterns. However, if there are changes in HRF between groups whilst subjects undergo a task, then any change in activation may be age-related and alterations in neurovascular coupling. One study found decreased SNR despite similar shape HRFs in old and young subjects on a simple motor task (D'Esposito, Zarahn, Aguirre, & Rypma, 1999). The decrease

was attributed to greater levels of noise on older participants which led to a decrease in the spatial degree of the BOLD signal. This suggests there are some neurovascular changes with age. Other studies have found similar reductions in the SNR and decreased activation patterns during behavioural imaging in ageing subjects (Hesselmann et al., 2001; Huettel, Singerman, & McCarthy, 2001; Taoka et al., 1998).

**Advantages and Disadvantages of fMRI**

Whilst advantages of fMRI are numerous and include that it is non-invasive; it does not require radioactive compounds; it can acquire high spatial scan resolution images and it can objectively measure brain activity (Glover, 2011). Some limitations are noteworthy.

Resolution images acquired from fMRI are limited by SNR. Due to the nature of noise in fMRI images, pixel size is normally increased even in higher field magnets to accommodate for this factor. Nevertheless, higher tesla magnetic neuroimaging also suffers from noise from motion artefacts, field inhomogeneity and scanner drift noise that all exponentially increase with higher tesla scanners (Boubela, Kalcher, Nasel, & Moser, 2014). However, analysis methods do offer regression methods to control for noise factors. Spatial smoothing may also allow inferences to be made in adjacent voxels from other nearby voxel activations (Wang, Wang, Aguirre, & Detre, 2005). Nonetheless, the signal acquired after all these processes is relatively small and difficult to interpret without computational algorithms (Glover, 2011).

Another shortcoming of fMRI includes that temporal resolution is restricted by the hemodynamic duration (Glover, 2011). This is because the BOLD HRF has an initial width of more than three seconds with the peak of this response occurring at five to six seconds after commencement of a neural event. The HRF is therefore slower than the underlying neural mechanism and temporal information is as a result, greatly skewed. Nevertheless, through paradigm jittering of event related stimuli, and various analysis methods, temporal interpretations can be attained in the range of 100ms (Ogawa et al., 2000).

**Arterial Spin Labelling**

Until a direct and non-invasive method is created where adenosine 5 triphosphate (ATP) consumption and production can be measured, researchers must rely on correlates such as CBF as an indirect measure of neuronal activity. Many methods of measuring CBF use tracer kinetics which yield models that define the changing aspects of the tracer as it passes through arterial architecture and into cerebral microvasculature. An advantage of arterial spin labelling (ASL) is that it uses endogenous arterial bolus as a tracer and does not require invasive injection of tracers which could be harmful or uncomfortable. Moreover, it is safe to repeat and can be used to track changes over time. Absolute blood flow can be measured through ASL which can be conveyed as meaningful units. Furthermore, it can produce higher quality spatial and temporal resolutions than any other techniques (Borogovac & Asllani, 2012).

The theoretical framework of ASL involves the labelling of arterial water before reaching the imaged volume. Labelling denotes the inversion of magnetic spins in protons. After a time delay, the labelled spins exchange with cerebral tissue and a labelled image is acquired (Alsop & Detre, 1996). This image has arterial water (bolus) which is in an altered magnetisation from static tissue water. This can be modelled as static tissue = +1 and labelled water as -1 (inversion). Signals acquired from a voxel in a labelled image indicates a sum over the blood and tissue spins. A control image is also acquired in addition to the labelled image in which the arterial spins have not been labelled through inversion. The control image is then subtracted from the labelled image at each voxel and is measured relative to the amount of CBF delivered to that voxel. This can be shown as an equation:

$$ASL_{signal} = \frac{(M_C - M_L)}{M_C}$$

Where $M_C$ is the control image and $M_L$ is the labelled image. Cerebral perfusion is calculated by applying a set of estimated physiological and MR factors on the ASL signal image. This is used to acquire voxelwise flow values in total physiological units. Several pairs of labelled and control images are acquired to ensure an average of multiple outputs have been acquired (Borogovac & Asllani, 2012). The two main types of ASL which exist are continuous arterial

spin labelling (CASL) and pulsed arterial spin labelling (PASL). Moreover, many software permutations have been designed within these categories. These variations methodologies allow greater background suppression of static tissue signal to improve SNR of the ASL signal (Garcia, Duhamel, & Alsop, 2005). Pulsed arterial spin labelling is used in the current investigation of patients and controls. This is because it is more easily implemented and requires only 10-15ms short intermittent pulses to invert spins in a specific region and referred to as the inversion slab (Golay, Hendrikse, & Lim, 2004). As aforementioned, a time delay is allowed until the bolus (inverted spins) reaches cerebral tissue where a $T_1$ image is acquired as the labelled image. A control image is also acquired for perfusion subtraction in the analysis (Golay et al., 2004). The PASL method of ASL is widely used as it is straightforward compared to CASL and also requires shorter labelling pulses.

In contrast, the CASL method uses long inversion durations which can lead to decreased signal. This is because of magnetization transfer (MT) effects which is when spin-spin cross relaxations occur in nearby protons. This can greatly reduce $T_2$ signal acquired due to very fast dephasing which shortens $T_2$ relaxation times (Wolff & Balaban, 1989). Therefore, an advantage of PASL is that it is less susceptible to magnetization transfer (MT) effects due to short inversion times which can in turn increase signal to noise ratio when labelled images are acquired (Wolff & Balaban, 1989). Nevertheless, PASL does have some drawbacks including low SNR, slice artefacts that limit brain coverage and sensitivity to bolus transit times. However, transit time confounds are worth noting and imperative to acquire ASL labelled images. This is because if arterial blood is labelled and a labelled image is acquired immediately following labelling, then CBF would be mis-estimated. Whereas if the image was acquired to late then voxels where bolus has left would be underestimated or overestimated. Therefore, blood destined for another voxel passes the intended voxel and reaches another voxel at washout. Hence, arterial transit time (ATT) which is the average time it takes for bolus to cross vasculature in the region of interest, and tissue transit time (TTT) which is the time required for the bolus to exchange with regional tissue, requires careful calibration. Nevertheless, a concession must be made between loss of signal due to relaxation processes and length of the delay transit (Alsop & Detre, 1996). This is important when whole brain imaging is required because transit times to specific regional destinations will have distinct transit times.

For studies when labelled blood is assumed to be in microvasculature, careful ATT estimation must be employed as in the current study. However, if multiple ASL images are

acquired at multiple delays with inversion bolus, then an estimate can be estimated using a kinetic curve model which can be fitted to CBF measurements. This model describes cerebral perfusion and assumes that very little bolus will be present in microvasculature at first. The bolus increases exponentially in cerebral architecture which is followed by a venous tracer washout period where the curve exponentially declines (Buxton & Frank, 1997). However, ATT values are normally assumed to be homogenous throughout the brain and vary linearly with ascending slices (Borogovac & Asllani, 2012).

**Resting State Functional Connectivity**

Resting state fMRI (rsfMRI) is commonly used as a functional brain imaging method to evaluate spontaneous regional interactions when a subject is not performing a task (Biswal, 2012; Buckner, Krienen, & Yeo, 2013). Any regional activations are measured using fMRI in which the BOLD signal is acquired at rest (Biswal, 2012). Resting state connectivity can be defined as temporal correlations of neural activity patterns between spatially distinct brain areas and neuronal groups (Biswal et al., 1997). More specifically, this refers to the level of co-activation of functional time sequences measured when a subject is resting (Lowe et al., 2000). Examining these integrative networks provides insights about neuronal group activity. Moreover, it allows investigation of networks when they function normally and can be used to discover altered patterns in neurodegenerative disease (Bullmore & Sporns, 2009; Greicius, 2008). Researchers have located a number of networks which are commonly activated using rsfMRI where it is thought that co-activations in these regions denotes communication between brain regions (Biswal, Yetkin, Haughton, & Hyde, 1995; Damoiseaux et al., 2006; Greicius et al., 2003; Salvador, Suckling, Schwarzbauer, & Bullmore, 2005). The neuronal basis of resting state activity comes from the observation that brain regions co-operate with each other to form a functional linkage during rest. These regions are correlated even though they are anatomically separated to form a network (Biswal et al., 1995; Damoiseaux et al., 2006; Lowe et al., 2000; Salvador et al., 2005).

Model dependent methods are commonly used to analyse functional resting state data. This is a simple way to examine functional connections. The technique involves the correlation time series in a particular region at rest against the time series of other regions which results in a functional connectivity map (fcMAP) (Biswal et al., 1997; Cordes et al., 2000). This defines the functional connections of the region of interest (Biswal et al., 1997; Cordes et al., 2000). The predefined region is called the seed region and can be selected in an a priori fashion from

a task-dependent activation map in a separate fMRI session. The seed forms the basis to measure connectivity with other identified regions. However, this approach is limited to functional connections from the seed region only, making it difficult to interpret connectivity on a whole brain scale (van den Heuvel & Hulshoff Pol, 2010).

Many studies have shown the development of functionally connected sub-networks that are robustly detected during rest and are frequently denoted as resting state networks (Damoiseaux et al., 2006; Fox & Raichle, 2007). Whilst these regions are anatomically distal, they show high levels of functional connectivity when at rest (Beckmann, DeLuca, Devlin, & Smith, 2005). These networks include the default mode network (DMN) which is of interest in the current study consisting of the medial prefrontal cortex, inferior parietal lobe, precuneous and temporal regions (Buckner & Vincent, 2007; Fox & Raichle, 2007; Greicius et al., 2003; Raichle & Snyder, 2007). Most identified networks also seem to share and overlap with putative resting functional networks during task activation (van den Heuvel & Hulshoff Pol, 2010). The DMN has elevated level of neuronal activity which can sometimes be higher than functional activity in distinction to other resting state networks. This indicates that this network reveals the default state of neuronal activity and makes it a good network to measure seed connectivity from predefined regions (Raichle & Snyder, 2007). Activity and connectivity of the DMN have been associated with core process including integration of emotional processing (Greicius et al., 2003); global monitoring (Gusnard et al., 2001); divergent thinking (Heinonen et al., 2016) and mind drifting (Mason et al., 2007). These complex higher level process related to the DMN indicate that it can provide a unique perspective in examining resting state connectivity, in neurological disorders and underlying cognitive thinking concepts (Bullmore & Sporns, 2009; Garrity et al., 2007; Harrison et al., 2007).

However, the mechanisms behind functional connections are difficult to understand without considering structural connections between regions. This refers to white matter tracts that are bundles of lengthy axons that interconnect with large groups of spatially distal neurons. Due to these properties, they allow the transfer of vast functional data between spatially distinct brain areas (Damoiseaux & Greicius, 2009). Diffusion tensor imaging (DTI) research illustrates that regions with higher levels of structural integrity, have greater functional connectivity to the DMN and other resting state systems (Greicius, 2008; Hagmann et al., 2008). Moreover, combined DTI and resting state data indicate that the integrity of the

cingulum tract is imperative to the interconnectivity of key regions involved in the DMN (Lawes et al., 2008; Schmahmann et al., 2007). Other white matter tracts have also been found to be interconnected to the DMN (van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). This suggests that there is a more common relationship between the DMN and other resting state networks (van den Heuvel et al., 2009). Nevertheless, the exact nature of this relationship is largely unknown (Bullmore & Sporns, 2009).

Advantages of resting state analysis include that resting state activity requires almost 20 percent of the body's energy expenditure and is used to facilitate ongoing neural signals at rest (Ames, 2000; Raichle & Mintun, 2006). Nevertheless, a majority of fMRI research is aimed towards functional task activations which require less than five percent of the bodies energy (Raichle & Mintun, 2006). Moreover, when participants complete a task, only 20% of total BOLD activity is task related which suggests that 80% is of signal is discarded as noise (Fox & Greicius, 2010; Fox & Raichle, 2007; Fox, Snyder, Vincent, & Raichle, 2007). However, BOLD modulations during resting state activity are comprised of most of the signal allowing a higher SNR (Daliri & Behroozi, 2013). This implies that most knowledge of brain activations come from studies attempting to understand only small components of functional activity (Daliri & Behroozi, 2013). Another benefit of resting state connectivity is that it can be used in clinical settings where patients or participants are unable to perform a task. Therefore, resting state activity can be a good indicator of brain function in the absence of task related neural measures. Limitations of task related activity is that there task activity is contaminated with noise. This can be related to physiological interactions or motion. Since resting state activity does not require any parameter adjustments, there are fewer instances of noise which increases SNR.

Confounds to resting state methods are that there is limited knowledge about neural interactions and their correlation to individual cognitive ability or skill. Therefore, task related functional activations must be used to characterise and inform the underlying brain regions at rest which inform inferences made of a structural network (Daliri & Behroozi, 2013). Additionally, between group variability such as tiredness, anxiety etc. are difficult to control during resting state scans which can limit analysis outcomes (Behroozi, Daliri, & Boyaci, 2011). Another noteworthy finding revealed that cardiac pulses and respiratory BOLD signal changes have been known to resemble the DMN (Birn, Diamond, Smith, & Bandettini, 2006). Caution must therefore be taken to separate DMN activity from noise

through regression or to measure pulse and respiratory rate during resting state activity. Finally, there is no control on what subjects are thinking during resting state analysis, therefore it may not be clear if the subject is truly resting (Daliri & Behroozi, 2013).

**Ageing and Resting State Connectivity**

Ageing has been associated with cognitive decline. However, researchers also speculate that not only does ageing lead to specific regional declines, but that functional connectivity integration underlie age related impairments. One study exploring age related deficits in a group of healthy younger (mean age 22.8 $\pm$ 2.3) and older (mean age 70.7 $\pm$ 6) participants measured intrinsic functional connectivity of the DMN (Damoiseaux et al., 2008). All participants also underwent an extensive neuropsychological assessment containing attention, information and motor speed, working memory and executive function tasks. Outcomes showed decreased activity in older participants compared to the younger cohort. Additionally, correlations between tasks of executive function and the anterior DMN regions were weakened in the older group. Limitations to the study were that the sample size was small especially in the young group and does not allow researchers to infer when age related deficits occurred along an age continuum. Nevertheless, these findings are of importance in ageing because the DMN has highly correlated with Alzheimer's disease due to amyloid-β deposition in DMN areas which can disturb DMN connectivity (Buckner, Andrews-Hanna, & Schacter, 2008). Many studies in general also suggest that ageing is associated with reduced resting state connectivity (Hafkemeijer, van der Grond, & Rombouts, 2012; Mevel et al., 2011; Prvulovic, Bokde, Faltraco, & Hampel, 2011).

Only one study has found improvements and declines with age and another study showed no age related declines in DMN resting state connectivity (Jones et al., 2011; Zuo et al., 2010). However, this may be because samples consisted of only middle age and older participants and suggests that age related decline may begin earlier than thought in middle age which resembles activity of the older group. However, this speculation remains inconclusive without a younger group for comparison concluding the study may be underpowered (Onoda, Ishihara, & Yamaguchi, 2012). Differences in study outcomes may stem from variations in model-free and model dependent methodologies and inconsistent analysis procedures between studies.

Many hypotheses have been made to determine the cause of reduced function connectivity with age. One of these theories is the loss of white matter integrity. Many studies support this notion showing that white matter integrity declines as a function of age. Weakening of white matter fibres has been correlated with decreased functional connectivity within specific brain networks in older populations (Chen, Chou, Song, & Madden, 2009; Davis, Kragel, Madden, & Cabeza, 2012; Teipel et al., 2010). White matter hyperintensities are also common in elderly participants (Soderlund, Nyberg, Adolfsson, Nilsson, & Launer, 2003). An investigation in subjects with severe white matter hyperintensities showed they also had decreased functional connectivity associated with the dorsolateral prefrontal cortex (Mayda, Westphal, Carter, & DeCarli, 2011). An increasing amount of research supports this disconnection theory in healthy ageing (O'Sullivan et al., 2001). One study found both increases and decreases in functional connectivity in patients with multiple sclerosis which is known to severely impair white matter tracts (Hawellek, Hipp, Lewis, Corbetta, & Engel, 2011). This may facilitate or impede affected regions that have become less efficient over time.

In conclusion, the mechanisms behind fMRI may allow comprehensive assessments of functional and structural integrity. However, caution must be taken when interpreting outcomes from ageing populations since numerous deductions can be made on the basis of under or over activations. Nevertheless, multi-model fMRI imaging approaches can help separate these factors to allow for a better understanding of brain activity in older populations.

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## Appendix 10: Pilot Study

### Background

Measures employed in the present study were the stop signal (SS) task and mental rotation (MR) task. The SS paradigm has been employed because response inhibition is considered a key component of executive function (Andrés, 2003; Aron, 2007; Stuphorn & Schall, 2006) and is suitable for assessment in a laboratory setting (Vince, 1948). The task employs the ability to supress inappropriate or no longer required responses (Verbruggen & Logan, 2009). Response inhibition impairments have been linked to disorders including attention deficit hyperactivity disorder (Nigg, 2001), obsessive compulsive disorder (Penades et al., 2007), and clinical disorders such as cancer (Chao et al., 2012). The paradigm typically involves a reaction time response on go trials (no stop) and inhibition of responses to stop trials. Performance on the stop signal task can be modelled as an independent horse race model which describes the processes between the probability of responding p(respond|signal) and the probability of inhibiting p(inhibit|signal) on stop signal trials (Logan & Cowan, 1984). The p(respond|signal) depends on three factors including the stop signal delay (SSD), go reaction time (go RT), and stop signal reaction time (SSRT). When the SSD increases, the p(respond|signal) increases. This is because the stop process starts later and finishes later in comparison to the go process which has already begun thereby forcing a response. As subsequent SSDs are presented the p(respond|signal) decreases and go RT increases. This is because of the increased probability that the stop process finishes before the go process (Verbruggen & Logan, 2009). Furthermore, SSRT increases for subsequent SSDs because the probability that the stop process finishes after the go process increases. The calculation of SSRT using p(respond|signal) is aptly explained in a model by
The SS task of the pilot studies below employed a fixed SSD design opposed to a performance adjusted SSD design. Both fixed and performance adjusted SSD tasks have been shown to activate the same brain regions in past studies. Previous investigations have found the inferior frontal gyrus (IFG), middle frontal gyrus (MFG), and anterior cingulate cortex (ACC) to be activated when inhibitory activity occurs through various paradigms (Ragozzino, 2007). Moreover, this is supported through findings of slower SSRT speed in participants with damage to the IFG and/or MFG (Aron et al., 2003). This confirms IFG and MFG involvement in monitoring during SS go tasks whilst simultaneously preparing for inhibition if a stop signal occurs. (Kringelbach & Rolls, 2004). The ACC has been correlated with post error monitoring, outcome monitoring, and post error slowing (Li et al., 2008). Research points to a neural network between the ACC and dorsolateral prefrontal cortex (DLPFC) (IFG/MFG) during subsequent task trials (Kerns et al., 2004).

The SS task is preferred over other tasks such as the go/nogo task since it requires intricate sensorimotor map representation, odd ball consideration, task switching, inhibition, and post error processing (Li et al., 2006). Neural network maps associated with the SS task and go/nogo task have

found activation of overlapping regions in the medial, mesial, and parietal and inferior frontal cortices (Rubia et al., 2001). The go/nogo task has been associated with lateralisation to the left hemisphere DLPFC areas specialising in motor planning and response selection (Rubia et al., 2001). The SS task has been correlated with right hemispheric laterization in the anterior cingulate cortex, inferior prefrontal, and parietal cortices which are known for specialising in inhibition (Rubia et al., 2001). These areas are predominantly associated with executive function, attention, and high working memory load (Rubia et al., 2001). Lesion studies furthermore supports right hemisphere dominance in which localised damage to the right IFG and MFG can lead to loss of inhibitory control (Drewe, 1975; Verfaellie & Heilman, 1987). This validates the SS task as a suitable measure to assess inhibitory executive function, because it requires higher inhibitory loads when stop signals are presented compared to the go/nogo task. In one study, prostate cancer patients undergoing androgen deprivation therapy (ADT) were assessed on the SS task compared to control patients who were not receiving ADT (Chao et al., 2012). No difference in SS task performance was observed at six months compared to baseline between or within groups. However, decreased connectivity was observed between the medial prefrontal cortex and dorsolateral prefrontal cortex (DLPFC), right insula, right superior temporal gyrus, and ACC in ADT patients compared to the non-treatment group (Chao et al., 2012). As aforementioned, these cortical regions have been implicated as being central to executive function. Therefore, lowered testosterone levels from ADT may contribute to brain impairments during therapy compared to control participants. Limitations of the study were that of a small sample size and patients were only exposed to ADT for a short duration. In light of the above study, the pilot study below uses brain imaging (fMRI) to assess neural correlates associated with the SS task, to find consistent activation in areas of the IFG/MFG (inhibition), and ACC (post error monitoring) so that the task can be implemented in a baseline to longitudinal study with ADT patients and healthy controls.

The mental rotation (MR) task was implemented in the study below to assess spatial reasoning ability. The task is considered as assessing spatial reasoning due to the requirement of participants to spatially manipulate, monitor, and update information as the task progresses (Potvin et al., 2013). The task necessitates mental rotation of shapes using executive function control and spatial orientation (Potvin et al., 2013). Past animal studies have found brain activation of bilateral occipital parietal lobes and supramarginal gyri, thought to be associated with spatial map generation to mentally code locations of targets (Andersen & Buneo, 2002). This is apparent through electrophysiological research of the superior parietal cortex in primates (Snyder et al., 1998). Individual cells in the area are known to be responsible for eye-centred coordinates modulated by primate head or body position (Snyder et al., 1998). Conversely, monkeys with spatial neglect in one part of space often have lesions in the parietal cortex (Karnath et al., 2001). Motor cortex activation during mental spatial manipulation is much debated throughout neuroimaging literature. This is because task activated regions must dissociate

between regions activated due to motor planning or motor demands of the task (e.g. button response pressing).

The assessment of spatial reasoning is of paramount importance in patients undergoing ADT since testosterone levels are exhausted (Green et al., 2002; Green et al., 2004; Joly et al., 2006). Researchers have explored the effects of testosterone on cognition with findings leading to the conclusion that testosterone does affect cognition, and especially spatial reasoning. For example one study assessed the effects of normal endogenous serum testosterone levels and normal free testosterone levels on neuropsychological performance (Moffat et al., 2002). Outcomes revealed a correlation between high visual memory, verbal memory, visuospatial function, and visuo motor scanning performance and high free testosterone levels. Higher mean free testosterone levels and longitudinal free testosterone levels were concomitantly associated with a decreased rate of visual memory decline (Moffat et al., 2002). Significantly impaired spatial reasoning performance was associated with visual memory decline in hypo-gonadal males (Moffat et al., 2002). The study therefore suggests a possible beneficial effect of free testosterone on cognition. A shortcoming was that participants classified as hypo-gonadal were on average eight years older than those participants with normal testosterone levels. Older participants have been known to have cognitive difficulties due to older age which may be a confounding factor (Lunenfield, 2003). Another study found that participants substituted with testosterone for six weeks had better spatial memory scores, and constructional ability scores compared to a placebo group (Cherrier et al., 2005). The above behavioural literature illustrates that much research from the past has found associations between testosterone levels and impaired spatial reasoning ability (Barrett-Connor et al., 1999; Fonda et al., 2005; Moffat et al., 2002). The MR task has also been assessed in a longitudinal nine month neuroimaging (fMRI) study with ADT patients compared to matched healthy controls (Cherrier et al., 2010). Reductions in right parietal occipital lobes were apparent when ADT patients performed the MR task compared to controls (Cherrier et al., 2010) leading to worse performance. This suggests ADT patients may suffer from spatial deficits during ADT treatment. Therefore, testosterone deprivation could lead to neuronal impairments which will be assessed in the current research.

Neural substrates associated with both tasks aforementioned are speculated to be areas of activation in the present study of healthy participants, but less so in ADT patients due to ADT treatment. Previous imaging research has often assessed young samples with an increased difficulty in fMRI paradigms (Lineweaver et al., 2005; Potvin et al., 2013). These paradigms could lead to floor effects indicating high task difficulty in elderly patients and participants. To account for slower reaction times of senior participants (Lewis & Brown, 1994), longer duration stimulus presentation times and inter stimulus

intervals will be incorporated. The pilot study below aims to assess if the MR and SS tasks elicit brain activation in areas analogues to past research, so that tasks can be incorporated into a longitudinal study in ADT patients compared to healthy controls.

**Pilot Study**

**Pilot 1: Imaging task Development**

Pilot one was trialled in healthy controls to assess executive function performance on the stop signal (SS) task, and to assess if activated brain regions were consistent with past research. Stimuli and inter stimulus intervals (ISIs) were presented with extended durations relative to SS paradigms of previous literature.

Higher levels of performance on go trials were expected compared to stop trials in which more commission errors were predicted. The inferior frontal gyrus (IFG), middle frontal gyrus (MFG), and anterior cingulate cortex (ACC) were hypothesised to be active on stop trials (successful inhibition) and ACC during commission errors. Activation of the motor cortex and cerebellum were speculated to be active during go trials

The mental rotation (MR) task was trialled to assess spatial reasoning ability. Participants with hypo gonadal testosterone levels have been found to have lower levels of activation during MR performance.

Participants were hypothesised to make more errors on shapes which have been rotated (experimental) compared to shapes which have not been rotated (control).

This is because the number of correct responses are expected to decrease as the number of degrees needed to rotate shapes increase due to increased task difficulty. Conversely, reaction times are expected to increase as the number of degrees required to rotate shapes increase. This is because more time is required to compare shapes when they are rotated to a greater extent. Areas of precentral gyri, precuneous cortices, lateral occipital lobes, and supramarginal gyri are expected to be activated during experimental tasks.

## Methods

### Design

The SS task was assessed with a within subjects fMRI event-related design and the MR task was assessed with a within subjects fMRI block design. This was to ensure that tasks functioned correctly and to confirm that brain regions activated were in line with previous research. Participants were tested once only.

### Participants

Three participants were recruited (females = 2: males = 1; Age: 22.4 $\pm$ 4.2) for the SS task. Three participants were separately assessed on the MR task (females = 2: males = 1; Age: 27.2 $\pm$ 3.1). Participants were recruited on the basis that they were healthy, had no previous medical conditions, and had normal to corrected vision.

### Brain imaging task

*Stop signal task.*

In the stop signal task, participants were presented with a white fixation cross (500ms) followed by a yellow hash (#) symbol in the centre of the screen. Hash symbol timings were separated into no flicker (350ms), short flicker (800ms), medium flicker (900ms), and long flicker (1000ms) durations. The hash symbol was always yellow in colour and presented on a black background. After each presentation of a flicker or no flicker trial a blank screen was presented (inter stimulus interval) (ISI) which was randomly jittered between 2800ms, 3000ms, or 3200ms. The ISI started at the offset of an ending stimulus and ended at the onset of the white fixation point. The ISI was jittered to make the task more unpredictable so that the BOLD response did not habituate, and to avoid co-linearity in BOLD responses. This thereby increased the efficiency of the design. Go trials (no stop signals) consisted of no flicker trials in which the hash symbol did not flicker. Participants were instructed to press their right index finger onto a 2 x 2 NATA response pad on these trials. Stop signals (inhibition trials) consisted of flickering hash symbols which was composed of short, medium and long durations in which participants were instructed to refrain from responding. Participants were primed and provided with instructions before completing the task from the researcher. The task was split into two runs each containing 100 trials with 200 trials in total. Ordering of no flicker and flicker hash symbols were randomised (For durations of hash symbols see figure 1). No flicker signals comprised 60 per cent of these trials and stop signals made up 40 per cent of trials [short flicker (15), medium flicker (15) and long flicker (10)]. Apart from randomisation of stimuli, each run was identical. The duration of each run was 7 minutes 21 seconds

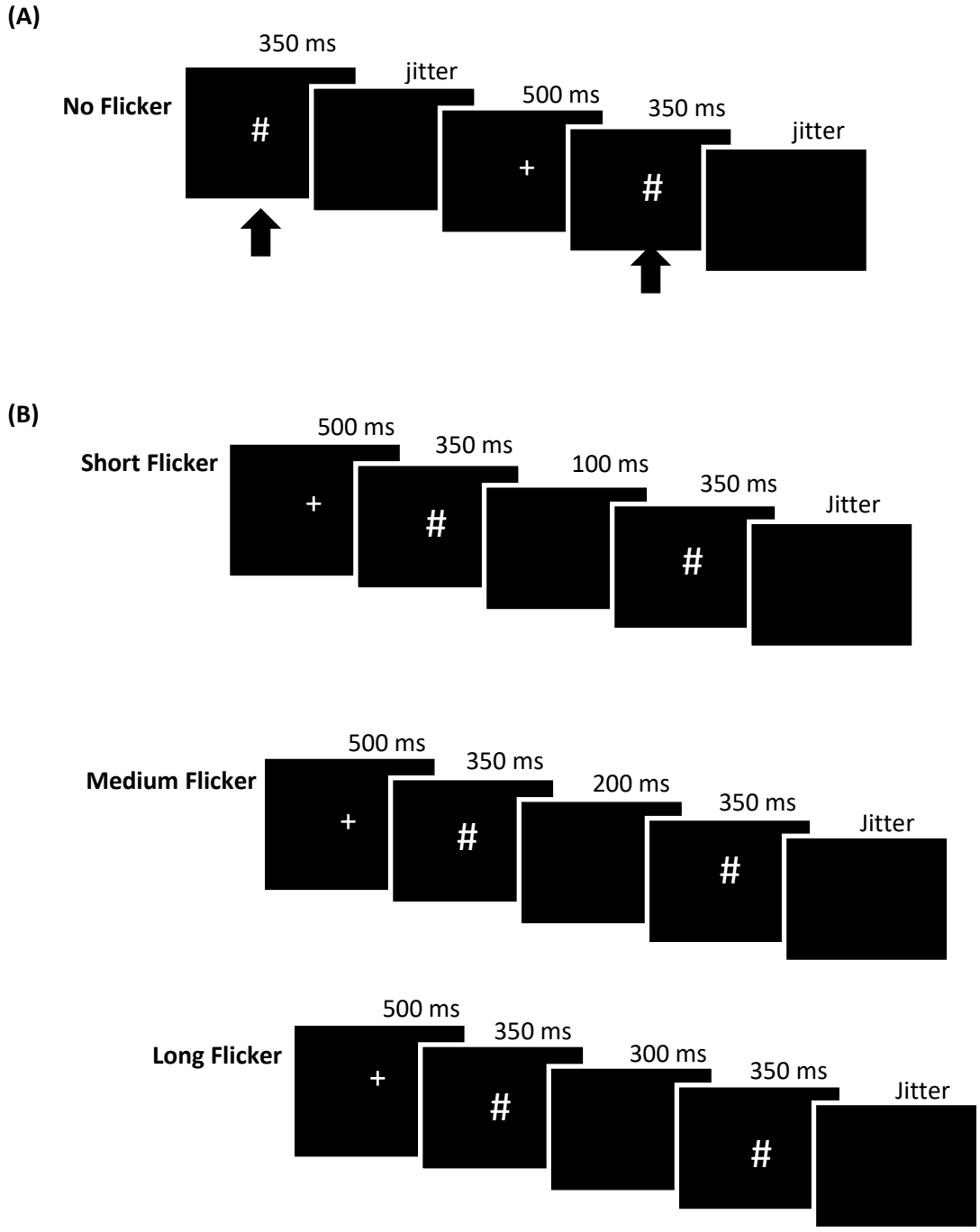


**Figure 1**: *fMRI illustration of the stop signal task. (A) No flicker timings (go trials) in which participants must respond on a response pad. Arrows under hash symbols represent when participants must respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 1, blank screens labelled as 'jitter' were jittered around the temporal resolution of 3 seconds. Jittered durations varied between 2800ms, 3000ms and 3200ms in a random order. Parts (A) and (B) were presented in random order. Abbreviations: ISI, interstimulus interval; ms, milliseconds.*

*Mental rotation task*

The MR task consisted of a 7.5 minute run with eight alternating 38 second blocks separated by 20 second rest periods. Blocks comprised of experimental and control conditions each repeated four times during the functional run. Eight shapes were presented per block. The task was adapted from Shepard and Metzler (1971) MR task in which 3D shapes are displayed on a blank screen. In experimental blocks, one shape from each pair was rotated along its vertical axis at 60, 120, 180 or 240 degrees and contained identical shapes whilst the other half contained rotated mirrored shapes. Un-rotated identical or mirror shapes were presented in the control condition. Participants had to determine if shapes were identical or mirror images by pressing either their right index or middle finger respectively in both conditions. Slides were presented for 3750ms followed by a fixation point on a blank screen for 750ms.

Both SS and MR tasks were designed using E-prime 2.0 which triggered MR image acquisition and collected behavioural data consisting of responses and reaction times.

**Imaging Acquisition.**

Sagittal $T_1$ structural images were obtained using a 3.0 T scanner (Philips). Blood oxygenation level dependent (BOLD) signals were acquired with a gradient echo planar imaging (FEEPI) sequence in the sagittal plane (thickness of 1mm), repetition time (TR) = 8.4s, echo time (TE) = 3.8ms, flip angle = 7.16°, field of view = 230 × 175 mm, matrix size = 288 x 288. Voxel dimensions were 1mm x 1mm x 1mm isotropic. 184 dynamics (images) were acquired for SS tasks with forty contiguous axial slices (3mm thickness) per image. These were acquired with TR = 2.5s, TE = 3.80s, flip angle = 90°, field of view (FOV) = 240 × 240, matric size = 68 x 68. A tilted acquisition plane ($30^{o}$ to the rostral > caudal tilt) was used and adjusted individually to reduce signal dropout in frontal brain regions (orbitofrontal cortex).

**FSL analysis**

Rawdata in Par Rec from the Philips scanner was converted to Neuroinformatics Technology initiative (Niftii) format. Structural scans were reoriented using a reorient script in a Linux terminal. Structural T1s were then BET extracted using FSL 5.0.8 (Smith et al., 2004) BET brain extraction software (Smith, 2002). The point of interest on scans were corrected to determine the centre of the cortex for skull stripping. T1s were checked with FSL view to assess quality of skull extraction. Pre-processing steps included motion correction of images using FSL MCFLIRT. Regular slice timing correction was applied with a spatial smoothing full width by half maximum (FWHM) of 8mm. A high pass filter was applied to remove low frequency noise thereby allowing better signal to noise ratio (SNR). Functional images were co-registered to structural T1s and then co-registered images

were registered to standard MNI space. For functional scans Dummy scans were discarded and only voxels with significant ($p < 0.05$) functional activation were accepted as voxels with substantial signal change.

Statistical analysis at the subject level on the SS task was carried out at a single subject level first using the general linear model (GLM) in FSL. Stop signal task durations were separated into four conditions: go correct, go incorrect, stop correct, and stop incorrect which were convolved with the GLM. Trials associated with gin and sc runs were paired together due to low trial power on gin trials. Moreover, both trials required inhibition. The stop signal task was additionally decomposed into trials of long (correct/incorrect), medium (correct/incorrect) and short (correct/incorrect) and run as contrasts. Long flicker trials were compared against short flicker correct trials and medium flicker correct trials to determine which trials produced maximum inhibition activation. Long flicker correct trials were also run against go correct trials separately as this allowed comparison of maximum inhibition activation compared to response (go trial) activation.

The mental rotation task was spilt into experimental and control task trial duration which were convolved with the GLM. Experimental conditions were contrasted against the control condition. Contrasts were run with the general linear model in FSL and the canonical hemodynamic response function was used to model activation. For both tasks, first level analysis was performed with an uncorrected z-threshold with $p < 0.05$ for voxel activation. Analysis of single subjects were combined using a higher level analysis and mixed Flame effects stats were implemented to correct for multiple comparisons with a z-threshold of 2.3 and 1.3. A cluster significance threshold of $p < 0.05$ was used to locate any voxel clusters surviving multiple comparisons.

## Results

*Stop signal task*

First level analysis showed slight activation of IFG and MFG. However, group level analyses with mixed flame did not survive multiple comparison correction (cluster z-threshold 2.3). A lower cluster threshold was implemented but the chance of making type7 1 errors (false positives) using these methods were high, and did not confirm any reliable activation. Supplementary contrasts were run between short correct, medium correct, and long flicker correct response trials on stop signal runs. Activation of inhibition areas during long flicker runs were typically in the IFG, MFG and paracingulate gyrus. A contrast of long flicker correct minus go correct trials run at a minimum Z threshold corrected for multiple comparisons of 1.3 and showed most activation in the right MFG (MNI coordinates: $x = 34$; $y = 18$; $z = 30$; cluster size = 220 voxels; local maxima Z = 1.67; $p < 0.05$), right IFG (MNI coordinates: $x = 58$; $y = 18$; $z = 22$; cluster size = 140 voxels; local maxima Z = 1.53; $p < 0.05$), and paracingulate gyrus (MNI coordinates: $x = -4$; $y = 30$; $z = 34$; cluster size = 51 voxels; local maxima Z = 1.74; $p < 0.05$). This contrast was undertaken to clarify that inhibition was contained to primarily long flicker trials. Medium correct or short flicker trials did not activate any typical stop signal areas when contrasted with long flicker or go correct trials. Behavioural performance indicates lack of commission or omission errors.

*Mental rotation task*

Activation was significant at an uncorrected threshold ($P < 0.05$) in bilateral supramarginal gyri, precentral gyri, supramarginal gyri, and lateral occipital cortices (MNI coordinates table 1). Activated areas were consistent with past research; however, no activation was present at Z = 2.0 or 2.3 thresholds. Therefore, these findings were not considered to be reliable due to high probability of type 1 errors. Behavioural performance showed a higher proportion of incorrect responses were made when discriminating between task pair shapes which had been rotated compared to control shapes which were unrotated.

**Table 1. Regional brain activations for mental rotation task (uncorrected; $p < 0.05$)**

| | | | MNI coordinates | | | Z |
|---|---|---|---|---|---|---|
| **Contrast** | **Side** | **Region** | **X** | **Y** | **Z** | **value** |
| Task - Control | L | Supramarginal gyrus | -50 | -38 | 50 | 3.03 |
| | R | Supramarginal gyrus | 48 | -34 | 50 | 3.34 |
| | L | Lateral occipital cortex | -26 | -64 | 46 | 4.52 |
| | R | Lateral occipital cortex | 32 | -64 | 50 | 3.38 |
| | R/L | Juxtapositional lobule | -2 | 6 | 46 | 3.23 |
| | L | Precentral gyrus | -40 | 4 | 44 | 3.19 |
| | R | Precentral gyrus | 40 | -4 | 44 | 3.29 |
| | L | Superior frontal gyrus | -22 | 0 | 64 | 3.46 |

Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right.

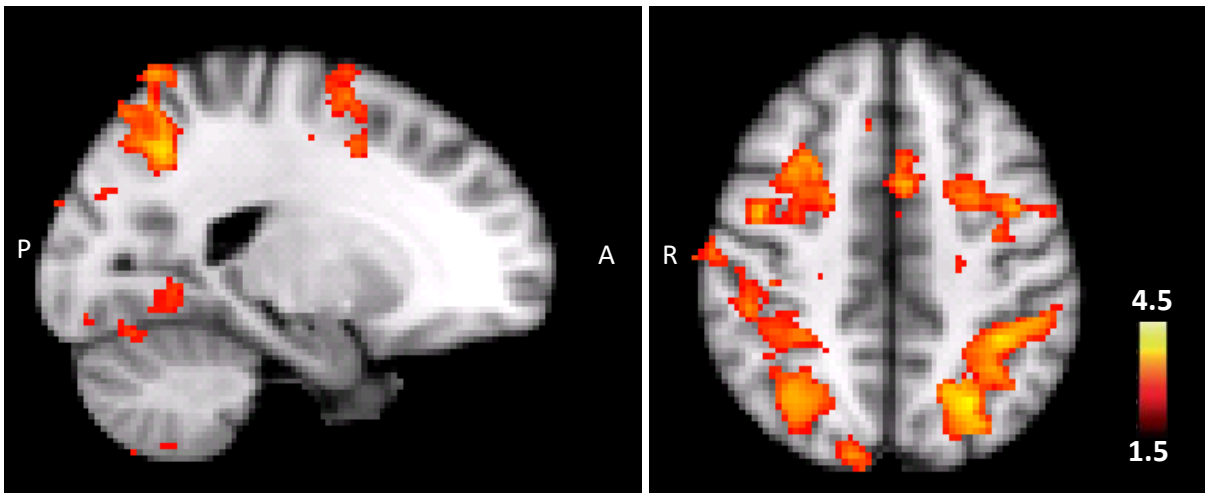


**Figure 2**: *Task condition compared to the control condition contrast. Axial and left hemisphere sagittal whole brain activation view (p < 0.05; uncorrected). Colour bars represent least (red) to most (yellow) activation on brain activation maps. Abbreviations: A, Anterior, P, posterior; R, right.*

**Discussion**

Stop signal (SS) task activation was not analogous to previous literature and to the present studies hypothesis (Menon, Adleman, White, Glover, & Reiss, 2001; Rubia et al., 2001). Motor cortex activation was not apparent in pilot study one but may be more widespread in succeeding trials if inter stimulus intervals (ISI) between go and stop signals were shorter in length. This could reduce reaction time responses leading to forced motor activation. A subsequent study was run with a third participant using short ISI jitter periods (1800ms, 2000ms, 2200ms) but no motor cortex activation was found. The cerebellum was activated in the aforementioned study and has been associated with motor control, fine motor movement, accuracy and timing (Van Mier & Petersen, 2002). Nevertheless, activation at low thresholds made the task unreliable thereby making the task invalid for further use. A weakness of pilot study one may be of low power issues due to fewer trials. Past research has incorporated a high proportion of total trials with high overall power. This allows for reliable activation at high thresholds (Bissett & Logan, 2011; Li et al., 2006; Li et al., 2008). With this in mind, increased trial numbers in further pilots could activate recognised SS task areas compared to pilot study one.

Participants in pilot study one made every few commission errors which may have led to inactivity of the anterior cingulate cortex (ACC), or insula in conjunction with past research (Ramautar, Slagter, Kok, & Ridderinkhof, 2006). Researchers speculate ACC and insula activity may be due to error feedback processing (Ramautar et al., 2006). Error feedback allows response adjustments to be made on subsequent trials from faults made on previous trials (Ridderinkhof, Guido, & Gordon, 1999). The above pilot study did not account for this phenomenon due to low frequency of commission errors made. Furthermore, the majority of commission errors were made on long flicker correct trials which led to the most inhibition activity related to the IFG, MFG, and ACC when contrasted with other flicker durations. Inactive BOLD signal in areas hypothesised to be activate led to a further behavioural pilot study. The design of the SS task was altered so that participants were more likely to make commission errors. If behavioural performance is in line with past research then it may be plausible that the IFG and MFG are activated due to increased monitoring and unpredictability of subsequent trials on the task. This is referred to as the new SS task. Unpredictability of trial durations could engage participants in inhibition behaviour rather than prediction behaviour on subsequent trials.

Activation on the mental rotation (MR) task was consistent with past research (Zacks, 2008) at an uncorrected threshold ($p < 0.05$). One shortcoming to this is that any activation could be due to noise

rather than actual signal. Participant feedback suggests a floor effect in which rotation task shapes were highly demanding to mentally manipulate. This likely disengaged participants from the task or led to increased errors. Interestingly, one participant achieved an 80% correct response rate which coincided with BOLD activation in regions typically associated with the MR task. This led to the speculation that a higher percentage of correct responses could indicate internal mental rotation, eliciting activation in brain regions typically associated in the MR task. In support of this assumption, MR task performance in literature of the past reports 94.6% accuracy in healthy controls compared to patients diagnosed with schizophrenia (76.7%) (Potvin et al., 2013). With this in mind, behavioural tasks in the second behavioural pilot aimed to replicate the above findings in healthy control participants. This should lead to brain activated regions consistent with previous MR tasks in a following fMRI study.

## Pilot Study 2: Behavioural Study.

The stop signal (SS) task and mental rotation (MR) task were piloted behaviourally with the contingent hypothesis being that if behavioural performance is consistent with past research, then it is plausible to suppose that brain areas activated are in typically task associated regions.

The new SS task inter stimulus interval (ISI) durations were decreased. This was to make stimuli presentation less predictable. Long and short flicker trial frequency were increased whilst medium flicker trials were decreased. These is because the majority of task activations were found to be from long flicker trials compared to short flicker trials in pilot study one. Fixed SSD durations were altered in which the initial stop duration was varied between short, medium, and long flicker trials.

Participants were hypothesised to make more commission errors on inhibition (stop) trials due to variable ISI periods and because initial stop trial durations were altered to make flicker presentation unpredictable. Errors were expected to increase due to inconsistency of stimuli presentation lengths, and higher frequency of long flicker trials. Instructions to participants clearly stated that go trials (no flicker) were reaction time tasks to decrease reaction times.

Shapes in the MR task were altered so that fewer blocks were used per shape and no oblique projecting shapes were presented. Previous research has employed these methods allowing increased mental rotation efficiency.

The altered version of the task is speculated to allow better mental rotational ability. Participants are expected to have significantly better performance on the new MR task compared to the task of pilot study one.

**Study Design**

The new SS task was assessed with a within subjects design in which participants completed the old and new SS task in one session. There were two IVs (new or old stop signal task) with two levels. These were short flicker correct and long flicker correct trials. Old and new SS tasks were each run twice (figure 1) and in a counterbalanced order.

The MR task similarly had two IVs of old or new tasks with two sub levels (rotated and un-rotated shapes).

**Participants**

Twenty-one participants (male = 12, females = 9; mean age: 20.2 $\pm$ 3.01) were assessed on the new SS task. Two participants were excluded due to unforeseen circumstances and being performance outliers. The final analysis was conducted on 19 participants (mean age: 20 $\pm$ 2.98). A separate cohort of 23 participants (male = 4, females = 19; mean age: 19.7 $\pm$ 2.33) were assessed on an altered version of the MR task. The only inclusion criterion was that participants should be healthy, have corrected vision, and have no history of serious or major medical conditions.

**Procedure**

Subjects were assessed individually on an Acer aspire laptop in controlled conditions limiting interference from external environmental factors. Ethical consent was taken (Appendix 2 & 3) after which instructions for either the SS task or MR task were administered verbally. Practice trials of each task was administered and consisted of similar trials to the new versions of paradigms. Instructions were presented but only 10 stimuli from each task were undertaken to avoid practice effects. Instructions were repeated a maximum of twice if necessary. If further instruction was required then they were administered verbally only without stimuli practice to avoid practice effects. One group undertook a 45 minute session of both versions of the old and new SS tasks and another separate cohort undertook a 45 minute session of both the old and new versions of the MR task.

*Stop signal task*

Participants were presented with a white fixation cross for 500ms followed by a yellow hash (#) symbol in the centre of the screen. Hash symbol durations were separated into go trials (no flicker) and stop trials (short flicker, medium flicker, and long flicker) presentation timings (figure 3). The hash symbol was always yellow in colour and presented on a black background. All stimuli durations were kept equal (700ms) and a jittered blank screen duration (ISI) was presented subsequently after

each stimulus to make trial occurrence unpredictable. Jitters (0ms, 200ms or 400ms) occurred at the end of each ISI and before the fixation point. An algorithm was used to create ISI durations which were contingent upon the timing of the jitter at the beginning of each trial. This allowed trial length including hash trial jitters and ISIs to always be 2.5 seconds in length. Subjects were required to respond on a keyboard to no flicker trials and to inhibit responses on stop trials. Go trials comprised 60 per cent of all trials and stop signals encompassed 40 per cent of trials [short flicker (15), medium flicker (5) and long flicker (20)]. Ordering of no flicker and flicker hash symbols were randomised (For durations of hash symbols see Figure 3). Apart from randomisation of stimuli each run was identical. The duration of each run was 5 minutes 14 seconds and experiments were split into two runs each consisting of 100 trials with 200 trials in total. The task was made using E-prime 2.0 which collected behavioural data consisting of keyboard responses (correct, inhibited, incorrect and reaction time) and reaction times.

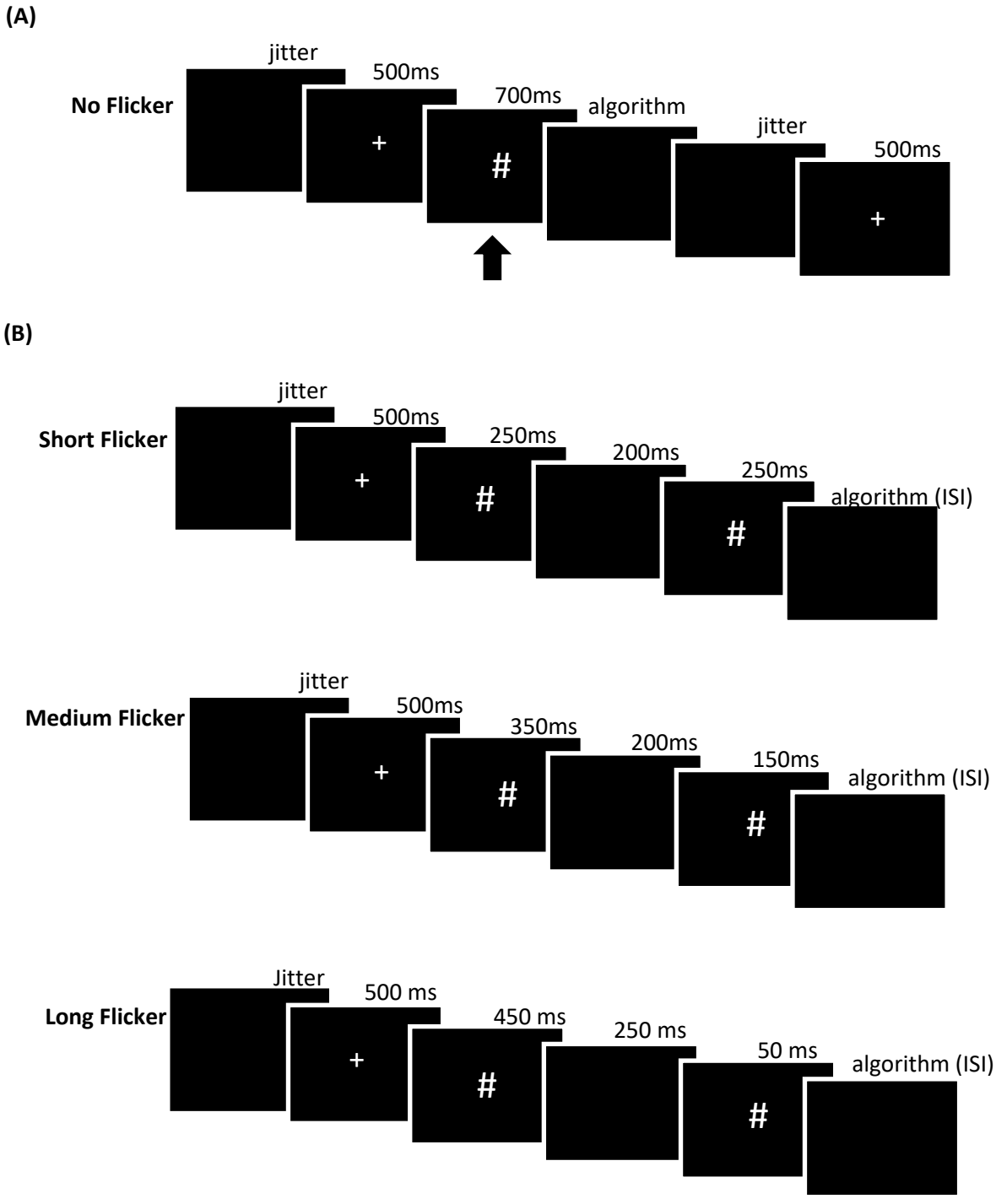


**Figure 3**: *fMRI illustration of the Stop signal task. (A) No flicker timings (go trials) in which participants must respond. Arrows under hash symbol figures represent when participants should respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 1, blank screens labelled as 'jitter' were jittered around 0, 200 & 400ms. Blank screens labelled 'algorithm (ISI)' had durations which were contingent on jitter durations. These intervals varied between 1100ms, 1300ms and 900ms. Parts (A) and (B) were presented in a randomised order. Abbreviations: ISI, interstimulus interval; ms, milliseconds.*

*Mental rotation task*

The mental rotation task was as described in pilot study one. Complexity of shapes were reduced by decreasing the number of blocks per shape. Moreover, 3D blocks were altered to exclude blocks projecting in oblique directions (figure 4).

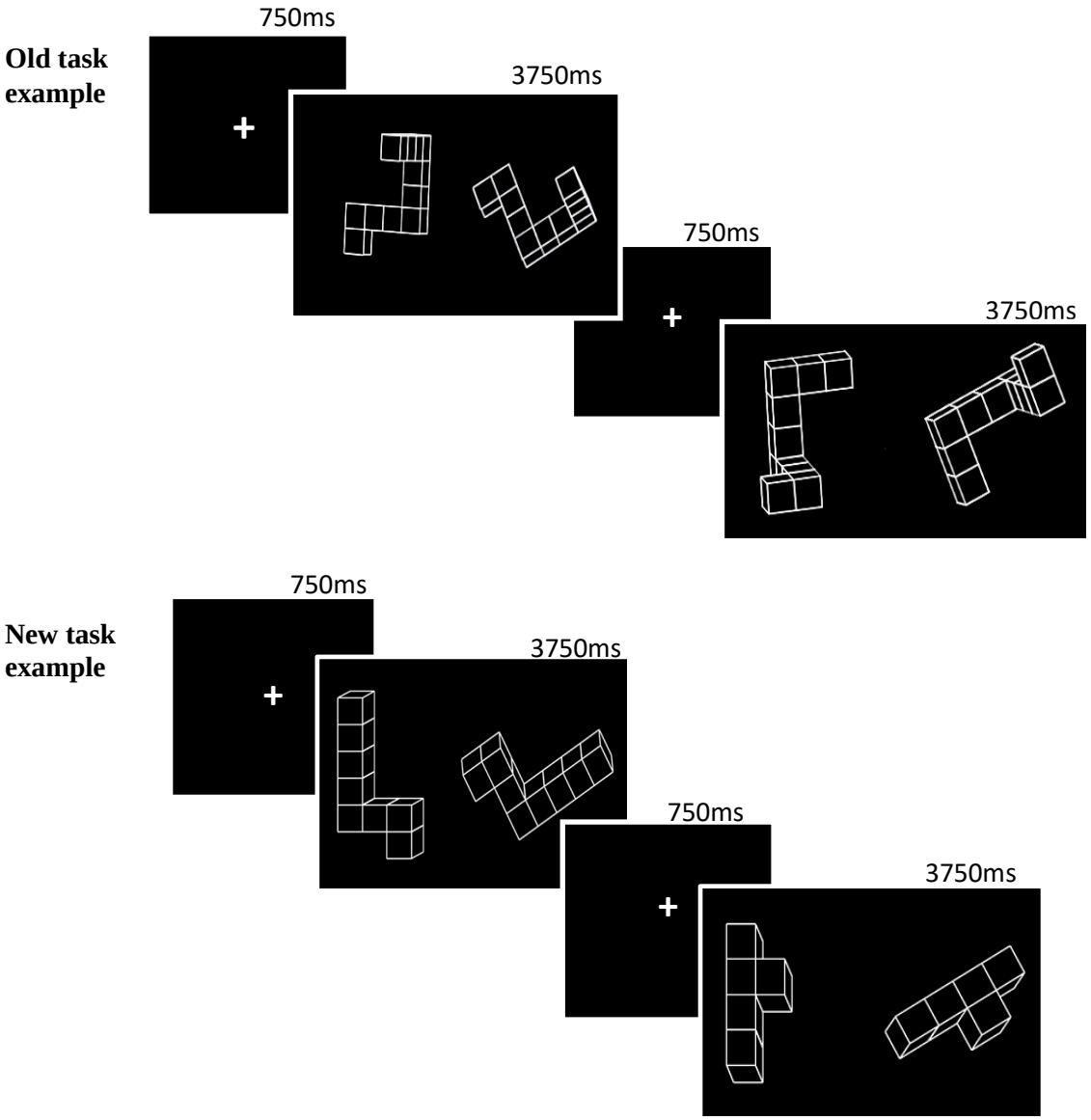


**Figure 4:** *Shows change and modification of task from pilot study one to the new task of study three. The old task used many oblique angles which were removed from the new task of study two.*
*Abbreviations: ms, milliseconds.*

**Findings**

Assumptions for a parametric test were met. These were that the data were interval level, independent of each other and normally distributed.

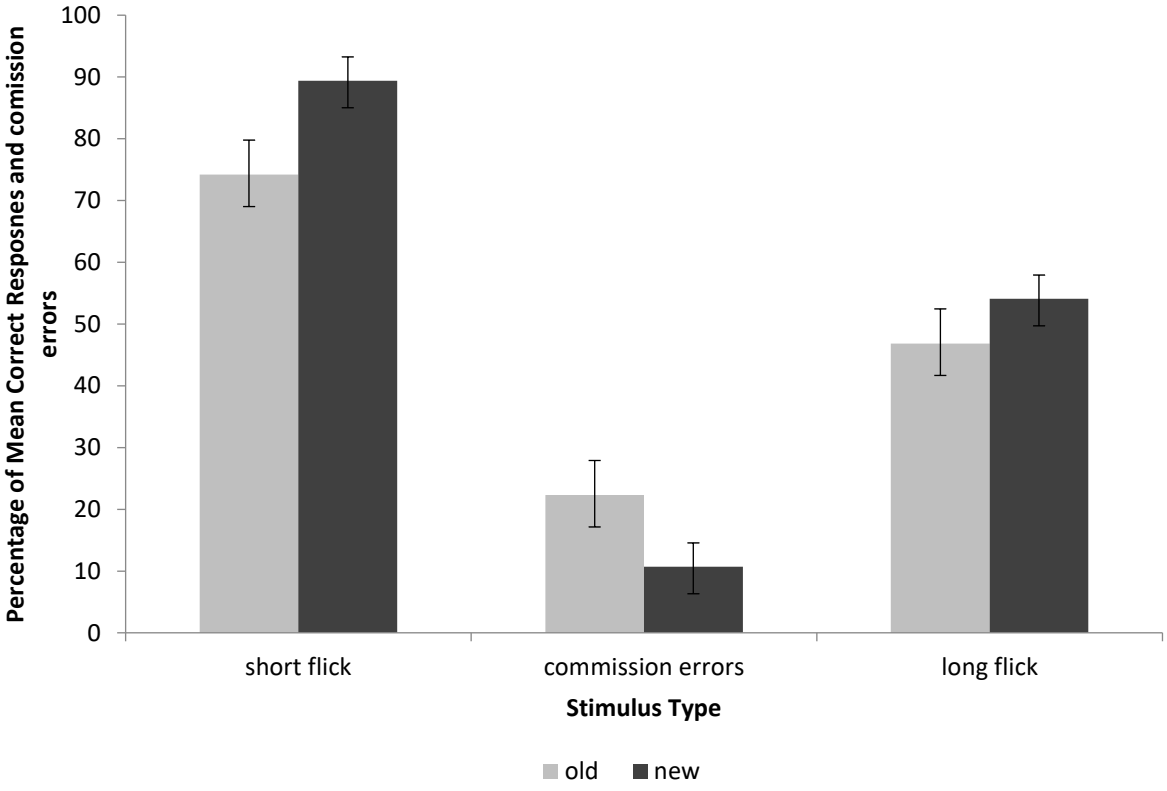


**Figure 5**: Percentage of *mean correct responses and commission errors made by participants to short and long flicker stimuli in old and new stop signal tasks.*

*Stop signal task*

Participants made significantly more commission errors on the old SS task compared to the new task (10.53 $\pm$ 3.17), $t(18) = 2.41$, $p = 0.03$, $r = 0.43$).

A two way ANOVA was conducted with one IV being task type (old or new). The other IV was the flicker trial (short flicker and long flicker) with the dependent variable being the number of commission errors made. Main effects revealed a significant difference in commission errors depending on the type of task this being old or new: $F(1,18) = 8.71$, $p = 0.009$, partial $\eta^2 = 0.33$). The main effect of flicker trial (short or long) was also significant: $F(1,18) = 126.97$, $p < 0.001$, partial $\eta^2 = 0.87$). However, the interaction between task type and flicker trial was not significant: $F(1,18) = 0.215$, $p > 0.05$).

Pairwise Bonferroni corrections between the old and new task revealed a non-significant but trending difference in mean number of commission errors made (old: 38.37 $\pm$ 4.29) (new: 29.31 $\pm$ 3.72) ($p$ = 0.09, confidence interval: 2.90 to 17.22). Significant differences were found in the mean number of commission errors made on short flicker trials (16.48 $\pm$ 4.02) compared to long flicker trials (50.20 $\pm$ 3.83) ($p < 0.01$, confidence interval: -40.00 to -27.43). However, this finding could not explain differences in mean number of commission errors made between short and long flicker trials in the new task compared to the old task, illustrated by the non-significant interaction.

*Taken together, these results suggest that differences in the number of commission errors made between the old and new tasks were significant. This suggest that more commission errors were made in the old task compared to the new task and that more errors were made on long flicker trials compared to short flicker trials. However, no significant interaction emerged suggesting no difference in the number of commission errors made between old and new task short flicker trials and between old and new task long flicker trials.*

*Mental rotation task*

A paired sample t-test showed significantly more mean correct responses in the new MR task (22.55 $\pm$ 4.81) opposed to the old MR task (16.40 $\pm$ 4.02): $t(19) = -5.37$, $p < 0.001$. A further repeated measures one-way ANOVA revealed that the number of correct responses decreased as the number of degrees needed to mentally rotate shapes increased in the old MR task: $F(3, 66) = 4.17$, $p < 0.01$, partial $\eta^2 = 0.27$. Posthoc Bonferroni pairwise comparisons indicated more correct responses in the 60 degree condition (5.17 $\pm$ 0.36) compared to the 240 degree condition (3.83 $\pm$ 0.35) in the old MR task ($p$ = 0.04, confidence interval: 0.05 - 2.64). A trend to sacrifice reaction time for accuracy was found as mental rotation required increased: $F(3, 66) = 2.62$, $p$ = 0.06, partial $\eta^2 = 0.10$.

Analysis on the new MR task demonstrated decreased performance as the number of degrees required to rotate shapes increased: $F(3, 66) = 6.70$, $p < 0.001$, partial $\eta^2 = 0.23$. Bonferroni correction revealed significantly better performance in the 60 degree condition (mean correct: 6.30 $\pm$ 0.34) compared to the 180 degree condition (mean correct: 4.83 $\pm$ 0.31) ($p < 0.001$). Again, a trend towards sacrificing reaction time for accuracy was found with increasing rotation required: $F(3, 36) = 0.56$, $p > 0.05$ ).

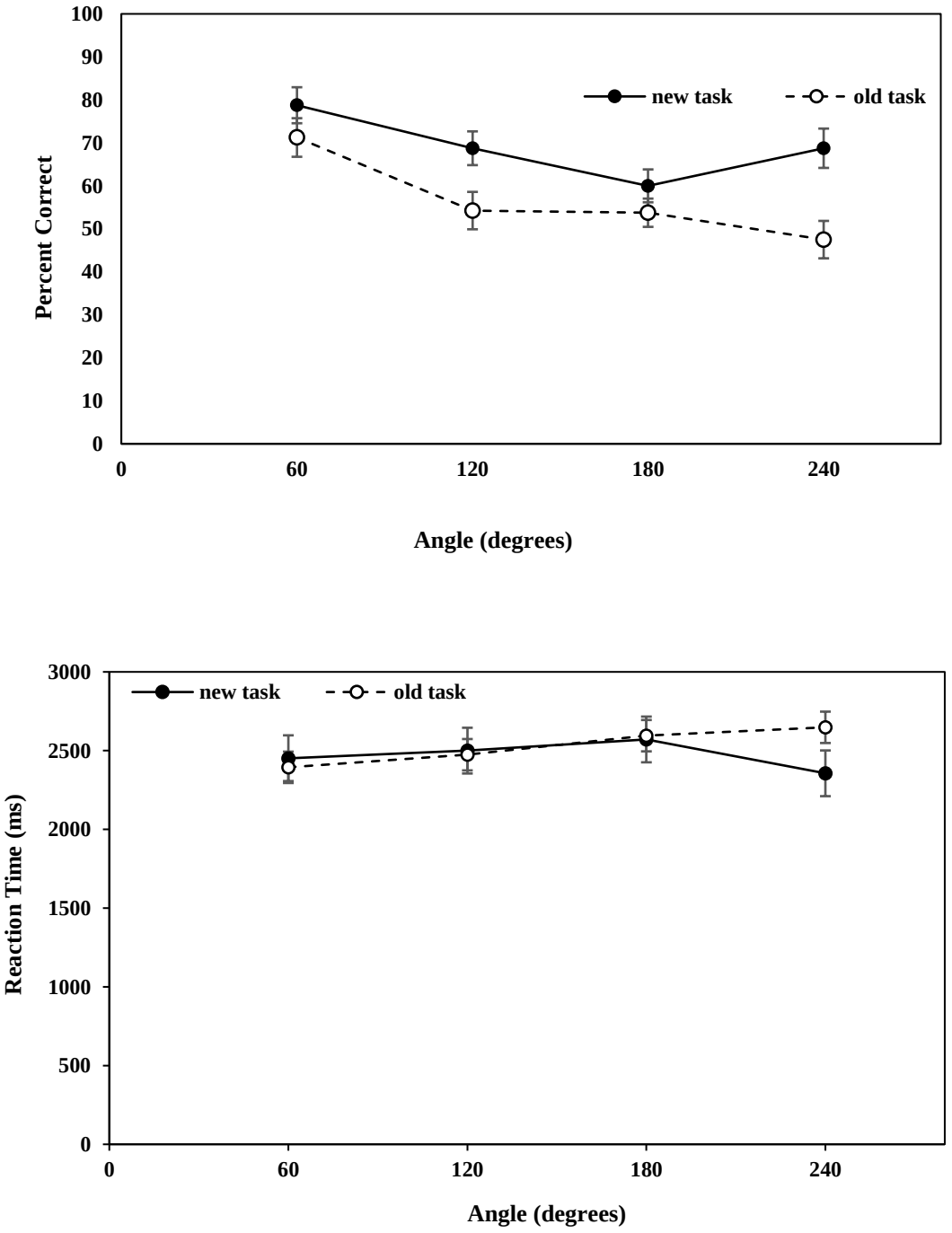


**Figure 6:** *Mean percentage of correct responses (upper panel) and mean reaction time (lower panel) on the mental rotation test as a function of degree of orientation from vertical. Participants' performance decreased and reaction time increased as the number of degrees needed to mentally rotate shapes increased. This was more evident in the old task compared to the altered MR task. Abbreviations: ms, milliseconds.*

**Discussion**

Significant differences were observed between the old SS task and new SS task and between flicker trials. However, this was in the opposite direction to the prediction of pilot study two. Fewer commission errors were made on the new task compared to the old task. Further analysis of short and long commission errors revealed the majority of errors were made on long flicker trials. This suggests the old SS task of pilot study one demanded more inhibitory ability compared to the new task. This is in contrast to what was predicted.

Better performance was found in the new MR task in line with past research (Potvin et al., 2013) compared the old task. Moreover, performance decreased as a function of degree of orientation from vertical. This was more apparent on the old task compared to the new task leading to the assumption of better internal mental rotation ability with new task shapes. The new task will be implemented in further pilot assessments and is speculated to elicit brain activation consistent with past research. This is due to higher performance rates as a function of degree orientation from vertical, analogues to the performance of the participant in pilot study one.

## Pilot study 3

A longitudinal three month study was conducted to assess activation consistency of the MR task from pilot study two and a new SS task. Two SS tasks failed to activate brain regions consistent with past research in pilot studies one and two. As above-mentioned, this may be due to the lack of commission errors made. The subsequent pilot was undertaken to establish if areas previously associated with the SS paradigm were activated with further alteration to the SS task. Brain activity elicited from the MR task in pilot study two was also trialled to measure activation consistency over the three month period.

### Participants

Four participants were recruited (females = 3, males = 1; Age = 28.2 $\pm$ 2.3). Participants were neuro-imaged with the SS task from baseline to three month follow-up period. All participants were assessed on the MR task at baseline and one participant (female; Age = 33) was assessed at the three month follow-up period.

### Brain imaging task

Stop signal task

Task presentation durations were similar to those of pilot study two in which stop signal delays (SSD) were fixed. However, the ISI was presented for 3000ms initially and either increased if a correct inhibition was made in the previous trial, or decreased by 50ms if a commission error was made in the previous trial. The ISI duration increased to a maximum of 3400ms and did not increase further even if correct inhibitions were made. The minimum ISI duration was 2600ms and did not decrease further even if further commission errors were made.

*Mental rotation task*

The MR task design was as described in pilot study two with the new task design from pilot two.

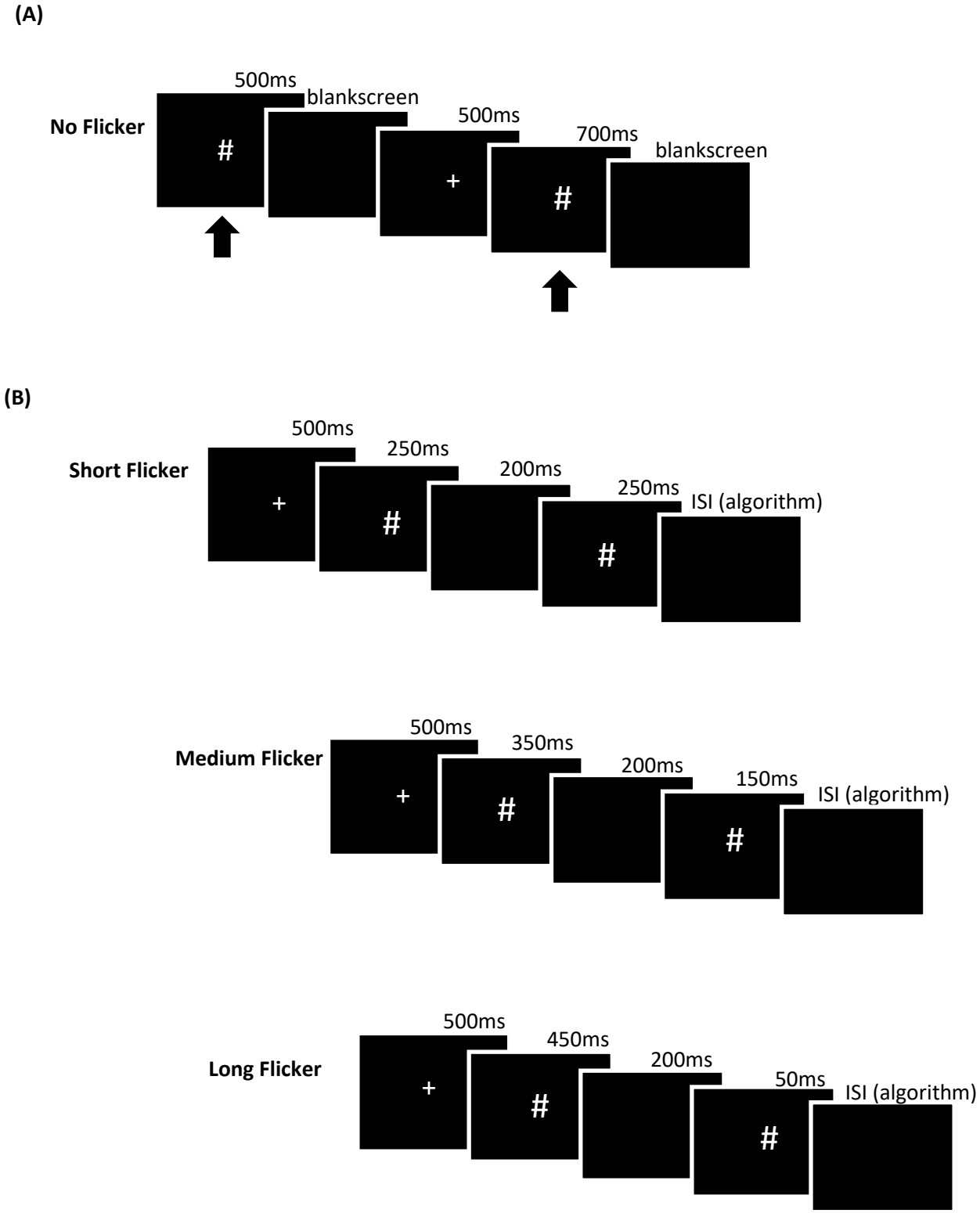


**Figure 7***: fMRI illustration of the Stop signal task. (A) No flicker timings in which participants must press the button on response pad. Arrows under hash symbol figures represent when participants must respond. (B) stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 2, blank screens labelled as ISI algorithm were varied in their duration and increased or decreased in duration depending on if the participant responded or inhibited correctly.*

**Imaging Acquisition.**

fMRI scanning sequences were as described in pilot study one.

**FSL analysis**

Analysis was as described in pilot study one.

**SPM Analysis**

Rawdata par rec files produced by the Philips scanner were first extracted and converted into Neuroinformatics Technology initiative (Niftii) format for use in SPM5 (Welcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK (SPM5: http://www.fil.ion.ucl.ac.uk/spm) on Matlab version 7.9.0.529 (R2009b). Volumes were slice time corrected for the two SS task runs and one MR task run. Slice time correction was undertaken on 184 slices acquired per run in both tasks. The repetition time (TR) was inputted as 2.5 seconds and the TA calculated as TR/(TR/number of slices). Slice order was entered in a regular up ascending order from slices 1 to 184. Volumes were then realigned and estimated. Images were realigned to the first image of each run, and then all images of each run were aligned to the first volume of the first run. Estimation was run with higher $5^{th}$ degree spline interpolation. Image realignments were assessed for any motion in either the X, Y, or Z directions or in terms of pitch, roll, and yaw rotations. A pre-established exclusion criteria was a correlation between movement parameters and task regressors greater than 0.5 or excessive movement. These standards have been associated with best practice based on previous fMRI studies (Whalley, Harris, & Lawrie, 2007). No subjects had to be excluded using the criteria. The anatomical image (T1) was first viewed to change the origin as the anterior commissure for each subject. This was undertaken to increase co-registration and normalisation accuracy. Images were subsequently co-registered to the mean reference epi image from the realignment procedure. The source image was the anatomical (MPRAGE) image which was revolved to be in alignment with the reference epi image. All functional images were realigned to the reference epi image with trilinear interpolation and then normalised to the standard SPM5 MNI EPI template. Normalization parameters were estimated using the mean image for each run, and applied to all volumes of that run. An $8mm^3$ full width by half maximum (FWHM) Gaussian kernel was used to spatially smooth normalised images. Smoothing involves the notion that nearby voxels with significant activation may have BOLD activation compared to those voxels distant from a specific voxel region. This allows amplification of true signals and increases signal to noise ratio.

Statistical analysis at the subject level was carried out using the general linear model (GLM). Default settings were implemented from SPM5 with an SS task design matrix consisting of four conditions

including go correct, go incorrect, stop correct, and stop incorrect trials. The mental rotation task was composed of two conditions which were the task condition and control condition. Regressors were convolved with the traditional canonical hemodynamic response function model (hrf) to model data. A high pass filter of 100s cut-off was implemented to remove low frequency noise components such as drift.

The main SS task contrast was of stop correct trials and go incorrect trials together verses go correct trials. This was the most efficient combination of inhibition trials against go trials. The contrast of interest in the MR task was the task condition against the control condition. A script was generated using SPM's jobman to automate the entire preprocessing and statistical analysis processes.

**Reproducibility (intra class coefficient).**

Intra-subject reliability was calculated using intra-voxel reliability at specific regions of interest (Raemaekers et al., 2007). The reliability tool box used for this was designed by (Caceres, Hall, Zelaya, Williams, & Mehta, 2009) (http://www neuroimagingsciences.com/). The toolbox analysis measures the total amount of variance explained by intra voxel variations, thereby assessing the stability of spatial distribution of the BOLD signal in each subject in a selected region. Consequently, this method can be used to assess between subject differences. The median and standard deviation were attained with a bootstrap method of 1000 resamples of subjects. Spatial smoothing can critically affect findings of intra-voxel ICC (Caceres et al., 2009). Therefore, rawdata was pre-processed using an 8mm smoothing kernel which has been demonstrated as being an optimal method in past research (Caceres et al., 2009). A one sample t-test was used to obtain the activation network of both baseline and follow-up sessions at a group level. Peak voxel region of interests (ROIs) were extracted and masked for further intra-voxel ICC analysis. The rIFG, right inferior parietal gyrus and ACC were extracted for analysis from SS task network activation. Regions with activation were significant at a cluster level FDR corrections ($p < 0.05$) and obtained with a voxel-wise extent threshold K = 20 voxel extent (Table 4 and 5). The MR task showed significant activation in areas of the left and right supramarginal gyri, and superior parietal lobe which were extracted. Masks were separately extracted using MarsBar toolbox (Brett, Anton, Valabregue, & Poline, 2002). Reliability measures were implemented into Matlab toolbox with SPM5.

**Behavioural findings**

*Stop signal task*

**Table 1. Descriptive statistics for go and stop trials (pilot study three)**

| Trial type | Baseline | Follow up |
|---|---|---|
| Go | | |
| Accuracy (%) | 76.11 ± 1.92 | 75.00 ± 2.83 |
| Reaction time (ms) | 602.61 ± 29.21 | 618.75 ± 30.36 |
| Stop | | |
| Commission errors (%) | 32.08 ± 2.92 | 27.92 ± 5.42 |
| Failed RT (ms) | 525.24 ± 6.22 | 523.19 ± 66.05 |
| Critical SSD (ms) | 350 | 350 |
| SSRT (ms) | 267 ± 314.67 | 338 ± 331 |

Abbreviations: ms, milliseconds; RT, reaction time; SD, standard deviation; SSD, stopsignal delay; SSRT, stop-signal reaction time

*Task performance*

Performance on go and stop trials are separately shown in table one. Baseline to follow-up session performance and reaction times were assessed with paired sample t-tests. However, no significant differences were found longitudinally. Stop signal reaction time (SSRT) was acquired with the most commonly used integration method (Logan & Cowan, 1984). This method is estimates the SSRT by subtracting the SSD from go RT distribution. The go RT distribution can be acquired by rank ordering all go trial reaction times. The nth go RT is then selected and subtracted from the median SSD. This process is repeated for each participant and then averaged. For example in a session of 20 trials from which go RT can be measured, there are four stop trials (one correct stop and three incorrect stop). The probability of correctly stopping is 0.25, therefore, the probability of responding is 0.75. The stop signal is presented 350ms after the initial go onset of the go stimulus. Go RT are 300, 320, 340, 380, 400, 410, 420, 460, 500, 520, 540, 550, 590, 600, 700, 750ms.

*n* = number of go RTs (which can be measured) x probability of response on stop trials.

*n* therefore = 16 x 0.75 = 12

the $12^{th}$ reaction time is 550ms.

Therefore, the stop process finishing estimate is 550ms after the onset of the go stimulus. Subtraction of this from the SSD which is 350ms is 550-350 revealing an SSRT of 200ms (example from Eagle et al. (2008)

**Table 2. Descriptive statistics for go and stop trials between pilot study one and pilot study three.**

| Trial type | pilot 1 | pilot 3 |
|---|---|---|
| Go | | |
| Accuracy (%) | 91.6 ± 4.33 | 79.44 ± 3.76* |
| Reaction time (ms) | 1256.89 ± 14.66 | 618.75 ± 30.38** |
| Stop | | |
| Commission errors (%) | 18.10 ± 6.85 | 30.33 ± 5.51* |
| Commission errors RT (ms) | 695.55 ± 24.66 | 524.22 ± 34.79 |
| Critical SSD | 900ms | 350ms |
| SSRT (ms) | 112 ± 23.25 | 290.83 ± 103.54* |

Pilot three means and SD were averaged between baseline and follow-up assessments. Abbreviations: ms, milliseconds; RT, reaction time; SD, standard deviation; SSD, stopsignal delay; SSRT, stop-signal reaction time; *p<0.05; **p<0.01.

Table two shows SS task performance on go and stop trials between pilot studies one and three. An independent samples t-test revealed differences in go trial accuracy between study one and three. Findings show a higher percentage of accuracy in study one compared to study three: $t(4) = 3.67$, $p = 0.02$. Reaction times in study three were also significantly quicker compared to study one: $t(4) = 32.77$, $p < 0.001$. Commission error reaction times were significantly quicker in study one compared to study three $t(4) = 6.96$, $p = 0.02$. No significant differences emerged between the percentages of commission errors made in study one compared to study three. However, a trend was found in which more commission errors were made in study three in line with past research and the hypothesis of pilot three. An independent samples t-test was employed to assess SSRTs between pilot study one and three. This revealed significant increases in SSRT in pilot three compared to pilot one $t(4) = 2.91$, $p = 0.04$.

*Mental rotation task.*

**Table 3. Descriptive statistics. Pilot studies one and three**

| Trial type | pilot 1 | pilot 3 |
|---|---|---|
| **MR task performance** | | |
| Accuracy (%) | 52.09 ± 17.21 | 67.71 ± 20.81 |
| Reaction time (ms) | 2457.44 ± 64.45 | 1922.59 ± 103.55** |
| **Divided degrees** | | |
| *60 degrees* | | |
| Accuracy (%) | 54.16 ± 14.43 | 79.16 ± 14.43 |
| RT (ms) | 2386.80 ± 315.35 | 1783.30 ± 267.67 |
| *120 degrees* | | |
| Accuracy (%) | 37.50 ± 0.00 | 58.33 ± 14.43 |
| RT (ms) | 2382.07 ± 119.07 | 1907.01 ± 239.92 |
| *180 degrees* | | |
| Accuracy (%) | 33.33 ± 19.09 | 62.50 ± 12.50 |
| RT (ms) | 2509.36 ± 371.06 | 2223.07 ± 313.02 |
| *240 degrees* | | |
| Accuracy (%) | 62.50 ± 25 | 62.50 ± 33.07 |
| RT (ms) | 2596.06 ± 79.46 | 1949.78 ± 299.91 |

Abbreviations: ms, milliseconds; RT, reaction time; SD, standard deviation; **p<0.01

An independent samples t-test revealed no significant differences between the average MR percentage accuracy of pilot one compared to pilot three. However, faster reaction times were apparent in study three compared to study one: $t(2) = 23.46$, $p = 0.002$ in line with the hypothesis of pilot three. Further analyses were undertaken to evaluate differences between the percentages of correct responses and mean reaction time as a function of degree orientation from vertical. A Freidman's ANOVA was employed since data were non parametric and were not normally distributed due to the small sample sizes. However, no significant differences were found in study one or study three using this analysis.

**Functional fMRI findings**

*Stop signal task*

The contrast of interest assessed was of inhibition trials minus go correct trials (go incorrect trials plus stop correct trials minus go correct trials). Go incorrect and stop correct trials were combined as they increased overall inhibition power. BOLD signal activation areas was predominantly found in the ACC and right IFG extending into the MFG in line with past literature (Chikazoe et al., 2009; Jahfari et al., 2012; Zandbelt et al., 2013). Activation survived correction for multiple comparisons and was consistent across participants when analysed individually, and amalgamated at a group level.

**Table 4. Whole brain maximum voxel activations for stop signal task FDR corrected (p<0.05).**

| Contrast | ROI | MNI coordinates X | Y | Z | t-max | $ICC_v$ med (STE) |
|---|---|---|---|---|---|---|
| | L Insula | -33 | 14 | -1 | 6.62 | 0.41 (0.33) |
| | L inferior parietal lobe | -48 | -34 | 44 | 6.84 | 0.47 (0.39) |
| | Anterior cingulate cortex | 6 | 11 | 53 | 7.54 | 0.63 (0.12) |
| | R cerebellum | 30 | -73 | -55 | 6.54 | 0.55 (0.29) |
| | R middle frontal gyrus | 39 | 38 | 29 | 9.66 | 0.58 (0.18) |
| | R postcentral gyrus | 48 | -34 | 53 | 7.89 | 0.43 (0.31) |
| | R inferior frontal gyrus | 48 | 20 | -4 | 7.31 | 0.45 (0.31) |
| | Superior frontal gyrus | 6 | 11 | 53 | 7.54 | 0.45 (0.20) |

Abbreviations; $ICC_v$ med, intra-voxel reliability medians; L, left; MNI, Montreal neurological institute; R, right; ROI, region of interest; STE, standard error. Appendix one for $ICC_v$ reliability per subject and for each ROI.

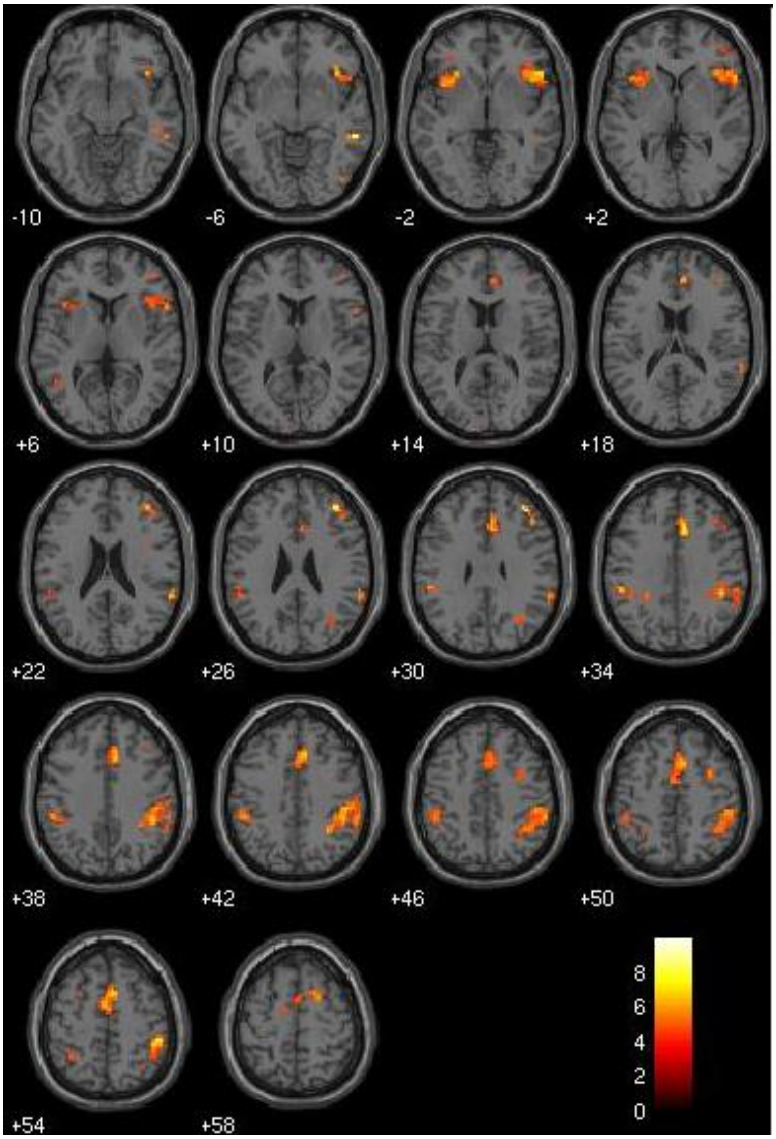


**Figure 7:** *Stop signal task brain regions of activation in the contrast of successful inhibitions and unsuccessful go trials compared to successful go trials. BOLD contrasts superimposed in T1 structural image in axial sections from z = -10 to z = +58. Adjacent sections are 4mm apart. Colour bar represents voxel t-values. Colour bars represent t-values which correspond from least (red) to most (yellow) activation on brain activation maps.*

*Mental rotation task*

Areas of activation were compared by contrasting the task condition minus the control condition. Consistent neural activation was found in line with past research (Table 4). When baseline and follow up sessions were combined, activation was significant at an uncorrected threshold $p < 0.001$ (Table 4).

**Table 5. Whole brain peak activations for mental rotation task ($p < 0.001$) uncorrected. Participant one intra-voxel reliability in region of interests.**

| Contrast | ROI | MNI coordinates X | Y | Z | t-max | $ICC_v$ med |
|---|---|---|---|---|---|---|
| Task - control condition | L pyramis (cerebellum) | -12 | -67 | -40 | 13.52 | 0.52 |
| | L cerebellar tonsil | -15 | -58 | -52 | 17.40 | 0.34 |
| | R precuneous | -3 | -58 | 65 | 12.97 | 0.40 |
| | L supramarginal gyrus | -42 | -52 | 29 | 14.33 | 0.87 |
| | R inferior semi-lunar lobe | 12 | -73 | -46 | 18.37 | 0.56 |
| | R superior frontal gyrus | 2 | 13 | 57 | 12.47 | 0.43 |
| | R inferior frontal gyrus | 30 | 35 | -10 | 13.46 | 0.49 |

Abbreviations; $ICC_v$ med, intra-voxel reliability medians; L, left; MNI, Montreal neurological institute; R, right; ROI, region of interest; STE, standard error. Appendix one for $ICC_v$ reliability per subject and for each ROI.

The table shows median intra-voxel ICC for ROIs with peak voxel activation with most reliability found in posterior regions. Frontal regions were not expected to be active however, activation in these regions are consistent with past research (Potvin et al., 2013; Zacks, 2008). The range of $ICC_v$ values for both tasks between baseline and follow-up fall between 0.34 - 0.87. Established conventions classify values of ICC of less than 0.4 as poor, between 0.4 - 0.6 as fair and between 0.6 – 0.8 as good (Cicchetti & Sparrow, 1981). Furthermore, a review of brain activations amongst a variety of task condition found the majority of ICC values amongst studies to be fair (0.50). Moreover, the majority of studies reported median ICC values between 0.33 – 0.66 (Bennett & Miller, 2010).

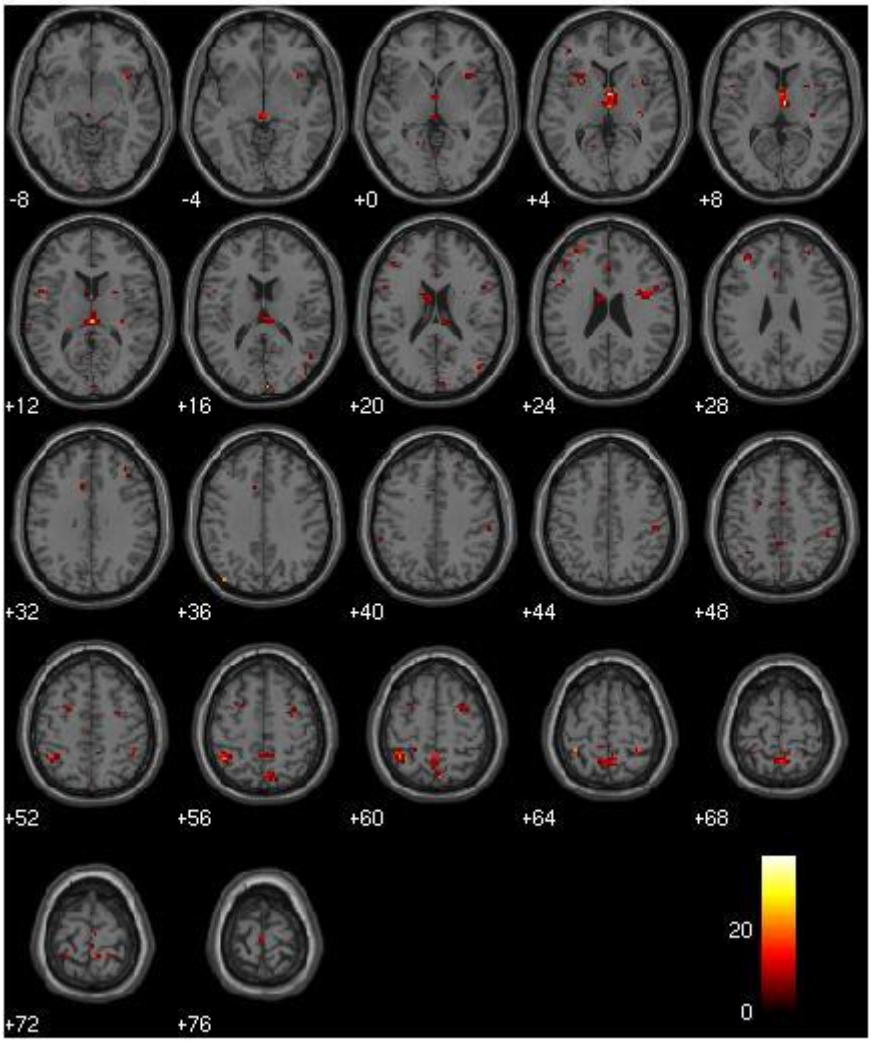


**Figure 8:** *Mental rotation task brain regions of activation in the contrast of the task condition compared to the control condition. BOLD contrasts superimposed in T1 structural image in axial sections from z = -8 to z = +76. Adjacent sections are 4mm apart. Colour bars represent t-values which correspond from least (red) to most (yellow) activation on brain activation maps.*

**Discussion**

Pilot study three aimed to first ascertain if activation was consistent with past research and secondly, to determine if activation was reliable over a longitudinal study interval. Both the SS task and MR task failed to activate neural networks that matched past research in pilot study one. However, pilot study three was successful in producing activation over a longitudinal period in line with earlier literature on the SS task (Aron, 2007; Aron & Poldrack, 2006; Chevrier et al., 2007; Li et al., 2006) and MR task (Lineweaver et al., 2005; Potvin et al., 2013; Zacks, 2008). One constraint of the pilots above was of low multiple comparison thresholds required to detect activation. This may be attributable to small sample sizes. However, both tasks did show reliable activation across a three month baseline to follow-up period despite low thresholds. Peak voxel activations were used to assess reliability, supporting that both activations and paradigms trialled were robust. Reliability of the longitudinal period leads to the conclusion that the tasks can be incorporated further with larger clinical and healthy sample sizes. The MR and SS task elicited activation in putative areas and will be incorporated in the future longitudinal clinical study. Both tasks showed activation of typically fronto-parietal network regions characteristic of executive function tasks requiring higher order computations. More importantly, these networks are areas in which ADT patients have difficulty during and after treatment.

**General Discussion and Plans for Future Research**

Pilot study one did not elicit regional brain activation on the SS task and MR task consistent with past research and of the studies hypothesis. Pilot study two was conducted with altered versions of both tasks to assess behavioural performance which if analogues to findings in past research, was speculated to activate brain regions comparable to the main studies hypothesis. An altered MR task with reduced difficulty was made and behaviourally tested with findings indicating fewer errors were made on the new task. This led to mental rotation related activity in brain regions analogues to past research in pilot three. The SS task was altered to contain a higher frequency of long flicker trials and short ISI jitter periods between go and stop signals. This was hypothesised to reduce trial predictability and increase commission error percentage. If commission errors significantly increased, then it was proposed that inhibitory brain regions were activated. Findings indicated that more commission errors were made on the old task compared to the new task in contrast to what was predicted. Pilot study three was conducted with variable ISI periods and fixed SSDs were altered in their duration. Participants made more commission errors in pilot study three and brain activation was analogous to earlier research.

The new SS task generated a higher percentage of errors which could be due to long ISI durations. Long flicker trials reduced predictability of subsequent stop signal trials evidenced higher commission errors during these trials in pilot study two. Furthermore, most brain activation in pilot study one was contained to long flicker trials regardless of high commission errors. Participant feedback also informed that long flicker trials reduced predictability in the task forcing inhibitory behaviour. Pilot study two incorporated a higher frequency of long flicker trials but failed to increase the overall percentage of commission errors and therefore the SS task of study two was not incorporated. Pilot study three was altered with variable ISI periods that dynamically changed between trial presentations leading to high commission errors. Furthermore, variable ISIs were used because participant feedback from pilots one and two reported that long ISIs led to the task being challenging, and subsequent trials were unpredictable compared to pilot study two. This prevented habituation to the task. Instructions were also altered to emphasise the task was a reaction time experiment to reduce post error slowing. Delayed reactions in previous tasks allowed the accommodation and delay for the primary duration of a go period in a stop signal trial, thus slowing responses on go trials in conjunction to increase accuracy. Internal inhibitory reaction times increased verified by reduced SSRTs in pilot three compared to pilot one. This induced internal higher order executive function processing and inhibitory activity due to task unpredictability.

Another consideration was that the SS task incorporated was chosen with fixed stop signal delays. The staircase procedure was piloted in one participant however it did not stimulate typical SST neural activation. This could be due to the time limitation set on the current paradigm. Whilst some studies

claim that performance adjusted delay (staircase procedural) SSDs produce more reliable activation in putative inhibitory areas (inferior frontal gyrus, rMFG inferior parietal gyri etc.) (Fauth-Buhler et al., 2012). These studies incorporated multiple runs with a higher frequency of trials thereby increasing total task duration. This created sufficient power through trials alone to detect neuronal activation. Other experimental trials have also adopted this methodology with the SSD staircase procedure with task durations typically being between 19 – 40 minutes (Elton et al., 2014; Hu & Li, 2012; White et al., 2014). The current paradigm was restricted to small durations due to the number of scan runs required in the given time frame. This made scanning periods practical as subjects were elderly and some had added comorbidities. Elderly participants are known to have more movement artefacts during fMRI scans due to head movement especially in long duration scans (Power et al., 2014). Therefore, due to these concerns, the task had to be made practical in the given time frame and produce brain activation in typically found SST neural areas. Fixed SSDs were used since they have been found to elicit similar brain activations compared to staircase procedure SSTs (Fauth-Buhler et al., 2012). Moreover, fixed SSDs were successful in eliciting SST brain activations consistent with past research in a shorter duration task paradigm.

Neural activity was consistent with past research in typical areas including the IFG, MFG, inferior parietal cortex (IPC) (angular and supramarginal gyri), ACC, and superior frontal gyrus (SFG) in pilot study three (Eagle et al., 2008; Li et al., 2006; van den Wildenberg et al., 2006; Zandbelt et al., 2013). The IFG and MFG have been associated with proactive control behaviour and inhibition (Braver, Barch, Gray, Molfese, & Snyder, 2001). Activation of the right IPC has been correlated with rule violation such as when a stop signal does not occur, thereby nullifying the stop rule which was expected (Zandbelt et al., 2013). Alternatively, IPC involvement could indicate unexpected inhibition during an expected go signal leading to activation when correctly inhibiting (Corbetta, Patel, & Shulman, 2008). From the above findings it may be proposed that the right IPC, IFG, and MFG are a neural network concerned with bottom up processes (updating expectations) rather than a top down processes such as expectation of a signal (Corbetta et al., 2008; Corbetta & Shulman, 2002). The ACC was involved in the task which may be active due to commission or omission errors according to past research (Aron & Poldrack, 2006). The SS tasks of the pilot above did not incorporate feedback which could increase ACC activity. However, feedback displays serve as a hindrance in task duration especially between TR excitations.

The MR task was designed according to the Shepard and Metzler (1971) task. However, the paradigm failed to activate brain regions analogues to past literature in pilot one (Zacks, 2008). This could be because shapes were presented in 3D projected oblique angles requiring more time to process.

Nevertheless, stimuli presentation and block durations were similar to fMRI tasks previously reported (Butler et al., 2006; Potvin et al., 2013; Zacks, 2008). Furthermore, neural activity may not have been present due to loss of interest in the task because of high task difficulty. Difficulty of the task was verified by very poor performance rates inconsistent with past research (Potvin et al., 2013). However, one participant in pilot study one had close to 80 percent correct response rates and had activation in well-known MR associated regions. With this in mind a subsequent behavioural study with block shapes were assessed to decrease task difficulty. Performance increased in pilot two on the altered MR task compared to pilot one. It was therefore plausible to assume the altered version of the task activated putative MR task associated neural correlates. Pilot study three demonstrated that this assumption was met and brain regions activated were similar to those reported in previous studies (Lineweaver et al., 2005; Potvin et al., 2013). The behavioural performance in pilot study three demonstrated increased reaction time, and there was a decreasing trend in the number of correct responses as a function of shape orientation from vertical. However, reaction time decreased and the number of correct responses slightly increased when mentally rotating 240 degrees in the new MR task. This could be due to counter clockwise rotation in the altered MR task which was difficult to undertake in the old MR task of study one due to high task difficulty. Clockwise and counter clockwise rotation in study three could therefore elicit activation in MR associated regions which were not found to be activate in study one (Just, Carpenter, Maguire, Diwadkar, & McMains, 2001).

Brain regions activated were subdivisions of the parietal lobes. These areas included the superior parietal lobes, lateral occipital lobes, and supramarginal gyri. Activation of the superior parietal lobe converges with neuropsychological data and has been known to be important in visuospatial image transformations (Ratcliff, 1979). The superior parietal cortex is associated with implementation of spatial maps which code the location of objects to be manipulated (Andersen & Buneo, 2002). This is most clearly seen in patients with hemi-spatial neglect with parietal cortex lesions in which space in the contralateral side to the lesion is disregarded (Karnath et al., 2001; Malhotra & Russell, 2015). Activation of motor regions in the precentral cortex is widely debated in literature during mental rotation of shapes (Potvin et al., 2013; Vingerhoets et al., 2001). However, it has been argued that motor region activity could reflect computation of motor recreations in relation to joint, or torque perspectives (Michelon, Vettel, & Zacks, 2006). This could assist in solving mental rotation problems through for example mentally rotating shapes and simulating hand movements (Zacks, 2008). Alternatively, motor activity during MR could simply be due to manual button presses (Zacks, 2008).

Limitations of the current study and tasks to be incorporated in a longitudinal study of prostate cancer patients were that age of participants were not in parallel to prostate cancer patients. Elderly participants may have decreased executive functioning than younger subjects. Furthermore, activations in the tasks assessed were thresholded liberally for multiple comparisons at Z threshold < 1.8, $p < 0.05$ and in SPM $p < 0.001$ (uncorrected threshold or corrected for multiple comparisons in

SS task only). The putative stringent level is normally Z threshold < 2.3 corrected for multiple comparisons. Thresholds at 2.3 or greater are implemented to discount type 1 errors in significant clusters thereby ruling out noise due to head motion, biological artefacts or scanner drift. Therefore the current pilot tasks have a higher probability of presenting activation with type 1 errors due to noise rather than true signal. Nevertheless, past research with high Z or t scores typically have greater sample sizes and are able to safely group findings. The current pilot study only assessed up to four participants per task. Therefore, activation areas rather than being conclusive are considered to be preliminary and trending in the direction of past research. A further shortcoming of the above pilot is of gender differences in the MR task (Parsons, 2004). The conducted MR pilot task used an opportunity sample with the majority being female participants. In the above pilot study, females tended to have more activation compared to males. This may be due to a larger female sample size or due to rotation abilities of female participants. Another explanation may be the ability of males to discriminate between two different objects without mental rotation. A study by Hooven, Chabris, Ellison, Kieveit, and Kosslyn (submitted for publication) assessed 123 male and female participants. Findings presented that males scored higher than females and males were better able to distinguish between same and different shapes without mental rotation, compared to female participants. Females reported a higher effort load to mentally rotating shapes and objects compared to males. This could account for higher fMRI activation in females compared to males in the pilot studies conducted above. However, no differences in fMRI activation areas were noted in the pilot above. Finally some journals report no sex differences in MR task performance and that context and instructions delivered to participants greatly affect their performance (Moè, 2009). Intra-voxel reliability in specific ROIs in pilot study three was stable and spatial distributions across voxels during the three month period was consistent in recognised areas for both tasks. Some between subject variability was present but reliability was consistent with fMRI studies of the past (Bennett & Miller, 2010).

The current tasks are feasible in assessing executive function and spatial reasoning. The SS and MR task elicited activations with neural correlates in line with past research. Movement during scanning was at minimal levels although it is questionable if older patients require separate sessions to account for physical conditions affecting movement. These may be arthritis, osteoporosis or other health conditions requiring separate scanning sessions.

In conclusion the results suggest both tasks in pilot study one did not elicit brain activations in areas parallel to past research. The MR task did achieve behavioural performance according to previous findings but the SS task did not. Pilot study three assessed the altered MR and SS task which elicited neural correlates associated with both tasks. Future research suggests tasks are feasible and can be

incorporated in longitudinal cognitive assessments of ADT patients with prostate cancer compared to matched healthy controls.

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## Appendix 11: Within subjects statistical analysis of healthy controls

The main purpose of the within subjects analysis was to assess cognitive change over time and to assess if any change was accounted for by covariates (testosterone or psychosocial factors). Primary outcome measures were scores on a battery of executive function and spatial reasoning measures. A p value of either 0.05 or less was accepted as statistically significant (two-tailed). Paired samples t-tests were conducted to assess the level of psychosocial (anxiety and depression) change and testosterone level change that occurred from baseline to follow-up. A further t-test was conducted to assess change in testosterone levels between time points. A repeated measures multivariate analysis of variance was undertaken with contrast scaled scores on the D-KEFS as dependent variables (TMT-A, VF, DF, CWI). The Tower test scaled score was added as a scaled score dependent measure since contrast were not available for this measure. The repeated factor was time (baseline and six months). Contrast scaled scores allow assessment on primary measures whilst controlling for task performance on basic items. Scores were also scaled to provide normative age classification performance. A similar MANOVA was conducted on the spatial working memory CANTAB task with number of between and double errors in the three conditions as dependent variables (4 boxes, 6 boxes and 8 boxes errors made). CANTAB strategy scores were compared in a paired samples t test.

Further MANOVAs were conducted with testosterone levels, HADS anxiety and depression at baseline and follow-up as covariates (MANCOVAs) with neuropsychological dependent variables. The working memory Index from the Wechsler Adult Intelligence scale (WAIS) was added as a covariate so that it could be adjusted for in the spatial working memory index CANTAB task. This was to exclusively gauge spatial reasoning performance of healthy participants on the CANTAB without the influence of working memory.

The BRIEF-A assessment was modelled using similar MANOVAs where the dependent variables were all scaled scores on the self-report questionnaires (Inhibition, self-shift, self-emotional control, self-organisation, ability to initiate, working memory, monitor, behavioural regulation index, metacognition and global executive function scores). The grouping variables were questionnaire type (self) and time (baseline and follow-up), which were used to model an interaction. Covariates (anxiety and depression) were added to the MANOVA in a MANCOVA if differences were found between time points only. This was to gauge if covariates contributed to any variance in differences between time periods. Testosterone was added to the model regardless of group change to assess if it had any mediating effect as it was a key component of the current assessment of participants.

The use of MANOVAs and MANCOVAs allows the entry of many dependent variables in unison which benefits the management of family-wise error rates and reduces type 1 errors. Moreover, MANOVA analyses are resilient to violations of homogeneity which is not the case with other statistical methods including ANOVAs etc. (O'Brien & Kaiser, 1985).

Psychosocial covariates to be added were assessed first and showed the anxiety and depression factors were correlated in healthy participants $r = 0.41$, $p = 0.002$. Although this relationship was found, the covariates were not correlated to the extent that they were excluded from the analysis. Past research has found that correlations of $r = 0.50$ or even 0.80 or above could cause multicollinearity issues (Vatcheva et al., 2016). A further MANOVA was conducted to test for homogeneity of regression slopes where interactions were modelled between the fixed factor, time and covariates. No significant differences were found, concluding that subsequent MANCOVAs conducted did not violate the assumption of homogeneity of regression slopes.

Individual change was calculated for each neuropsychological measure per participant with the Reliable change index (RCI) according to the methodology of Jacobson and Truax (1991). This method allows the measurement of reliable performance changes across time (i.e. baseline and follow-up) (Jacobson & Truax, 1991). Calculation of RCI in the current study first required that the test-retest reliability coefficient was computed for each measure ($r_{xx}$). The standard error of each measure $SE_m$ was then calculated as ($SD_1$ ($\sqrt{[1-r_{xx}]}$) where $SD_1$ was the standard deviation of group performance at baseline. Then the standard error of the difference ($SE_{diff}$) was calculated using $SE_m$ and the equation for this was $SE_{diff} = \sqrt{[2(SE_m)^2]}$ which represented the spread and distribution of change scores expected if no change occurred. A 90% confidence interval was set by multiplying the $SE_{diff}$ by $\pm$ 1.645 (Kneebone, Andrew, Baker, & Knight, 1998). Practice effects were controlled for by subtracting the follow-up group mean scores by baseline group mean scores, then adding the result to the newly calculated $SE_{diff}$ (Sawrie, Chelune, Naugle, & Luders, 1996). The equation for the 90% CI was therefore RCI = $SE_{diff}$ x ($\pm$ 1.645) + practice effect. Finally, each subject's follow-up score was subtracted from their baseline score (T2-T1) so that a difference score could be obtained. If the difference score fell outside of the RCI, then a significant and reliable change in cognitive performance was thought to have occurred in the subject.

A chi-square test was conducted to assess the effects of the proportion of participants that increased or decreased in testosterone levels affected the proportion of participants that reliably improved or declined in performance on cognitive measures. Any relationship between proportions was considered significant by using the $p < 0.05$ alpha value (two-sided). The cross tabulation of any significant chi-square was assessed to determine the cross-tabulation cells that had standardised residuals (std. residuals) above or below +/-1.96 as the alpha level of 0.05 which were then reported.

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## Appendix 12: FSL and SPM analysis

FSL analysis

Region of interest analysis allowed the assessment of small clusters of voxels in task related regions thereby reducing the amount of error due to multiple comparisons of voxels that could be prone to type-I errors and chance findings during whole brain analysis between baseline and follow-up. Therefore, the selection of a small number of voxels from a regional spatial distribution minimized the chance of errors due to chance findings (Logan et al., 2008). Moreover, temporal signal to noise ratio (tSNR) over the spatial distribution has been known to show true change that may not be visible during whole brain analysis (Logan et al., 2008). The quantification of the BOLD signal as a percent signal also allowed change across a time course to be calculated allowing comparisons to be made. Thus the analysis allowed assessment of task related regions without the requirement of stringent criteria to assess if voxels were activated and to assess their consistency in activation between time periods. While ROI analysis may reduce chances of errors, outcomes from the analysis should still be interpreted with caution due to issues of multiple testing since many factors were compared increasing the chances of type-I errors. This may lead to increases of chance findings where the null hypothesis was rejected for the alternate hypothesis to increase familywise errors. Consequently, signal change differences between time points may be susceptible to error.

**SPM Analysis**

SPM analysis was undertaken to investigate intra-class correlations (ICC) in voxel regions of interest. The ICC method is an analysis technique used to measure test-retest reliability of the spatial distribution of BOLD fMRI voxel activation across separate sessions (Caceres et al., 2009). It uses baseline fMRI activation in selected ROIs to predict consistency of intra-voxel activation in subsequent sessions in the same region. This allows the measurement of a within subjects analysis of repeatability of observations by calculating within subject error variance (Zandbelt et al., 2008). Reliable activations are those observations that have less than the agreed limit of within subject variance threshold (Bland & Altman, 1986). Correlations are specifically employed to measure the strength of reliability between sessions with high correlation indicating greater reliability (Raemaekers et al., 2007). Therefore, in the current study, ICCs were employed to measure the reliability of activation in the healthy ageing sample across baseline and follow-up. This allowed inferences to be made on the integrity of activation in specifically selected regions across a longitudinal period. Regional activated voxels were selected from putative SST and MRot task activation areas at baseline to predict activation at six months. These regions were selected through first level and group level analysis performed with SPM that generated task related contrasts. Regions

of interest (ROIs) for voxel ICC reliability were selected through maximal activation voxels in baseline group activation maps. These regions were masked and extracted through the SPM Marsbar software (Matthew et al., 2002). Masks were implemented into the ICC toolbox designed by (Caceres et al., 2009).

Rawdata par rec files produced by the Philips scanner were first extracted and converted into Neuroinformatics Technology initiative (Niftii) format for use in SPM12 (Welcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK (SPM)) on Matlab version 7.9.0.529 (R2009b). The two SS task runs and one MRot task run were first slice time corrected (184 slices per run). The repetition time (TR) was inputted as 2.5 seconds and the TA calculated as TR/(TR/number of slices). Slice order was entered in a regular up ascending order from slices 1 to 184. Volumes were then realigned and estimated. Images were realigned to the first image of each run, and then all images of each run were aligned to the first volume of the first run. Estimation was run with higher $5^{th}$ degree spline interpolation. Image realignments were evaluated for any motion in either the X, Y, or Z directions or in pitch, roll, and yaw rotations. A pre-established exclusion criteria was a correlation between movement parameters and task regressors greater than 0.5. These standards have been associated with best practice based on previous fMRI studies (Whalley, Gountouna, et al., 2007). No subjects had to be excluded using the criteria. The anatomical image ($T_1$) was first viewed to change the origin as the anterior commissure for each subject. This was employed to increase co-registration and normalisation accuracy. Images were subsequently co-registered to the mean reference epi image from the realignment procedure. The source image was the anatomical (MPRAGE) image which was revolved to be in alignment with the reference epi image. All functional images were realigned to the reference epi image with trilinear interpolation and then normalised to the standard SPM12 MNI EPI template. Normalization parameters were estimated using the mean image for each run, and applied to all volumes of that run. A $8mm^3$ full width by half maximum (FWHM) Gaussian kernel was used to spatially smooth normalised images.

Statistical analysis at the subject level was estimated using the general linear model (GLM). Default settings were implemented from SPM12 with an SS task design matrix consisting of four conditions including go correct, go incorrect, stop correct, and stop incorrect trials. The MRot task was composed of two conditions which were the task condition (exp) and control condition (cont). Regressors were convolved with the traditional canonical hemodynamic response function model (hrf) to model data. A high pass filter of 100s cut-off was implemented to remove low frequency noise components such as drift.

The main SS task contrast was of go incorrect (gin) and stop correct (sc) trials together compared to stop incorrect (sin) and go correct (gc) trials. The contrast therefore was go incorrect and stop correct compared to stop incorrect and go correct (gin+sc-sin+gc). This was the most efficient combination of

inhibition trials against go trials. The contrast of interest in the MRot task was the task condition (exp) compared to the control condition (cont) (exp-cont). A script was generated using SPM's jobman to automate the entire preprocessing and statistical analysis processes.

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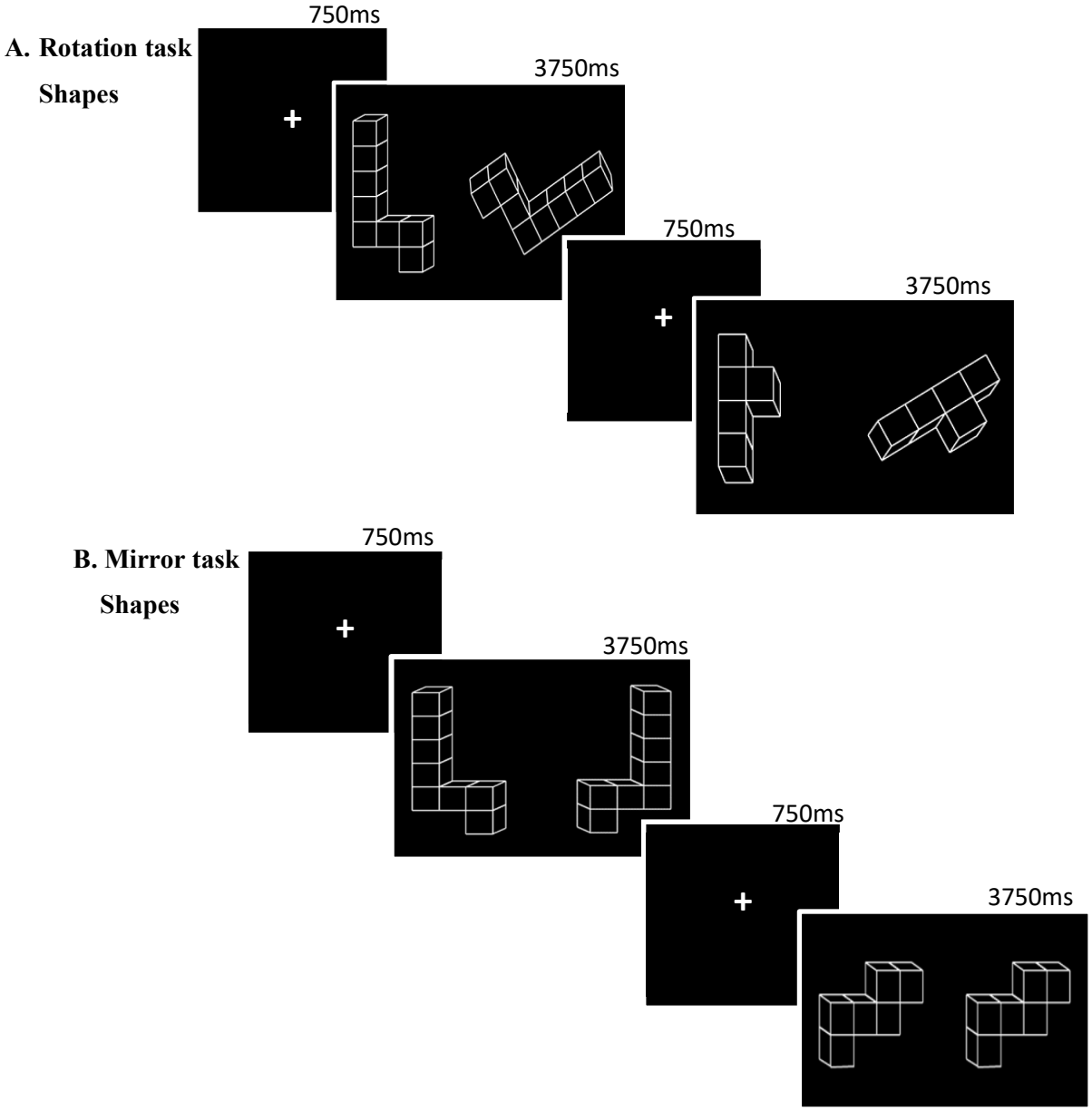


**Figure 3.2:** *Shows MRot task parameters and examples of presented shapes. A. Task rotated shape examples where subjects were required to make a response after mentally rotating shapes clockwise or anti-clockwise to judge if shapes were in the same orientation or mirror images of each other. B. same or mirror oriented shape pairs that require no rotation to distinguish if they were the same or different. Abbreviations: MRot, mental rotation task; ms, milliseconds.*

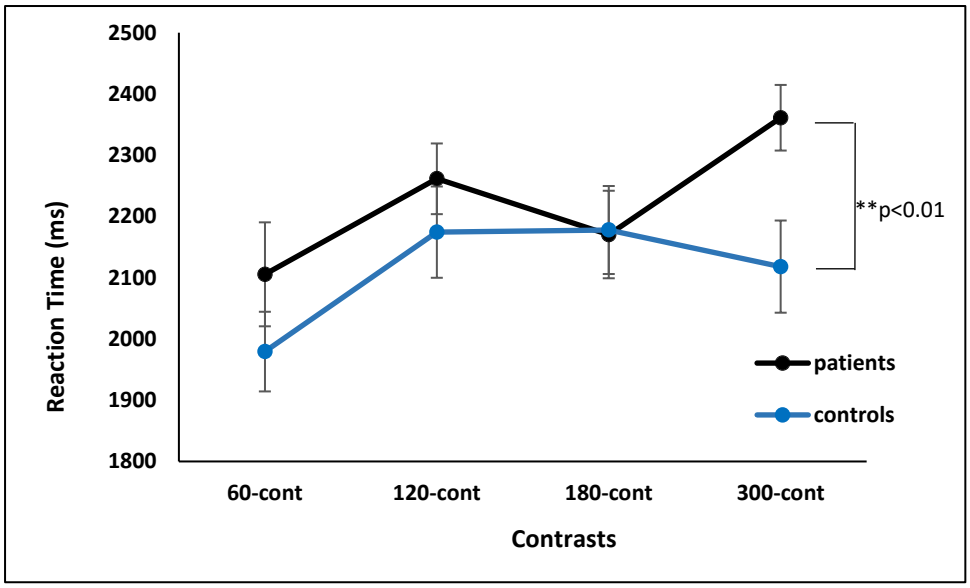


**Figure 3.3:** *MRot task average reaction time between patients and controls. Abbreviations: cont, control condition; MRot, mental rotation task; ms, milliseconds.*

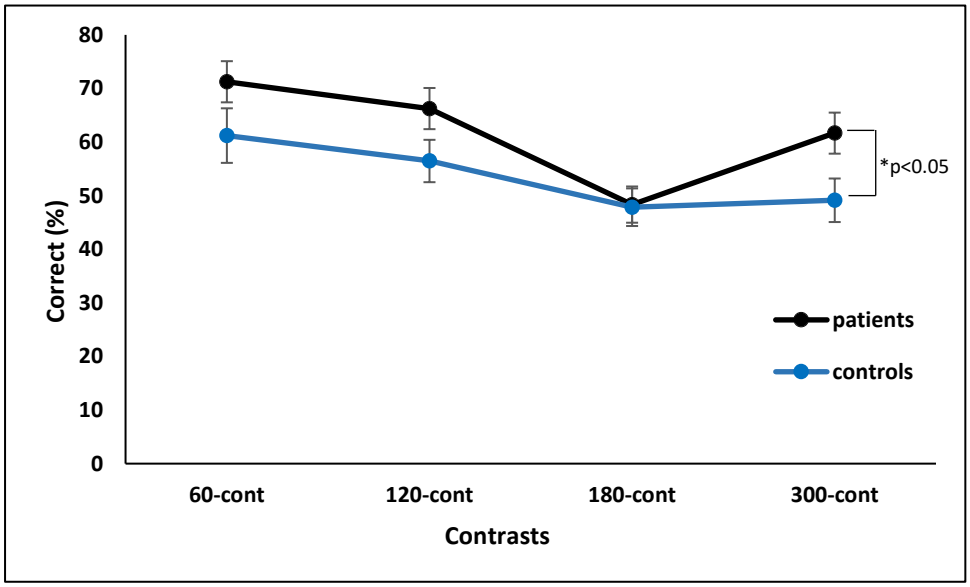


**Figure 3.4:** *MRot task average percentage correct between patients and controls. Abbreviations: cont, control condition; MRot, mental rotation task; *p<0.05; **p<0.01.*

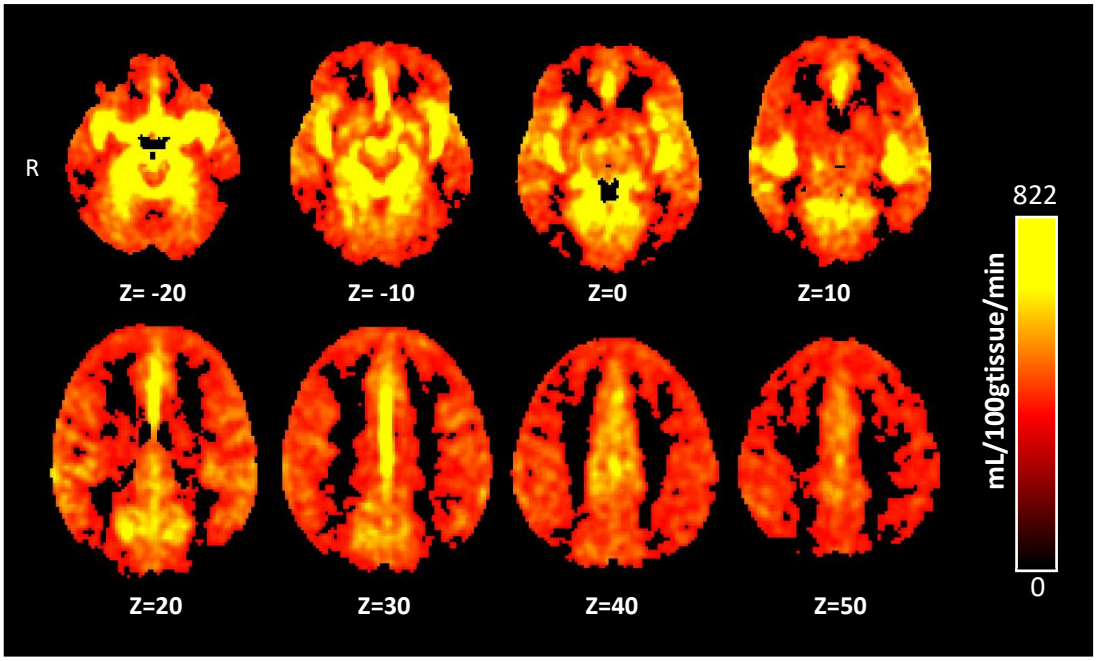


**Figure 3.5:** *Healthy Controls (n=26) ASL Group Analysis. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres.*

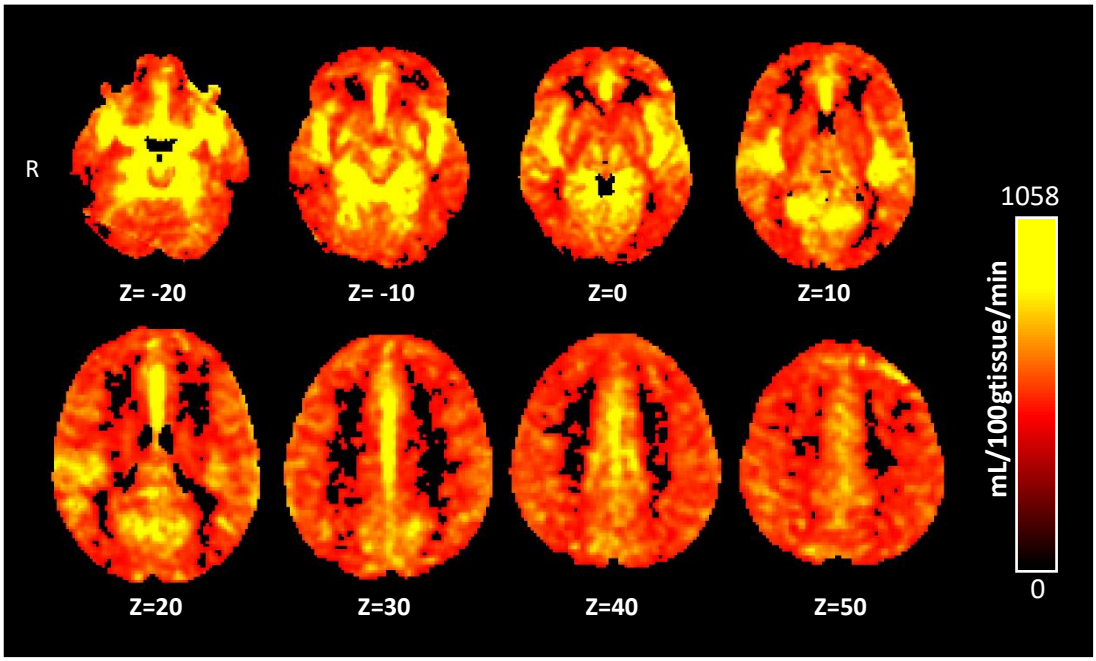


**Figure 3.6:** *PC patients ASL (n=24) Group Analysis. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres R, right.*

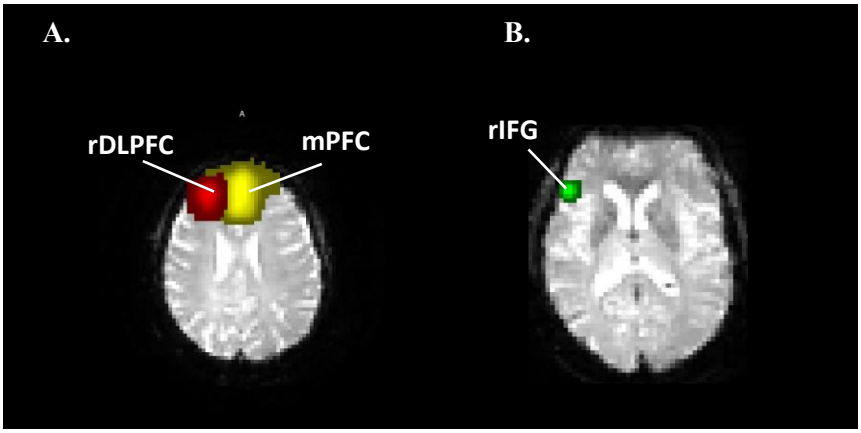


**Figure 3.10:** *Resting state analysis masks of seed regions and regions of interest. (A) Masks showing the mPFC which was seeded to create a functional connectivity map of the default mode network. The strength of connectivity from the DLPFC was measured to the DMN. (B) The strength of connectivity was similarly measured from the rIFG to the DMN. Abbreviations: A, anterior; DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; mPFC, medial prefrontal cortex; r, right.*

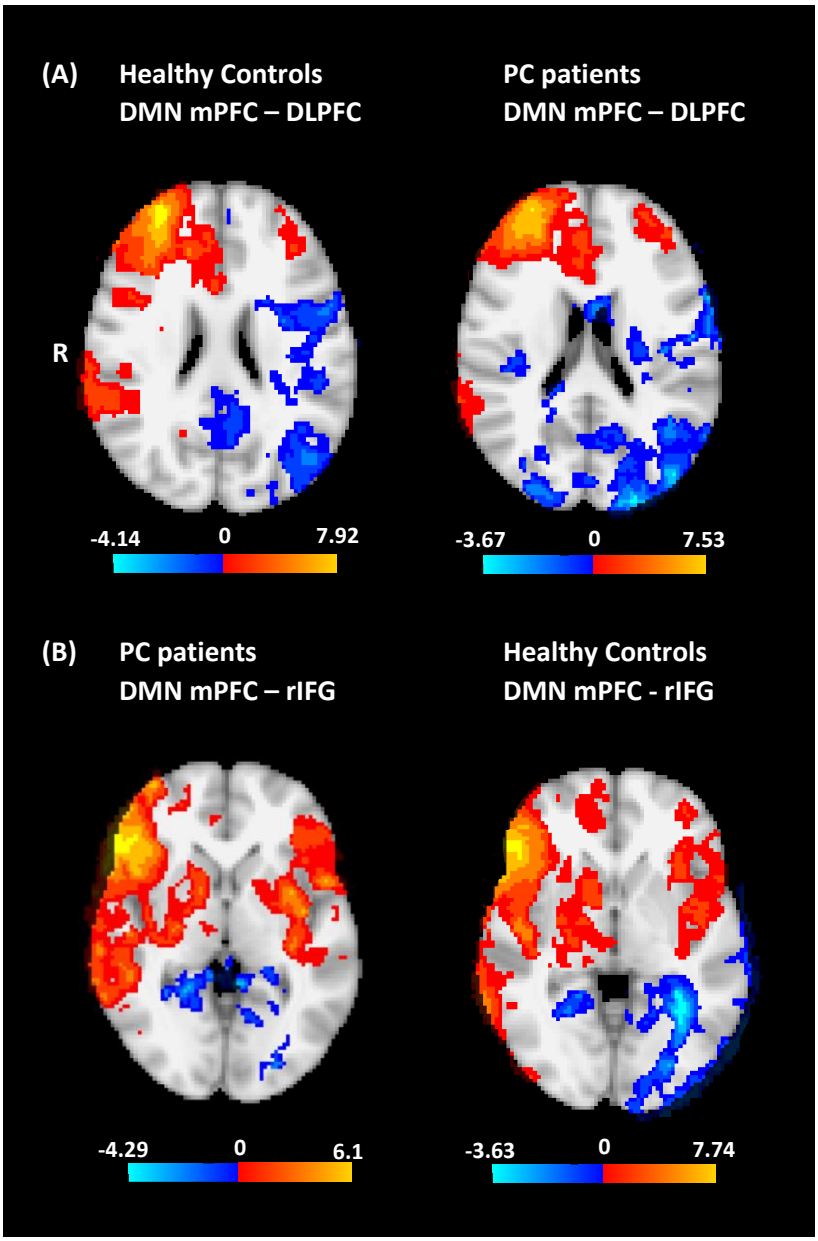


**Figure 3.11:** *Resting state analysis of patients and controls. (A) Patient and control resting state connectivity map with rDLPFC as region of interest to DMN. (B) Patient and control resting state connectivity map with rIFG as region of interest to DMN (Corrected for multiple comparisons, Z > +/-2.3; Cluster-wise threshold, p < 0.05). The colour bar represents negative BOLD in light blue to dark blue areas of brain maps and greater BOLD activation in red to yellow areas of brain maps. Abbreviations: DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; mPFC, medial prefrontal cortex; r, right.*

## 3.9 Regional ASL perfusion

Figure 3.13 shows CBF values in regions of interest of controls and patients. Regions were selected based on activation areas of the stop signal and MRot task. Regional ASL analysis was conducted subsequently to ensure that putative task regions were first active before conducting the analysis. Perfusion assessments were employed in these regions to assess the integrity of BOLD signal activation found in patients and controls. Hypoperfusion has been known to lead to reductions in the BOLD signal which may lead to reduction in SST activation. The rIFG pars triangularis and pars opercularis were both masked and combined so that a regional estimate of the rIFG could be gauged. Bilateral supramarginal gyri were also masked to measure CBF in these regions that were related to the MRot task.

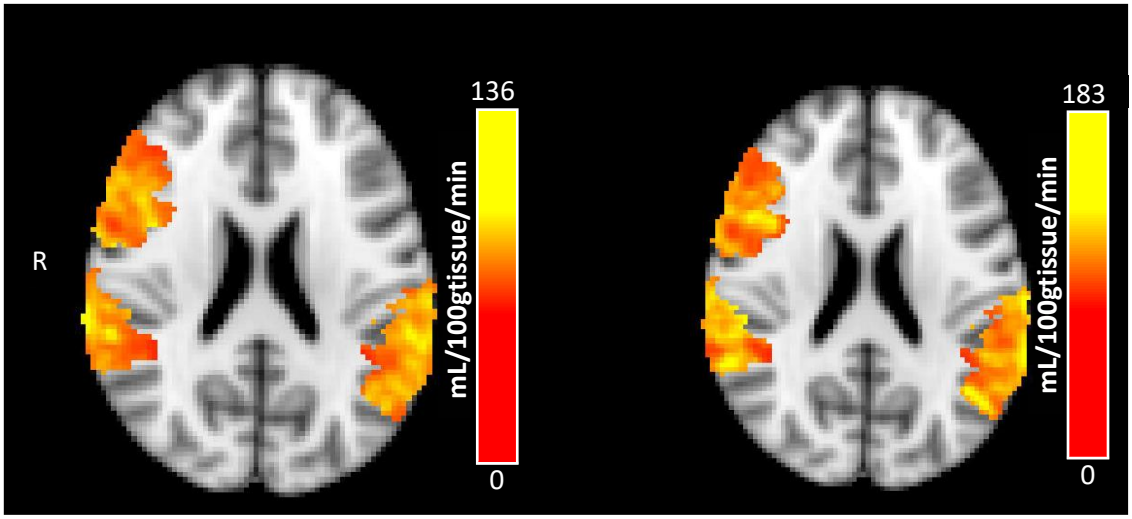


**Figure 3.13: (A)** *Healthy controls rIFG, right supramarginal and left supramarginal gyrus ASL group level perfusion maps*. **(B)** *PC patients Controls rIFG, right Supramarginal and left Supramarginal gyrus ASL Group Level perfusion maps. The colour bar represents least perfusion in black to red areas of the CBF map and greatest perfusion in yellow areas of the CBF map. Abbreviations: g, grams; min, minute; mL, millilitres.*

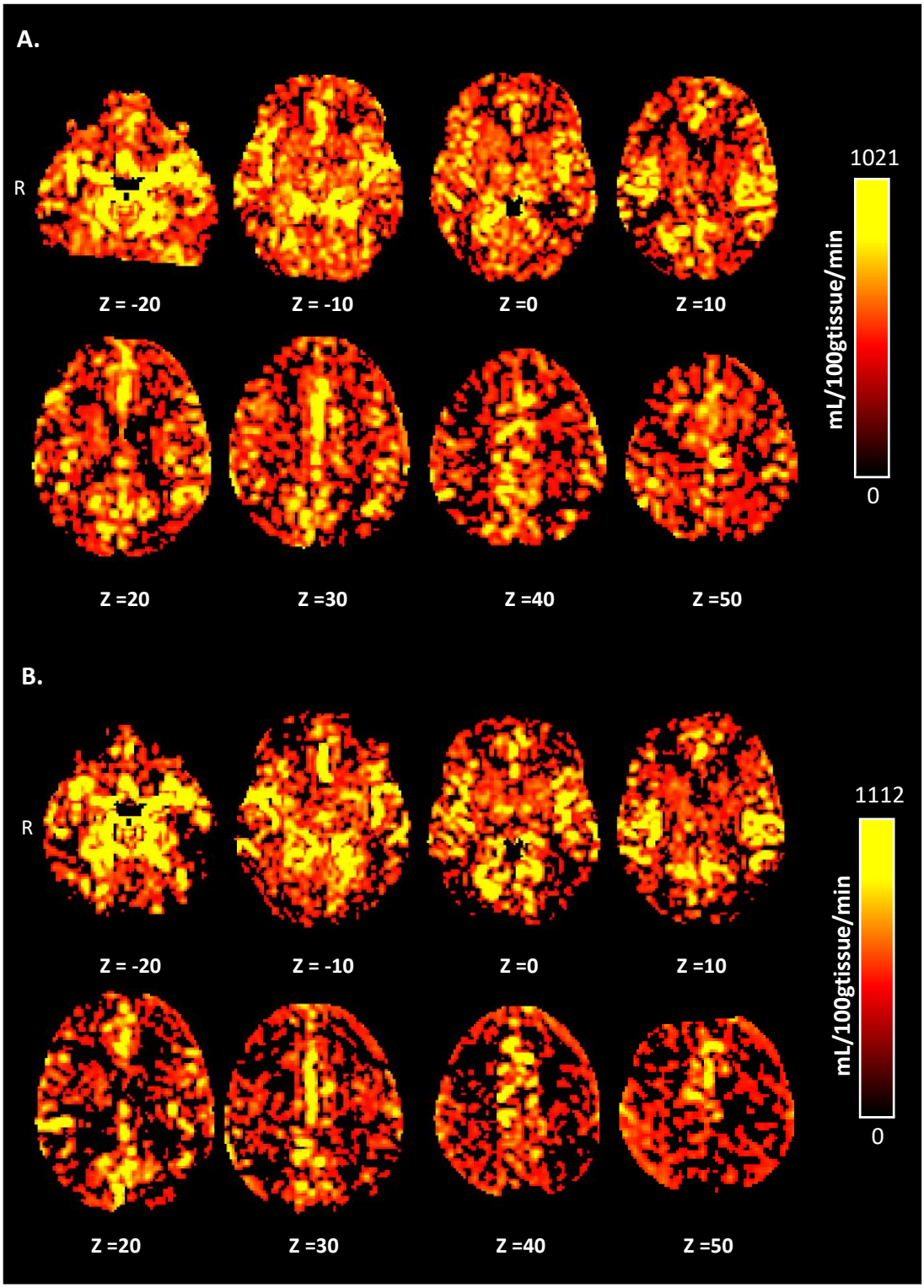


**Figure 4.3:** *Whole brain ASL maps of healthy subjects at: (A) Baseline (n = 8) (B) Six Months (n = 8). The colour bar represents least perfusion in black to red areas of CBF maps and greatest perfusion in yellow areas of CBF maps. Abbreviations: g, grams; min, minute; mL, millilitres, R, right.*

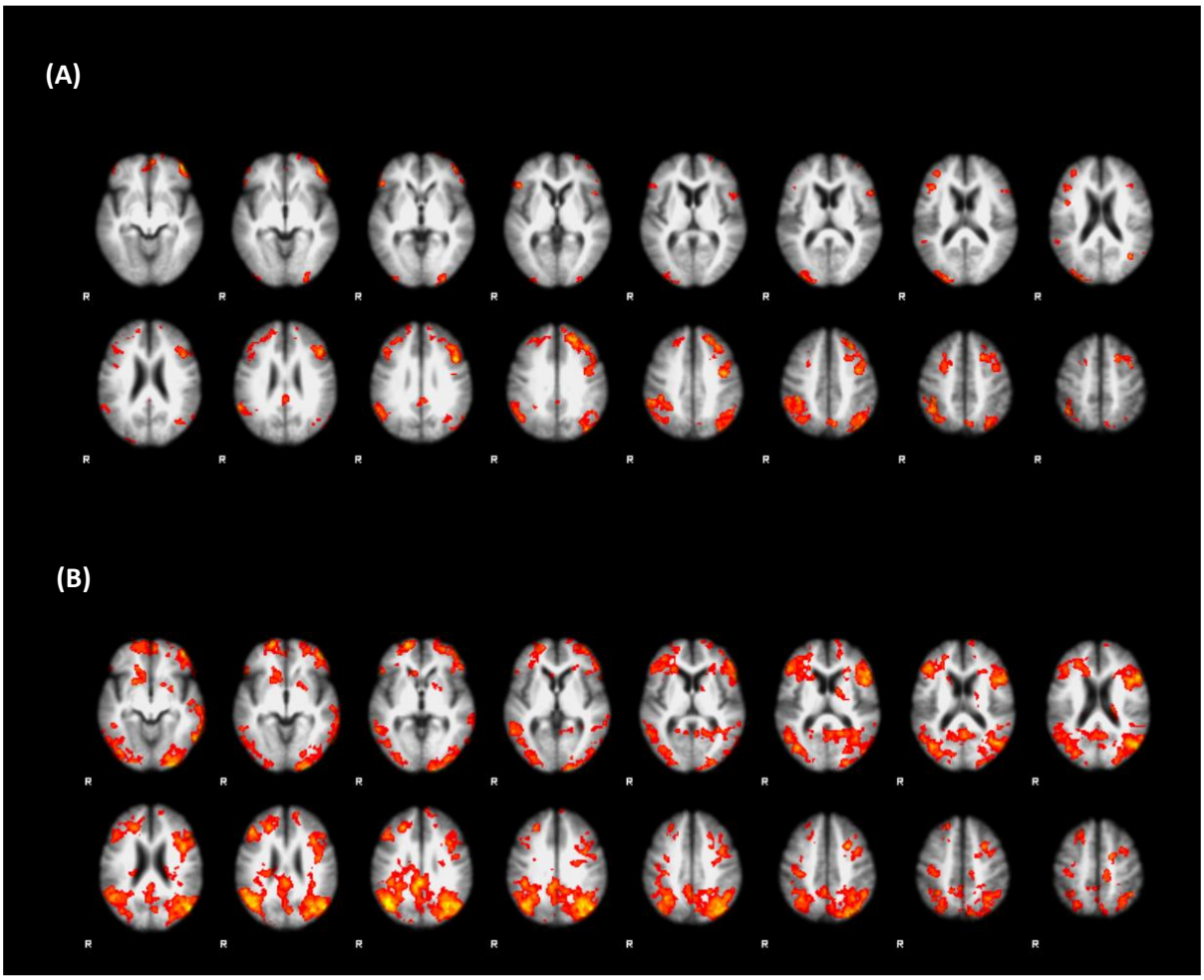


**Figure 4.4:** *SST whole brain group activation. A. baseline group activation. B. Six month group activation. Abbreviations: R, right; SST, stop signal task.*

Overlapping of distinct flicker contrasts trials in figure 4.6 show areas contributing to activation on the separate flicker contrasts. This provides a better view that can be visually inspected. Activation seemed to be apparent in areas including the rIFG and RMFG. Furthermore, parietal areas were activated supporting a fronto-parietal network that is in line with an executive function network at baseline and follow-up. Additional areas of activity were present in the left IFG and left MFG. The lc-gc and mc-gc contrasts showed most activation.

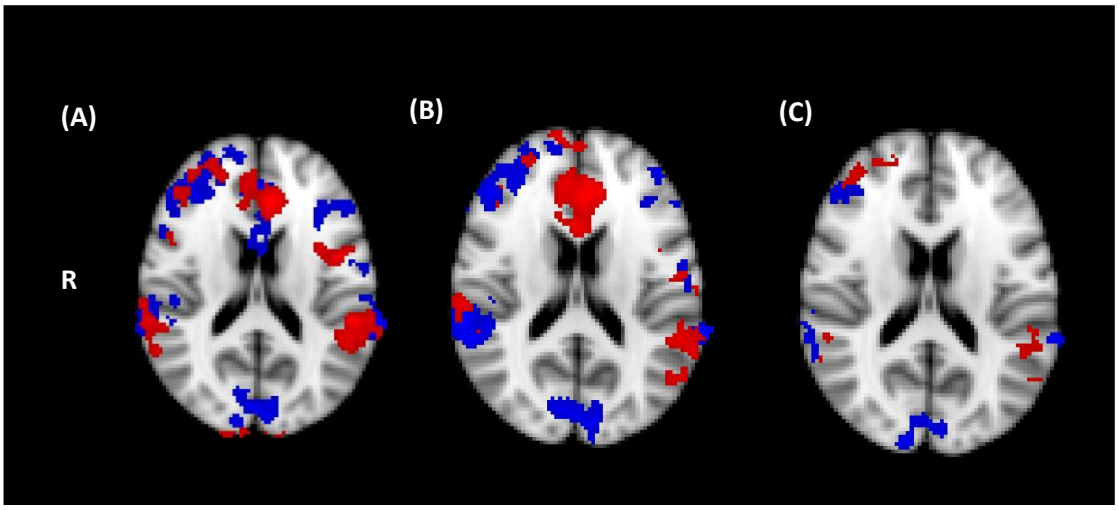


**Figure 4.6:** *SST overlap images of healthy participants at baseline and six-month follow-up where red activation colour maps indicate activation of participant group activation at baseline and purple activation group maps at six months. (A) Group activation maps on the contrast lc-gc. (B) Group activation maps on the contrast mc-gc. (C) Group activation maps on the contrast sc-gc (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations*: *lc, long correct; mc, medium correct; R, right; sc, short correct; SST, stop signal task.*

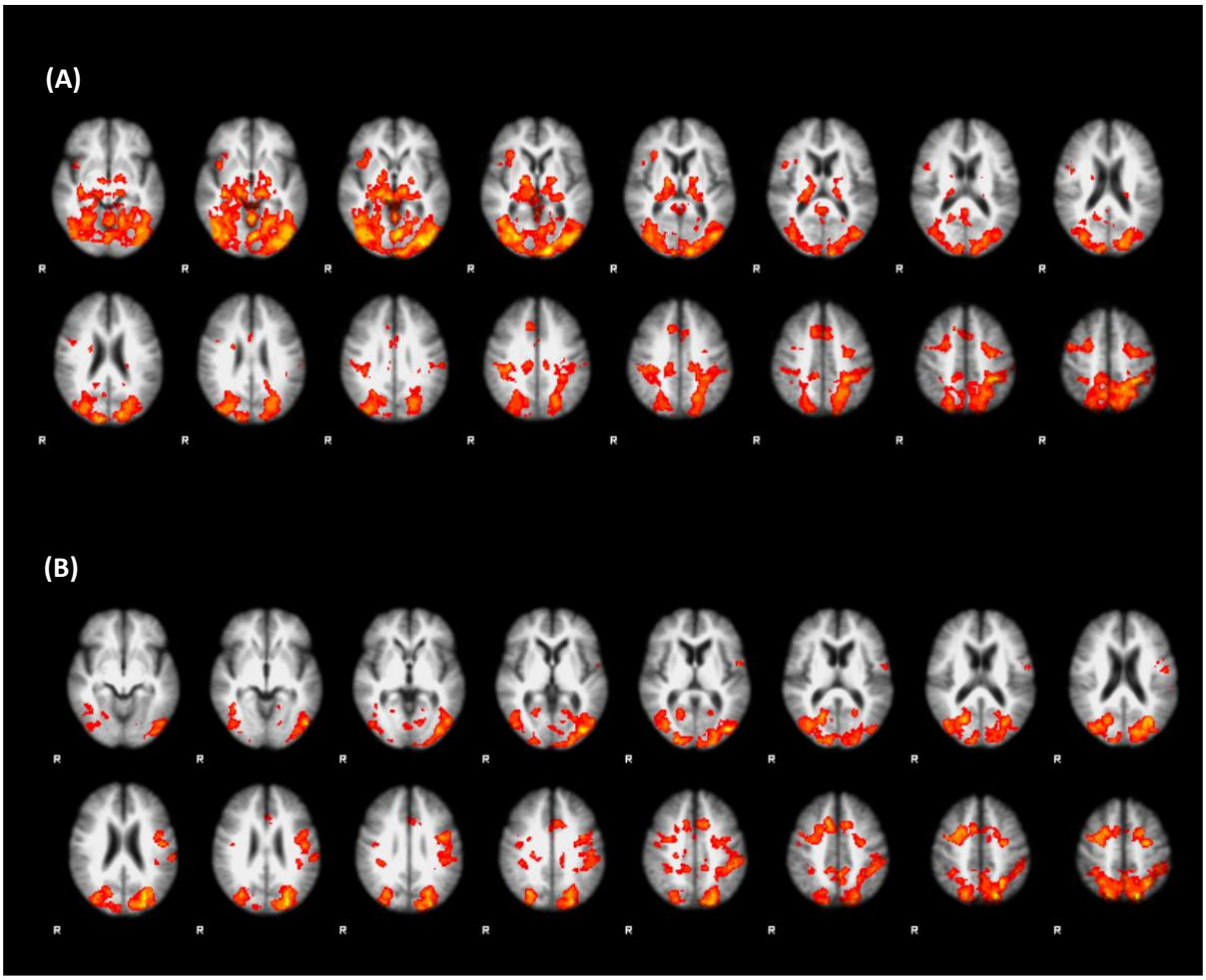


**Figure 4.7:** *MRot task whole brain activation. (A) Baseline MRot activation. (B) six month MRot activation. Abbreviations: MRot, mental rotation task; R, right.*

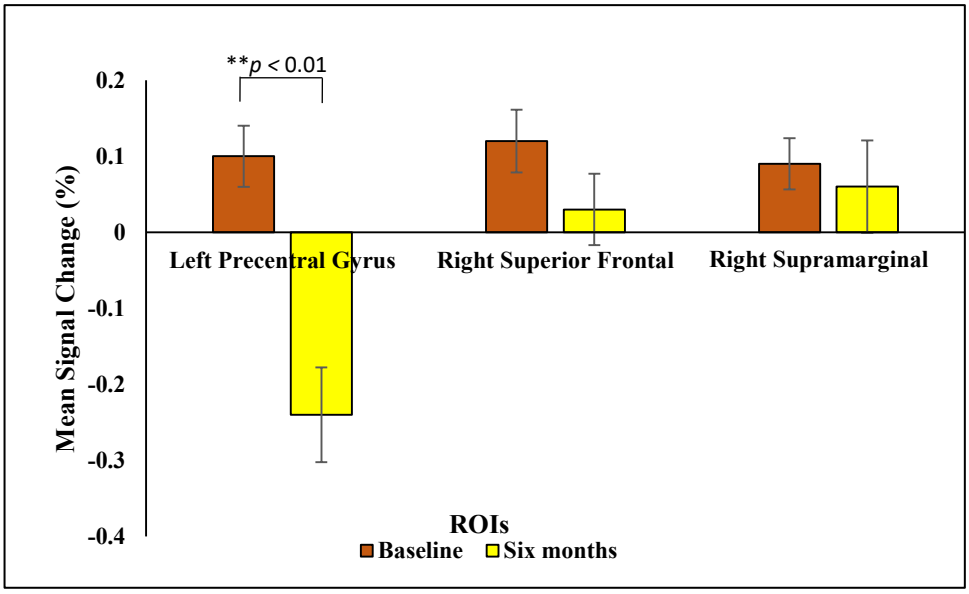


**Figure 4.8:** *MRot mean signal change on 60-cont contrast showing a significant difference in the left precentral gyrus. Abbreviations: MRot, mental rotation task; ROIs, regions of interest.*

*Contrast 120-cont*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 1.25$, $p > 0.05$). A main effect was shown between the signal changes of ROIs when controlling for time ($F(2, 48) = 3.54$, $p = 0.04$, $\eta^2 = 0.13$). No significant effect was found on the interaction term of signal change in ROIs between baseline and six months. Mauchly's test of sphericity was violated ($X_2(2) = 7.96$, $p = 0.02$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of sphericity ($\varepsilon=0.77$) ($F(1.54, 37.14) = 0.89$, $p > 0.05$). Change in testosterone did not covary with any differences between signal change between regions and time ($F(1.54, 37.14) = 3.39$, $p > 0.05$).

*Contrast 180-cont*

No significant main effect of averaged signal change across all ROIs between time points was found ($F(1, 24) = 1.55$, $p > 0.05$). No main effect was shown between the signal changes of ROIs when controlling for time. Mauchly's test of sphericity was violated ($X_2(2) = 10.32$, $p < 0.01$), therefore, degrees of freedom were modified using Greenhouse Geisser estimates of

Overlap images assist in demonstrating activation visually in healthy participants at baseline and follow-up (figure 4.9). No activation was apparent in contrasts requiring least rotation at six months, however, activation was present at baseline (60-cont and 120-cont). The 180-cont contrast showed most activation in putative MRot areas including the bilateral supramarginal gyri, precentral gyri and parietal lobe areas.

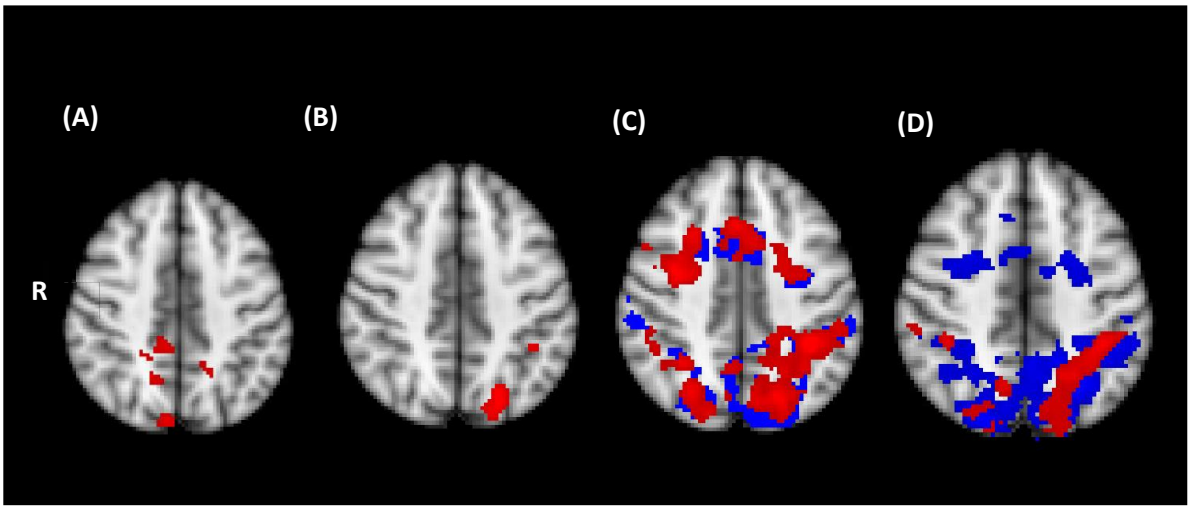


**Figure 4.9:** *MRot overlap images of healthy participants at baseline and six-month follow-up where red activation colour maps indicate group activation at baseline and blue activation group maps show activation at six months. (A) Group activation maps on the contrast 60-cont. (B) Group activation maps on the contrast 120-cont. (C) Group activation maps on the contrast 180-cont. (D) Group activation maps on the contrast 180-cont. (Corrected for multiple comparisons, Z >2.3; Cluster-wise threshold, p < 0.05). Abbreviations: cont, control condition; MRot, mental rotation task; R, right.*

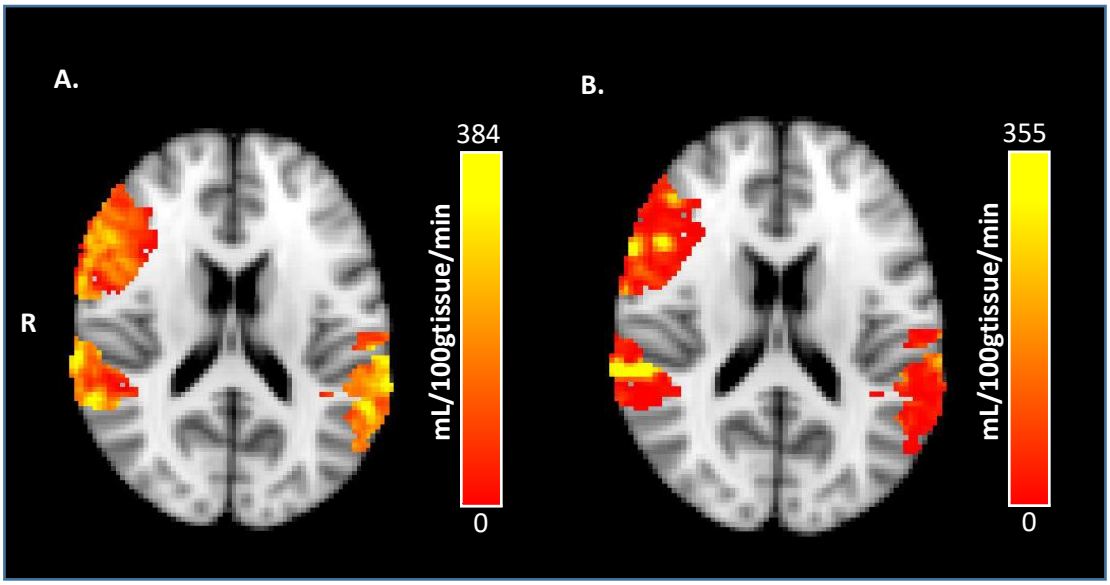


**Figure 4.10:** *rIFG gyrus and right and left supramarginal gyri masked ASL perfusion of: (A) baseline (n = 8) (B) six months healthy participants (n = 8). The colour bar represents least perfusion in red areas of the CBF map and greatest perfusion in yellow areas of the map. Abbreviations: g, grams; min, minute; mL, millilitres; R, right.*

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## GENERAL MRI INFORMATION SHEET FOR RESEARCH PARTICIPANTS

**Dear Research Participant**

Thank you for volunteering to undergo a research magnetic resonance imaging (MRI) brain scan at the Birmingham University Imaging Centre (BUIC).

Your participation at this research centre will help benefit understanding in the areas of knowledge about the central nervous system, in neuroscience and medicine. This information sheet broadly describes the MRI procedure, and answers some common questions. If you have any further questions, please do not hesitate to contact your researcher.

You can find out more information about what we do from our web site www.buic.bham.ac.uk

**What is MRI?**

MRI is a relatively recently developed technique which combines the use of magnetic fields and radio-waves to image the body. MRI does not use *any* ionizing radiation or X-rays and there are *no* known side-effects or cumulative risks.

For your safety you will be asked to fill out an MRI Safety Screening Questionnaire, and to remove all metallic items before you enter the magnet room. FMRI (functional MRI) uses similar methods to conventional clinical MRI to obtain 'functional brain images'. The technique relies on indirectly identifying small changes in blood flow/oxygenation in different parts of the brain.

**The MRI Scanner**

As you can see from the picture opposite, the scanner is a large cylinder which has a tube (bore) running through the middle, open at both ends.

You will enter the scanner tube on a moveable bed, laid down on your back, head-first, with your lower legs remaining outside the magnet's bore.

UNIVERSITY OF BIRMINGHAM

**Birmingham University**

**Imaging Centre**

**www.buic.bham.ac.uk**

BUIC Birmingham University Imaging Centre

## PARTICIPANT INFORMATION SHEET

**Title of project:**

**Part 1**

**Introduction to the research and invitation to take part:**
You are being invited to take part in a research study. It is important that you understand why the research is being done and what it will involve before you decide whether or not to take part. Please read the following information carefully, and please discuss this with others if you wish. Feel free to ask us if there is anything that is not clear or if you would like more information.

**What is the purpose of the study?**
The study will be examining the relations between brain structure and brain function using magnetic resonance imaging.

**What kinds of stimuli will be presented?**
The stimuli will be visual images (pictures, words), sounds, tactile input or smells. Any stimuli that might potentially be distressing will be shown to you beforehand to enable you to judge if you feel distress. You will be able to withdraw from the study at any time (below).

**Do I have to take part?**
No. It is up to you whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. Withdrawing from the study will not affect you in any way (e.g., your future medical treatment).

**What will happen to me if I take part?**
You will undergo an MRI scan and you may also be asked to carry out a task while in the scanner. As you carry out the task we will measure changes related to brain activity which will inform us about how brain areas operate while a task is being undertaken. The scanning session will last about 45 min, during which time you will be asked to lie still.

**What is magnetic resonance imaging?**
Magnetic resonance imaging involves changes the gradient of a magnetic field to produce shifts in the alignment of atoms in the body of the person being scanned. The changes in alignment can be used to measure the structure and function of the tissues. When the brain is scanned we can derive information about both brain structure and function. The procedure is non-invasive and carries no known harm outside of safety issues for operating in a high magnetic field (e.g., if you have a cardiac pacemaker). For this reason you will be asked

to go through a safety questionnaire with a scan operator prior to being allowed to proceed into the scanning environment.

**What are the possible benefits of taking part?**

By learning more about how the brain works, by using MRI, we will be able to develop better ways of diagnosing changes in brain function, and we will learn about how to improve brain function to optimize performance.

**What happens at the end of the research study?**

The results will be written for scientific publication. In addition we will report them in a newsletter that we will distribute to all participants and to hospitals. All data will be reported anonymously.

**Using your data in other research** - we would ask you in the *consent form* if you are willing to share the data we collected from you with other researchers. If you agree we would upload your anonymized data on a server and make it available for research use. There are two levels of data sharing: sharing only with researchers associated with the University of Birmingham; or sharing it as an open access resource. In the latter case we would make the data available on the web as an open access resource. The latter adhere with current government and international policies on scientific data. ***You can choose to allow sharing your data in any or neither forms.*** In case of brain imaging data, you should be aware that there are software that can generate the skeleton shape of the face based on MR imaging data, hence by having access to your data one can potentially reconstruct your face. Furthermore, similar to fingerprint brain are individually unique, thus in the future technology may become available enabling to recognize an individual from the brain structure. If you are happy to share your data we would request you to fill up a short questionnaire about your general health and demographic. The data will never contain any personal details about you (name, address, etc.).

**What if there is a problem?**

It is possible that lying in the scanner might cause some back or neck pain, and it is possible to feel a burning sensation. If you experience any discomfort you can press the emergency buzzer and you will be brought out of the scanner immediately. Any complaint about the way the study has been conducted or any possible harm you may have suffered will be addressed. The detailed information on this is given in Part 2.

**Will my taking part in the study be kept confidential?**

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

***Contact Details* - BUIC Management:**

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

**Appendix 6: Consent Form**

THE UNIVERSITY
OF BIRMINGHAM

School of Psychology
The University of Birmingham
Edgbaston
Birmingham B15 2TT
United Kingdom

# Consent form

Study name: **The Effects of Androgen Deprivation Therapy on Cognition**

Participant identification number:

Name of researcher spoken to:

*Please write your inititals in the box*

1. **I confirm that I have read and understand the information sheet dated..... (version.....) for the above study.** ☐
2. **I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.** ☐
3. **I understand that my participation is voluntary and I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.** ☐
4. **I agree to have a blood test.** ☐
5. **I agree to my GP being informed of my participation in the study** ☐
6. **I agree to be contacted at a later date up to six months from now.** ☐

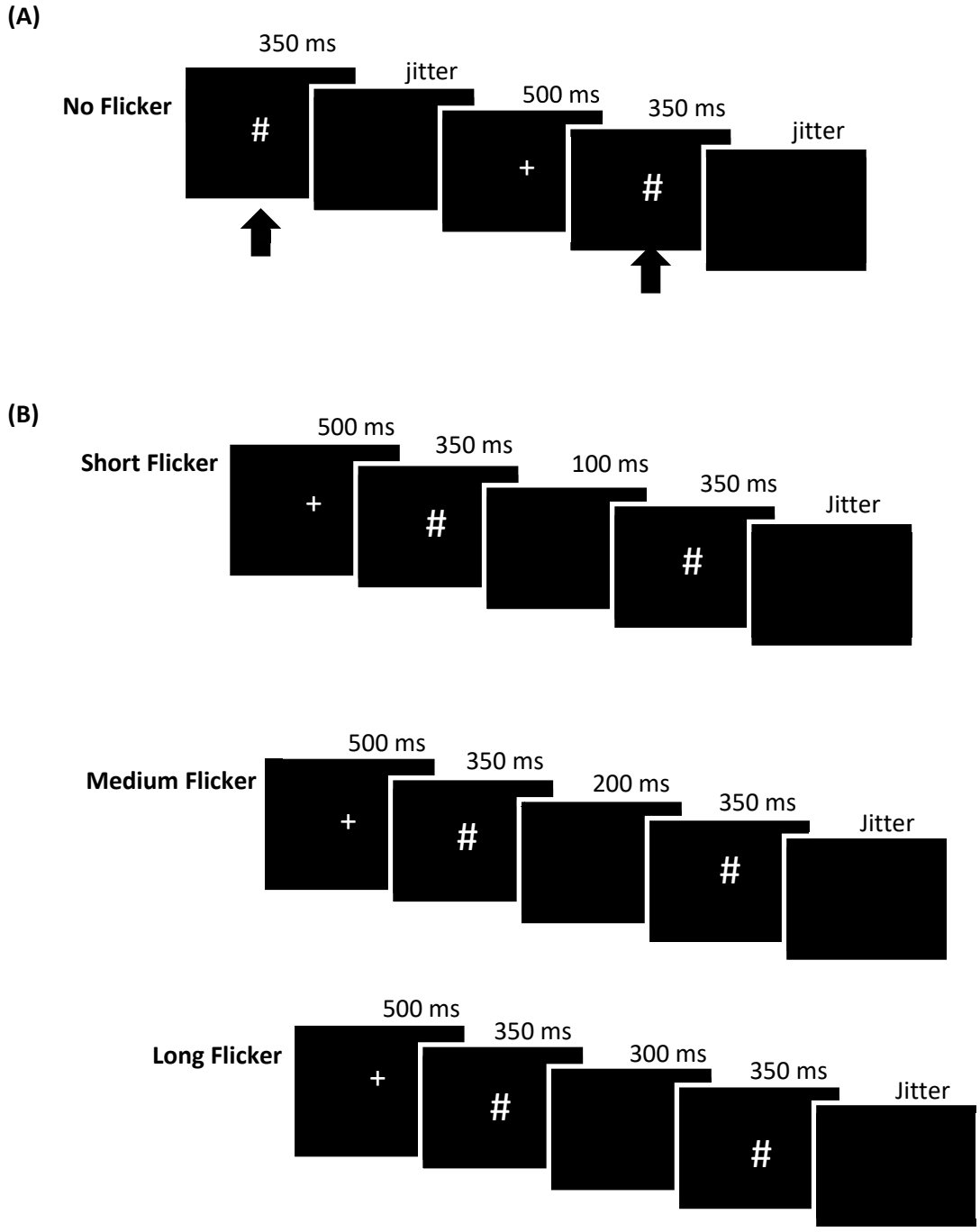


**Figure 1**: *fMRI illustration of the stop signal task. (A) No flicker timings (go trials) in which participants must respond on a response pad. Arrows under hash symbols represent when participants must respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 1, blank screens labelled as 'jitter' were jittered around the temporal resolution of 3 seconds. Jittered durations varied between 2800ms, 3000ms and 3200ms in a random order. Parts (A) and (B) were presented in random order. Abbreviations: ISI, interstimulus interval; ms, milliseconds.*

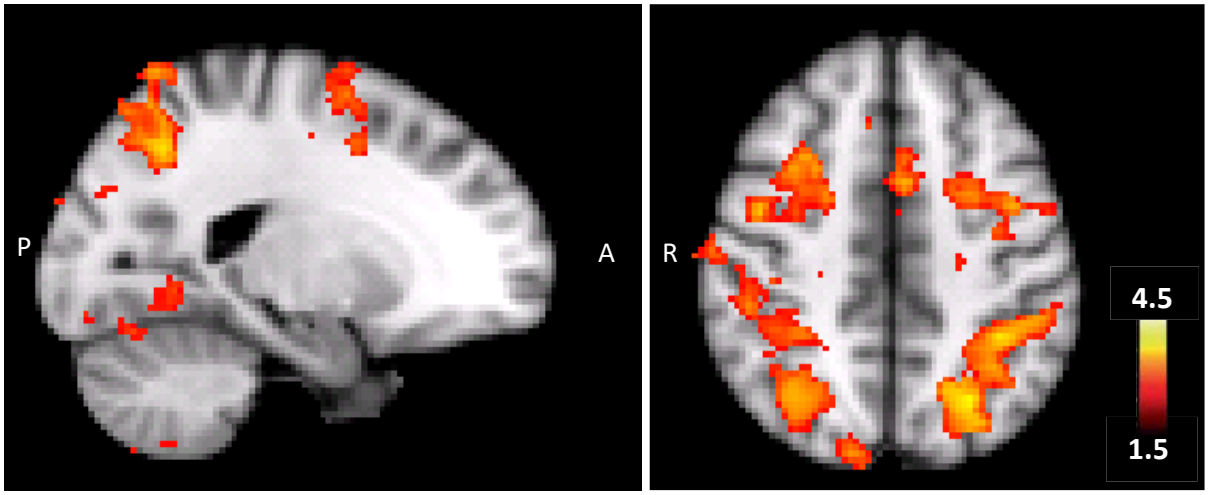


**Figure 2**: *Task condition compared to the control condition contrast. Axial and left hemisphere sagittal whole brain activation view ($p < 0.05$; uncorrected). Colour bars represent least (red) to most (yellow) activation on brain activation maps. Abbreviations: A, Anterior, P, posterior; R, right.*

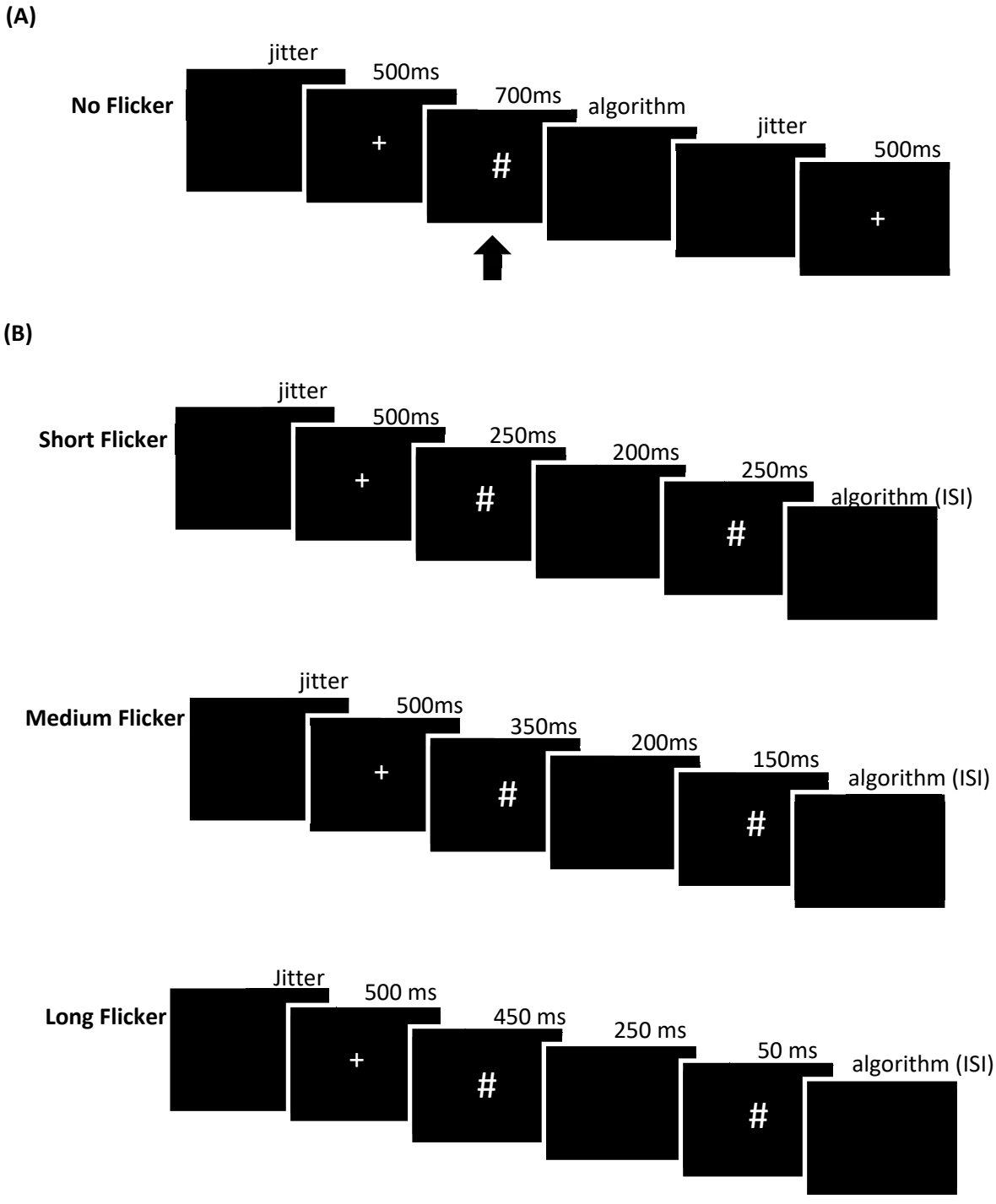


**Figure 3**: *fMRI illustration of the Stop signal task. (A) No flicker timings (go trials) in which participants must respond. Arrows under hash symbol figures represent when participants should respond. (B) Stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 1, blank screens labelled as 'jitter' were jittered around 0, 200 & 400ms. Blank screens labelled 'algorithm (ISI)' had durations which were contingent on jitter durations. These intervals varied between 1100ms, 1300ms and 900ms. Parts (A) and (B) were presented in a randomised order. Abbreviations: ISI, interstimulus interval; ms, milliseconds.*

*Mental rotation task*

The mental rotation task was as described in pilot study one. Complexity of shapes were reduced by decreasing the number of blocks per shape. Moreover, 3D blocks were altered to exclude blocks projecting in oblique directions (figure 4).

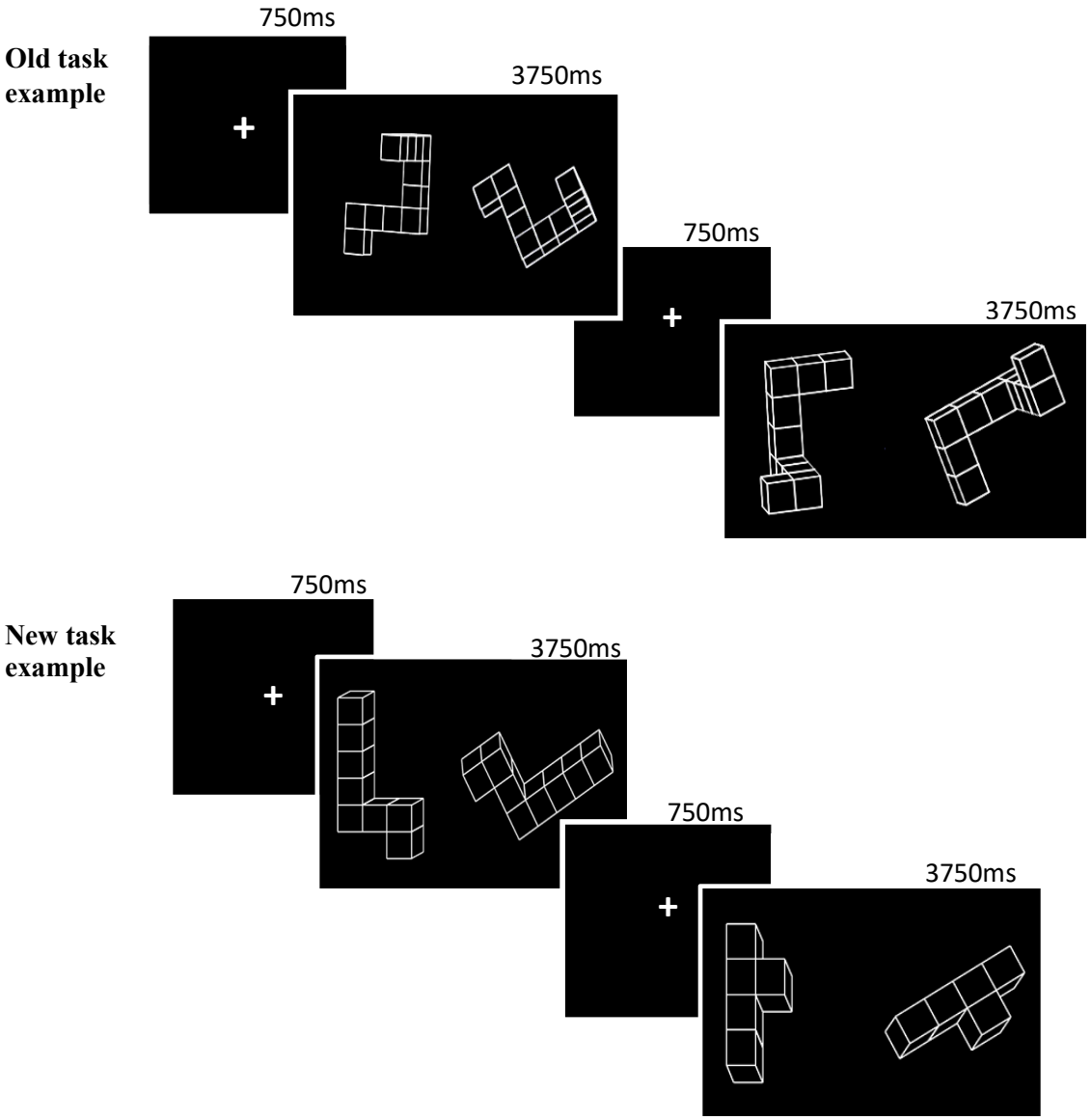


**Figure 4:** *Shows change and modification of task from pilot study one to the new task of study three. The old task used many oblique angles which were removed from the new task of study two.*
*Abbreviations: ms, milliseconds.*

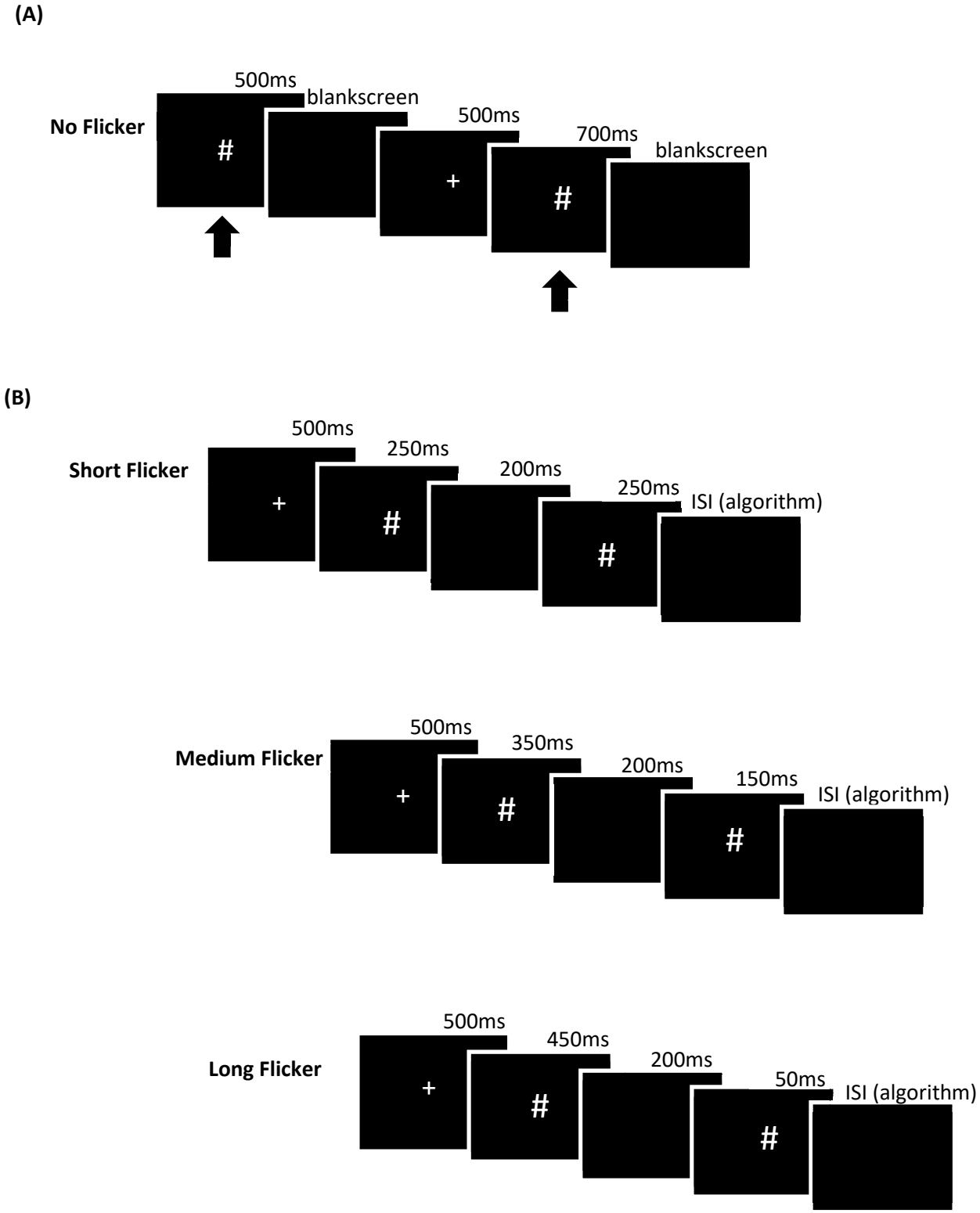


**Figure 7***: fMRI illustration of the Stop signal task. (A) No flicker timings in which participants must press the button on response pad. Arrows under hash symbol figures represent when participants must respond. (B) stop trials were separated into short, medium, and long trials. In parts (A) and (B) of figure 2, blank screens labelled as ISI algorithm were varied in their duration and increased or decreased in duration depending on if the participant responded or inhibited correctly.*