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Cognitive measures and structural indices of 'brain sex'
a neuropsychobiological investigation

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COGNITIVE MEASURES AND STRUCTURAL INDICES OF 'BRAIN SEX': A NEUROPSYCHOBIOLOGICAL INVESTIGATION

By

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Abstract

Cognitive sex differences are consistently observed in cognitive development, cognitive performance and cognitive degeneration; however, the exact factor(s) that lead to these differences is yet to be discovered. The organizational-activational hypothesis suggests that gonadal hormones may be the underlying factors that lead to cognitive sex differences; however, this hypothesis does not suggest any direct links between gonadal hormones or specific brain structures and cognition. This thesis aimed to explore potential theoretical and experimental links between gonadal hormones, cognition and specific brain structure / function. Thus, the association between cognitive performance as well as cognitive brain type with individual masculinization / feminization levels were initially explored. Finally, the association between individual masculinization / feminization levels and brain activity was explored in order to locate a link between masculinization / feminization levels and specific brain function. In order to achieve this, three experiments were designed. The first experiment explored sex differences in a multi-trial verbal free recall test and male / female allocation on a cognitive brain type scale according to an individual's cognitive brain type. Cognitive brain type was measured by the difference between a female-biased task (free recall) and a non-female biased task (productive vocabulary). For exploring sex differences in free recall, a two-way mixed ANCOVA was used, with the dependent variable being free recall and the independent being 'sex', which was used as a general index of individual masculinization / feminization levels. The covariates were factors that previous studies have indicated as significant determinants of free recall, engaging frontal lobe function such as executive function and working memory. Male / female allocation on the brain type scale was explored via a Fisher's exact test of independence, which was used in order to explore the existence of an association between male / female group and brain type group

membership. The second experiment explored the association between cognitive performance as well as cognitive brain type with individual masculinization / feminization levels. The utilized variables as well as the study design was the same as of the first experiment, while an indirect measurement of pre-natal hormone effects (2D:4D), a direct (saliva) measurement of circulating testosterone and estradiol and a measure of brain type via systemizing-empathizing (S-E) were added. Thus, individual masculinization / feminization levels were indicated by sex (as a general index), and pre-natal and post-natal indices / levels of gonadal hormones. The S-E brain type measurement was used as way to confirm the effectiveness of the cognitive brain type measure that was used in this experiment. In the third study the association between individual masculinization / feminization levels with Theta reactivity was explored. The results confirmed previous studies indicating a female advantage in verbal free recall. Moreover, masculinization / feminization levels appeared to be associated with cognitive brain type classification. S-E brain type classification was associated with individual masculinization / feminization levels only when sex was included as a factor; while S-E brain type showed a positive trend across, and an association with, cognitive brain types. Finally, Theta reactivity appeared to be sexually differentiated in the left frontal lobe; while individual masculinization / feminization levels were associated with Theta reactivity. These results were interpreted to suggest that measures of masculinization / feminization can replace 'sex' as a dissociating factor in verbal free recall and potentially other sex-biased cognitive tasks. That is, cognitive brain type appears to be linked to individual masculinization / feminization levels and both measures of brain type possibly rely on the same underlying biological factors. Sex differences as well as individual masculinization / feminization level differences may exist in hippocampal function.

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Chapter 1: Literature Review

1.1 Introduction

The exact nature of sex differences in cognition is an issue that has been addressed by many researchers, through the exploration of behavioural and/or neurological differences (for a review see Zaidi, 2010). Sex differences have been reported by various studies, both in specific cognitive tasks (Adreano & Cahil, 2009) and in brain anatomy (Zaidi, 2010). However, the literature lacks a unifying theory that can connect behavioural and neurological findings, and explain the presence of sex differences in cognitive performance (Kaushanskaya, Marian, Yoo 2011; Krueger & Salthouse, 2010; Adreano & Cahil, 2009), cognitive development (Ozcaliskan & Goldin-Meadow, 2010; Nagy, Kompagne, Orvos & Pal, 2007; Iverson & Goldin-Meadow, 2005), and cognitive degeneration (Alzheimer's Association, 2014; Spampinato, Weininger, Vavro, Parker, Patrick & Rumboldt, 2012; Cuetos, Herrera & Ellis, 2010; Ripich, Petrill, Whitehouse & Ziol, 1995; Ott & Cahn-Weiner, 2005).

Existing literature has stressed the importance of learning and memory[1] as main factors that affect cognitive performance (Gold, 2003; 2004). Through this line of studies the importance of cholinergic transmission is stressed in regard to the functionality of specific brain areas and memory formation (Gold, 2003) as well as cognitive degeneration (Bartus, Dean, Beer & Lippa, 1982; Coyle, Price & De Long, 1983). Gold (2003) indicated that the hippocampus and amygdala are part of a cognitive system that is closely related to memory and learning while this system is significantly affected by cholinergic modulation. More specifically, this cognitive system appears to be closely related to the acquisition of information and memory formation at a

[1] Learning, retaining and recalling information is described by Luine (2014) as the most basic form of cognitive function.

basic level while it is significantly affected by the neurotransmitter acetylcholine (Gold, 2003; for a review on this subject see Hasselmo, 2006). However, both the hippocampus and amygdala are significantly sexually differentiated[2] (for a review Giedd, Raznahan, Mills & Lenroot, 2012; Zaidi, 2010); indicating a link between brain structure and function, cognitive sex differences, learning and memory. Consequently, if it is argued that learning and memory are linked to the functionality of the hippocampus and amygdala then it can also be argued that the observed cognitive sex differences in learning and memory may also be linked to the hippocampus and amygdala, since these two areas are significantly differentiated by sex (Zaidi, 2010) and are also linked to cognition (Gold, 2003). Although sex differences in learning and memory are reported in developmental (Ozcaliskan & Goldin-Meadow, 2010) as well as cognitive performance (Adreano & Cahil, 2009) studies, there is no study or theory to date that has attempted to explore the connection between the effects that learning and memory might have on the development and appearance of cognitive sex differences. In line with the above rationale, sex differences in learning and memory that are linked to hippocampus and amygdala will be the main focus of this thesis, attempting to address both theoretically and experimentally how these two brain areas may contribute to the appearance of cognitive sex differences. Thus, in the following sections, the effects of sex on the structure and function of hippocampus and amygdala will be explored. This is done in order to explore the basis of sexual differentiation on brain areas that are significantly related to the cognitive aspects of learning and memory. Then, the exact function of hippocampus and amygdala will be explored briefly by reviewing studies with rodents in an attempt to understand how the hippocampus-amygdala system works on simple cognitive systems. Next, studies that have indicated cognitive sex differences in humans will be analysed in an attempt to fit the observed function of the hippocampus-amygdala system in more complex

[2] This subject will be extensively explored in the following sections of this Chapter.

examples of cognitive performance. Having established how the hippocampus-amygdala system affects learning and memory, the development (in both neuro-typical and non neuro-typical populations) and the degeneration of the human memory system will be reviewed in an attempt to locate the theoretical links between hippocampus-amygdala function and learning and memory in human cognition. Finally, factors that may affect this sex differentiation will be explored in an attempt to understand the factors that limit / reduce cognitive sex differences and lead to the formation of an individual's cognitive phenotype; a cognitive phenotype that existing studies (Goldenfeld, Baron-Cohen, Wheelwright, 2005) have indicated surpasses the strict boundaries of sex.

To summarise, this review aims to identify potential links between cognitive, developmental and neuro-degenerative sex differences using research evidence as well as theoretical models from cognitive psychology, neuro-biology and endocrinology. The cognitive model presented here was used to explore the long-term effects that basic-level information processing ability[3] may have on brain structure and function. The explanatory capacity of the developed model was assessed via behavioural, endocrinological, psychological and neuro-functional methods in the empirical chapters of this thesis, allowing some predictions regarding the biological underpinnings of cognition and consequently cognitive sex differences to be made. The cognitive model assessed within this thesis is based around the idea that basic-level information processing ability significantly affects the structure of the memory system, while the memory system in conjunction with basic-level information processing ability determines cognition. All these elements are hypothesized to be responsible for observed differences in neuro-developmental

[3] Basic information processing ability refers to the rate by which information (either visuo-spatial or language-based) enters the memory system (that is, learning and memory formation at a basic level). The exact properties of this function will be analyzed in the following sections.

and neurodegenerative diseases as well as inconsistencies in the effectiveness of specific therapies (such as hormone replacement therapies and Acetylcholine-related drugs). Moreover, it is argued that the relationship between basic-level information processing ability and memory structure is a key factor affecting an individual's cognitive phenotype. In the following section the basic elements that lead to the formation of the central theoretical model will be presented, starting from the main factors that differentiate the brain.

1.2 The effects of sexual hormones on the brain

One way to start investigating the neural underpinnings of cognitive sex differences is to look at specific features of brain formations that appear to be sexually differentiated[4]. This is a practice that has been adopted by several researchers and has provided valuable information regarding brain's sexual differentiation; albeit not always directly relatable to cognitive performance. Thus, the focus of this thesis is on brain areas that appear to be affected structurally and functionally by gonadal hormones (pre-natal and post-natal) and their relationship with specific aspects of cognition. To begin with, structural sex differences in the human brain are well-established with respect to size, connectivity between different areas of the brain, neuronal innervations and volume of specific areas including hippocampus (which is larger in women) and the amygdala (which is larger in men; for a review see Zaidi, 2010; Giedd et al., 2012). The main factors responsible for these structural differentiations are considered to be sexual (gonadal) hormones, which initiate this differentiation prenatally and continue to affect the brain post-natally (Zaidi, 2010).

[4] According to Ropers and Hamel (2005) sex is considered as the major differentiating factor in biology.

1.2.1. Sexual (gonadal) hormone effects on brain structure

1.2.1.1 Sexual (gonadal) hormones and feminization-defeminisation of the brain

Maternal circulating oestrogen (MCE) during pregnancy has the most significant role in the initiation of the de-feminization or feminization in the brain of the foetus, at least in rodents (Bakker, Mees, Douhard, Balthazart, Gabbant, Szpirer, & Szpirer, 2006; Mees, Bakker, Szpirer & Szipirer, 2006; Gabant, Forrester, Nichols, Van-Reeth, Mees, Pajack, Watt, Smitz, Alexandre, Szpirer, & Szpirer, 2002). This role is controlled by alpha-fetoprotein (AFP), which reacts with MCE and stops the de-feminization of the brain. The exact method by which this reaction is performed has not yet been established; however there are two prevailing theories. The first theory suggests that AFP binds with MCE and prevents it from passing through the blood brain barrier (BBB), and consequently this action prevents the de-feminization of the brain (Bakker et al., 2006). The other theory states that instead of completely blocking MCE from passing through BBB, AFP actually directs it towards specific brain areas (Bakker et al., 2006). Although there is relatively little extended research regarding the function of AFP in humans, there are enough indications of a role for AFP in rodents (Vakharia & Mizejewski, 2000; Mizejewski, 2004; Mizejewski, Smith, Butterstein, 2004) that do not allow us to reject the possibility of a similar function in humans.

1.2.1.2 Sexual hormones and masculinization of the brain

A significant factor that is related to brain masculinisation is foetally-produced sexual hormones; which masculinization is initiated from pre-natally and is continued during post-natal development (Rosselli, Liu & Hurn, 2009). More specifically, testosterone produced by the foetus passes through the blood brain barrier and begins the masculinisation of the brain through

the process of aromatization, where testosterone is turned into estradiol (Rosselli et al., 2009). This process appears to continue during post-natal development, while aromatization capacity appears to be linked to age, sex and physiological status (Rosselli et al., 2009). Aromatization does not take place in every brain area indiscriminately; rather, it is location-specific (Rosselli et al., 2009). The activity of aromatization is mainly observed in the hypothalamus and amygdala. To a lesser degree parts of the hippocampus (hippocampal dentate gyrus granule cells) appear to be able to aromatise testosterone to estradiol, while this function is linked to the regulation of hippocampal synaptic plasticity (Kretz, Fester, Wehrenberg, Zhou, Brauckmann, Zhao, Prange-Kiel, Naumann, Jarry, Frotscher, Rune, 2004).

Consequently, since the male foetus produces more testosterone than the female foetus, brain masculinisation is enhanced in males compared to females (Lutchmaya, Baron-Cohen, Raggatt, Knickmaeyer & Manning, 2004). With regards to foetus-produced oestrogen, research indicates that there are significant differences in the effects of self-produced oestrogen in each sex (i.e. self-produced oestrogen affects male and female nervous systems differently). Furthermore, this differentiation occurs as a result of sex differences in brain structure that occurred before the sexual hormones were produced by the foetus (for a review see Gillies & McArthur, 2010). Thus, it would be useful to examine in more depth the exact effects of gonadal hormones on brain structure.

Several researchers have examined the structural effects that gonadal hormones have on the brain, focusing on either the effects of pre-natal hormones or post-natal hormones on post-natal brain structure. Chura, Lombardo, Ashwin, Auyeung, Chakrabarti, Bullmore and Baron-Cohen (2010) were the first to examine a potential link between pre-natal testosterone levels and brain structure in humans. Specifically, Chura et al. (2010), using high-resolution structural magnetic

resonance images (MRI) of the brain and foetal testosterone levels obtained via amniocentesis during the second trimester of pregnancy, reported a significant positive relationship between foetal testosterone and asymmetry of the corpus callosum. That is, higher levels of foetal testosterone were related to a greater rightward isthmus[5] asymmetry. This finding enabled a link to be made between a series of existing studies that explored corpus callosum asymmetry and foetal testosterone in animals (Phoenix, Goy, Gerall, Young, 1959; Arnold & Breedlove, 1985) with humans; supporting an 'organizational' role of foetal testosterone on the brain.

These findings were taken a step further by Lombardo, Ashwin, Auyeung, Chakrabarti, Taylor, Hackett, Bullmore, Baron-Cohen (2012), who examined the effects of pre-natal testosterone on grey matter volume. Specifically, Lombardo et al. (2012) examined the relationship between testosterone levels, collected via amniocentesis during the third trimester of pregnancy, and grey matter volume of pre-adolescent males via MRI. Their results indicated a positive predictive relationship between foetal testosterone levels and the volume of grey matter in the left ventromedial amygdala, bilateral somatosensory areas, motor cortex, pre-motor cortex and right temporo-parietal junction / posterior superior temporal sulcus. On the other hand, they also reported a negative predictive relationship between foetal testosterone levels and grey matter volumes on bilateral sylvian fissures. In the second part of their study, Lombardo et al., (2012) related their findings to a larger set of existing MRI data and reported the existence of sexual dimorphism in the amygdala and hypothalamus, with males having greater volumes in both structures. Interestingly, the part of the amygdala which was found to be sexually dimorphic was not the same as the part that was predicted by foetal testosterone. Based on these results, the

[5] Posterior subsection of corpus callosum

potential importance of current (circulating) testosterone levels in the development of sexually dimorphic brain structures was highlighted.

The effects of circulating testosterone levels as well as circulating oestrogen levels on brain volume was explored by Neufang, Specht, Hausmann, Gunturkun, Herpertz-Dahlmann, Fink and Konrad (2009). In this study, Neufang et al. (2009) indicated the existence of sexual dimorphism in grey matter volume in the left amygdala, striatum and hippocampus. Specifically, larger grey matter volume in the left amygdala was observed in males, whereas larger grey matter volume was observed in the striatum and hippocampus for females. Moreover, a significant positive relationship between circulating testosterone and grey matter volume on the amygdala was indicated, while oestrogen levels were related to higher para-hippocampal grey matter volume. On the other hand, hippocampal grey matter volume was negatively correlated with circulating testosterone levels; this effect was found to be less observable in females.

The existence of a difference between males and females on the effect size of circulating testosterone and estradiol levels on brain volume was further supported by Peper, van de Heuvel, Mandl, Hulshoff Pol, van Honk (2011) in their review of neuroimaging studies. That is, estradiol levels were found to be negatively related to brain volume of thalamus, hypothalamus, amygdala and hippocampus in females, while in males there was a positive association between testosterone levels and brain volume of the same areas. Peper et al. (2011) concluded that the association between circulating hormones and medial temporal structures (such as hippocampus and amygdala) are closely related to an individual's sex.

The main reason underlying the relationship between sex and the effects of gonadal hormones on brain volume was argued to be the effects of sex chromosome differences, which appear to affect

at least a part of the apparent relationship (Peper et al., 2011). Existing studies have stressed the importance of genetic configuration on brain structure, indicating a close relationship between sex chromosome gene dosage and gonadal hormones (Lenroot, Lee, Giedd, 2009). More specifically, according to Carrel and Willard (2005), there is a significant difference in the quantity of genes carried by the X chromosome (that carries substantially more genes) compared to the Y chromosome. This difference in gene quantity in relation to brain volume and to gonadal hormones was reviewed by Lenroot et al. (2009). Lenroot et al. (2009) reviewed studies that explored brain volume of individuals with a genetic condition named chromosome aneuploidies. This condition is characterized by a variation in the quantity of sex chromosomes (either due to extra X chromosomes or Y chromosomes or both). Lenroot et al. (2009) in their review concluded that there is a notable association between the X chromosome and brain volume; that is, X chromosomes appears to be negatively related to brain volume (with an enhanced effect on temporal lobe areas). In addition, it was reported that testosterone supplementation appears to have a positive effect on brain volume of individuals with an additional X chromosome (i.e. XXY instead of XY). That is, in individuals with XXY chromosomes, who have by default smaller brain volumes, testosterone supplementation reduced volumetric differences compared to controls (i.e. testosterone increased brain volume). It is noteworthy that amygdala volume was found to be significantly lower in XXY males compared to XY males, whereas hippocampal areas were not found to be affected significantly. Thus, since sex is closely related to XX / XY chromosomal differences, and X / Y chromosomes appear to be associated with brain volume and gonadal hormones, then it can be argued that the relationship between sex and the effects of gonadal hormones on brain volume appears due to sex chromosome differences.

In summary, sex differences in brain volume as seen from MRI studies are mostly observed in the caudate nucleus, cerebellum, amygdala and hippocampus (for a review see Giedd et al., 2012). Moreover, MRI studies indicate that the hippocampus and amygdala are two brain structures whose structure and function is significantly affected by gonadal hormones (both pre- and post-natally; for a review see Heany, van Honk, Stein, Brooks, 2016). Thus, hippocampus and amygdala volume is significantly affected by both chromosomal and hormonal differences; establishing these two brain structures as two of the most sex-affected brain areas.

The reported studies support the hypothesis for both 'organizational' and 'activational' effects of gonadal hormones on the brain, with sex (due to its relationship to X and Y chromosomes as well as with its relationship with gonadal hormone production and effects) having a catalytic role on these effects. Thus, it can be argued that there are three factors that are significant determinants of brain organization; these are X and Y related genes, pre-natal gonadal hormones and post-natal gonadal hormones. All these factors appear to co-affect each other while the most significant effect[6] of their interaction is located on hippocampus and amygdala. Consequently it can be argued that sex is a valuable dissociating factor in terms of brain structure and function only because it incorporates general information regarding an individual's chromosomal profile (XX or XY), pre-natal masculinization / feminization (due to AFP as well as foetal produced hormones) and post-natal hormones (post-natal hormone production).

1.2.1.3 Gonadal hormone effects on neuronal function

Besides from causing structural differences, sex hormones are also responsible for alterations in the function of neuronal cells, which consequently affect their developmental course and are

[6] An effect that is closely related to cognition

strongly related to cognitive development (Zaidi, 2010). For example, the neurotransmitter acetylcholine (ACh) is linked to memory formation and function through its relationship to hippocampal and amygdala function (Gold, 2004). Interestingly, testosterone is the main cause of high levels of 'insulin-like Growth factor-I' (IGF-I), which is responsible for reducing the levels of ACh deriving specifically from the hippocampus, without affecting ACh deriving from other areas including the amygdala (Cherrier, Plymate, Mohan, Asthana, Matsumoto, Bremner, Peskind, Raskind, Latendresse, Haley & Craft, 2004; Seto, Zheng, McNicoll, Collier, Quirion & Kar, 2002). Since male testosterone levels are higher than those found in females then the functionality of the hippocampus in males is potentially impaired compared to females, by implication.

In conclusion, the hippocampus and amygdala are closely related to cognitive function (Hummond, Tull & Stackman, 2004; Schroeder & Packard, 2004; Gold, 2003; Kosaki & Watanabe, 2012) and their differentiation due to sexual hormones is both structural (Zaidi, 2010; Giedd et al., 2012) and functional (Cherrier et al., 2004; Heany et al., 2016). This can be used as the basis for exploring sex differences in cognition, by investigating the exact role of the hippocampus and amygdala in cognitive functions and their relationship to memory formation and function. They are part of a cognitive system that is closely related to memory and learning, while this system is significantly affected by cholinergic modulation (Gold, 2003). Thus, at this point it would be useful to examine how cholinergic transmission affects the functionality of these two brain structures.

1.3 Hippocampus, amygdala and acetylcholine function

1.3.1. Studies exploring acetylcholine function in rodents

Studies with rodents[7], where the hippocampus and / or amygdala were either neutralized or completely removed, have indicated effects on different aspects of memory function (Chan, Morell, Jarrard & Davinson, 2001; Micheau & Marighetto, 2010; Kosaki & Watanabe, 2012). For example, Pych, Chang, Colon-Rivera, Haag and Gold (2005) performed an experiment in order to measure acetylcholine (ACh) efflux by the hippocampus during a response-type four-arm maze task. Pych et al. (2005) implanted into each rat a microdialysis guide cannula in order to obtain direct measurements of ACh levels before and after each trial under two conditions; a 'cue-rich' and a 'cue-poor' condition.

Pych et al. (2005) reported that hippocampal ACh efflux levels were positively correlated with successful and rapid learning only in the cue-rich condition. In contrast, the results from the cue-poor condition revealed that ACh levels were negatively correlated with successful and rapid learning. Therefore, these results indicate that hippocampal ACh facilitates memory formation through enabling associations to be made between the available sensory-input. However, in a situation where the available sensory-inputs are limited (cue-poor condition), then high levels of hippocampal ACh impede memory formation, since 'associational' learning relies in the presence of multiple cues (for a review of similar studies see Micheau & Marighetto, 2011).

These findings were further supported and expanded by Oliveira, Hawk, Abel and Havekes (2010). Results from an 'object-place recognition task' indicated a significant impairment in

[7]Rodents and humans sustain similar cognitive deficits (or performance alterations) when specific areas of their brain, also common between the two species are compromised (McIntyre, Pal, Marriott & Gold, 2002). Consequently, studying rodent-brain, at least regarding its very basic functions that resemble human brain's functions, can constitute a base for the understanding of the higher levels of human cognition.

performance of mice with blocked hippocampal function (referred to as 'hippocampal mice') compared to controls, thus supporting Pych et al.'s (2005) findings that the hippocampus facilitates learning through enabling the creation of associations between sensory inputs. The results from a 'novel object recognition' task, where mice have to 'remember' which object they have seen before and consequently spend significantly less time exploring it, indicated superior performance in 'hippocampal' mice compared to controls. However, this superiority disappeared when the task was performed after familiarization with the experimental environment (Oliveira et al., 2010) suggesting that memory formation by creating relational associations between sensory-inputs competes with memory formation that relies on the strength of a specific input's properties.

A different function can be attributed to the hippocampus from research conducted by Flaherty, Coppotelly, Hsu and Otto (1998), where two groups of mice were trained to run fast in a straight line in order to reach food. The results indicated that mice with an intact hippocampus significantly reduced their running-speed in the second phase of the experiment where the food quantity was significantly lower than it was in the first phase of the experiment. On the other hand, mice with a neutralized hippocampus did not show any significant change in their running speed during the second phase of the experiment (Flaherty et al., 1998). In other words, 'hippocampal' mice were unable to refrain from executing the learnt procedure. Therefore, it can be suggested that the hippocampus is involved in functions where a form of re-learning is required i.e. relating new 'memories' to an existing memory base and adjusting it accordingly (for a review of similar studies see Chan et al., 2001).

An experiment of consummatory behaviour was designed by Gilbert and Kesner (2002) in order to specify the exact roles of the hippocampus and amygdala in this experimental situation. Three

groups of mice were used; one with hippocampus excision, another with amygdala excision and a third group, which was used as control. Gilbert and Kesner's (2002) results indicated that the group that had undergone an amygdala excision showed a deficiency in memorizing the difference between solutions and consequently presented reduced consumption compared to 'hippocampal' and control groups. The importance of this experiment lies in the finding that 'amygdala' mice were significantly impaired compared to control and 'hippocampal' mice in distinguishing between two relatively similar sensory-inputs. Thus, amygdala function enhances a cognitive system's ability to distinguish between the 'intensity' of similar sensory inputs; while lack of amygdala function leads to recognizing both inputs as equivalent.

The effect of amygdala function on the intensity of a sensory input is also observed in the study of Holland, Han and Gallagher (2000), which tested amygdala-lesioned mice with a much simpler stimulus-input using a multiple-choice reaction time task (MRCT). In a MCRT, mice are trained to choose among three to five ports and push the one that is illuminated by their muzzle. In Holland et al.'s (2000) experiment, the target-port was illuminated for 0.5 seconds in the first phase and for 0.25 seconds in the second phase. The results indicated that in the first phase of the experiment, the amygdala-lesioned mice were impaired in learning the experimental procedure, and only after learning had taken place were performance differences with control mice equilibrated. However, in the second phase, lesioned mice were significantly impaired compared to the control group in both learning the experimental procedure and performance after learning had taken place. Therefore, based on the studies of Holland et al. (2000) and Gilbert and Kesner (2002), it can be suggested that the amygdala works as a 'stimulus-enhancer', which facilitates the preservation of a sensory-input to enable it to be integrated into memory (for a review see Jacobs, Renken, Aleman & Cornelissen, 2012).

An intact amygdala appears to be necessary for good performance on specific tasks while intact hippocampal formations are not (McIntyre et al., 2002). For example, a conditioned place preference task (CPP) is a task where rodents have to decide between two different locations based on the food reward that is placed in one of them, and is considered to be an amygdala-dependent task (McIntyre et al., 2002). McIntyre et al. (2002) utilized this task and measured ACh efflux from hippocampal areas during task performance on rodents. Moreover, a hippocampus-dependent task was also employed (a spontaneous alteration task) in order to examine the relationship between hippocampal activation and task performance. McIntyre et al.'s (2002) findings indicated a significant negative correlation between hippocampal ACh and CPP performance; suggesting the existence of an antagonistic relationship between the amygdala and hippocampus when it comes to cognitive performance on an amygdala-dependent task. Regarding the hippocampal-dependent task, although ACh levels increased significantly during testing they did not appear to correlate with task performance (McIntyre et al., 2002). McIntyre, Marriot and Gold (2003) in a follow-up study, measured ACh amygdala efflux during a hippocampal-dependent task (spontaneous alteration task). Interestingly, they found a positive correlation between amygdala ACh efflux and performance on a hippocampal-dependent task, suggesting the existence of an synergistic relationship between the hippocampus and amygdala when it comes to cognitive performance on an hippocampus-dependent task (McIntyre et al., 2003; for a review on this issue see Gold, 2004).

In summary, the amygdala appears to work as a stimulus enhancer; and as such amygdala functionality is critical in experiments where novelty is a significant factor[8]. On the other hand,

[8] Because incorporation of novel stimuli in the memory system depend on 1. repetition of the input 2. effect size of the input on the engaged neural structures (Bailey, Giustetto, Huang, Hawkins & Kandel, 2000; see also Section 1.5.2). In these experiments repetition of the process is not possible thus the difference is located on the effect that

the hippocampus appears to facilitate associations between available stimuli; and consequently its functional integrity is vital when it comes to tasks where multiple cues are available and an association between these cues is beneficial. Moreover, hippocampal inputs appear to have priority over amygdala inputs (blocking amygdala inputs); however this relationship is not reciprocal. On the contrary amygdala inputs appear to enhance hippocampal inputs, optimizing hippocampal-based cognitive performance.

1.3.2. Studies exploring acetylcholine function in humans

The main effect of acetylcholine in human cognition appears to be mainly on information encoding (Atri, Sherman, Norman, Kirchhoff, Nicolas, Greicius, Cramer, Breiter, Hasselmo, Stern, 2004). The role of acetylcholine on human cognition is usually explored through studies that use drugs to enhance or avert the action of acetylcholine (a review on this subject for human and non-human studies in Hasselmo, 2006).

Scopolamine and methylamine are two types of drugs that bind on the muscarinic and nicotinic receptors respectively and, in doing so, prevent acetylcholine from binding on them, which consequently affects the synapse. Interestingly, the effects of scopolamine are focussed on impairing performance on tasks such as verbal learning but leave intact performance of tasks such as digit span, suggesting a link between acetylcholine availability and associative learning (Atri et al., 2004). For example, Voss Thienel, Reske, Habel and Kircher (2010) manipulated the effects of scopolamine and methylamine on cognitive ability and argued that the only affected aspects of cognition were the ability to recall previously given words in any order (free recall) and also the ability to recognize given words. As it can be seen from Voss et al.'s (2010)

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the sensory input has on the engaged neural structures and subsequently this process is left to be linked to amygdala function.

experiment, the lack of acetylcholine prevents the brain from temporarily holding novel information (i.e. free recall) long enough to trigger existing memory patterns (i.e. recognition). It is noteworthy that Moscovitch and Winocur (1992; see also see Davachi & Wagner, 2002) argued that free recall is a cognitive task that is directly linked to hippocampal function. Specifically, Moscovitch and Winocur (1992) reported that hippocampal damage in humans leads to a severe impairment on free recall performance; while other memory-related functions are spared (such as digit span performance). So, it can be concluded that scopolamine and subsequently acetylcholine affect hippocampal and para-hippocampal areas; a conclusion that has also been drawn by other researchers (Atri et al., 2004).

Summarizing the findings of the above studies, the hippocampus seems to be closely related to learning by facilitating associations between the available sensory-inputs as well as relating and connecting new stimuli to the existing memory base; a function mostly applicable on conditions were multiple stimuli are available and helpful for a task (Chan et al., 2001; Oliveira et al., 2010). In contrast, the amygdala seems to facilitate learning by enhancing the properties of a specific sensory-input (Holland et al., 2000; Gilbert & Kesner, 2002). Bearing in mind the structural and functional sex differences between the hippocampus and the amygdala (Zaidi, 2010; Cherrier et al., 2004) and the specific functions of these two brain areas, a cognitive profile can be created for each sex. Therefore, based on Oliveira et al.'s (2010; Gold, 2004) argument that hippocampal function 'competes' with amygdala function, and as males have a smaller hippocampus (Zaidi, 2010) and their hippocampal ACh is reduced due to testosterone (Cherrier et al., 2004), it might be expected that due to reduced hippocampal-amygdala competence (compared to females), a 'male' cognitive system would be based on acuity and should be less able to collect and exploit multiple stimuli-inputs compared to a 'female' system. Conversely,

due to a larger hippocampus and more hippocampal ACh, a female cognitive system, (Zaidi, 2010; Cherrier et al., 2004) might be expected to rely less on acuity (due to stronger hippocampus-amygdala competence) and to be significantly more capable of collecting many stimuli-inputs, relating them together to form 'informational units', and consequently will function better in situations where multiple stimuli are present and beneficial for a given task.

Based on research that has addressed hormonal effects on brain structure and function, it is argued that the hippocampus and amygdala are two key-structures in sexually differentiated cognitive function (see Section 1.2). Moreover, according to experimental studies that manipulated and explored the exact cognitive functions that are related to the hippocampus and amygdala it is suggested that these two brain structures affect two basic cognitive functions; acuity and memory formation[9]. Interestingly, acuity and memory formation appear to be two cognitive abilities that are sexually differentiated in humans also, suggesting a similar relationship between hippocampus and amygdala function and acuity and memory formation in humans[10]. Thus, the exploration of cognitive sex differences on tasks that relate to acuity and / or memory formation is important for supporting and understanding the hypothesis regarding the hippocampus-amygdala system and its effects on the development of the memory system.

[9] These two cognitive functions are significantly interrelated; however differences in the balance of functionality between hippocampus and amygdala is accused for creating phenotypic differences in acuity and memory formation.
[10] It is not implied that these two categories are neurologically or cognitively separate or that they depend on cognitive mechanisms that do not affect each other.

1.4 Cognitive sex differences in humans

1.4.1 Sex differences in acuity

There have been a number of studies that have examined sex differences in cognition. Studies that explored sex differences using the Embedded Figures Task indicated a male superiority in the ability to locate a specific target in a complex background (Elliot, 1961 cited in Baron-Cohen, 2002). In the same line, Voyer et al. (1995 cited in Baron-Cohen, 2002) argued for a male advantage on detecting features of a target that is either moving or fixed. Additionally, Kimura (1996) argued that a female disadvantage in aiming accuracy it is present from the early years of a child's life. Consequently, there is an observable male advantage in tasks where 'acuity' is a significant performance-determining factor.

These very early studies provided a basis for studies like Abramov, Gordon, Feldman and Chavara (2012), who investigated sex differences in simple visual functions. Abramov et al.'s (2012) findings indicated a significant male-superiority on contrast sensitivity function (CSF) as well as on visual acuity. More specifically, Abramov et al. (2012) argued that sex differences in visual functions are based on the sensitivity of each sex to high and low spatial frequencies. Both sexes were found to be equally sensitive to low frequencies. However, males presented a significant superiority on their sensitivity for high spatial frequencies, which determines the ability to detect and discriminate the details of a target. In addition, males were found to be superior in detecting fast-moving stimuli compared to females. Interestingly, these results cannot be explained by any reported structural brain differences between the two sexes (Abramov et al., 2012). Therefore, the findings of Abramov et al. (2012) are in line with the above mentioned

animal studies (see Section1.3.1), which argue for a male dependence on acuity due to a structurally-caused lower function of hippocampal activity in relation to amygdala function.

Higher male acuity was also reported by Zundorf, Karnath and Lewald (2011) in an auditory spatial localization task which was created in order to explain the 'cocktail party' effect. In Zundorf et al.'s (2011) experiment, the task was to detect the target-sound and point towards its source manually and verbally under two conditions: without or with the presence of four distracter sounds. Each participant was initially familiarized with the five potential sounds and the potential locations of their sources under both conditions. As Zundorf et al. (2011) argued, male superiority was found only in the second condition, where participants had to distinguish the target sound from the distracters. In other words, in a multi-cue environment where the associations between available cues are not beneficial for task-performance, males present a significant advantage over females when it comes to recognizing single-stimulus properties and therefore being able to locate it in space. The significance of these results is even greater if we consider that familiarization weakens the competence of the hippocampus over the amygdala as Oliveira et al. (2010) has indicated. This type of experiment can be related to studies with rodents (see Section1.3.1), where similar types of cognitive functions were directly related to the hippocampus and amygdala, implying a functional relationship between amygdala function and acuity (regarding the sensory-related properties of a stimulus) and a consequent male-advantage in tasks that are benefited by this ability.

1.4.2 Sex differences in acuity

A relative female inability to ignore background information is demonstrated by studies that used tests such as *The Rod and Frame test*. In this type of test the participant is asked to judge the

orientation of a rod, which is placed on a frame and the frame's angle is changed. Females were found to be significantly affected by the angle of the frame (Witkin et al., 1962 cited in Baron-Cohen, 2002). The above finding supports the argument formed by animal-studies (see Section 1.3.1) regarding the existence of a higher female dependence on 'associational' memory formation and consequently a higher hippocampal function. This female dependence on 'associational' memory formation was later supported by Andersen, Dahmani, Konishi and Bohbot (2012), who managed to determine the degree of landmark exploitation by each sex during a virtual navigation task, through an eye-tracking measurement. According to Andersen et al.'s (2012) results, both sexes performed equally well under cue-rich conditions. However, as the available cues decreased, female performance also decreased leading to a male superiority effect (Andersen et al., 2012), which is more or less established in this type of task (Adreano & Cahil, 2009). Andersen et al. (2012) reported that this reduction in female performance was due to a high dependence on environmental cues, as indicated by the eye-tracking measurements. Additionally, males were found to decrease their eye-fixations to environmental cues as time passed, compared to females who did not indicate any reduction. In contrast, females' increased cue-fixations were related to the need for additional time to complete the task (Andersen et al., 2012); supporting a general female dependence on 'associational' learning, which is favoured in cue-rich conditions and is facilitated by hippocampal acetylcholine (Micheau & Marighetto, 2011). Previous studies that investigated sex differences, not only in virtual but also in 'live' navigation, reported similar findings, stressing the importance of time availability, number of repetitions (thus, raising exposure time to available stimuli) and available cues, as fundamental factors for the appearance of sex differences in this type of task (Piccardi, Bianchini, Iasevoli, Giannone & Guariglia, 2011; Picardi, Risetti, Nori, Tanzilli, Bernardi & Guariglia, 2010;

Piccardi, Iaria, Ricci, Bianchini, Zompanti & Guariglia, 2008). In other words, given more time or cues, sex differences are eliminated; pointing towards the cognitive mechanisms that control stimuli collection[11] as the main factors responsible for the observed sex differences.

The suggested female dependence on 'associational' learning can also be indicated through event-related potential (ERP) studies such as that reported by Feng, Zheng, Zhang, Song, Luo, Li and Talhelm (2011), where male and female brain voltage and activation amplitude was measured during a cued versus a non-cued condition task. According to Feng et al. (2011), females presented significantly higher brain activation when they processed related stimuli (cued condition), while their processing speed was also higher than that of males for this type of stimuli as indicated by their faster reaction times. Feng et al. (2011) suggests that these results are indicative of a larger recruitment of brain cells in females in tasks where related stimuli need to be processed, providing further support for the suggested cognitive profiles.

All the above studies were focused at a very basic functional level, such as visual resolution (Abramov et al., 2012), processing of a simple sound (Zundorf et al., 2011), collecting and exploiting landmarks (Andersen et al., 2012) and reaction to simple cues such as a single letter (Feng et al., 2011). These functions are very similar to functions that were discussed in Section 1.3, as related to the hippocampus and amygdala. Thus, it is argued that the hippocampus and amygdala affect cognitive function at a very basic information-processing level which produces sex differences in specific cognitive functions such as acuity and memory formation [12]based on associations. But are these functional differences accompanied by any neurobiological differentiation or do they just exist at a purely functional / performance level?

[11] This refers to the Hippocampus-amygdala system.
[12] It is reminded that these two cognitive functions are regarded as interdependent.

1.4.3 Neurobiological sex differences

Alarcón, Cservenka, Rudolph, Fair and Nagel (2015), using functional magnetic resonance imaging (fMRI), explored the existence of sex differences in functional connectivity between the amygdala and other areas of the brain. More specifically, Alarcón et al. (2015) measured functional connectivity during a resting state (i.e. participants being still, with eyes-open and fixating their gaze on a stable point) on a sample consisting of 51 females and 71 males with ages ranging from ten to sixteen years old. According to Alarcón et al.'s (2015) findings, there was an apparent difference between males and females in relation to the areas that showed functional coupling. In particular, the left superficial amygdala and ventromedial pre-frontal cortex were functionally related in males, whereas the right superficial amygdala and dorsomedial pre-frontal cortex showed functional coupling in females.

In addition, sex differences regarding the maturation course of the brain were evident. That is, functional coupling between the superficial amygdala and parieto-occipital cortex appeared to gradually reduce with age in males only, while in females that was not the case. However, functional coupling between the baso-lateral amygdala and parieto-occipital cortex appeared to reduce with age in females only, whereas males showed the exact opposite pattern. At this point it is useful to note that the baso-lateral amygdala is the specific part of amygdala nuclei that is related to learning and memory (Alarcón et al., 2015) and consequently the above result can be interpreted as a neurobiological support for a female advantage in learning and memory functions.

In summary, it appears that there are specific sex differences at a neuronal level between the two sexes. As indicated above (see Sections 1.4.1; 1.4.2), sex differences were located at a very basic

functional level and were associated with the hippocampus and amygdala. Consequently, the appearance of a neurobiological differentiation allows an argument to be made regarding a potential interconnection between these two sex-based differences (i.e. basic functional differences and neurobiological differences). In particular, it can be argued that sex differences at a basic functional level can potentially cause sex differences at a neurobiological level. Stated differently, sex differences in basic information processing ability may cause sex differences in neuronal connectivity.

But how does information processing in such a basic level can affect neuronal connectivity? Although that this is a question that has not yet been answered experimentally, it can be approached theoretically. However, in order to achieve that, it is essential first to explore how information is processed and stored at a neural level and then see if there are developmental extensions of this issue.

The following section will explore how information is processed at a neural level as well as the structural effects that information processing has on neural function and on the formation of memory networks. This will be done in order to provide some theoretical basis to explore the argument made in Section 1.3 regarding the potential existence of a causal relationship between information processing at a basic level and long-term differences in patterns of neuronal connectivity. That is, in order to understand how an individual's cognitive sex is formed, it is necessary to have a basic understanding of how information enters the memory system and how it is incorporated by the memory system at a neuronal level.

1.5 The human memory system

1.5.1 Synaptic memory formation

Modern neuroscience states that when an information-stimulus enters the brain, it affects certain neurons and the synapses between them (Bailey et al., 2000). In particular, if the information-stimulus affects, for example, two neurons and activates them closely in time, the weight of the synapse between them will be enhanced (Hebb, 1949 cited in Sandberg, 2003). Therefore, when the same or a similar stimulus activates one of these two neurons, the other neuron will probably be activated too (Bailey et al., 2000). Consequently, the recording or not of novel information depends on the repetition and the intensity of the same information (Bailey et al., 2000).

Through this procedure a network of memory patterns is created, such that when an external stimulation similar to a stored pattern is provided, this pattern is activated and memory retrieval occurs (Wang, Aihara & Fan, 2007). The repetition of an information-stimulus is therefore a key element of memory construction. The human brain can reproduce a novel stimulus by reactivating the neurons that this particular stimulus initially activated (Hebb, 1949 cited in Sandberg, 2003). This results in the co-activation of the closest parts of neuronal patterns that are similar to the initially affected neurons, thereby creating short memory triggers (Hebb, 1949 cited in Sandberg, 2003).

These memory triggers are actually neurons sensitized to the new information and can be easily activated again. This reactivation can initiate a chain reaction, which keeps activating the rest of the parts of the affected neuronal pattern until every part of the pattern is activated or the electric charge of the initial stimulus is depleted (Hebb, 1949 cited in Sandberg, 2003). The repetition of

this procedure in conjunction with the intensity of the initial stimulus, results in the consolidation of the new information with the existing memory patterns (Bailey et al., 2000).

During the process of incorporation of new information with an already existing memory pattern, changes are caused in the synaptic weights of the whole pattern, resulting in an alteration of the whole pattern in order to include the new information (Cuetos et al., 2010). Therefore, every time that new information is incorporated in a memory pattern, it activates and consequently strengthens the synaptic weights of every other previously connected piece of information in this pattern. Consequently, a hierarchical memory structure is created, where the previously recorded information is the most strongly connected and well preserved (Cuetos et al., 2010).

1.5.2 Memory system formation

The memory system develops either by relating novel information to pre-existing networks or by creating new networks (Besnard, Caboche & Laroche, 2012; McKenzie & Eichenbaum, 2011; Cuetos et al., 2010). According to Besnard et al. (2012), there are two mechanisms that dictate memory system formation; the first works by integrating novel information in an existing memory network, and the second works by creating new, independent networks. Besnard et al. (2012) argued that these mechanisms depend on two neuronal properties. The first property is the capability of a network to undertake only a finite number of readjustments before it 'locks' (i.e. becomes incapable of readjusting), where this 'locking' also occurs after a period of inactivity (Besnard et al., 2012). The second refers to a function whereby if a novel stimulus causes a neuronal activation that is similar to a stored network, then this network is reactivated and incorporates the new information according to the Hebbian rule (Hebb, 1949 cited in Bailey et al., 2000). If the novel information is not similar to an existing network, then a new independent

network is created (Besnard et al., 2012). Consequently a memory system is created; composed of either many or few different networks that include a high or low variety of information. Thus, it can be argued that there are two crucial factors that affect memory formation, which are novelty of the information and prior memory for similar information. Relating these studies to the hippocampus-amygdala hypothesis, it can be seen that a small alteration in the rate by which information is being collected and incorporated into the memory system (that is basic information processing ability) may cause significant variances in the structure of the memory system. In other words, differences in basic information processing ability can lead to differences in the configuration of the memory system.

But how much effect does basic information processing ability have on memory system structure? The case study of an agnosic patient named HJA, tested by Riddoch, Humphrey, Gannon, Blott and Jones (1999) provides a strong indication of a direct effect of basic information processing ability on brain structure. Riddoch et al. (1999) tested HJA's perception and recognition ability 16 years after his first testing. Apparently, HJA's perception ability remained the same as it was during his first testing (16 years ago; Riddoch et al., 1999). However, Riddoch et al. (1999) reported a significant improvement in HJA's ability to name objects that were presented visually to him. Riddoch et al. (1999) argued that this improvement was due to a re-formation of HJA's existing memories, done in accordance to his impaired visual abilities. Stated differently, HJA's impaired visual abilities manage to re-structure his existing memories and adapt top-down functions to accommodate his (limited) bottom-up activations. Further support for this argument comes from an observation of Riddoch et al. (1999) who indicated that HJA's detailed memory of objects that he knew on his first testing (16 years ago by Riddoch & Humphrey, 1987) was lost. Thus, it can be argued that there is an interactive

relationship between perception[13] and the memory system. That is, the memory system appears to be adjusted based on bottom-up activations. This argument supports the existence of a direct relationship between basic information processing and memory structure as described and argued for in the previous sections of this chapter.

In conclusion, it can be argued that basic information processing ability can both affect the formation of the memory network and alter existing memory structure and function. These conclusions add to the theoretical argument introduced in Section 1.3, regarding the potential long-term effects of basic information processing ability on neural connectivity and subsequently to an individual's cognitive profile. In particular, these studies indicate that a) altering basic information processing ability has a direct effect on encoding memories and b) both basic information processing ability and encoded memories affect cognitive performance, at least in visual recognition tasks. Thus, these studies support a bidirectional relationship between basic information processing ability and memory structure in regard to cognitive performance. Consequently, it can be argued that the formation of an individual's cognitive profile (cognitive sex) is closely linked to basic information processing ability; a function that it is closely related to the hippocampus-amygdala system.

1.5.3 The development of human memory system

In order to explore the argued relationship between basic information processing ability, memory structure, cognitive performance and cognitive sex, it is necessary to understand how the memory system is configured. Thus, this part of the thesis will discuss how the memory system develops. As explored in Section 1.4, the memory system develops by either adding novel

[13] In this thesis the word perception is associated to an individual's basic information processing ability.

information into pre-existing memory networks or by creating new memory networks, at least at a very basic neural-functioning level. Moreover, it was argued that the transition from bottom-up to top-down processing is essential for the proper function of the cognitive system; stressing the importance of pre-existing memories for the integrity of a cognitive system. But how exactly do existing memories assist the cognitive system when confronted with novel information? Garbarini and Adenzato (2004) highlighted the importance of motor development as a binding component of visuo-spatial codes. In particular, Garbarini and Adenzato (2004) argued that motor-memories act as a base on which visuo-spatial information is attached and stressed the importance of specific neurons that assist this process.

That is, Garbarini and Adenzato (2004; see also Rizzolatti & Arbib, 1998; Rizzolatti, Fogassi & Gallese, 2006) argued for the existence of two different neuron types one responsible for bottom-up activation (canonical neurons) and the other responsible for top-down activation (mirror neurons). Most importantly, Garbarini and Adenzato's (2004) findings indicate that the memory system appears to develop by adding on known / concrete information (such as motor memories), which is used as reference in order to easily relate to more abstract information, such as visuo-spatial stimuli.

1.6 Linguistic development

The use of simple / concrete information as a base in order to add-on more complex / abstract information is observed as the human memory system grows, and gradually engages with more complex information; this can be observed through the acquisition and development of language.

This observation adds to the significance of a potential relationship between basic information processing ability, memory structure and cognitive performance.

In particular, if the memory system is structured as described in Section 1.4, then that would mean that memory networks containing complex information are connected to networks containing simple information and vice-versa. Moreover and in line with the above rationale, tasks that utilize language possibly engage a large part of the memory system since language is on the top of the informational chain[14]. Thus, it is important to see how language develops throughout life span and most importantly to explore conditions that include language impairments and the cognitive impairments that accompany them.

1.6.1 Sign language

The transition from the use of known / concrete information as a reference point towards the use of abstract information is supported by the way that sign languages have developed. According to Gentilucci and Corballis (2006) sign language has been initially developed by 'pantomimes' of movements, gradually accompanied by gestures which corresponded with the whole to-be-described motion or object. Eventually, these gestures lost their precise motion-representational features and became more abstract, thereby forming an abstract reference system that binds novel information with the memory base, i.e. sign language (Gentilucci & Corballis, 2006). Thus, it can be seen that the development and evolution of sign languages follows the rule of neural memory formation; that is using concrete/simple memories as a base on which to attach on more

[14]Based on the theoretical background of memory structure the informational chain develops from motor memories > visual-spatial memories (attached on motor-memories) > language-based memories (attached to visual-spatial memories)

complex information. That way the memory system is constantly evolving, allowing the processing of more information at the same time[15].

1.6.2 Verbal language

The same developmental pattern can be seen in the transition from gestural coding to the linguistic coding of information (Iverson & Goldin-Meadow, 2005). Children are able to use gestures from the age of 9 months in order to indicate an action or an object (Iverson & Goldin-Meadow, 2005). Furthermore, in the study of Iverson and Goldin-Meadow (2005), who examined the relationship between gestures and language development in children, it was found that the first linguistic codes that a child acquires are words that existed previously in their repertoire as gestures. Interestingly, children gradually evolve their communicational skills from a gesture and speech combination (point + word) towards a speech and speech combination (word + word) (Iverson & Goldin-Meadow, 2005). Thus, gestural coding, which derives from motor-memory and visuo-spatial memory construction (Gentilucci & Corballis, 2006) is a necessary base on which the first linguistic codes are built (Iverson & Goldin-Meadow, 2005).

The gradual transition from motor to linguistic coding, which was indicated in pre-school children in the study of Iverson and Goldin-Meadow (2005) is also verified in older children and adults by the study of McKenzie, Bull and Cray (2003). According to McKenzie et al. (2003), six to seven-year-old children and adults are affected differently by phonological or visual disruption during a mathematical task. Specifically, McKenzie et al. (2003) argued that children, in comparison to adults, are more susceptible to visual disruption rather than phonological disruption and vice-versa. Additionally, McKenzie et al. (2003) argued that susceptibility to

[15] In the case of sign language information-processing capacity is enhanced as the signs evolved from strictly descriptive to abstract and consequently shorter.

phonological disruption gradually increases with age, supporting the aforementioned arguments for a gradual transition from visuo-motor coding towards linguistic coding.

In other words, for younger children whose memory systems are not yet fully evolved, visuo-spatial memory networks are the main networks responsible[16] for cognitive performance. Thus, when children use their cognitive system (in this case via a mathematical task) only a visuo-spatial distracter severely affects their processing capacity. On the other hand, for adults whose memory systems are fully evolved and mainly dictated by language-based memory networks, only phonological distraction has severe effects on their cognitive performance.

In conclusion, evidence presented in this section describes a gradual transition from motor-memory, to visuo-spatial memory and finally to language-based memory during development. Most importantly, it is shown that within each developmental stage the cognitive system is affected differently by distracters. This finding highlights that cognitive performance is related to memory structure, supporting the theoretical relationship between memory structure and cognitive performance.

As was argued in the previous sections, there seems to be a bi-directional relationship between basic information processing ability and memory structure; and this relationship appears to affect cognitive performance and also affect the formation of cognitive phenotype. Previous sections provided indications of the existence of this relationship through studies that explored normal development. Thus, it would be useful to examine whether the above relationship is evidenced in studies that explore conditions where the development is impaired due to neuro-developmental and neuro-degenerative issues.

[16] At this stage of development the memory system is mainly composed by visuo-spatial networks on which language-based information is attached on.

1.7 Disorders associated with the development and the degeneration of human memory system

1.7.1 Autism

Individuals with Autism present with an impaired development of their ability to communicate and an engagement in limited activities that are very repetitive (Baron-Cohen & Hammer, 1997). This symptomatology is evident after the child has completed the Piagetian 'sensory-motor' stage (thus has passed the second year of its life), where executive function and linguistic skills are compromised (Russell, Jarrold & Hood, 1999). Russell et al. (1999) argued that in children with autism executive function is not compromised in tasks where only visuo-spatial information was needed to be processed automatically, i.e. without having to articulate in their mind a novel rule.

Also, even in tasks where a linguistic response was needed but processing was based on visuo-spatial codes and no novel rules needed to be articulated, children with autism presented with normal executive function (Russell et al., 1999). According to Bishop and Norbury's (2005) experimental findings, executive function is compromised in tasks where a rule needs to be followed; that is, tasks where complex verbal information needs to be held in conscious memory and there is a congruent demand to process either visuo-spatial or verbal information. This is supported by Hughes, Russell and Robbins (1994), who argued that executive functioning in children that fall within the Autistic spectrum (also in Alzheimer's disease and in Specific Language Impairment) is gradually compromised as the information load to be processed is increased. Bishop and Norbury (2005) concluded that executive dysfunction in children that fall

within the Autistic spectrum is not related to any symptoms of autism but only with verbal ability and attention.

Although studies on autistic spectrum disorders are mainly focused on deficiencies related to the disorder, some studies have also addressed the advantages that are observed in specific tasks (for example Ring, Baron-Cohen, Wheelright, Williams, Brammer, Andrew & Bullmore, 1999). More specifically, individuals who fall within the Autistic spectrum show an advantage over neurotypical populations on cognitive tasks such as the embedded figure task (Ring et al., 1999), visual search tasks (Joseph, Brandon, Connolly, Wolfe & Horowitz, 2009), and the block design task (Shah & Frith, 1993). The common factor between all these tasks is the need for searching and processing of specific features / objects which are 'hidden' within others that serve as distracters. This advantage is referred as the 'local advantage phenomenon' and is has been attributed to either a superior ability to process specific / local features or to an inferior ability to process unspecific / global information of individuals within the Autistic spectrum (see Van de Helm, 2016). Van de Helm (2016) proposed that both of the above possibilities regarding the cause of the 'local advantage phenomenon' may indeed be plausible as well as related.

The relationship between 'local' and 'global' processing has been demonstrated indirectly through EEG studies (Peters, Taguet, Vega, Jeste, Fernandez, Tan, Nelson, Sahin, & Warfield, 2013). More specifically, Peters et al. (2013) compared functional connectivity through the EEG of individuals within the Autistic spectrum to neuro-typical individuals and found that they differed in their proportion of short-range functional connections in relation to long-range functional connections; with individuals who fall within the autistic spectrum having enhanced short-range functional connections and reduced long-range functional connections. Thus, it can be argued that the advanced 'local' processing that is observed in ASD, may be related to

enhanced short-range functional connections and reduced long-range functional connections (for a similar finding see Anderson, Nielsen, Froehlich, DuBray, Druzqal, Cariello, Cooperrider, Zielinski, Ravichandran, Fletcher, Alexander, Biqler, Lange & Lainhart, 2011; Barttfeld, Wicker, Cukier, Navarta, Lew & Sigman, 2011).

To summarise, the cognitive system of individuals with Autism appears to be negatively affected by the informational load of a task. In other words, basic information processing ability appears to be reduced as the informational load increases in individuals within the Autistic spectrum. Moreover, the development of memory networks containing motor-memories and visuo-spatial memories appears to be sufficient; since there are no cognitive performance deviations compared to neuro-typical individuals when only visuo-spatial information is being processed.

However, when language-based information is processed then individuals on the Autistic spectrum differentiate from neuro-typical individuals. Thus, it can be argued that the difference between individuals on the Autistic spectrum and neuro-typical populations is located on the development and functionality of language-based memory networks. In particular, following the rationale of this thesis, in a case where basic informational processing ability is compromised, then memory development would be delayed, followed by extremities in its structure. As described in Section 1.4.1 and 1.4.2, slow pace of collecting information (i.e. low basic information processing ability) results in networks being open in alterations for long time periods and consequently leading to the development of large networks. Thus, in case of Autism, it would be expected that the first two memory network types [17] (that is motor-memory and visuo-spatial memory[18] networks) to be enlarged. However, as motor-memory is created from the

[17] The memory system is built on the following order: motor-memory> visuo-spatial memory>language-based memory (see Chapter 5)
[18] Visuo-spatial memory records the position, the direction and the colour of an object (see Section 4.1.3)

movements that a person can do, by default the range of this memory is predetermined since the movements that a person can do are limited. Consequently, only visuo-spatial memory networks are expected to be enlarged. Enlarged or extended visuo-spatial memory means that a visuo-spatial input will cause an enhanced/high activation of the memory system (see Section 1.4). This 'enhanced' activation would be observed through an enhanced ability to process visuo-spatial information; which is the case for individuals who fall within the Autistic spectrum[19].

Finally, whatever the exact reasons for the Autistic phenotype might be, the facts are that the Autistic phenotype includes a deviation from neuro-typical phenotypes on basic information processing ability (either this results in impairment or an advantage) and this differentiation is accompanied by differences in neural connectivity. Thus, basic information processing ability appears to have a direct effect on brain structure, supporting the theoretical argument of this thesis.

According to Tomasi and Volkow (2011) sex differentiates the ratio of short / long range connectivity, with neuro-typical males having more short-range connections in relation to long-range connections compared to females in specific brain areas. Thus, the Autistic phenotype seems to be an extreme form of the male phenotype, at least at a neural-connectivity level. Consequently, at this point it would be useful to examine two theories that argue for a close relationship between Autism and sex. These theories are both supported by cognitive and biological evidence. However both of them present specific weaknesses that can be resolved by the theoretical base of this thesis that argues for a relationship between hippocampus and amygdala interaction and cognitive function.

[19] Refers to the 'local advantage phenomenon' (Van de Helm, 2016)

1.7.1.1 The extreme male brain theory of autism

Baron-Cohen and Hammer (1997) placed the autistic spectrum on the far side of a continuum of brain types that are determined by pre-natal masculinisation levels, arguing that Autism is a form of an extreme male brain. This theory was built on studies that indicated a consistent sex-advantage on specific cognitive tasks, such as verbal fluency tasks for females and mental rotation tasks for males, on which individuals within the autistic spectrum appeared to be even worse (on female-biased tasks) or better (on male-biased tasks) than the normal population (for a review see Baron-Cohen, 1999).

Demographic evidence supports the extreme-male brain theory claims for the existence of a sex-bias in Autism since the male to female ratio of individuals that fall within the autistic spectrum disorder is almost 9 (males) to 1 (female; Baron-Cohen & Hammer, 1997). It is noteworthy that this male prevalence is observed in a wide range of neurodevelopmental disorders including autism, intellectual disability, and attention deficit hyperactivity disorder (Jacquemont, Coe, Hersch, Duyzend, Krumm, Bergmann, Beckmann, Rosenfeld & Eichler, 2014). However, recent findings indicated a persistence of cognitive sex differences within the autistic spectrum (Baron-Cohen, Cassidy, Auyeung, Allison, Achoukhi, Robertson, Pohl & Lai, 2014), which according to Baron-Cohen and Hammer (1997) should not exist; suggesting a need to reconsider aspects of the theory.

1.7.1.2 The amygdala theory of autism

The amygdala theory of autism argues that abnormal amygdala size is the biological factor, responsible for the autistic cognitive phenotype and specifically accounting for disabilities in ‘social intelligence’ (Baron-Cohen, Ring, Bullmore, Wheelrght, Ashwin & Williams, 2000).

Although studies have indicated a significant relationship between amygdala size and disabilities in 'social intelligence' in males under five years old who are on the autistic spectrum, the lack of an equivalent relationship in females raises doubts regarding the exact role of the amygdala in Autism[20] (Schumann, Barnes, Lord & Courchesne, 2009; for a review see Lai, Lombardo, Auyeung, Chakrabarti & Baron-Cohen, 2015).

As it was discussed in Section 1.2 and 1.3, the hippocampus and amygdala affect two basic cognitive functions; that is acuity and memory formation. Moreover, hippocampal function appears to interfere with amygdala function while amygdala function appears to assist hippocampal function (see Section 1.2). Consequently, higher hippocampal function would impair amygdala function but this impairment would be relative to the difference between hippocampus and amygdala functionality. That is, an abnormally developed amygdala is expected to cause different level of impairment in males compared to females since male and female hippocampi differ by default (see Sections 1.2; 1.3).

This rationale provides a strong explanation for the inconsistencies between amygdala size and autistic severity between males and females indicated by Schumann et al. (2009) and provides support for the Amygdala theory of Autism as stated by Baron-Cohen et al. (2002). Consequently, if Autism affects only the amygdala, then the severity of Autistic symptomatology in males would normally be related to amygdala size, since the male cognitive system is by default dominated by amygdala function. On the other hand, in females hippocampal functionality is expected to be able to compensate for the amygdala over-growth and subsequently 'blur' the relationship between amygdala size and autistic severity.

[20] Amygdala appears to be enlarged in children within the Autistic spectrum while this difference is counterbalanced during adolescence and even reversed (Schumann et al., 2009). Here it is only stressed the importance of abnormal amygdala size in children in order to address the effects that amygdala volume may have on the development of the memory system and consequently to cognition.

Taken together with the amygdala theory of Autism, this rationale offers a reasonable explanation regarding the 'persistence of normative sex differences despite the clinical diagnosis of Autism' (p. 8, Baron-Cohen et al., 2014) which according to Baron-Cohen et al. (2014) seem not to fit with the extreme male theory of Autism. That is, if the extreme male-like cognitive phenotype that is observed in individuals within the Autistic spectrum is the result of abnormal amygdala function, then sex differences may reasonably appear even within the spectrum due to default hippocampal sex differences.

1.7.2 The degeneration of the human memory system

As argued in Sections 1.4 and 1.5, the cognitive system evolves hierarchically, proceeding from motor-memory towards visuo-spatial memory and finally language-based memory. This hierarchy appears to be consistent even in the degeneration of the memory system as indicated by studies that examine neurodegenerative conditions such as Alzheimer's and Parkinson's disease.

1.7.2.1 Alzheimer's disease

The degeneration of the memory system in the most common type of dementia (i.e. Alzheimer's disease; A.D.) is signified by gradual neuronal death, indicated by a gradual loss of brain tissue (for example see Cairns, Brannstom, Khan, Rossor & Lantos, 2003). This degeneration is followed by cognitive decline, which gradually develops from loss of complex and recently acquired abilities towards loss of simple and early-learned abilities (Rainville, Amieva, Lafont, Dartigues, Orgogozo & Fabrigoule, 2002). Similarly, the loss of linguistic ability in individuals with A.D. follows the same pattern; starting from losing access to recently acquired vocabulary, followed by loss of vocabulary that was acquired after the eighth year of the patient's life and

finally, in the progressed stages of the disease, there is a loss of vocabulary that was acquired before the sixth year of life (Cuetos et al., 2010).

Focusing on the way that linguistic loss progresses, it can be argued that there is a convergence with the aforementioned developmental milestones of language that were indicated by the studies of Iverson and Goldin-Meadow (2005) and McKenzie et al. (2003). Interestingly, linguistic loss follows the same pattern in individuals with A.D. who are multilingual, starting from the most recent, and progressing towards the earlier (i.e. language 3 to language 2 to language 1; Paradis, 2008). Additionally, recent studies have indicated that bilingualism can delay the onset of A.D. symptoms (Sweiser, Ware, Fischer, Craik & Bialistok, 2012). Sweiser et al. (2012) argued that bilingualism is the cause of an observed divergence between cognitive performance and expected brain atrophy in individuals with Alzheimer's disease. More specifically, bilingual individuals with A.D. presented with significantly greater brain atrophy compared to the brain atrophy that was expected based on their cognitive performance (Sweiser et al., 2012). Thus, the continuous transition from simple towards more complicated coding mechanisms increases the integrity of cognitive performance against decay. Further evidence for the importance of *transition* comes from the results of Costa, Calabria, Marne, Hernandez, Juncadella, Gascon-Bayarri, Leo, Ortiz-Gil, Ugas, Blesa and Rene's (2012) study, which indicated a concurrent loss of L1 and L2 in patients with A.D.. Interestingly their participants had acquired both languages concurrently (Costa et al., 2012); a detail that stresses the importance of *transition* from one coding mechanism to another and supports the notion of a hierarchically built memory system.

1.7.2.2 Parkinson's disease

The same pattern of degeneration in cognitive skills can be observed in patients with Parkinson's disease (PD), although following a different direction. More specifically, the degeneration of cognitive performance begins backwards, starting with motor symptoms (tremor, bradykinesia), and mild cognitive dysfunction (bradyphrenia) accompanied by linguistic deficits, which are concentrated on a loss of fluency in verb production (Holtgraves, McNamara, Cappaert & Durso, 2010). Since 'verbs' are action-words closely related to visuo-spatial codes (Ozcaliskan & Goldin-Meadow, 2010; Gentilucci & Corballis, 2006; Iverson & Goldin-Meadow, 2005) then it can be suggested that the degeneration proceeds from simple codes towards more complex codes. This is in line with the findings of Owen, Beksinka, James Leigh, Summers, Marsen, Quinn, Sahakian and Robbins (1993) who argued that in Parkinson's disease a growing impairment of visuo-spatial memory and recognition is observed. Further support comes from studies with bilingual P.D. patients, where it was indicated that that the deterioration of linguistic features is more prevalent in P.D. patients' first acquired language (L1), whereas P.D. patients' second language (L2) is preserved (Zanini, Tavano & Fabbro, 2010; Paradis, 2008). Thus, these studies support the argument of a specific hierarchy existing in memory deterioration and consequently in memory formation.

1.7.3 Neurobiological factors that may lead to neuro-degeneration

According to Francis et al. (2005) the cause of neuronal death is the process of *apoptosis* triggered by *beta-Amyloids*. *Apoptosis* mainly affects neurons containing the neurotransmitter *acetylcholine*, which has a fundamental role on neuronal functionality (Francis et al., 2005). A major consequence of neurodegenerative diseases is the loss of neuronal tissue, which reduces

the communication between neural networks (Cairns et al., 2003). However, when neurotransmission is enhanced by the use of ACh-regulating drugs, this ability is effectively balanced (Auld et al., 2002). Moreover, as AD progresses, the ability of the brain to relate novel information to existing memory- networks, as well as comparing them with other new or old ones (a function referred as *priming* according to Fleischman, 2007), becomes even more dependent on *cholinergic transmission* to the point where the neuronal loss is too expanded to be counterbalanced (Auld et al., 2002). In addition, Jang et al. (2010) indicated that the use of ACh-regulating drugs may have deleterious effects on memory neurons. That is, although these drugs improve cognitive performance by enhancing information-holding and *priming ability* (Auld et al., 2002), they also impede memory formation and consequently contribute to the development of AD (Jang et al., 2010). Based on the above it can be concluded that the ability to form new memories is linked to acetylcholine (which is related to hippocampus-amygdala system) and is a major factor of neuronal viability.

This is partially supported by reports indicating the importance of constant mental stimulation throughout a person's lifespan, introducing education as a possible protective factor for AD (Bornebroek & Breteler, 2004). Bearing in mind that every new piece of information that is incorporated in an existing memory pattern causes the reformation of the whole pattern (Deianna, Platt, Riedel, 2010), it can be argued that constant incorporation of new information manages to keep the existing neuronal networks active and consequently prevents them from dying. The importance of constant feedback in order for memory to be preserved was highlighted in Section 1.4.4 through the case of HJA. In particular, HJA appeared to have lost memories of objects that due to his condition could not re-activate. Thus, things that HJA could

not see ceased to exist; that is, memory networks containing this information gradually deteriorated.

In summary, it is argued the degradation of the memory system (indicated by the loss of neuronal tissue) can be counterbalanced by heightened functionality of the cholinergic system (up to a certain limit). Moreover, the importance of constant re-activation of the existing memory networks on memory system preservation was highlighted. Thus, cognitive function depends on both structure and function of the memory system since structural degradation can be counterbalanced by enhanced functionality, at least up to a certain limit. Finally, the importance of re-activation on memory system preservation relates to the central theoretical argument of this thesis, since re-activation is based on basic information processing ability (see Section1.4). Thus, problematic 'basic information processing ability' results in memory degradation (see also HJA case in Section 1.4.4), which supports the hypothesis of the current thesis regarding a relationship between basic information processing ability, memory structure and cognitive phenotype.

1.8 Sex differences on the development and degeneration of the human memory system

As it was indicated in Section 1.2, sexual (gonadal) hormones, both pre- and post- natal, affect the production of ACh from the hippocampus. In addition, in Section 1.3, it was argued that the hippocampus and amygdala belong to a system that is responsible for basic information processing ability, while the idea of a causal relationship between basic information processing ability and memory structure was introduced. Since gonadal hormones as well as X / Y linked

genes are the basic differentiating factors of the human brain (see Section1.2), then at this point it would be useful to see the available evidence regarding a sex-based differentiation of basic information processing ability. More specifically, a potential difference in the basic information processing ability by which each sex strengthens a specific 'memory network', leads to the formation of a large or small memory base (i.e. the sum of different memory networks) and consequently determines the ability of each sex to acquire new information. Thus, based on this hypothesis, a female advantage is expected for collecting new information and therefore females are expected to be able to 'learn' (acquire) more complex information faster than males.

1.8.1 Sex differences in the development of the human memory system

In support of this hypothesis is the appearance of such sex differences from a very early age, as indicated by the study of Nagy et al. (2007). According to this study, new-born females are significantly better imitators of hand movements than new-born males. This early female advantage is also followed by a female advantage in later communicative ability (Ozcaliskan & Goldin-Meadow, 2010). More specifically, Ozcaliskan and Goldin-Meadow (2010) argued that males develop speech that is based on gestures (gesture + speech) and due to the aforementioned hierarchical development of communication ability (Iverson & Goldin-Meadow, 2005) develop speech that is based on speech (speech + speech) significantly later than females. This study concludes a series of studies that have identified sex differences in the developmental course of language, where a female time-advantage was specifically verified on first-word and sentence production, whereas the same advantage was also spotted in the magnitude of female children's vocabulary and the diversity that female children present in their sentences (Ozcaliskan & Goldin-Meadow, 2010; Iverson & Goldin-Meadow, 2005). Thus, females develop their memory base faster and begin the development of their memory system significantly earlier than males.

The female advantage in the acquisition of new information is also visible during adulthood. There is a plethora of studies indicating a female advantage in verbal memory tasks; however, the exact causes of these differences are not specified (Kaushanskaya et al., 2011; Krueger & Salthouse, 2010; Adreano & Cahil, 2009). Krueger and Salthouse (2010) argued that the appearance of sex differences in verbal memory tasks is located only in the increased capability of females in acquiring new words while both sexes' ability to withhold acquired words is similar. Additionally, a study by Kaushanskaya et al. (2011) stressed the importance of familiarity as a causal factor for the female advantage in acquiring novel words. In further detail, Kaushanskaya et al. (2011) argued that sex differences in novel word acquisition diminish when the to-be-remembered novel words do not present any phonological familiarity with any known words. The findings of these studies support the notion that pre-existing knowledge influences the ability to 'acquire' novel stimuli more for females (Kaushanskaya et al., 2011), while 'acquisition' ability is the only ability that differentiates the two sexes (Krueger & Salthouse, 2010). Thus, female advantage in verbal memory fits the profile of an individual with an expanded linguistic memory base, which allows the acquisition of more novel words compared to males. Linking this argument back to the hippocampus-amygdala hypothesis, it can be seen that developmental studies provide support for a higher female basic information processing ability, which may eventually lead to a different memory structure (observed via an expanded linguistic memory base).

1.8.2 Sex differences in the degeneration of the human memory system

1.8.2.1 Alzheimer's disease

Interestingly all the above observations regarding sex differences in memory structure and function are also witnessed in cases of neuro-degeneration. The existence of sex differences in the appearance of neurodegenerative diseases is well documented, with Alzheimer's disease appearing in females more often than in males (Alzheimer's Association, 2014). However, there also seems to be a substantial sex difference in the progression speed of the disease. Spampinato et al. (2012)[21] revealed that cognitive decline progresses significantly faster in male A.D. patients compared to female A.D. patients. In particular, Spampinato et al. (2012) examined the progress of mild cognitive impairment to A.D. in 109 patients and observed that females present with higher grey matter loss much earlier than males. However, males appear to catch-up, pointing out that the development of the disease was significantly more severe. Additionally, a significant divergence was observed between females' cognitive impairment and expected brain atrophy; indicated by a greater brain atrophy in females, which was not congruent with their preserved cognitive level (Spampinato et al., 2012).

This sex difference in brain atrophy becomes even more interesting considering that it is concentrated on the hippocampus. Spampinato et al. (2012) reported that males suffered greater and more rapid loss of hippocampal volume than females. Furthermore, as was previously mentioned, cognitive decline in A.D. is accompanied by a decline in linguistic skills (Cuetos et al., 2010). Interestingly, Ripich, Petrill and Ziol (1995 cited in Ott & Cahn-Weiner, 2005) reported sex differences in the progress of language decline in A.D. patients, indicated by a faster

[21]This study was part of a large longitudinal study that followed healthy individuals and individuals with mild cognitive impairment and Alzheimer's disease over a period of five years called Alzheimer's Disease Neuroimaging Initiative.

linguistic loss in females specifically on the Boston Naming Test and on word recognition (for a wider task spectrum of higher female cognitive deterioration compared to male see Laws, Irvine & Gale, 2016).

Summarizing sex differences in Alzheimer's disease, females are more likely to develop A.D. and lose specific linguistic abilities faster; on the other hand, females are better able to preserve their cognitive integrity in more developed stages of A.D. which is related to a slower-developing atrophy of their hippocampus[22] (Spampinato et al., 2012; Ripich et al., 1995 cited in Ott & Cahn-Weiner, 2005). Thus, at this point it can be suggested that the argued higher functioning female hippocampus may be responsible for the observed progress-rate of the AD related hippocampal atrophy.

1.8.2.2 Parkinson's disease

Contrary to Alzheimer's disease, Parkinson's disease is most common among male populations with a ratio of male to female reaching almost 2:1 (Gillies, Pienaar, Vohra & Qamhawi, 2014). In the U.K. these numbers translate to 30.9 per 10,000 for males and 24.1 per 10,000 for females; that is 69,850 male individuals and 57,043 female individuals (Parkinson's U.K., 2009). Parkinson's disease appears a different age onset between sexes, where according to Haaxma, Bloem, Borm, Oyen, Leenders, Eshuis, Booij, Dluzen and Horstink (2007) males develop Parkinson's disease around 2.2 years earlier than females.

[22]This conclusion is challenged by a meta-analysis from Law et al. (2016). However, Law et al. (2016) did not included Spampinato et al. (2012) study although it is one of the few longitudinal studies on this field (using MRI) with 109 participants (with more males than females, avoiding that way effect-inflation as Law et al., 2016, p. 59 argued that occurs in most of the studies), record of pre- and post-disease cognitive data as well as 5-year observation of cognitive declination and brain atrophy.

At a neuronal level, post-mortem studies exploring *dopaminergic neurons* have indicated sex differences in the increase of gene expressions that are related to Parkinson's disease; that is, males appear to be prone to genetic mutations that may lead to Parkinson's disease (Gillies et al., 2014). Interestingly, the mechanisms that are used for the preservation of *dopaminergic neurons* appear to be differentiated by sex, leading to the argument that treatment should also be sex specific (Gillies et al., 2014).

According to Haaxma et al. (2007), the delay of Parkinson's onset in females occurs mostly due to a 'higher striatal dopamine transporter binding' that is observed in females compared to males; this difference is observed in normal populations and is not related to Parkinson's disease (Haaxma et al., 2007). Furthermore, Haaxma et al. (2007) argued that except for the disease's onset delay, the most common symptoms that females show (tremor), are symptoms that are usually observed on older individuals with Parkinson's disease. Interestingly, these types of symptoms are related to a slower developmental pace of the disease. Regarding cognitive symptoms that accompany Parkinson's disease, females appear to be less affected in tests of verbal fluency as well as recognition of facial emotions in comparison to males; however, females with Parkinson's disease show a reduction in visuo-spatial performance.

Summarizing sex differences in Parkinson's disease, males show greater risk for developing Parkinson's disease and lose abilities that are related to language faster than females. On the other hand, females show a resistance to Parkinson's disease development and this resistance may be attributed to differences in the dopaminergic system. Interestingly, Parkinson's disease has also been linked to the hippocampus based on findings that relate the function of dopaminergic neurons to synaptic plasticity and therefore memory (Calabresi, Castrioto, Filippo & Picconi, 2013). Thus it may be suggested that female resilience to Parkinson's development

might be related to hippocampal function and consequently to sex differences in memory structure.

In conclusion, it can be seen that the argued effects of sex differences on the development of the memory system that are also observable in the degeneration of the memory system. Luine (2014) was also driven to this conclusion, arguing that: 'An important aspect of the programming of sex differences during development is that these differences may contribute to the development and patterns of adult cognitive loss seen in some neurodegenerative and psychiatric diseases' (p.6, Luine, 2014). Thus, it can be argued that there are specific sex differences in the development and degeneration of the cognitive system and the link between these differences is basic information processing ability; an ability / function that it is argued to be linked to the hippocampus-amygdala system.

1.9 The 'cognitive sex' of the brain

Summarizing findings and theories on sex differences, females seem better able to exploit large amounts of available information (Section 1.8). Thus, applying Besnard et al.'s (2012; Section 1.5.1; 1.5.2) model to the above evidence, it can be argued that the female memory system may develop more rapidly than males, due to a relatively high functioning hippocampus. However, due to a finite number of neuronal alterations, each memory network would 'lock' relatively quickly and consequently this would prompt the creation of many separate networks in females. On the other hand, in males, a reduced ability to exploit available information would result in their memory systems developing more slowly, which would leave each network susceptible to

alterations. Consequently, this results in a memory system composed of relatively few different memory networks.

However, informational availability through the developmental stages of a person's life may differ from person to person. In addition, individual genetic differences, hormone levels and individual differences in the testosterone-aromatization process (McCarthy, 2009; McCarthy & Arnold, 2011; Section 1.2) may cause within-sex differentiations. As a result, the formation of a memory system within a 'female' range, or a memory system within a 'male' range, may not be sex-specific. More specifically, there are factors that affect the feminization (hardwiring the brain towards a 'female' range system) or de-feminization (towards 'male' range system) of the brain, as well as the masculinisation (towards a 'male' range system) or de-masculinisation (towards a 'female' range system) of the brain. In other words, there are factors that seem to set the potentials for certain developmental pathways. These factors potentially determine the cognitive phenotype of the human brain.

1.9.1 Biological indices of brain sex: From sex-biased tasks to cognitive profiles

Gonadal hormones appear to be the main factors that set the potentials for specific developmental pathways; however, the interaction of this hormonal set-up with the environment (in terms of informational input) appears to be the factor that forms / shapes the cognitive system (McCarthy & Arnold, 2011). The differences that emerge due to hormonal set-up and environmental interaction are quite evident since they appear to directly affect the structure of the neural system. More specifically, McCarthy and Arnold (2011) argued that, at a neural level, there is only one system type that is either weighted towards a female-like or a male-like configuration, while the weight is determined by pre-natal hormone set-up (organizational

hormone effects), post-natal hormonal effects (activational hormonal effects) and environmental input effects. In addition McCarthy and Arnold (2011) argued that sexual hormones directly affect a few, specific hormone-responsive neurons, as well as indirectly through other nearby neurons.

Based on McCarthy and Arnold's (2011) point of view, it can be argued that as we move away from brain cells that are directly affected by gonadal steroids, the differences between functional phenotypes diminish. In practice, this could mean that cognitive functions controlled by cells that are developed away from steroid-sensitive cells would produce a rather neutral cognitive phenotype (i.e. not sexually specific). Similarly, cognitive functions that are controlled by hormone-sensitive cells are expected to reflect a cognitive phenotype that is shaped by prominent sexual hormones. That is, in cases where high masculinisation and / or low feminization occurs we expect to see a male-like phenotype; while in cases where high feminization and / or low masculinisation occurs, a female-like phenotype is expected. Thus, in order to be able to define brain sex, it is critical to measure the deviation in functionality between brain cells that are directly (or indirectly) affected by gonadal hormones, and those that are unaffected.

A study that can be seen as an attempt to address McCarthy and Arnold's (2011) ideas regarding cognitive profile is the study of Satterthwaite, Wolf, Roalf, Ruparel, Erus, Vandekar, Gennatas, Elliott, Smith, Hakonarson, Verma, Davatzikos, Gur and Gur (2015). Satterthwaite et al. (2015) provided a link between specific cognitive profiles and functional connectivity profiles. In this study, Satterthwaite et al. (2015) defined each sex's cognitive profile via cognitive tasks that are generally acknowledged to be sex-biased (i.e. spatial tasks and non-verbal reasoning tasks). Moreover, they measured functional connectivity during a resting state and pointed out specific patterns of connectivity that were sex specific. That is, individual's cognitive sex (i.e. someone

better in male biased tasks and worse in female biased tasks has a 'male' cognitive sex) predicted individuals' patterns of connectivity and vice versa (Satterthwaite et al., 2015). Satterthwaite et al. (2015) identified a significant sex difference in the functional segregation of the brain, with males having significantly more functionally interconnected brains; concluding that these differences are stabilized at least from late childhood. Satterthwaite et al. (2015) argued that cognitive sex differences have a direct relation to neuronal functionality; focusing on 'androgen-dependent' in-uterus programming or early environmental influences as the main candidates for causing the apparent neuronal functional differentiation between sexes. This study was the first to indicate a relationship between cognitive phenotype and patterns of brain connectivity. That is, cognitive phenotype (cognitive sex) measured by sex-biased cognitive tasks was related to specific patterns of functional brain interconnectivity. Most importantly, the qualities of 'sex' as a factor were all projected on the factor 'cognitive profile / sex'; while the factor 'cognitive profile / sex' acted as a more accurate dissociative factor than the factor 'sex' alone. That is, cognitive sex appears to surpass the strict boundaries of sex and seems to be a better index of the underlying neural connectivity patterns. This finding links back to the conclusion drawn in Section 1.2, where it was argued that sex is a valuable dissociating factor in terms of brain structure and function only because it incorporates general information regarding an individual's chromosomal profile, pre-natal masculinization / feminization and post-natal hormone levels[23]. Consequently, it can be argued that an individual's chromosomal profile, pre-natal masculinization / feminization and post-natal hormones (combined) may be the link between an individual's cognitive profile and observed structural and functional individual differences.

[23] It is reminded that all three factors are interdependent (see Section 1.1.2)

1.9.2 Psychological indices of brain sex: Systemizing and empathizing

Approaching the issue of cognitive sex differences from a psychological perspective, some researchers have argued for the existence of sex-specific mental states that are related to sexual (gonadal) hormones (Baron-Cohen 2002; 2014; Goldenfeld et al., 2005; Auyeung, Baron-Cohen, Chapman, Knickmeyer, Taylor & Hacket, 2006). Specifically, they introduced *systemizing* and *empathizing* as two mental states that compete in the brain, and based on this competition, brain sex, and by extension cognitive style, is determined. *Systemizing* is described as the drive to understand, create and function based upon rule-governed systems, while *empathizing* is described as the drive to understand, predict, adjust and respond accurately to social situations.

Goldenfeld et al. (2005), as well as Baron-Cohen et al. (2014), used systemizing and empathizing to define an individual's brain sex type, resulting in a five-level-classification. This five-level-classification comprised: extreme type E (individuals with overdeveloped or normal empathizing and underdeveloped systemizing), type E (individuals with higher empathizing than systemizing), type B (individuals with balanced empathizing and systemizing), type S (individuals with higher systemizing than empathizing) , and extreme type S (individuals with overdeveloped or normal systemizing and underdeveloped empathizing). One major aspect of the empathizing-systemizing (E-S) theory is that there is a disproportional allocation of males and females on the type S category and type E category. Specifically, more males tend to be allocated to the type S category compared to females, while more females tend to be allocated to the type E category compared to males (Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003). Although the exact nature of the relationship between systemizing and empathizing is still unclear, particularly in relation to the biological basis of these two constructs (Wheelwright Baron-Cohen, Goldenfeld, Delaney, Fine, Smith, Weil & Wakabayashi, 2006), an indication

regarding the biological basis of E-S can be provided through the extreme male brain theory of Autism; since an extremely enhanced *systemizing* drive in combination with an extremely poor *empathizing* drive is related to individuals who fall within the Autistic spectrum (Baron-Cohen et al., 2014; Goldenfeld et al., 2005).

According to the extreme male brain theory of Autism, extreme pre-natal masculinisation is a major factor in the development of the Autistic spectrum condition while the autistic spectrum is placed on the far side of a continuum of brain types (Baron-Cohen & Hammer, 1997). The link between systemizing and pre-natal sexual hormones was explored by Auyeung et al. (2006), where a strong relationship between foetal testosterone and systemizing was evidenced in typically developing children. Additionally, Manning, Baron-Cohen, Wheelwright and Fink (2010), linked 2D:4D[24] with systemizing (negative correlation) and empathizing (positive correlation, albeit non-significant) in a large scale study, thus providing a connection between indirectly-measured pre-natal hormone effects and brain sex type. In addition, Lai, Lombardo, Chakrabarti, Ecker, Sadek, Wheelwright, Murphy, Suckling, Bullmore, MRC AIMS Consortium and Baron-Cohen (2012) indicated a link between systemizing and empathizing ratio with differences in neurobiological elements such as grey matter volume as well as areas of the brain that are related to the dopaminergic and the endocrine system using MRI. This study provided a basis for a potential connection between brain sex and specific neurobiological factors. The authors concluded that E-S brain sex type is related to cognitive systems related to reward processing and neuroendocrine control, which eventually affects each brain sex type's learning mode. In particular, it was suggested that type S learning mode may be based on probabilistic learning that is not socially mediated, while type E learning mode may be based on reward-

[24] 2D:4D (the ratio between the length of the second and fourth finger of the hand) is regarded as an indirect measure of pre-natal gonadal hormone effects. Its exact function is discussed extensively in Section1.9.3.

dependent learning that is socially mediated. In conclusion, it can be argued that brain sex type differences are found to be linked to learning and consequently memory; a function that through this thesis it is argued to be significantly related to the hippocampus-amygdala system.

Some indications regarding the relationship between the hippocampus-amygdala hypothesis and E-S type can be found in animal studies that have explored the interaction of the dopaminergic system with hippocampus and amygdala. The integration of a reward with contextual information is a function that the hippocampus is significantly engaged in (Abraham, Neve, Lattal, 2014). According to Abraham et al. (2014), associative learning is significantly affected by the meso-cortico-limbic pathway, which links dopaminergic neurons to the hippocampus and amygdala. Abraham et al. (2014) argued that a potential function of the hippocampus is to encode contextual information and affect dopamine levels of the meso-cortico-limbic pathway. Thus, Lai et al.'s (2012) finding that socially mediated reward-dependent learning is linked to type E characteristics may suggest a close relationship between type E brain sex and hippocampal activity.

On the other hand, amygdala is also engaged in associative learning; albeit in a different way (Abraham et al., 2014; Costa, Monte, Lucas, Murray, Averbeck, 2016). According to Costa et al. (2016) amygdala is engaged in associative learning as a mechanism of selection between provided stimuli. Specifically, amygdala engages in facilitating associative learning from positive stimulation. Within that context, the finding that non-socially mediated probabilistic-dependent learning is linked to type S profiles may suggest a close relationship between type S brain sex and amygdala activity.

In conclusion, the argument regarding brain sex type and learning modes via the dopaminergic system can be linked to the hippocampus-amygdala system and provide some indications regarding an underlying relationship between the hippocampus-amygdala system and brain sex. However, the above arguments are based on animal studies and consequently can be used only as indications regarding a potential relationship of the above-mentioned structures.

1.9.3 Second to forth digit ratio: A somatometric index of brain sex?

Zhengui and Cohn (2011) proposed that individual susceptibility to pre-natal hormonal influences can be indicated through the somatometric index of the second to fourth digit ratio (2D:4D ratio). In particular, Zhengui and Cohn (2011) argued that 2D:4D may serve as an index of the level of pre-natal exposure to androgens (see also Knickmeyer Woolson, Hamer, Konneker & Gilmor, 2011) and oestrogen.

Moreover, several studies (Honecopp, Bartholdt, Beier & Liebert, 2007; McIntyre & Alexander, 2011; Hampson & Sankar, 2012) have shown that the relationship between the second and the fourth finger length (2D:4D) in humans has non-significant or no direct connection with circulatory sexual hormones. Additionally, the relationship between the length of the second and the fourth finger seems to be stabilized by 12 weeks of gestation (Malas, Dogan, Evcil & Desdicioglu, 2006) even after intra-testicular testosterone reaches peak levels, at around the fifteenth week of gestation (Scott, Mason & Sharpe, 2009). Therefore, it can be argued that the relationship between the length of these fingers is a rather poor index of foetal-produced sexual hormones; an argument also raised by Hurd, Vaillancourt and Dinsdale (2011). Thus, the relationship between the lengths of these fingers can be better regarded as a relative index of the effects of maternal circulating sexual hormones on the foetal brain, and specifically as an index

of the level of feminization or de-feminization of the brain. However, as it was argued in Section 1.2.1, the effects of maternal circulating hormones and specifically maternal circulating oestrogen seem to be mediated by alpha-fetoprotein (AFP), which is significantly related to sex. Thus, in order for 2D:4D to be an accurate index of the level of feminization de-feminization of the brain, sex (or AFP function if possible) must also be taken into account.

As previously argued, the development of an individual's cognitive phenotype depends on the interaction between three factors; individual genetic profile, pre-natal hormones (including AFP action) and post-natal hormones. Thus, taking into account the factor sex we also gain some information regarding X / Y related genes, which as it was indicated in Section 1.2, have significant effects on brain volume and on the effects of pre- and post- natal hormones on the brain structure and function.

1.9.2.1 Second to forth digit ratio correlates

2D:4D has been related to various aspects of human anatomy (Meindl, Windhager, Wallner & Schaefer, 2011; Schaefer, Fink, Mitteroecker, Neave & Bookstein, 2005), cognitive ability (Kempel, Gohlke, Klempau, Zinsberger, Reuter & Hening, 2005), structural brain differences (Kalai, Csatho, Kover, Makany, Nemes, Horvarth, Kovacs, Manning, Nadel & Nagy, 2005), physical fitness (Honekopp, Manning & Muller, 2006) sport achievement (Manning & Taylor, 2001) reproductive success (Manning, Barley, Walton, Lewis-Jones, Trivers, Singh, Thornhill, Rohde, Bereczkei, Henzi, Soler & Szwed, 2000) sex-role identity (Csatho, Osvath, Bickak, Karadi, Manning & Kallai, 2003) and aspects of psychology (Manning, et al., 2010).

In brief, high (feminine) 2D:4D is positively correlated with longer and bigger forehead, leaner eyebrows, narrow and long nose and small lower face (and vice-versa; Meindl et al., 2011).

Moreover, low (masculine) 2D:4D places the performance spectrum of females towards the lower male performance spectrum in male biased tasks; that is females with low 2D:4D perform better than females with high 2D:4D in spatial ability tasks (Kempel et al., 2005). Furthermore, the size of the posterior and the middle part of the hippocampus appears to correlate with 2D:4D in females (Kalai et al., 2005). Regarding physical fitness, Honekopp et al. (2005) argued that 2D:4D is a negative correlate in males and females; which is in line with Manning and Taylor (2001) who argued for a sport achievement-related and 2D:4D trend, with high (feminine) 2D:4D being a negative correlate of sport achievement.

In another study, Manning et al. (2000) argued for a significant relationship between 2D:4D and reproductive success in both sexes; with low (masculine) 2D:4D being a positive correlate of reproductive success in males while the opposite holds true for females. In addition, low (masculine) 2D:4D appeared to be a significant correlate of a masculinised individually-perceived identity (Csatho et al., 2003). Finally, Manning et al. (2010) reported a significant relationship between 2D:4D and the psychological measures of Systemizing and Empathizing[25]. In particular, low (masculine) 2D:4D appeared to be significantly correlated with high systemizing and low empathizing while the opposite was true for high (feminine) 2D:4D (Manning et al., 2010).

In summary, low 2D:4D appears to be related with many key features of masculinity while the opposite holds true for high 2D:4D, which appears to be related with many key features of femininity. Thus, masculinity and femininity[26] are closely affected by pre-natal hormone sensitization / desensitization to sexual hormones and this is observable in various aspects of

[25]Systemizing and Empathizing are two psychological constructs initially proposed by Simon Baron-Cohen (2002; 2014) and it is thought to be measuring brain sex type; with systemizing being male-biased and empathizing being female-biased.

[26] Referring to both physiological and psychological aspects of masculinity and femininity

human behaviour. Thus, it can be concluded that 2D:4D appears to be linked to brain structure and function, cognitive ability and various behaviours that are linked to masculinization / feminization. Consequently, 2D:4D can be seen as a valuable tool for the exploration of brain sex.

1.10 Summary and conclusions

The evidence discussed in this chapter explored the potential role of sex hormones on brain development and function from a behavioural, biological and a psychological perspective. Behavioural and biological data lead to the observation that sexual hormonal effects are potentially a strong candidate for the most deterministic role in cognition although their exact function is still unknown. Thus, based on existing research it was argued that pre-natal and post-natal hormones along with individual genetic variations are responsible for the masculinisation or feminisation of the hippocampus and the amygdala which, in turn, affect basic information processing ability[27]. Moreover, it was argued that basic information processing ability in conjunction with environmental influences[28] alter the structure of the memory system. Finally, it was argued that the structure of the memory system determines individual's cognitive phenotype and subsequently affects cognitive performance.

By reviewing existing literature it was possible to propose a model linking sexual (gonadal) hormones, brain structure and function, memory formation and cognitive performance. Based on this review it is concluded that sexual hormones affect the hippocampus and amygdala system,

[27] Basic information processing ability refers to the rate by which information (either visuo-spatial or language-based) enter the memory system.
[28] Environmental influences refer to the availability of information to be processed

which determine basic information processing ability, which in turn affects the structure of the memory system, finally affecting cognitive performance. Following the above argument, a high-functioning hippocampus and low-functioning amygdala (feminized hippocampus / amygdala) are expected to produce a memory system composed of many different memory networks, which are sparsely interconnected. Conversely, a low-functioning hippocampus and high-functioning amygdala (masculinised hippocampus / amygdala) are expected to produce a memory system composed by fewer memory networks, but which are highly interconnected. Considering the available literature to date, there is no any research or theory that offers a link between sexual (gonadal) hormones, specific brain structures and memory formation/structure (at a neuronal level). These three elements appear to be interdependent in a variety of research studies that were presented in this literature review. Based on the existing literature it is hypothesized that the 'rate' by which information is collected (i.e. basic information processing ability) has a direct effect on memory structure and function; which in turn (through development) determines an individual's cognitive phenotype.

Following the above rationale, it was argued that the main index that denotes the level of hippocampus and amygdala masculinisation (or feminization) is sex. This is due to the catalytic role that sex has on the effects of maternal circulating sexual hormones to the foetus (Scott, Mason, & Sharpe, 2009), as well as to the effects of foetal-produced oestrogen to the foetus (Gillies & McArthur, 2010), which in turn affects the way that gonadal hormones structure the pre-natal and later the post-natal brain; leading to the development of an individual's brain sex type. Based on the above, it is concluded that cognitive phenotype (or brain sex type) is the result of the combined effects of X / Y linked chromosomes, pre-natal and post-natal gonadal hormones on hippocampus and amygdala. These factors, along with environmental influences

are argued to be responsible for shaping an individual's brain sex type. Thus, in the following chapters the dissociating strength of the factor sex on brain sex type and brain activity will be tested against factors (such as 2D:4D and circulating gonadal hormone levels) that the literature review has indicated incorporate significantly more information regarding the hippocampus-amygdala masculinization / feminization levels than the factor sex only. This approach will allow a tentative examination of the biological basis of brain sex type, and specifically for a potential link between brain sex type and individual levels of masculinization / feminization.

Chapter 2: Verbal free recall & productive vocabulary – A behavioural measure of cognitive configuration (Experiment 1)

2.1 Introduction

A female advantage in performance on verbal memory tasks as well as a consistent utilization of verbal cues (when available) in various tasks from females has been reported in several studies (Hyde & Linn, 1998; Voyer, Voyer & Bryden, 1995; for a review see Andreano & Cahill, 2009). However, there are inconsistencies in reports of this female verbal advantage, mostly due to differences in the assessments and tasks used (e.g. multi-trial verbal learning tasks, story recall tasks), such that it is not possible to identify the exact aspect of test performance that is sex (female)-biased.

In Table 1, first presented in Adreano and Cahil (2009) some key studies that tested verbal memory are presented along with sample sizes and effect sizes. In this table it can be seen that sex differences in verbal memory are observed from moderate samples (N=36, Yonker, Eriksson, Nilsson & Herlitz, 2003) to large samples (N>1000, Youngjohn Larrabee & Crook III, 1991). Moreover, the majority of these studies are paradigms of multi-trial verbal learning tasks, such as California Verbal Learning test (CVLT; for example: Chipman & Kimura, 1998, N=49, Cohen's d=.97), Rey Auditory Verbal Learning Test (RAVLT; for example: Bolla-Wilson & Bleecker, N =45, Cohen's d=.85), and verbal recall (for example: Yonker et al., 2003, N=36, Cohen's d=.77, task: free recall). Multi-trial verbal learning tasks are tasks where lists of words are presented to

each participant and he / she is asked to recall as many words as possible, and this procedure is repeated over several trials.

Table 1. Reported p-values (P), effect sizes (Cohen's d) and sample sizes (N) of previous studies in verbal memory

Notes. RAVLT: Rey Auditory Verbal Learning Test; CVLT: California Verbal Learning Test
Source: Adreano and Cahil (2009)

Looking at studies that produced large effect sizes with moderate samples it can be argued that the appearance of sex differences could be linked to the nature of the tasks used in the studies. That is, small differences in task assessment may be responsible for enhancing or reducing sex differences in performance on these types of task.

In order to understand how task assessment may affect performance and consequently impact our understanding of how sex differences affect performance, it might be useful to consider sex differences in basic cognitive functions and how these functions relate to brain structures.

As was discussed in Section 1.4, sex differences in cognition are related to memory formation: the sex differences appear to relate to the way that memory is structured. As was indicated by animal studies, and supported by human studies, the hippocampus and amygdala appear to have a significant role in memory performance and formation (e.g. see Eichenbaum, 2004). As argued in Chapter 1, the hippocampus and amygdala form a system, which is engaged when collection and recollection of information is needed. Specifically, it was argued that the hippocampus facilitates the formation of associations between available stimuli (Eichenbaum, 2004; Davachi & Wagner, 2002; Moscovitch & Winocur, 1992; Moscovitch, 1992). In contrast, the amygdala enhances the incoming stimulus / stimuli in terms of neural activation (that is, amygdala enhances the neural stimulation caused by a stimulus). The main neurotransmitter that is related to the function of amygdala and hippocampus is acetylcholine and it appears to affect performance on both hippocampus-related and amygdala-related tasks in rodents. In humans, enhancing or averting the action of acetylcholine via drugs appears to affect performance in free recall tasks (Voss et al., 2010). As rodent and human brains appear to be similar in terms of simple processes (McIntyre, Marriott & Gold, 2002) it can be argued that the hippocampus and amygdala may also be engaged in a similar way, in tasks that require processing of same level stimuli; that is, tasks that do not depend on learning and applying complex rules but instead they are based on simple operations such as utilizing associations between stimuli. These are tasks where the participant needs to retain in memory a simple stimulus (or an association among simple stimuli), such as for example the location of an object in space. Thus, it is argued that

since free recall is affected by variations in acetylcholine levels, and acetylcholine levels are significant in impacting cognitive performance related to the hippocampus and amygdala, then the hippocampus and amygdala may have a significant role in free recall performance.

Given that free recall task performance potentially implicates both the hippocampus and amygdala, there is a need to employ a variant of the free recall task which will enable the separate consideration of performance based on hippocampal function alone versus performance based on the amygdala and hippocampus working together. However, existing studies do not allow the exclusion of a synergistic action between hippocampus and amygdala in order to have a free recall trial score, isolated from the effects of the amygdala. For example, Krueger and Salthouse (2010) explored the effects of sex on a multi-trial, word-based, free recall paradigm. In this study, a list (list A) of fifteen words was presented and immediate recall was requested for three consecutive trials (list presentation and immediate recall); then another list of fifteen words (list B) was introduced and immediate recall of that list was required; after this distraction, the participants were asked to recall the first list once again. Krueger and Salthouse (2010) were primarily interested in determining whether sex is associated with performance differences in learning new items from trial to trial or in retaining previously learned items. Based on their results, Krueger and Salthouse (2010) argued that sex differences in free recall related to a specific sex difference in learning new items from trial to trial; with females having a significant advantage. Looking at this study through the hippocampus-amygdala hypothesis, it can be argued that this female advantage on learning new items it might be due to a higher functioning hippocampus. More specifically, given that the hippocampus assists the formation of associations between stimuli, the presentation of list A from trial one to trial three benefited this (hippocampal) process and provided females with the observed advantage (for information

regarding the importance / function of hippocampus on free recall paradigms see Davachi & Wagner, 2002). Thus, female superior word-learning ability may be attributed to a superior hippocampal function. However, this free recall paradigm does not allow us to exclude the synergistic action of the amygdala on the free recall trial score.

In order to have a free recall trial score that is isolated from the effects of the amygdala a three-step free recall task was employed in this the study reported in this chapter[29]. The first step required participants to listen to and immediately recall a word list. The second step involved listening to and immediately recalling a second (distracter) word list. The third step required delayed recall of the first list of words. Thus, first verbal recall was assessed without any prior demands on the related cognitive mechanisms (first free recall); then recall was assessed while the related cognitive mechanisms were engaged (second free recall); and finally the effects of distraction on the participants' ability to retain information was assessed (i.e. via recall of the original list).

Placing the above free recall paradigm within the hippocampus-amygdala hypothesis context it can be argued that in the recall of the first list and the recall of the second list (distracter) performance cannot be attributed to hippocampus[30] or amygdala[31] only. Both mechanisms / systems (may) contribute to this recall and performance is achieved through a synergistic action. However, in free recall of the first list after the distraction, the effects of amygdala are (theoretically) excluded due to the distracter (see Appendix 1). This occurs because if the theory

[29] Verbal free recall is an umbrella term used to describe many different verbal recall tasks that require the verbal recall of word lists, lists of numbers or lists of letters. The focus of this experiment is on the specific task apparatus that is used including the use of words that are commonly used. This is done in order to explore the activation and inhibition (achieved by the distracter) of existing memory networks where these words are included.

[30] That is, recall is due to a formation of association between the words-stimuli (see Chapter 1, Section 1.3.2, page 16)

[31] That is, recall is due to a longer-lasting stimulus-based activation of the related sensory networks (see Chapter 1, Section 1.3.2, page 16)

behind the function of amygdala holds true, the distracter leads to the amygdala-based activation that was caused by list A to switch off in order to be replaced by amygdala-based activation caused by list B (distracter). Thus, performance on free recall after distraction must be based on the hippocampal-based associations that were formed / achieved during free recall one; that is, free recall after distraction may be used as a proxy of hippocampal functionality.

In Chapter 1, Section 1.8, it was argued that due to sex differences in the way that information is collected and incorporated into the memory system, structural differences are created in the memory system. Moreover, it was argued that structural differences in the memory system are the main cause of sexually differentiated cognitive phenotypes that can be collectively referred to as 'brain sex'. Brain sex may not be sex-specific (in terms of biologically male / female) and can potentially be measured by calculating the deviation in functionality between brain cells that are directly (or indirectly) affected by steroids, and those that are unaffected. Thus, in order to measure brain sex, a sex-biased cognitive task is needed (as an index of sex-affected brain cells; in this case free recall was used as such) as well as a neutral task (as an index of unaffected brain cells). For that reason, a test that is not significantly related to sex (or it is not female-biased; see Snow & Weinstock, 1990) was also administered as an index of non-female-biased cognitive function. Thus, it can be argued that this test engages cell assemblies that are affected by female sexual hormones or affected by non-female sexual hormones. Using these two tests, a calculation of each individual's functional deviation of their female steroid-sensitive brain cells from their non-female steroid-sensitive cells was attempted, in order to create an index of brain sex. In terms of the hippocampus-amygdala hypothesis, the female-biased task is an indicator / proxy of hippocampal functionality while the neutral task is used as a proxy for non-hippocampal based

performance[32]. In other words we compare a hippocampus-affected task versus a non-hippocampus affected task. Thus, looking at the performance deviation of these two tasks we attempt to determine a system that depends mostly on hippocampus in order to perform (that is a female-like cognitive system) or a system where the hippocampus is not the main factor that determines performance (that is a male-like cognitive system).

Based on the above, it was hypothesised that performance on a verbal recall task (a female-biased task) would be heightened in females while performance on a productive vocabulary test will not demonstrate a significant advantage for female participants. Moreover, an individual's cognitive phenotype created by the calculation of the deviation of verbal free recall scores (female-biased test) from the productive vocabulary scores (non-female-biased test) will enable the replication of Goldenfeld et al.'s (2005) 5-level brain-type-classification. Specifically, Goldenfeld et al. (2005) measured brain masculinization / feminization by calculating the deviation between the psychological measures of empathizing (female-biased trait) and systemizing (male-biased trait) resulting in five categories comprising *extreme empathizers, empathizers, balanced, systemizers* and *extreme systemizers*. In Goldenfeld et al.'s (2005) study, extreme empathizers were classified as individuals with extreme brain feminization, empathizers as individuals with brain feminization, individuals with equal systemizing and empathizing scores were classified as having a balanced brain (that is, their brain is neither more masculinized or more feminized), systemizers were classified as having brain masculinization and extreme systemizers were classified as having extreme brain masculinization. According to their findings, the presence of females gradually reduced as they moved from the extreme empathizers category

[32] Because if in this task hippocampal function was critical could provide an advantage to certain cognitive configurations; which advantage would be expressed as a sex difference. Since this task is not sex biased it consequently means that the hippocampus-amygdala system do not engage in a performance-determinant way; thus this task (productive vocabulary) can be used as a proxy for non-hippocampus-amygdala function.

towards the extreme systemizers category; indicating that in the extreme systemizers category only males were identified. Thus, it was concluded that an extreme masculinized brain is related to extreme systemizing ability. In their study, Goldenfeld et al. (2005) included a sample of individuals within the Autistic spectrum, who scored high in systemizing and low in empathizing (relative to neuro-typical individuals) and who were classified as individuals with extreme masculinized brains. Goldenfeld et al. (2005) argued that 'this result lends support to the extreme male brain theory of Autism at the psychological level, and confirms that autism spectrum conditions arise from a cognitive deficit in empathizing.' (p. 344); pointing out the need of a link between the extreme male brain and direct neural relates.

The current study was an attempt to recreate the same categorization but instead of using psychological measures it was based on the use of cognitive performance (that is free recall and productive vocabulary). This was done because if brain sex can be assessed through the use of these cognitive measures, then it will support at least some speculation regarding the relationships between specific cognitive functions and underlying neural relates that are heavily affected by sex[33]. More specifically, if brain sex can be calculated via cognitive measures that are proxies for certain brain functions that are related to the masculinization / feminization of the brain (such as free recall) then brain sex could be used as a proxy for specific brain structure / function configuration.

In free recall tasks, the engagement of the hippocampus (as a mechanism for associational memory processes) is supported by a growing number of neuroscientists in both typically developed individuals (see Davachi & Wagner, 2002) and those on the autistic spectrum (see

[33] As it was indicated by Voss et al. (2010) experiment free recall is related to the neurotransmitter acetylcholine which significantly affects hippocampal functionality.

Maister, Simons & Plaisted-Grant, 2013). In addition, Moscovitch and Winocur (1992; Moscovitch, 1992) argued that free recall performance is the result of a frontal lobe and hippocampus synergistic action. More specifically, Moscovitch and Winocur (1992) argued that performance on free recall depends on the synergistic action of 1. a hippocampus-related part that is responsible for creating associations between stimuli and retrieve them automatically; and on 2. a frontal-lobe related part that is responsible for strategic, self-initiated retrieval. In that way, frontal-lobe function appears to have a significant role in free recall performance. That might be particularly true because recent findings indicate that even after controlling for general differences in tasks that engage other mechanisms that may contribute / affect word-list free recall performance (i.e. episodic verbal memory ability measured by tests of paired associates and story recall tests) sex differences in word-list free recall remain significant (Krueger and Salthouse, 2010). Moreover, free recall performance is found to be susceptible to executive function deficiencies (Taconnat, Baudouin, Fay, Raz, Bouazzaoui, El-Hage, Isingrini & Ergis, 2010). Thus, the frontal lobe and by extension, executive function[34] must be taken into consideration when exploring the underlying mechanisms of free recall.

Tower of Hanoi is a task widely utilized by neuropsychologists in clinical and / or in experimental fields as a proxy of frontal lobe functionality (Welsh, Satterlee-Cartmell & Stine, 1999). For that reason, a task of Tower of Hanoi was assessed in order to control for differences in cognition that tap executive function including planning and learning (see Rainville et al., 2002; Welsh & Huizinga, 2005; Sergeant, Geurts & Oosterlaan, 2002; Vakil, Hassin-Baer & Karni, 2014). Moreover, it was considered essential to control for specific executive function /

[34] Executive function is an umbrella term that is used to describe various cognitive processes that among others include planning, attentional capacity, resistance to interference and working memory are affected by frontal lobe damage (Chan. Shum, Toulopoulou & Chen, 2008; McCabe, Roediger, McDaniel, Balota, Hambrick, 2010)

working memory differences within the verbal domain, since free recall is a verbal task. So, In order to control for short-term and working memory differences we added a measurement of forward and backward digit span (see Jones & Maken, 2016; Rosenthal et al., 2006). Forward and backward digit span test is considered to be a good proxy for primary attentional capacity and mental tracking within the verbal domain (Parsons, Rizzo, van der Zaag, McGee & Buckwalter, 2005). Finally, since vocabulary ability and age have been previously indicated as mediators of free recall performance (see Krueger & Salthouse, 2010) these variables were used as a control for free recall performance.

The female verbal recall advantage is observed systematically in almost every type of verbal memory task that has included a moderate number of participants (Yonker et al., 2003, N=36, Cohen's d=.77, task: free recall; Chipman & Kimura, 1998, N=49, Cohen's d=.97, task: CVLT; see Table 1). Looking at previous studies it can be observed that this is particularly true if we exclude studies that used story recall and object memory. Moreover, if we only look at studies that used a paradigm that was developed around a type of verbal-list as a basic measure, we see that as the number of participants grows, the effect size gets smaller. Thus, this may be an indication of an effect that it is actually small but inflated due to the number of participants. Moreover it is noteworthy that we are attempting to explore an effect that we already know exists and it is relatively robust. Consequently, it is possible that as we manipulate experimental conditions in order to highlight this effect, a higher number of participants will be more likely to lead to a significant result. However, this result may not be interpreted correctly due to a potential inflation (see Sullivan & Feinn, 2012). Sullivan and Fein (2012) stressed the importance of, as well as the imminent dangers associated with the interpretation of significant findings in experiments where the effect, although present, is due to a large sample size and

consequently gets inflated and overestimated. In order to overcome this potential issue, a pilot study was performed with small sample (which previous studies have used and found sex differences with a good effect size; see Table 1).

In conclusion, the aim of this study was to see whether it was possible to observe a significant female advantage in verbal free recall after distraction, supported by a large effect size within a small sample in order to avoid a Type I error. This pilot study was also intended to develop and test the adequacy of this specific version of verbal recall and make a first attempt at combining it with productive vocabulary performance and that way assess the cognitive brain sex type of participants. In this way, this pilot study was mainly concerned with the development of the procedure as well as the practical difficulties that may occur during the experimental process in order to record, adapt and plan the main study.

2.2 Method

2.2.1 Participants

For this experiment an opportunity sample of 42 Greek-speaking participants was recruited, aged between 19 and 54 years old (M= 34.88, SD= 9.610). More specifically, 22 males aged between 25 and 52 years old (M = 36.36; SD = 9.03) and 20 females aged between 19 to 54 years old (M = 33.25; SD = 10.18) were recruited. This sample consisted of members of the general public accessed by a snowball sampling technique. The snowball sampling was initiated via the researcher's network of colleagues and acquaintances. An opportunity sample of the general public was preferred over a sample of university students as this would enable a broader age-range to be obtained. The use of a Greek sample was not an intentional part of the design.

2.2.1.1 Inclusion / Exclusion criteria

All participants were asked to self-rate their prior experience of the tower of Hanoi puzzle. Participants with prior experience of the tower of Hanoi puzzle were excluded from the statistical analysis[35]. Moreover, efforts were made to recruit participants over 25 years old in order to reduce the effects of excessive hormone production on task performance[36]. Moreover, only monolingual participants were allowed to participate in this study (participants who were proficient and frequent users of a second language were excluded). This exclusion criterion was based on studies that have indicated significant differentiation in the rate of first and second language loss under conditions of neuro-degeneration, since this indication supports the idea that additional languages may lead to differences in memory structure (at least at a neuro-synaptic level) and consequently impact cognitive function. In total, 63 participants were assessed for eligibility on this study. From them 45 met the inclusion criteria and agreed to participate.

2.2.2 Materials

Free recall: To measure word free-recall ability, two lists containing 15 words each were adapted from the Greek version of the Rey Auditory-Verbal Learning Test (Rey, 1958). List 'A' and list 'B' contained everyday words such as «Τύμπανο» ("Drum") and «Βουνό» ("Mountain"). List A and B did not differ regarding their word-length ($t(28)=.359$, $p=.722$) or frequency ($t(28)=1.599$, $p=.121$; see Appendix 2, Table 1).

[35] Three participants were excluded for self-rating their tower of Hanoi experience in a scale from 0 to 10 with ratings above 0.

[36] According to Kelsey, Li, Mitchell, Whelan, Anderson, Wallace (2014) testosterone levels peak around the age of 19 years of life and then decreases gradually until the age of 40.

Productive vocabulary: To measure language comprehension, a Greek translation[37] of the vocabulary subtest of the "Wechsler Abbreviated Scale of Intelligence" battery (WASI) (1999) for ages from 8 to 89 years old was used (see Appendix 2). The WASI vocabulary subtest consists of 34 items of gradually increasing complexity from 'Bird' ('Πουλί') to 'Panacea' ('Πανάκεια'). Responses to each item were credited based on the General scoring principles of the UK WASI manual, varying from zero points, (for presenting incomplete to extremely low word comprehension), one point, (for presenting adequate to low word comprehension), to a maximum of two points, (for presenting good to perfect word comprehension).

Digit Span: The digit span test consists of two measurements of digit span: forward digit span and backward digit span. The forward digit span test consists of 36 sets of numbers, where the sets have increasing quantities of digits, arranged into blocks. The first block has two numbers per set, the second block has three numbers per set, and so on until the final block which has nine numbers per set. Similarly, the backward digit span test consists of 30 sets of numbers, with increasing quantities of digits per set arranged into blocks. The first block has two numbers per set, and the final block has seven numbers per set. For both tests, each set is read aloud in turn by the researcher, at a rate of two digits per second. After each set is read aloud, the participant is required to recall the numbers in the same order (forward digit span) or in the reverse order (backward digit span). Each correct recall of a set is credited with one point, and the test is terminated when participants fail to two sets in the same block.

Tower of Hanoi: A computerized version of Tower of Hanoi, named Tower Mania, version 1.1.16 (Zsolt, 2012), with two difficulty levels was utilized; where moves and time needed to

[37]The translation of the vocabulary subtest of the 'Wechsler Abbreviated Scale of Intelligence' battery (WASI) (1999) was undertaken by the author and has not been standardized.

complete each difficulty level were automatically recorded for post-test analysis. The first level comprised of a puzzle of three discs where the minimum number of moves required to solve the puzzle was seven. The second level comprised of a four-disc puzzle with a minimum of fifteen moves.

2.2.3 Design

The dependent variable was free recall scores derived from the Greek version of Rey Auditory-Verbal Learning Test (Rey, 1958). The independent variable was list type, which had three levels; List A (max 15), List B (max 15) and List A after distraction (that is, after List B is applied; max 15). A further independent variable was Sex, which had two levels; males and females.

A number of covariates were also assessed. Age of participant, measured in years was recorded, as was the number of moves taken to complete the Tower of Hanoi task and the time taken to complete it, measured in seconds (this was recorded for both levels of difficulty of this task). The participants' digit span score (forward digit span: maximum score 36, and backward digit span: maximum score 30) and productive vocabulary scores (maximum score 68) were also taken into account as potential covariates in the analysis.

Tower of Hanoi was used as a proxy for cognitive differences that tap executive function including planning and learning (see Rainville et al., 2002; Welsh & Huizinga, 2005; Sergeant, Geurts & Oosterlaan, 2002; Vakil, Hassin-Baer & Karni, 2014). Digit span was used as a proxy for verbal short-term and working memory differences (see Jones & Maken, 2016; Rosenthal et al., 2006).

2.2.4 Procedure

Ethical approval for this study was obtained from Coventry University's Ethics committee. A briefing was given to each participant prior to the experimental procedure, and written consent was obtained (Appendix 3).

In the first part of the free recall task, each participant was informed that he or she was going to listen to a list of words, and at the end of the listening he/she would have to recall as many as he/she could in any order. Each of the fifteen words (Appendix 2) was presented clearly at a level audible to the participant and at a pace of one word per two seconds. After the first list, the participant was informed that the same procedure would be repeated but with a different list, and the procedure was repeated with List 2. After this, the participant was asked again to recall as many words as he/she could from the first list that he/she had been presented with.

Thus, there were three measures that were scored:

1. Correctly recalled words from List 1; this variable was labelled *free recall 1*.

2. Correctly recalled words from List 2 (distracter); this variable was labelled *free recall 2*.

3. Correctly recalled words from List 1 after the distracter list; this variable was labelled *free recall 3*.

Following a five minute break, the productive vocabulary test was administered. The researcher provided the participant with directions for the test as described in the WASI (1999) manual. When an answer was not adequate, the researcher asked once "Can you please tell me some more about it?"(«Μήπως θα μπορούσατε να μου πείτε κάτι περισσότερο; ») or "Can you explain what you mean?"(«Θα μπορούσατε να μου εξηγήσετε τι εννοείτε; »; WASI, 1999). The final

score of language comprehension for each participant was produced based on the sum of each item's awarded credits.

Following a five minute break, the digit span task was administered as described above. The forward digit span test was administered first and the backward digit span second.

Finally, following a further five minute break, the Tower of Hanoi task was introduced. Participants who reported prior experience to this task they were excluded from the analysis. A Samsung Galaxy Note 10.1 inch tablet with 'Tower Mania' (Zsolt, 2012) loaded was handed to the participant followed by an introduction to the game by the researcher, explaining the goal and rules of the game. Each participant was asked to solve three puzzles, starting from a three-disk task first (easy / practice), followed by a four-disk task. After each task, the researcher noted the number of moves and time that the participant took to complete the task, which was automatically recorded on the tablet.

After the completion of the experimental procedure every participant was fully debriefed and ample time was provided for discussion with the researcher.

2.2.5 Data analysis

All data were analysed using IBM SPSS Version 22.0 (IBM Corp., Armonk, NY, USA). Simple independent samples t-tests were used to compare differences between males and females on the baseline scores on measured cognitive domains and demographic variables. A Pearson's correlation was used to explore the relationships between the measured cognitive domains and demographic variables, which previous literature had suggested might mediate free recall performance, in order to decide whether they should be included in ANCOVA analyses. A mixed factorial ANCOVA was used with free recall scores being used as a repeated measures outcome

(free recall 1, 2, 3) and sex being used as a between groups variable. This was performed to explore the effects of sex on free recall performance (in all levels) as well as performance on each task within the free recall paradigm.

The final step was to standardise and calculate the deviation between free recall after distraction and productive vocabulary scores in order to construct a cognitively-based index of potential 'brain sex'. The raw scores of productive vocabulary (PV) and free recall (FR) were standardised by subtracting the sample's mean (indicated by <…>) from each individual's score and then dividing it by the maximum possible score. Thus, for productive vocabulary we have: PV_{std}= (PV-<PV>)/ 68; and for free recall FR_{std}= (FR-<FR>)/ 15. The difference between the standardized productive vocabulary and free recall (this variable is termed as 'D') is calculated by *D= (PVstd-FR3std)/ 2*. Next, the entire sample was divided in quintiles based on the D scores (median positions). Thus, five brain type categories were identified, and were labelled as extreme feminized brain type (that is PV<<FR), feminized brain type (that is PV<FR), balanced brain type (that is PV≈FR), masculinized brain type (that is PV>FR) and extreme masculinized brain type (that is PV>>FR). Individuals who scored between the 14.3^{th} and 26.2^{th} percentile were classed as having a 'feminized brain type', those between the 26.2^{th} and the 66.7^{th} percentile as having a 'balanced brain type' and those between the 66.7^{th} and 92.3^{th} percentile as individuals with 'masculinized brain type'. Those, who fell into the lowest 2.5^{th} percentile, were classed as individuals with 'extreme feminized brain type' and those who fell in the highest 2.5^{th} percentile were classed as individuals with 'extreme masculinized brain type'. Differentiations between males and females on their allocation on the brain sex type scale are investigated using ANOVA and Fisher's exact test.

2.3 Results

2.3.1 Sex differences in verbal free recall

A series of independent samples t-tests were performed to assess baseline differences between males and females in key variables such as free recall, productive vocabulary, and other measures of cognitive function.

Table 2. Results of t-tests and descriptive statistics for cognitive scores and age (in years) of the sample by sex

Variable	Group				95% CI for Mean Difference	t	p	df	Hedge's gs
	Male (n=22)		Female (n=20)						
	M	SD	M	SD					
Free recall 1	7.05	1.93	8.3	2.25	-1.96, 0.06	-1.96	0.057	40	0.59
Free recall 2	5.82	1.46	6.7	1.62	-2.85, 0.08	-1.84	0.073	40	0.56
Free recall 3	2.91	2.46	4.8	2.44	-6.3, -0.37	-2.53*	0.015	40	0.75
Productive vocabulary	50.82	12.32	43.5	8.345	36.6, 13.83	2.23*	0.031	40	0.67
Forward digit span	23.73	4.108	22.8	4.008	13.49, 3.46	0.739	0.464	40	0.22
Backward digit span	18.23	5.468	19.7	4.692	7.36, 1.69	-0.932	0.357	40	0.28
Total moves (ToH)	49.22	14.67	51.65	33.56	25.08, 14.00	-0.308	0.76	40	0.09
Total time (ToH)	126.94	98.49	123.92	120.49	50.20, 72.05	0.089	0.929	40	0.02
Age (years)	36.36	9.03	33.25	10.18	22.63, 9.13	1.05	0.3	40	0.31

Note: first list free recall (free recall 1), second list free recall (free recall 2), free recall of the first list after distraction (free recall 3), productive vocabulary, forward digit span, backward digit span, total moves performed in the two difficulty levels of tower of Hanoi puzzle (total moves), total time for completing the two difficulty levels of tower of Hanoi puzzle (total time). Logarithmically transformed values for Free recall 1, 2, 3 were used for t-tests.

Next a Pearson's correlation analysis was performed among the variables that prior studies have indicated as potential mediators of free recall performance (Table 2).

Table 3. Simple correlations among age, tower of Hanoi moves (ToH moves), tower of Hanoi time (ToH time), forward digit spam (FDS) and backward digit span (BDS)

	Productive vocabulary	**ToH moves**	**ToH time**	**FDS**	**BDS**
Age	.323*	.288	.484*	-.197	-.266
Productive vocabulary		.001	.086	.175	.247
ToH moves			.737*	-.139	-.057
ToH time				-.140	-.097
FDS					.594*

* $p < 0.05$

Descriptive statistics (see Table 2) indicated that female scores were numerically higher in all free recall levels at baseline level; however, only free recall after distraction (free recall 3) reached statistical significance (p=.015). Moreover, males seemed to outperform females in productive vocabulary, scoring significantly higher (p=.031). In forward digit span and Tower of Hanoi (moves) males scored numerically better than females although these differences did not reach statistical significance. On the other hand, females scored slightly better in backwards digit span and Tower of Hanoi (time), but again these differences were not significant (Table 2). Looking at Table 3, the variable 'age' appeared to be significantly correlated with productive vocabulary (r=.323, p=.037) and Tower of Hanoi (time) (r=.484, p=.001). Tower of Hanoi (time) appeared to be significantly correlated with Tower of Hanoi (moves; r=.737, p<.001). Similarly, forward digit span was significantly correlated with backward digit span (r=.594, p<.001). The cut-off point was determined by the alpha level set at .05. Thus, the variables 'age', 'tower of Hanoi (moves)' and forward digit span were excluded from the model in order to preserve power for this moderate sample size.

The results of a Two-Way Mixed ANCOVA are presented below with all effects reported as significant at p<.05. In the first model the results showed that there was a significant main effect of free recall condition ($F(2, 80) = 54.963$, $p < .001$, $\eta_p^2 = .579$) on free recall scores, with first

list free recall scores (M= 7.64, SD= 2.162) being higher than second list free recall scores (M = 6.24, SD= 1.590) and the recall of the first list after distracter scores (M=3.81, SD=2.606). In addition, between-subject tests indicated a significant main effect of sex on free recall scores overall ($F(1, 40)= 7.967$, p=.007, $\eta_p^2= .166$). There was no significant within-subjects interaction between free recall condition and sex ($F(2, 40) = .960$, p = .387, $\eta_p^2= .023$).

Contrasts revealed that free recall of the first list scores achieved by each participant were significantly higher than the second list scores ($F(1,40)= 14.274$, p=.001, $\eta_p^2 = .263$) while the second list scores were significantly higher than the free recall of the first list after the distracter ($F(1,40)= 35.420$, p<.001, $\eta_p^2 = .470$). Finally, the free recall of the first list scores achieved by each participant were significantly higher than the free recall of the first list after the distracter ($F(1,40)= 140.770$, p< .001, $\eta_p^2 = .779$).

Based on the correlation matrix presented in Table 3 the covariates chosen for the analysis were: (1) Productive vocabulary, because there was a significant sex difference in baseline measurement and vocabulary is associated with free recall performance (Bolla-Wilson & Bleecker, 1986), (2) Backward digit recall, because this factor is highly correlated with forward digit recall (cut-off was set at p<.05) and is theory-wise related to working memory and (3) Tower of Hanoi time, because it was highly correlated with tower of Hanoi moves and participant age, and is a proxy for frontal lobe function. This was done in order to reduce the covariates entered into the model, in order to preserve power. Next the results from the adjusted model are presented, where the effects of first, second (distracter) and third free recall (free recall after distraction) and their interaction with sex were adjusted for productive vocabulary, digit span (backward) and tower of Hanoi (moves).

The results showed that the main effect of free recall condition was not significant (F(2, 74) = 2.475, p =.091 η_p^2 = .063) on free recall scores. Moreover, between-subject tests indicated a significant main effect of sex on free recall scores overall (*F*(1, 37)= 6.197, p=.017, η_p^2= .143). Finally, the interaction between sex and the main effect of free recall was not significant (F(2, 74)= 1.424, p=.247, η_p^2= .037).

2.3.2 Classification of brain sex type based on productive vocabulary (PV) scores and free recall (FR3)

Following the hypothesis that free recall after distraction may serve as an index of hippocampal performance, isolated from the synergistic action of amygdala, we used this variable in order to calculate a potential indicator of cognitive brain sex. Thus, performance deviation (for short referred as $D_{pv\text{-}fr}$) of free recall after distraction, from productive vocabulary (a neutral task or a non-female biased task) is hypothesised to be able to indicate / differentiate two different cognitive systems. That is, $D_{pv\text{-}fr}$ is hypothesized to be able to differentiate a cognitive system dominated by hippocampal function (that is, higher free recall performance compared to productive vocabulary performance) from a cognitive system which is not dominated by hippocampal function (that is, lower free recall performance compared to productive vocabulary performance).

Goldenfeld et al. (2005) argued that having either low empathizing or high systemizing or both, can lead to a high 'D'[38]; an indicative of an individual with male brain sex. They also calculated a 'C'[39] variable, which was designed to test whether the argued relationship between

[38] That is standardized productive vocabulary score minus standardized free recall score divided by 2. Based on Goldenfeld et al. (2005) a high D would be a characteristic of male brain sex while low D would be indicative of a female brain sex.
[39] That is standardized productive vocabulary score plus standardized free recall score divided by 2.

empathizing and systemizing is reciprocal or one-sided as Goldenfeld et al. (2005) argued. In this study, Goldenfeld et al.'s (2005) methodology was used but instead of using systemizing (a male biased trait) and empathizing (a female biased trait), productive vocabulary (a non-female biased task[40]) and free recall (a female biased task) were used. This was done in an attempt to measure brain sex via measures that may be proxies for specific brain structure / function. Achieving that can potentially enable at least some speculations regarding the underlying neural correlates of brain sex.

Similar to Goldenfeld et al.'s (2005) rationale, having either low hippocampal functionality or high amygdala functionality or both, can lead to a high 'D'[41]. The 'C'[42] variable is designed to test whether the argued relationship between hippocampal functionality and amygdala functionality is reciprocal, or one-sided.

Following the procedure described in Goldenfeld, Baron-Cohen and Wheelright (2005) the scores of productive vocabulary and free recall were standardized. Thus, PVstd= (PV-<PV>)/68, and FR3std= (FR3-<FR3>)/15 where <..> indicates current sample's mean scores divided by the maximum possible score. Descriptive statistics indicated a mean score of 47.33 for productive vocabulary (PV) and 3.81 for free recall (FR3). The next step was to normalize the two factors, resulting in the following two variables:

D= (PVstd-FR3std)/2 (i.e. the difference between the normalized PV and FR3 scores)

C= (PVstd+FR3std)/2 (i.e. the sum of the normalized PV and FR3 scores)

[40] A male prevalence in productive vocabulary tasks have been reported (Snow & Weinstock, 1990)
[41] That is standardized productive vocabulary score minus standardized free recall score divided by 2. Based on Goldenfeld et al. (2005) a high D would be a characteristic of male brain sex while low D would be indicative of a female brain sex.
[42] That is standardized productive vocabulary score plus standardized free recall score divided by 2.

Based on the theoretical framework of this study, pre-natal hormonal effects in conjunction with environmental informational availability affect the structure of the memory base. Consequently, it is argued that due to this pre-natal hormonal 'set-up', an advantage or a disadvantage may be observed for so-called sex-biased cognitive tasks, such as free recall, navigation and mental rotation. As it was argued in Chapter 1, sex-biased tasks engage steroid-sensitive brain cells. Consequently, measuring performance-deviation of female-biased tasks from neutral / male-biased tasks can potentially produce an index of feminization/masculinisation of the brain; that is, brain sex.

Table 4 shows *the mean scores* for the difference between standardized productive vocabulary test and the standardized free recall after distracter (D) and the combined scores (C) as well as the results of the comparisons of the tests between males and females. The means and standard deviations for $D_{pv\text{-}fr}$ for the different populations are: males (M= .056, SD= .109) females (M=-.062, SD= .109). Respectively, the means and standard deviations for $C_{pv\text{-}fr}$ for the different populations are: males (M= -.036, SD= .137) females (M=.004, SD=.095).

Table 4. Descriptive statistics and results of t-tests for the difference between standardized productive vocabulary tests and the standardized free recall after distracter (D) and the combined scores (C) by Sex

Test	**Group**									
	Male			**Female**			**95% CI for**			**Hedge's**
	M	**SD**	**n**	**M**	**SD**	**n**	**Mean Difference**	**t**	**df**	**g_s**
D(difference between scores)	.056	.109	22	-.062	.109	20	-7.71, 0.19	3.495*	40	1.06
C(sum between scores)	-.003	.137	22	-.004	.095	20	-8.14, 0.44	-.205	40	0.06

** $p < 0.05$.*

As can be seen in Figure 1, there is an observable differentiation of the data along the D axis while this is not the case regarding the C axis. Looking at Figure 1 in which the D axis runs from the bottom right to the top left corner, it can be seen that the majority of the male population

(marked as circles) lies within the positive side of the D axis (that is, top left corner), while the majority of the female population (marked as squares) lies within the negative side of the D axis (that is, bottom right corner).

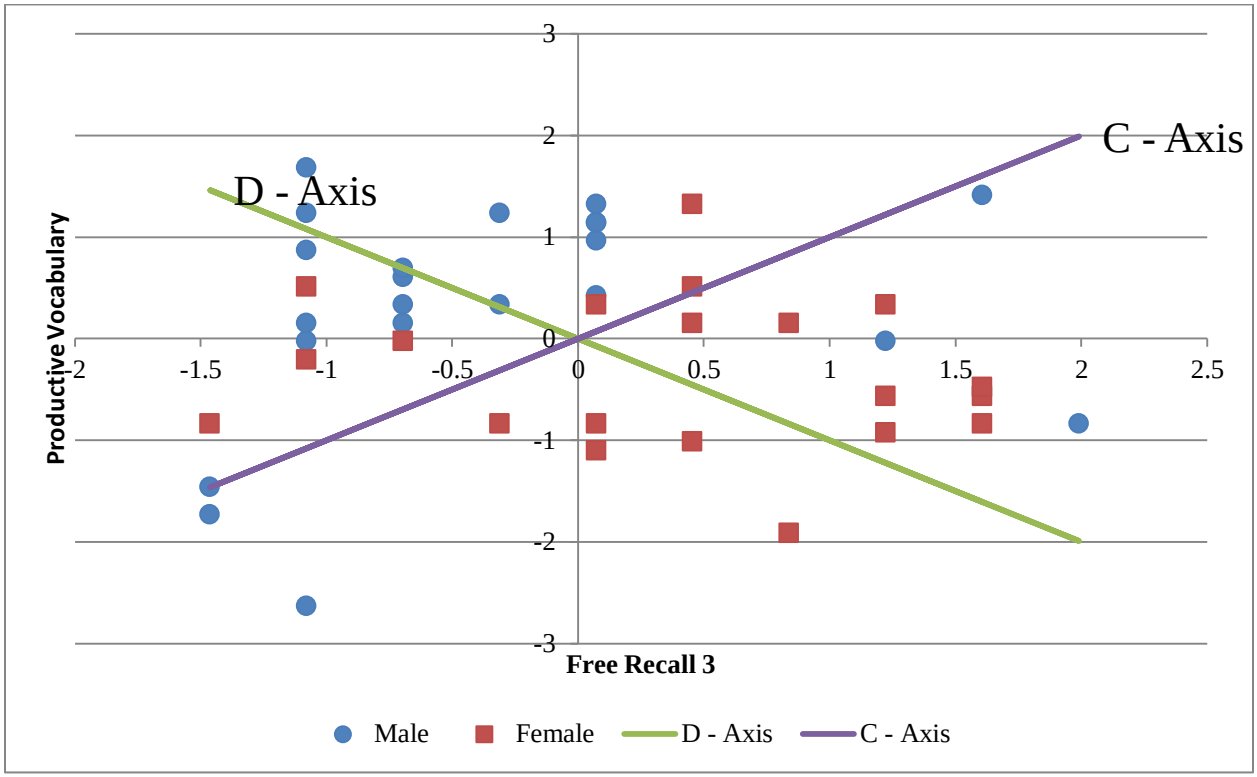

Figure 1. Scatter plot of the standardized productive vocabulary and free recall scores. D (difference between scores) and C (sum of scores) are also displayed on the figure.

Additionally, the cumulative distribution of the D and C values (see Figures 2, 3) was plotted. As it can be seen in Figure 2, there is a significant difference in the cumulative distribution between males and females, indicated by the spacing between the cumulative distributions of the two groups. This indication allows us to argue that these groups represent two distinct populations as defined by their D value. A one-way ANOVA was used in order to quantify the above observation, and this indicated a significant effect of sex on D scores ($F(1, 41)= 12.215$, $p=.001$). On the contrary, observing our groups based on the C values (Figure 3) it can be seen that the

distributions are intertwined, allowing us to argue that no clear categorization can be made based on the C value. A one-way ANOVA showed that the effect of sex on C scores was not significant (F(1, 41)= .042, p=.838). Thus, categorization of the data can be made based on D value only.

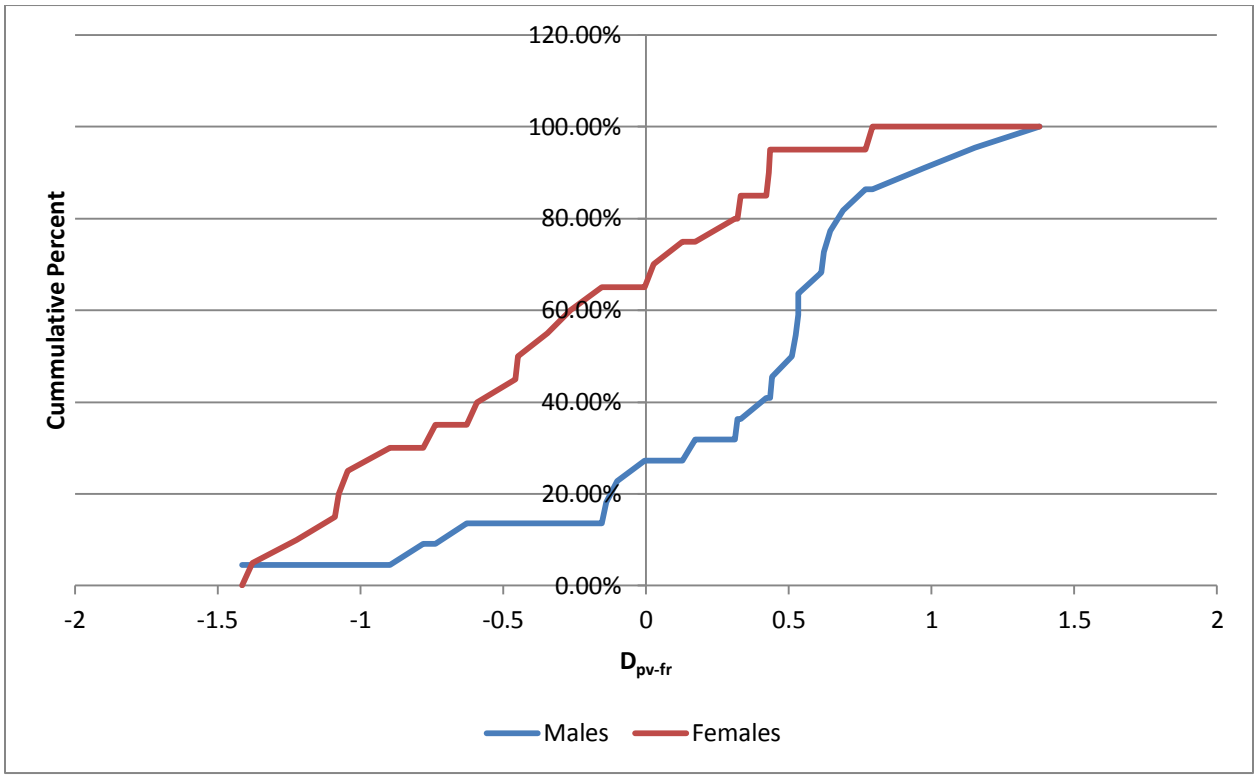

Figure 2. Cumulative distribution function of D. Categorization between males and females.

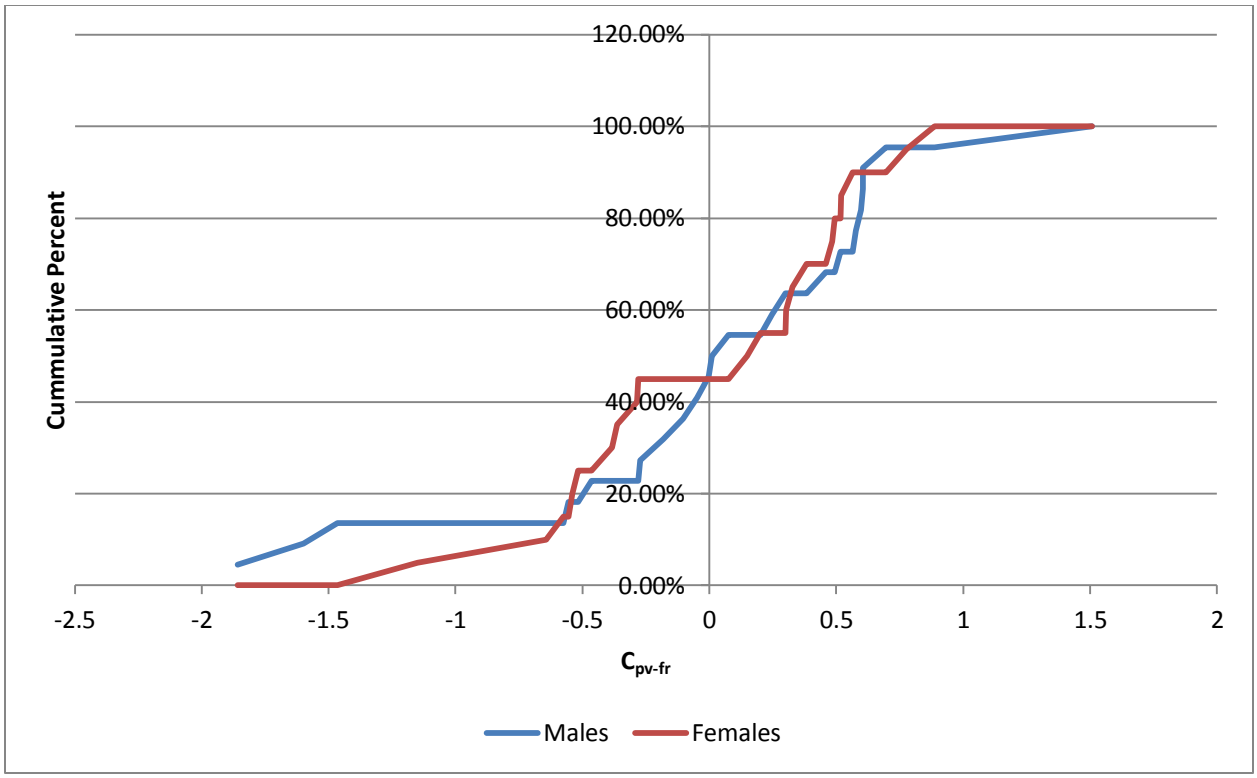

Figure 3. Cumulative distribution function of C. Categorization between males and females.

Categorization based on D values is shown in Table 5, where both groups are classified regarding their brain type, just as Goldenfeld et al. (2005) did in their study. Table 5 shows that only males are present in the 'extreme masculinized' brain type group, whilst in the 'masculinized' brain type group, the percentage of females remains low. Moreover, the numbers for males and females are almost equal in the 'balanced' brain type group, whilst in the 'feminized' and 'extreme feminized' brain types, the percentage of males is reduced. In order to quantify the above observation, that is to determine whether there are significant associations between group membership (i.e. the five brain type categories) and sex, a Fisher's exact test of independence was performed. The results indicated that the observed distribution of males and

females across the five brain type categories is significantly different from what we might expect (p=.026, Fisher's exact test).

Table 5. Classification of brain types based upon median positions and percentiles of males and females

Defining Characteristic	**PV<<FR3**	**PV<FR3**	**PV≈FR3**	**PV>FR3**	**PV>>FR3**
Brain types based on median positions of the two sub-populations Male, Female					
Brain Boundary (median)	D<-0.16	-0.16<D<-0.085	-0.085<D<0.085	0.085<D<0.16	D>0.16
Females (N) Males (N)	5 1	3 2	10 7	2 9	0 3
Brain Types Based on Percentiles of the two sub-groups Males, Females					
Brain Boundary Percentile	D<-0.16	-0.16<D<-0.085	-0.085<D<0.085	0.085<D<0.16	D>0.16
Percentile (Per)	Per<14.3	14.3<per<26.2	26.2<per<66.7	66.7<per<92.3	Per>92.3
Females Males	25% 4%	15% 9%	50% 32%	10% 41%	0% 14%

2.4 Discussion

2.4.1 Sex differences in verbal free recall

This study was set to assess sex differences in a multi-trial free recall paradigm. Based on the hippocampus-amygdala hypothesis it was predicted that the effect of sex would be stronger in free recall after distraction. Moreover a first attempt to assess brain sex via two cognitive measures instead of using psychological measures was made. The results indicated a significant sex difference in the multi-trial free recall performance overall. Although there was a numerical female advantage in free recall after distraction at the base-line level, there was no significant interaction between type of free recall task and sex. Regarding brain sex, when participants were categorized according to their performance on the two cognitive tasks, the distribution of males

and females appears to be similar to the pattern observed by previous studies (i.e. Goldenfeld et al., 2005; Baron-Cohen et al., 2014).

The results reported above are in line with previous studies that have indicated a significant female advantage in verbal free recall (Krueger & Salthouse, 2010; Bolla-Wilson & Blicker, 1986; Geffen, Moar, O'Hanlon, Clark & Geffen 1990; for a review see Andreano & Cahill, 2012). Females appear to outperform males in every level of the free recall paradigm as it was indicated by the baseline scores; with free recall scores after distraction being significantly lower than scores in the first recall condition. Looking at the results from the analysis of variance it can be seen that there is a significant effect of sex on free recall performance overall. This difference remained significant even after controlling for the influence of vocabulary ability, executive function and working memory. However, controlling for the above variables eliminated the main effect of free recall condition. Previous researchers have argued for the importance of these covariates, as they appear to account for a significant amount of variance in free recall performance. That is, Moscovitch (1992; Moscovitch & Winocur, 1992) argued for the significance of frontal lobe in free recall paradigms, stressing the importance of executive function and working memory in this type of tasks. Based on the findings of the current study it can be argued that sex differences in free recall performance cannot be simply attributed to differences in vocabulary ability, executive function or working memory.

The lack of an interaction between free recall condition and sex suggests that there may be no specific part of the free recall task that is mostly affected by sex and the covariates. Thus, at this point it can only be suggested that sex differences in this free recall paradigm are unlikely to be

due to frontal lobe function[43] since the role of frontal lobe was (at least at some level) controlled for.

The lack of an interaction between free recall condition and sex might be due to another factor that was not controlled in this study and may have affected the results. According to Siengthai, Kritz-Silverstein and Barrett-Connor (2008) handedness appears to be a factor that affects cognitive performance and, according to their experiments, performance on multi-trial learning tasks. Specifically, Siengthai et al. (2008) indicated that there is a significant differentiation in multi-trial word learning performance, verbal fluency and attention between left-handed and right-handed (and ambidextrous) females, with left-handed females performing significantly lower in those tasks. Consequently, left-handed females may act as a balancing factor in free recall performance, reducing male and female performance differences. Considering that handedness appears to be related to prenatal hormones (Kelso, Nichols, Warne, Zacharin, 2000), it can be argued that handedness (along with brain lateralization) might be potential mediators of verbal free recall performance. Thus, subsequent studies should at least control for this factor in order to be able to draw interpretable results.

2.4.2 Cognitive phenotype and brain sex

The next step of this study addressed the identification of individual cognitive phenotypes through score deviations between verbal free recall and productive vocabulary performance. The part of the free recall test that was used for creating the brain-sex variable was the free recall after distraction; since based on the hippocampus-amygdala hypothesis that was the part of the free recall task that is relatively isolated from the effects of the amygdala. The behavioural data

[43] Frontal lobe damage is related to a significant impairment on multi-trial free recall performance (Wheeler, Stuss & Tulnvin, 1995).

appeared to fit on the brain sex scale; indicated by the gradual reduction of male' presence as we move from the productive vocabulary (PV) >> free recall (FR) brain sex category towards the PV<<FR brain sex category while the opposite was true for females. Males were completely absent from the PV<<FR brain sex-type category; while the opposite was observed for females, which were completely absent from the PV>>FR brain sex-type category. Thus, brain sex can potentially be measured through behavioural tasks as well as from psychological (i.e. Baron-Cohen's systemizing-empathizing). Considering the identification of an individual's cognitive phenotype and his / her classification on the brain sex scale it can be argued that current findings support the hypothesis regarding a behavioural brain-sex type classification. Up to now, brain-sex type had only been explored and categorizations made through the psychological measures of systemizing and empathizing (Goldelfeld et al., 2005; Baron-Cohen et al., 2014). Attempting a brain sex categorization through cognitive tasks allows a better theoretical link between such categorisations the functionality of specific brain structures. However, to further substantiate such theoretical claims, it is first necessary to replicate such a categorisation in a study which also directly examines biological factors hypothesised to explain the observed pattern of association between sex and cognitive performance. This is undertaken in this study presented in the next chapter. The potential of these findings are interesting because they can be used as a stepping-stone towards a direct investigation of the biological factors that lie beneath the configuration of cognitive phenotype and consequently brain's sex. Thus, the next step is considered necessary to be focused on the biological factors that may affect brain sex.

Chapter 3: Gonadal hormones, brain type & systemizing-empathizing – A cognitive-biological investigation (Experiment 2)

3.1 Introduction

In Chapter 1, the hippocampus-amygdala hypothesis was introduced along with the theorised effects of pre-natal and post-natal hormone effects on these two brain structures. Chapter 2 explored the potential use of a verbal word free recall task as a proxy for hippocampus and frontal lobe function as suggested by previous researchers (Moscovitch & Winocur, 1992). Moreover, the results from Chapter 2 suggest that word-list free recall sex differences cannot be attributed to executive function, working memory[44], vocabulary differences or age only, since even after controlling for these factors sex differences emerged. Thus, the current chapter will explore sex differences in free recall performance in relation to pre-natal and post-natal hormone effects whilst also controlling for handedness. The pattern of sex distribution across the brain sex typology explored in Chapter 2 will be re-examined in this chapter in order to see if the result can be replicated. Crucially the relationship between brain sex type and individuals' masculinization / feminization level (determined by direct and indirect indices of gonadal hormones) will additionally be explored. Finally, an assessment of systemizing-empathizing will be included as a means of further validating the cognitive brain sex typology proposed in this thesis.

[44] Executive function and working memory are functions attributed to frontal lobe (Welsh et al., 1999)

As described in Chapter 1, an individual's cognitive phenotype is affected by the effects of pre-natal sexual hormones (organizational effects) as well as post-natal hormones (activational effects; McCarthy & Arnold, 2011). In particular, masculinisation or feminization of the foetus leads to the development of different memory systems. Consequently, that leads to a differentiation in memory system structures that may collectively form an individual's cognitive phenotype or, stated differently, an individual's brain sex. At this point it is crucial to recall the role of sex in relation to hormone effects. In Chapter 1 (Section 1.2) it was argued that the sex of the foetus affects the way that maternal circulating oestrogen acts on the foetus's brain. This function was argued to be related to alpha fetoprotein which appears to react with maternal circulating oestrogen and stops the de-feminization of the brain. Although the exact function of alpha fetoprotein has not yet been established in humans, its role still remains crucial. However, the importance of the above hypothesis lies on the conclusion that the effects of hormones are regulated by the sex of the foetus, thereby constituting the sex of the foetus as a factor which influences the effects of pre-natal hormones.

Previous studies that have assessed brain sex via the psychological assessment[45] of systemizing-empathizing have indicated that sex is related to specific advantages and disadvantages in specific cognitive areas. For example, an individual that has a Type S cognitive style (that is, higher systemizing than empathizing scores in the relevant tests, mostly observed in males) has a tendency to get involved with, and even to appear an advantage in situations that involve the creation and recognition of rule-based systems (Wheelwright et al., 2006). In an experiment with young adults Lai et al. (2012) indicated that Type S cognitive style is associated with the choice

[45] Brain sex measured by systemizing-empathizing is referred in the literature as a measure of cognitive brain sex. For reasons of dissociation between the current study and previous studies that used systemizing-empathizing brain sex in this thesis it is referred as a psychological measure of brain sex since systemizing and empathizing initially derived from a model of psychological sex differences (Baron-Cohen, 2002).

of science and mathematics as major university courses, while a Type E cognitive style (that is, higher empathizing than systemizing scores in the relevant tests) is linked to a preference for the humanities. Thus, from a certain point of view this can be interpreted as link between brain sex and a tendency towards specific academic preferences. Relating these findings to the hypothesis that gonadal hormones affect brain sex it is important to differentiate between advantages / disadvantages that are directly related to hormones and those that are not.

In order to examine which advantages / disadvantages are related to hormones and which are not, we can look at studies that have examined both the effects of sex as well as the effects of hormones. Post-natal circulating gonadal hormones appear to play a significant role in sex-biased tasks, enhancing or impeding performance. In particular, performance on verbal free recall is positively related to post-natal estradiol and testosterone levels in males only (Cherrier et al., 2003; 2004; Zimmerman, Lipton, Santore, McConnell, Derby, Katz, Baigi & Saunders-Pullman, 2011). However, the positive relationship between free recall and testosterone only appears when the process of testosterone aromatization to estradiol is not blocked, implying an instrumental role for estradiol in free recall performance (Cherrier et al., 2004). Conversely, estradiol is negatively related to performance on male-biased tasks (e.g. mental rotation), in males only (Kozaki & Yasukouchi, 2008).

Female performance on sex-biased tasks (verbal memory, mental rotation) appears to be different during different phases of the menstrual cycle. Specifically, during phases of the menstrual cycle where estradiol levels are low, females perform better on male-biased tasks[46] while females perform better on female-biased tasks[47] during phases of the menstrual cycle

[46] Male-biased tasks are tasks where a male advantage is observed systematically.
[47] Females-biased tasks are tasks where a female advantage is observed systematically.

where estradiol levels are high (Rossenbaum & Park, 2002; Hausmann, Slabbecoorn, Van Goozen, Cohen-Kettenis & Gunturkun, 2000). However, many researchers argue that this is not the case (for a review of research-to-date: Poromaa & Gingnell, 2014). Poroma and Gingell, (2014) argued that specifically in tasks including verbal cues, estradiol levels might be of importance but only in addition to genetic set-up and only in studies exploiting verbal working memory (for example see: Rossenbaum & Park, 2002). In addition, Hogervorst, De Jager, Budge and Smith (2004) reported a relationship between circulating estradiol and verbal free recall. However, in this study serum total estradiol was measured and not free / unbound estradiol, which is the type of estradiol measured in the studies reported above. The importance of the difference between the two types of estradiol lies on the fact that the biologically active form of estradiol is free / unbound estradiol, which is capable of passing through the blood-brain-barrier and consequently affect the brain (Ryan, Stanczyk, Dennerstein, Mack, Clark, Szoeke, Kildea & Henderson, 2012). Based on the above, it is concluded that the hormone of interest in sex-biased tasks is estradiol and specifically the relationship between estradiol and testosterone. Moreover, a clear relationship between sex-hormones and cognitive performance on sex-biased tasks in males can be observed.

Focusing on verbal free recall tasks, Moscovitch and Winocur, (1992; Chapter 2) argued that free recall performance depends on frontal lobe function and hippocampal function. The frontal lobe is responsible for strategic and self-initiated retrieval (see also Gershberg & Shimamura, 1995), while the hippocampus is believed to create associations and retrieve them through an automatic process. The frontal lobe is linked to executive function, constituting this function as a mediator of free recall performance (Moscovitch & Winocur, 1992). Interestingly, a study by LeBlanc et al.'s (2010) failed to indicate a relationship between executive function and either estradiol or

testosterone. Thus, when considering verbal free recall, gonadal hormones do not appear to affect executive function and consequently the hippocampus may be most affected by hormones and responsible for producing the observed sex-related advantages / disadvantages in these types of tasks. Consequently, in verbal free recall tasks the observed female advantage / male disadvantage is hypothetically linked to the function of hippocampus, since the other cognitive mechanism that is found to be responsible for free recall performance (i.e. executive function) is not linked to hormones (at least post-natal hormones). Thus, the function of hippocampus (if this hypothesis stands true) should be linked to gonadal hormones.

As it was explained in Chapter 1 (Section 1.3), when it comes to hippocampus-dependent tasks, the hippocampus acts in a synergistic way with the amygdala. Considering that amygdala function (and structure) is also linked to gonadal hormones but in an opposite way[48], even if executive function is controlled in an experiment of free recall, amygdala function will contaminate the results and consequently will make interpretation of findings difficult. Thus, it is essential to be able to reduce (if not eliminate) the effects of amygdala in free recall performance in order to produce interpretable results. In other words, in order to be able to identify a reliable link between gonadal hormones and free recall performance it is necessary to eliminate (as much as possible) the effects of the amygdala (always controlling for the effects of executive function).

Following the hippocampus-amygdala hypothesis, the reduction of amygdala contribution may be achieved through a three-step free recall paradigm (see Chapter 2 for more details). However, the results presented in Chapter 2 did not lead to a conclusive result. More specifically, in Chapter 2 (Experiment 1) it was hypothesized that in a multi-trial (three-step) verbal word-list

[48] Masculinization of the brain leads to lower (and smaller) hippocampal function and higher (and larger) amygdala function, while the opposite is true for feminization (Chapter 1, Sections: 1.2, 1.3)

free recall paradigm, sex differences will be apparent in the third part of the task; that is, free recall of a word-list after the recall of a distracter word-list (which, theoretically, is linked to relatively isolated hippocampal function). However, results indicated that although there was a sex difference in free recall task performance overall, these differences did not vary across in any specific level of the task used. Following the hippocampus-amygdala hypothesis, the above result could be attributed to a factor that was not controlled in this experiment; that is, handedness. As mentioned in Chapter 2, handedness has been found to be linked to multi-trial free recall performance (Siengthai et al., 2008) as well as pre-natal hormone effects (Kelso et al., 2000). Thus, it was argued that this factor might be related to free recall performance in a way that reduced male-female performance differences. Consequently, in the study reported in this chapter only right handed individuals took part.

Based on the above, the hypothesis from the study reported in Chapter 2 was re-tested and extended, by adding an indirect assessment of pre-natal sexual hormones[49] (2D:4D), a direct measurement of saliva free-unbound testosterone and free-estradiol levels, and a measurement of the psychological constructs of systemizing and empathizing only on right-handed participants.

Thus, it was predicted that:

1. Female participant will demonstrate significantly better performance than males on a word-based verbal free recall task. Based on the hippocampus-amygdala hypothesis it was further predicted that the part of the free recall task that would demonstrate the strongest sex differences would be free recall after distraction, since this part of the free recall task is hypothesized to be related to hippocampal function; .

[49] The ratio of the second to forth finger (2D:4D) was measured from the right-hand of right-handed participants only in order to control for handedness and the consequent brain lateralization.

2. Based on the hippocampus-amygdala hypothesis, cognitive sex differences appear due to differences in the function of the hippocampus and amygdala only, whose structure and function is related to pre- and post-natal hormones. Thus, it is predicted that measures of pre-natal hormones (2D:4D) and post-natal hormones (testosterone, estradiol) will have a significant effects on free recall performance.

3. An individual's cognitive phenotype created by the calculation of the deviation of verbal free recall (FR) scores (sex-biased test) from the productive vocabulary (PV) scores will enable the creation of a 5-level brain-type-classification based on the PV-FR scores (see Experiment 1, Chapter 2) featuring individuals with much lower productive vocabulary scores than free recall scores (PV<<FR), individuals with lower productive vocabulary that free recall scores; (PV<FR), individuals with relatively balanced productive vocabulary and free recall scores; (PV≈FR), individuals with higher productive vocabulary than free recall scores; (PV>FR), and individuals with much higher productive vocabulary than free recall scores; (PV>>FR). It was predicted that it would be predominantly females who will demonstrate the PV<<FR cognitive phenotype, while as we proceed towards PV<FR, PV≈FR and PV>FR cognitive phenotypes, the presence of females will be reduced. Conversely, there will be fewer males on the brain sex continuum as we proceed from PV>>FR to PV>FR cognitive phenotype.

4. Individual masculinization / feminization levels, as defined by (1) sex only[50], (2) a combination of sex and pre-natal hormone effects[51], and (3) by a combination of pre-natal and

[50] Sex carries information regarding (1) sex related genetic differences (XX-XY) that affect brain structure, (2) The mediation of pre-natal hormones effects on the brain, and (3) The levels of post-natal gonadal hormone production; which information is significantly related to hippocampus-amygdala system structure and function (Chapter 1, Section 1.2).

[51] Sex and 2D:4D can potentially be an index of (1) The effects of pre-natal hormones on the brain and (2) Provide a more accurate index for the structural differentiation of amygdala (since this structure depends on sex-related genetic information; for more details see Chapter 3, Section 3.2.5, page 106).

post-natal hormone effects (hormonal profile[52]), will be associated with an individual's cognitive phenotype (cognitive brain sex). That is, more masculinized individuals are expected to appear a cognitive phenotype of PV>FR and the opposite is expected for feminized individuals.

5. Brain sex type categorization defined by the psychological measures of Systemizing and Empathizing will be associated with levels of individual masculinization / feminization defined by sex only, a combination of sex and pre-natal hormone effects and a combination of pre-natal and post-natal hormone effects. That is, more masculinized individuals are expected to show a cognitive phenotype of S>E and the opposite is expected for feminized individuals.

6. Brain sex type defined by the psychological measures of Systemizing and Empathizing will be associated with brain sex type defined by cognitive phenotype (cognitive brain sex).

3.2 Method

3.2.1 Participants

This sample consisted of 48 English-speaking participants, aged between 23 and 57 years old (M= 35.87, SD= 9.309) who were recruited using an opportunity sampling technique with Coventry University staff and students. There were 21 males aged between 25 and 53 years old (M = 36.05; SD = 7.70) and 27 females aged between 23 and 57 years old (M = 35.74; SD = 10.53).

[52] Using the Hormonal profile although genetic information from sex is partially excluded we still have structural and functional information related to gonadal hormones, The partial exclusion of genetic information refers to the fact that since post-natal gonadal hormone production is significantly related to sex, using post-natal hormones there is still some information from the genetic background of the participant.

3.2.1.1 Inclusion / Exclusion criteria

Only right-handed participants were included into the research in order to reduce[53] the impact of handedness on cognitive function. All participants were asked to self-rate their prior experience to Tower of Hanoi puzzle. Participants with prior experience with the Tower of Hanoi puzzle were excluded from the statistical analysis. Moreover, an effort was made to test participants over 25 years old in order to reduce the effects of excessive hormone production[54]. Moreover, only native English speaking participants were allowed to participate in this study, while participants who were proficient and frequent users of a second language were excluded, as discussed in Chapter 2. In total, 49 participants were assessed for eligibility on this study. From them 48 (1 excluded due to Tower of Hanoi experience) met the inclusion criteria and consent to participate.

3.2.2 Materials

Free recall: To measure word free-recall ability, two lists containing 15 words each were formed by the four word lists that were used for immediate and delayed free recall in the English Longitudinal Study of Ageing (ELSA; Hupert, Gardener, McWilliams, 2004). List A and B did not differ regarding their word-length ($t(28)=1.20$, $p=.240$) or frequency ($t(28)=.472$, $p=.641$; Appendix 4, Table 2).

Productive vocabulary: Language comprehension was measured by the vocabulary subtest of the "Wechsler Abbreviated Scale of Intelligence" battery (WASI) (1999) for ages from 8 to 89

[53] The impact of handedness on brain asymmetry and cognitive function is a potentially critical factor since several studies have reported a significant relationship between handedness and cognition (Johnston, Nicholls, Shah, Shields, 2012; Siengthai et al., 2008; Johnston, Nicholls, Shah, Shields, 2009). According to Siengthai et al. (2008) self-reported handedness is not as accurate measure of handedness as grip strength. Thus, using self-report as a measure of handedness is not expected to fully control for handedness.

[54] According to Kelsey et al. (2014) testosterone levels peak around the age of 19 years of life and then decreases gradually until the age of 40.

years. The WASI vocabulary subtest consists of 34 items of gradually increasing complexity from ‘Bird’ to ‘Panacea’. Responses to each item are credited based on the General scoring principles of the UK WASI manual, varying from zero points, (for presenting incomplete to extremely low word comprehension), one point, (for presenting adequate to low word comprehension), to a maximum of two points, (for presenting good to perfect word comprehension).

Digit Span: The digit span test consists of two measurements of digit span: forward digit span and backward digit span. The forward digit span test consists of 36 sets of numbers, where the sets have increasing quantities of digits, arranged into blocks. The first block has two numbers per set, the second block has three numbers per set, and so on until the final block which has nine numbers per set. Similarly, the backward digit span test consists of 30 sets of numbers, with increasing quantities of digits per set arranged into blocks. The first block has two numbers per set, and the final block has seven numbers per set. For both tests, each set is read aloud in turn by the researcher, at a rate of two digits per second. After each set is read aloud, the participant is required to recall the numbers in the same order (forward digit span) or in the reverse order (backward digit span). Each correct recall of a set is credited with one point, and the test is terminated when participants fail to two sets in the same block.

Tower of Hanoi: A computerized version of Tower of Hanoi, named Tower Mania, version 1.1.16 (Zsolt, 2012), with two difficulty levels was utilized; where moves and time needed to complete each difficulty level were automatically recorded for post-test analysis. The first level comprised a puzzle of three discs where the minimum number of moves required to solve the puzzle was seven. The second level comprised of a four-disc puzzle with a fifteen move solution as a minimum.

Testosterone and Estradiol assays: For the collection and analysis of testosterone and estradiol, the 'Salimetrics Salivary Testosterone Enzyme Immunoassay Kit' and the 'Salimetrics Salivary Estradiol (17-beta-estradiol) Enzyme Immunoassay Kit' were used.

The 'Salimetrics Salivary Testosterone Enzyme Immunoassay Kit' has a .96 serum-saliva correlation and sensitivity of 1pg/mL. Sample test volume is set on 25mL; while the recommended collection volume is 75mL.[55]

The 'Salimetrics Salivary Estradiol (17-beta-estradiol) Enzyme Immunoassay Kit' has a .80 serum-saliva correlation and sensitivity of 0.1pg/mL. Sample test volume is set on 100mL; while the recommended collection volume is 225mL.[56] Directions from Salimetrics suggest that due to potential fluid loss during handling another 300mL are mandatory. Thus, 2ml were collected for safety.

Second and Forth digit length (2D:4D): The lengths of the participants' second and fourth fingers of their right hand were measured in millimetres using electronic callipers measuring to 0.1 millimetres. The length of each finger was measured from the ventral surface of the hand, from the lowest line at the base of the finger (basal crease) to the tip of the finger. Two consecutive measures were made by the researcher and the mean value was used as the 2D:4D variable. Only a direct measure of 2D:4D was the measure of choice instead of using both direct (by the researcher) and indirect (from photocopies of the measured hand) measurements. This choice was made based on the paper of Manning, Baron-Cohen, Wheelwright and Fink (2010) who argued that 2D:4D measured by photocopies may be an inaccurate measure due to

[55] For further details regarding the exact process of saliva analysis please see supportive document 'Salivary Testosterone immunoassay kit' at https://www.salimetrics.com/assets/documents/1-2402n.pdf.
[56] For further details regarding the exact process of saliva analysis please see supportive document 'Salivary Estradiol immunoassay kit' at https://www.salimetrics.com/assets/documents/1-3702n.pdf.

individual differences in the level of pressure that the participants apply on the photocopy machine (see also Manning, Fink, Neave, Caswell, 2004). Thus, Manning et al. (2010) concluded that direct measurement (by a researcher) may be a more accurate method. The above arguments raised by Manning et al. (2010) are supported by a meta-analysis by Honecopp and Watson (2010) who reviewed and compared 62 studies that used 2D:4D indirect and direct measurements and concluded that indirect measurement of 2D:4D distort the measurement upwards resulting in higher 2D:4D ratios.

3.2.3 Design

Similar to that used in Chapter 2, this study used a mixed design. The dependent variable was free recall performance (number of words recalled) as measured by a free recall assessment developed from items used in the English Longitudinal Study of Ageing (ELSA). This test itself was a repeated measures independent variable, comprising three levels: List A (max 15), List B (max 15), and List A after distraction (that is, after List B had been administered; max 15). The between groups independent variable was sex, with two levels; male and female.

There were a number of covariates included in the design of this study, which were as follows: age measured in years; the number of moves taken to complete the Tower of Hanoi task and the time taken to complete it (this was recorded for two levels of difficulty of this task); digit span score (forward digit span: maximum score 36, and backward digit span: maximum score 30); productive vocabulary scores (maximum score 68); pre-natal gonadal hormone effects (measured by the indirect index of 2D:4D; measuring finger length in millimeters); and circulating gonadal hormones (measured in Pg/mL).

The Tower of Hanoi was used as a proxy for cognitive differences that tap executive function including planning and learning (see Rainville et al., 2002; Welsh & Huizinga, 2005; Sergeant et al., 2002; Vakil et al., 2014). Digit span was used as a proxy for verbal short-term and working memory differences (see Jones & Maken, 2016; Rosenthal et al., 2006). The second to fourth finger ratio (2D:4D) was used as a relative index of the effects of maternal circulating sexual hormones on the foetal brain, and specifically as an index of the level of pre-natal feminization or de-feminization of the brain (see Section 1.9.3 or alternatively see Zhengui and Cohn, 2011). Finally, levels of circulating testosterone and estradiol were also taken under consideration since relationship between gonadal-hormones and cognitive performance on sex-biased tasks has been reported by previous studies (Kozaki & Yasukouchi, 2008; Rossenbaum & Park, 2002)

In addition, systemizing and empathizing were assessed in order to recreate the measure of brain sex reported in Goldenfeld et al. (2005). That was done in order to compare, and potentially validate, the cognitive measure of brain sex that was created from free recall and productive vocabulary performance of participants (previously explained and demonstrated in Chapter 2).

3.2.4 Procedure

Ethical approval for this study was obtained from Coventry University's Ethics committee. A briefing was given to each participant prior to the experimental procedure and written consent was obtained (Appendix 5).

The procedure that was followed was exactly as described in Experiment 1, Chapter 2, Section 2.2.4, page 76.

Regarding free recall, there were three measures that were scored:

1. Correctly recalled words from list 1; this variable was labelled *free recall 1*

2. Correctly recalled words from list 2 (distracter); this variable was labelled *free recall 2.*

3. Correctly recalled words from list 1 after the distracter list; this variable was labelled *free recall 3.*

Regarding productive vocabulary, the final score of *language comprehension* for each participant was produced based on the sum of each item's awarded credits.

Approximately 2ml of saliva was collected via unstimulated passive drooling. Each participant was provided with a plastic straw and a plastic sterilized tube (provided by Salimetrics), and he / she was advised to force saliva to flow from the straw to the tube until the desirable saliva volume was reached. Next, each tube was sealed, tagged and frozen at -25C at the time of the collection. Participants were asked to avoid eating or drinking anything except plain water for at least 45 minutes prior to saliva collection. Frozen saliva samples were sent to Salimetrics in order to be analysed.

Following a five minute break, the digit span task was administrated as described above. The forward digit span test was administered first and the backward digit span second.

Following a five minute break, the Tower of Hanoi task was administered. Participants who reported prior experience of this task were excluded from the analysis. A Samsung Galaxy Note 10.1 inch tablet with "Tower Mania" (Zsolt, 2012) loaded was handed to the participant followed by an introduction to the game by the researcher, explaining the goal and rules of the game. Each participant was asked to solve three puzzles, starting from a three disk task first (easy / practice),

followed by a four disk task. After each task, the researcher noted the number of moves and time that the participant took to complete the task, which was automatically recorded on the tablet.

Next, the length of the second (index finger) and fourth (ring finger) digit of the right hand of each participant was measured by the researcher, using the electronic callipers.

Finally, the questionnaires that assess systemizing and empathizing were administrated with this order.

3.2.5 Data analysis

All data were analysed using IBM SPSS Version 22.0 (IBM Corp., Armonk, NY, USA). Data analysis is divided into five parts. Each part begins with the descriptive statistics of the variables of interest that first appear in the respective part of the analysis. Specifically, mean values and standard deviation of the variables were computed and presented, while simple independent samples t-tests were used to explore differences between males and females in baseline scores on measured cognitive domains, demographic variables and hormone levels. In case of free recall scores, a Bonferroni correction was applied, which reduces the cut off for the significance level to p= 0.016. A further analysis was performed for the 2D:4D variable in order to explore if the current sample's scores and effect size of the observed sex-difference fall within the general population values. A meta-analysis of seven studies that used the same type of 2D:4D measurement with the current study was therefore performed for benchmarking purposes (Appendix 6). Saliva samples were analysed by Salimetrics, and raw data were provided in an excel file. All samples were measured in duplicates for each analysis. The intra-assay coefficient of variability (CV %) was less than 10%, thus the criterion for repeatability / precision of immunoassay test results was met.

Part 1: Exploration of sex differences in free recall task

Four models were formed to explore the effects of sex on free recall performance (in all levels) in order to indicate which types of free recall performance test were best differentiated by sex. Moreover, any interactions between factors, after controlling for the influence of variables which could contribute to the effect, were also investigated. Pearson's correlation was therefore used to check for any relationships between free recall and other variables that had been indicated by previous literature to be associated with free recall performance. These models are described below:

Model 1: Dependent variable is free recall scores, obtained across three conditions (FR1, FR2, FR3) and the between – subjects factor is the variable sex. The model was tested using mixed ANOVA.

Model 2: This analysis was the same as Model 1, but in this case relevant covariates were included. This model was tested using a mixed factorial ANCOVA.

Model 3: This was the same with Model 2, but included an additional variable that captured an individual's hormonal profile as covariate. In order to create this additional variable the participants' estradiol to testosterone ratio and 2D:4D were transformed into z-scores and added together. Since higher values of both ratios indicate higher feminization, and previous literature has indicated that both pre-natal and post-natal feminization / masculinization affect cognitive performance, then it is argued that the combination of these variables can provide more information regarding the effects of gonadal hormones on the brain. Specifically, the effects of post-natal gonadal hormones on the brain structure and function appear to be significantly related to the effects of pre-natal gonadal hormones (Chapter 1, Section 1.2). That is, post-natal

masculinization / feminization of the brain appears to be significantly linked to pre-natal masculinization / feminization of the brain. Thus, it is argued that in order to have a precise measure of the effects of gonadal hormones on the brain it is necessary to take into account pre-natal effects in relation to post-natal hormone effects[57]. Following the above rationale the variable below was created.

(Z-e/t ratio + Z-2D:4D ratio) /2 = Hormonal Profile

Model 4: This model is a follow up analysis of the previous ANCOVA analysis (Model 3). In this analysis the dependent variable is free recall scores (FR1, FR2, FR3) and the variables that have emerged from the correlation analysis or the theoretical background as well as hormonal profile were used as independent variables. No between subjects factor is included in this model.

Part 2: Classification of 'cognitive brain sex type' based on measures of productive vocabulary and free recall, and consideration of whether it is associated with degrees of masculinization / feminization based on (a) males/females (b) sex and 2D:4D, and (c) e/t ratio and 2D:4D classifications.

Following the procedure reported in Chapter 2, the difference between participants' performance on free recall after distraction and their productive vocabulary scores was calculated to achieve a categorisation of cognitive brain sex.

For defining masculinization / feminization levels, participants were grouped as follows:

(a) The first classification was based only on sex (males / females).

[57] As it was argued in Chapter 1, Section 1, the effects of gonadal hormones (pre- and post-natal) on the brain are affected by the genetic profile of each individual. Thus, ideally, an individual's hormonal profile would also include his/ hers genetic profile.

(b) The second classification (sex and 2D:4D) was based on the notion that 2D:4D is a proxy for individual susceptibility to pre-natal hormonal influences. Thus, the factors of sex and 2D:4D were combined in order to create a more accurate proxy for pre-natal hormone effects. In Chapter 1, Section 1.2.1, was suggested that maternal circulating hormone levels (pre-natal gonadal hormones) affect males and females differently. Thus, individual susceptibility to pre-natal hormones (2D:4D) along with the susceptibility to maternal circulating hormone levels (due to the sex of the foetus) can potentially provide a more accurate proxy for the effects of pre-natal hormones to the brain. For that reason, four sub-groups were created featuring males with 2D<4D (MM), males with 2D>4D (M), females with 2D<4D (F) and females with 2D>4D (FF). This sub-grouping was based on previous researchers that have indicated a general tendency of males for having prolonged forth finger compared to their second finger while in females both fingers are either equal or it is observed a prolonged second finger compared to their forth finger (Zheng & Cohn, 2011; Manning, Scutt, Wilson, Lewis-Jones, 1998). Moreover, previous studies (albeit within females only; Csatho et al., 2003), have indicated that females with male-type 2D:4D ratios perform better on a male-biased task (spatial navigation) compared to females with female 2D:4D ratio. Based on these results Csatho, et al. (2003) argued that the effects of prenatal steroids may also affect within-sex variation. Thus, it is expected that when this grouping is applied in both males and females (cognitive) differences will be enhanced (at least) between the two 'extreme' groups (that is, males with 2D<4D and females with 2D>4D).

(c) In a third classification, the estradiol to testosterone ratio and 2D:4D were used to create groups based specifically on individual hormonal profile. Specifically, the levels of e/t ratio where grouped as high / low based on the samples' median value (median=.0298); that is, e/t ratio values above median were labelled as 'high' and below or equal to median as 'low'. Then,

four groups were formed based on e/t ratio grouping and 2D:4D; featuring low e/t ratio and 2D<4D (extreme masculinized), low e/t ratio and 2D>4D (masculinized), high e/t ratio and 2D<4D (feminized) and high e/t ratio and 2D<4D (extreme feminized).

Part 3: Exploring systemizing / empathizing scores in relation to degrees of masculinization / feminization based on (a) males/females (b) sex and 2D:4D, and (c) e/t ratio and 2D:4D classifications.

Following the procedure described in Goldenfeld et al. (2005), systemizing scores and empathizing scores were standardised. Thus, Sstd= (S-<S>)/150, and Estd= (E-<E>)/80 where <..> indicates general population mean scores divided by the maximum possible score. Instead of using mean scores from this sample, the means from the Baron-Cohen et al. (2014) paper were used, since these means are more representative of the general population[58]. Thus, a mean score of 59.66 was used for systemizing and 44.87 for empathizing. According to Goldenfeld et al. (2005), score deviation (for short referred as D_{s-e}) of empathizing scores, from systemizing scores indicates / differentiate two different cognitive system configuration. That is, D_{s-e} is hypothesized to be able to differentiate a cognitive system that is more inclined to empathize (that is, an individual scoring lower in systemizing compared to sympathizing) from a cognitive system which is more inclined to systemize (that is, an individual scoring higher in systemizing compared to empathizing). Thus the following variable was created:

D_{s-e}= (Sstd-Estd)/2 (i.e. the difference between the normalized Systemizing and Empathizing scores)

[58] These means derive from a sample of 3.906 individuals

A Jonckheere-Terpstra test for ordered alternatives was then conducted to test the existence of a systematic increase / decrease between levels of masculinization / feminization, as measured by the three different groupings mentioned in Part 2, and the systemizing-empathizing assessment of brain sex.

Part 4: Associations between systemizing-empathizing and cognitive brain sex categorization.

This step is to consider whether the two measures of cognitive brain sex (derived from free recall / productive vocabulary scores and systemizing / empathizing scores respectively) are related to each other. This was explored through a Jonckheere-Terpstra test for ordered alternatives.

3.3 Results

Part 1: Exploration of sex differences in free recall task

A series of independent samples t-tests were performed in order to assess base-line differences between males and females in key variables such as free recall (all three levels of free recall), productive vocabulary, measures of cognitive function, pre-natal hormone levels as well as circulating hormone levels (Table 6).

Table 6. Descriptive Statistics and Results of t-tests by Sex for cognitive scores, age (in years), 2D:4D (in millimetres), estradiol to testosterone ratio (in Pg/mL) and hormone profile of the sample by Sex.

Variable	Group				95% CI for Mean Difference				Hedge's g_s
	Male (n=21)		Female (n=27)						
	M	SD	M	SD		t	P	df	
Free recall 1	7.67	2.05	9.11	1.98	-4.89,-0.01	-2.52	.02	46	.78
Free recall 2	7.24	1.67	8.11	2.15	-4.92,0.01	-1.50	.14	46	.42
Free recall 3	3.52	2.20	6.07	2.26	-5,19,-0.08	-4.03*	<.001	46	1.17
Productive vocabulary	43.62	12.07	43.80	9.98	29.35,6.381	-0.05	.965	46	.016
Forward digit span	25.10	4.39	23.85	4.45	14.81,3.83	.965	.339	46	.27
Backward digit span	17.05	5.39	16.52	3.99	6.52,3.53	.390	.698	46	.11
Total moves (ToH)	169.52	70.22	162.48	85.13	116.66,52.19	.306	.761	46	.087
Total time (ToH)	428.71	356.4	368.65	255.73	235.74,245.32	.680	.500	46	.19
Age (years)	36.05	7.704	35.74	10.53	23.04,5.61	.117	.911	46	.032
2D:4D[59]	.999	.045	1.021	0.45	-6.876,0.15	-1.217	.102	46	.48
E/T ratio	.0213	.007	-3.91	.366	-11.81,-.56	-7.470*	<.001	46	2.18
Hormonal Profile	-.5191	0.551	0.435	0.435	-8.54, -0.65	-6.46	<.001	46	1.91

Note: first list free recall (free recall 1), second list free recall (free recall 2), free recall of the first list after distraction (free recall 3), productive vocabulary, forward digit span, backward digit span, total moves performed in the two difficulty levels of Tower of Hanoi puzzle (total moves), total time for completing the two difficulty levels of Tower of Hanoi puzzle (total time). Logarithmic transformations were used for free recall 1, 2, 3 and e/t ratio. Bonferroni Correction was applied for free recall; alpha level was set at 0.016.

As it can be seen in Table 6, significant sex differences appear in free recall 3, e/t ratio and hormonal profile (all at p<.001), while no other sex differences were observed at baseline level.

A Pearson's correlation analysis was performed among the variables that prior studies have indicated to be potential 'mediators' of free recall performance and the indices of hormone levels (Table 7).

[59] An evaluation of the 2D:4D findings may be found in Appendix 6.

Table 7. Simple correlations among age, Tower of Hanoi moves (ToH moves), Tower of Hanoi time (ToH time), forward digit span (FDS), backward digit span (BDS), second to forth digit ratio (2D:4D), estradiol to testosterone ratio (e/t ratio) and hormone profile.

	Productive vocabulary	**ToH moves**	**ToH time**	**FDS**	**BDS**	**2D:4D**	**E/T ratio**	**Hormonal profile**
Age	-0.109	-0.074	0.105	-0.01	-0.047	-0.177	0.01	-0.09
Productive vocabulary		-0.191	-0.400*	0.318	0.433*	-0.058	0.081	0.016
ToH moves			0.749*	0.011	0.006	-0.159	0.074	-0.069
ToH time				-0.085	-0.168	-0.234	-0.004	-0.163
FDS					0.551*	0.154	-0.003	0.096
BDS						-0.158	0.002	-0.079
2D:4D							0.238	0.788*
E/T ratio								0.786*

* $p < 0.05$

Table 7 shows that there are six statistically significant correlations. Specifically, there is a statistically significant (1) weak and negative correlation between productive vocabulary and ToH time, (2) weak and positive correlation between productive vocabulary and BDS, (3) strong and positive correlation between ToH moves and ToH time, (4) moderate and positive correlation between FDS and BDS, (5) strong positive correlation between hormonal profile and 2D:4D, (6) strong positive correlation between hormonal profile the e/t ratio.

All the results of Two-Way Mixed AN(C)OVA's are presented below with all effects reported as significant at p<.05. The covariates chosen for the analysis were:

1. Backward digit span, because it appears to correlate with forward digit span and productive vocabulary, according to Table 3. The choice of BDS over FDS and PV is based on the ground that it includes the other two and theoretically is considered to be a measure of working memory.

2. Tower of Hanoi moves, because it was strongly correlated with Tower of Hanoi time, and is used here (following previous studies) as a proxy for frontal lobe function,

3. Age, since previous research has indicated age as a potential mediator of free recall performance (Krueger & Salthouse, 2010),

4. Hormonal profile (since it combines both hormone measures; pre- and post-natal).

The choice of the covariates was done in this way in order to reduce the number of covariates entered into each model, to preserve statistical power.

Table 8 provides a summary of the results from Model 1 (unadjusted) to Model 3 (fully adjusted). It can be seen that adding the covariates age, backward digit recall and Tower of Hanoi moves (Model 2) reduces the condition effect from η_p^2=.672 to η_p^2=.171; while the effects of group as well as the group*condition interaction are increased. Finally, it can be seen that all effects are eliminated in the fully adjusted model (Model 3), with only the effect of condition remaining significant; albeit being weaker than all the other models.

Table 8. Summary of models 1, 2, 3, displaying means and standard deviation (adjusted means and standard error for models 2, 3), the effect of sex (group effect), the effect of free recall overall (condition effect) and the interaction between sex and free recall overall (interaction effect).

	Grouping Variable		Group effect		Condition effect		Interaction effect	
	Males	Females						
	Mean Scores (SD or SE)		p	η_p^2	p	η_p^2	p	η_p^2
Model 1: Unadjusted								
Free recall 1	7.67 (2.058)	9.11 (1.987)						
Free recall 2	7.24 (1.670)	8.11 (2.154)	0.003	0.179	<.001	0.672	0.011	0.093
Free recall 3	3.52 (2.205)	6.07 (2.269)						
Model 2: Adjusted for Age, Backward Digit Span and Tower of Hanoi								
Free recall 1	7.69 (.445)	9.101 (.393)						
Free recall 2	7.253 (.420)	8.100 (.370)	0.003	0.191	<.001	0.173	0.007	0.116
Free recall 3	3.482 (.480)	6.107 (.423)						
Model 3: Model 2 + Hormonal Profile								
Free recall 1	7.929 (.507)	8.981 (.444)						
Free recall 2	7.318 (.483)	8.128 (423)	0.081	0.073	0.001	0.166	0.537	0.014
Free recall 3	4.110 (.522)	5.642 (.458)						

Model 1 (unadjusted)

In the first model (Table 9) the effects and interactions that appeared in the unadjusted model are presented. Mauchly's test indicated that the assumption of sphericity was met for the main effect of free recall, $\chi^2(2) = .899$, $p = .091$. Moreover, Levene's test indicated that the assumption of homogeneity of variance was met for free recall 1 (p= .832), free recall 2 (p= .117) and free recall 3 (p= .689).

Table 9. Model 1: Two-Way Mixed ANOVA summary table

	Grouping Variable			
	Total	**Males**	**Females**	**Difference**
N (%)	48 (100.00%)	21 (43.75%)	27 (56.25%)	
Mean Scores (SD)				
Free recall 1	8.48 (2.124)	7.67 (2.058)	9.11 (1.987)	-1.44 (0.587)*
Free recall 2	7.73 (1.987)	7.24 (1.670)	8.11 (2.154)	-0.87 (0.570)
Free recall 3	4.96 (2.560)	3.52 (2.205)	6.07 (2.269)	-2.55 (0.652)*
Differences between:				
Free recall 1 - Free recall 2	0.71 (0.276)*	0.43 (0.413)	1.00 (0.365)*	
Free recall 1 - Free recall 3	3.59 (0.236)*	4.14 (0.355)*	3.04 (0.313)*	
Free recall 2 - Free recall 3	2.88 (0.313)*	3.71 (0.470)*	2.04 (0.414)*	
Effects	**P-values**	**η_p^2**		
Group effect	0.003	0.179		
Condition effect	<.001	0.672		
Interaction effect	0.011	0.093		

Note. The difference between sexes is computed with males as reference. * $p < .05$

The results showed that there was:

- A significant main effect of free recall condition ($F(2, 92) = 94.202$, $p < .001$, $\eta_p^2 = .672$) on free recall scores (condition effect), with first list free recall (FR1) scores being higher than second list free recall (FR2) scores, while both scores are also higher than the recall of the first list after distracter (FR3) scores ($M_{FR1} > M_{FR2} > M_{FR3}$).
- A significant main effect of sex (group effect) on free recall scores overall ($M_{Males,FR} < M_{Females,FR}$; $F(1, 46) = 10.021$, $p = .003$, $\eta_p^2 = .179$).
- A significant interaction effect between free recall condition and sex ($F(2, 92) = 4.742$, $p = .011$, $\eta_p^2 = .093$). Specifically, females seem to score higher than males in cases of the first list free recall (FR1; $M_{Males,FR1} < M_{Females,FR1}$; $p = .018$, $\eta_p^2 = .116$) and the first list free recall after distracter (FR3; $M_{Males,FR3} < M_{Females,FR3}$; $p < .001$, $\eta_p^2 = .250$). Moreover, for males, there was a significant simple effect of free recall condition on free recall scores, with first list free recall

(FR1) scores and second list free recall (FR2) scores being higher than the recall of the first list after distracter (FR3) scores ($M_{FR1}=M_{FR2}>M_{FR3}$). For females, there was a significant simple effect of free recall condition on free recall scores, with first list free recall (FR1) scores being higher than second list free recall (FR2) scores, while both scores are also higher than the recall of the first list after distracter (FR3) scores ($M_{FR1}>M_{FR2}>M_{FR3}$).

Model 2 (adjusted for age, backward digit span and Tower of Hanoi)

Table 10 presents the effects and interactions that appeared in the second model adjusted for age, backward digit and Tower of Hanoi model. Mauchly's test indicated that the assumption of sphericity was not met for the main effects of free recall, $\chi^2(2) = .865$, $p = .047$. Moreover, Levene's test indicated that the assumption of homogeneity of variance was met for free recall 1 ($p= .762$), free recall 2 ($p= .097$) and free recall 3 ($p= .304$). These results showed that there was:

- A significant main effect of free recall condition ($F(1.761, 75.738) = 9.000$, $p <.001$, $\eta_p^2 = .173$) on free recall scores (condition effect), with first list free recall (FR1) scores being higher than second list free recall (FR2) scores, while both scores were also higher than the recall of the first list after distracter (FR3) scores ($M_{FR1}>M_{FR2}>M_{FR3}$).
- A significant main effect of sex (group effect) on free recall scores overall ($M_{Males,FR}<M_{Females,FR}$; $F(1, 43)= 10.177$, $p=.003$, $\eta_p^2= .191$).
- A significant interaction effect between free recall condition and sex ($F(1.761, 75.738) = 5.622$, $p = .007$, $\eta_p^2= .116$). Specifically, according to simple effect analysis, females seem to score higher than males in cases of the first list free recall (FR1; $M_{Males,FR1}<M_{Females,FR1}$; $p=.021$, $\eta_p^2 = .118$) and the first list free recall after distracter (FR3; $M_{Males,FR1}<M_{Females,FR1}$; $p<.001$, $\eta_p^2 = .281$). Moreover, for males, a significant simple effect of free recall condition on free recall

scores, with first list free recall (FR1) scores and second list free recall (FR2) scores being higher than the recall of the first list after distracter (FR3) scores ($M_{FR1}=M_{FR2}>M_{FR3}$). For females, a significant simple effect of free recall condition on free recall scores appeared, with first list free recall (FR1) scores being higher than second list free recall (FR2) scores, while both scores are also higher than the recall of the first list after distracter (FR3) scores ($M_{FR1}>M_{FR2}>M_{FR3}$).

- A significant interaction effect between free recall condition and Tower of Hanoi ($F(1.761, 75.738) = 4.001$, $p = .027$, $\eta_p^2 = .085$). Specifically, a statistically significant difference appears between the first list free recall (FR1) scores and the recall of the first list after distracter (FR3) scores ($F(1,43)=8.127$, $p = 0.007$, $\eta_p^2 = .159$) as well as between the second list free recall (FR2) scores and the recall of the first list after distracter (FR3) scores ($F(1,43)=5.339$, $p = 0.026$, $\eta_p^2 = .110$).

Table 10. Model 2: Two-way mixed ANCOVA summary table

		Grouping Variable		
	Total	**Males**	**Females**	**Difference**
N (%)	48 (100.00%)	21 (43.75%)	27 (56.25%)	
Adj.Mean Scores (Standard Error)				
Free recall 1	8.39 (.296)	7.69 (.445)	9.10 (.393)	-1.422 (0.594)*
Free recall 2	7.67 (.280)	7.253 (.420)	8.10 (.370)	-0.847 (0.561)
Free recall 3	4.79 (.319)	3.482 (.480)	6.107 (.423)	-2.625 (0.640)*
Mean Differences between:				
Free recall 1 - Free recall 2	0.71 (0.286)*	0.42 (0.426)	1.00 (0.376)*	
Free recall 1 - Free recall 3	3.59 (0.216)*	4.19 (0.325)*	2.99 (0.032)*	
Free recall 2 - Free recall 3	2.88 (0.302)*	3.77 (0.454)*	1.993 (0.00)*	
Effects	**P-values**	**η_p^2**		
Group effect	0.003	0.191		
Condition effect	<.001	0.173		
Interaction effect	0.007	0.116		

Note. The difference between sexes is computed with males as reference. Model controlling for age, backward digit span, Tower of Hanoi. Greenhouse-Geisser criterion applied.
* $p < .05$

Model 3 (adjusted for age, Tower of Hanoi, backward digit span and hormone profile effects)

Finally, the 'hormonal profile' variable was added to Model 2 where we controlled for cognitive differences (for a summary see Table 11). Thus, in this model (Model 3) the effects of age, Tower of Hanoi (moves), backward digit span and hormonal profile were controlled. Table 11 presents the effects and interactions for this model. Mauchly's test indicated that the assumption of sphericity was not met for the main effects of free recall, $\chi^2(2) = .856$, $p = .045$. Moreover, Levene's test indicated that the assumption of homogeneity of variance was met for free recall 1 ($p = .996$), free recall 2 ($p = .182$) and free recall 3 ($p = .165$). The results showed that there was:

- A significant main effect of free recall condition ($F(1.749, 71.696) = 8.170$, $p = .001$ $\eta_p^2 = .166$) on free recall scores (condition effect), with first list free recall (FR1) scores being higher than second list free recall (FR2) scores, while both scores are also higher than the recall of the first list after distracter (FR3) scores ($M_{FR1} > M_{FR2} > M_{FR3}$).
- No significant main effect of sex (group effect) on free recall scores overall ($F(1, 41) = 3.210$, $p = .081$, $\eta_p^2 = .073$).
- No significant interaction effect between free recall condition and sex ($F(1.749, 71.696) = .586$, $p = .559$, $\eta_p^2 = .014$).
- A significant interaction effect between free recall condition and Tower of Hanoi ($F(1.749, 71.696) = 4.786$, $p = .014$, $\eta_p^2 = .105$). Specifically, according to simple effect analysis, a statically significant difference appears between the first list free recall (FR1) scores and the recall of the first list after distracter (FR3) scores ($F(1,41) = 9.914$, $p = 0.003$, $\eta_p^2 = .195$) as well as between the second list free recall (FR2) scores and the recall of the first list after distracter (FR3) scores ($F(1,41) = 6.772$, $p = 0.013$, $\eta_p^2 = .141$),

Table 11. Model 3: Two-way mixed ANCOVA summary table

	Grouping Variable			
	Total	**Males**	**Females**	**Difference**
N (%)	48 (100.00%)	21 (43.75%)	27 (56.25%)	
Adj. Mean Scores (Standard Error)				
Free recall 1	8.45 (.296)	7.929 (.507)	8.981 (.444)	-.970 (0.741)
Free recall 2	7.72 (.282)	7.318 (.483)	8.128 (.423)	-0.706 (0.728)
Free recall 3	4.87 (.305)	4.110 (.522)	5.642 (.458)	-1.415 (0.794)
Mean Differences between:				
Free recall 1 - Free recall 2	0.73 (0.292)*	0.611 (0.499)	.852 (0.438)	
Free recall 1 - Free recall 3	3.57 (0.212)*	3.81 (0.363)*	3.33 (0.318)*	
Free recall 2 - Free recall 3	2.84 (0.295)*	3.20 (0.504)*	2.486 (0.442)*	
Effects	**P-values**	η_p^2		
Group effect	0.081	0.073		
Condition effect	0.001	0.166		
Interaction effect	0.537	0.014		

Note. The difference between sexes is computed with males as reference. Model controlling for age, Tower of Hanoi, backward digit span, hormonal profile (pre-natal and post-natal hormones). Greenhouse-Geisser criterion applied.
* $p < .05$

Model 4

Following the results obtained from Model 3, the next step was to explore the effects of hormonal profile on the model excluding the between subjects factor (i.e. sex). The elimination of the sex*free recall interaction after the insertion of the 'hormonal profile' that was observed in Model 3 suggests that the effect of the variable 'hormonal profile' differs across the two levels of the factor 'sex', which indicates a violation of the assumption of the 'homogeneity of regression slopes' (Field, 2009). However, since the main categorical variable is observed and not manipulated (i.e. the factor sex), the assumption of 'homogeneity of regression slopes' is not of relevance (Keppel & Wickens, 2004). Thus, the finding that the covariate 'hormonal profile' eliminated the effects of the between subjects factor (i.e. sex) can be interpreted as an indication that the covariate 'hormonal profile' provides largely the same information as the factor sex.

In order to explore the difference in effect size on the free recall task between the factor sex and the covariate hormonal profile a linear model was formed without the use of the between subjects factor (i.e. sex). Thus, in this model all covariates were entered (age, Tower of Hanoi, backward digit span and hormonal profile) without the between-subjects factor (i.e. sex).

Using a Greenhouse-Geisser correction, the results indicated that the main effect of free recall condition on free recall scores was significant ($F(1.752, 73.570) = 8.030$, $p=.001$ $\eta_p^2 = .161$). Moreover, the variable 'hormonal profile' produced significant between-subject effects ($F(1, 42) = 10.918$, $p=.002$ $\eta_p^2 = .206$). A significant interaction between 'hormonal profile' and free recall appeared also ($F(1.752, 73.570) = 7.956$, $p=.001$ $\eta_p^2 = .159$). In addition, the interaction between 'Tower of Hanoi (moves)' and free recall was also significant ($F(1.752, 73.570) = 4.918$, $p=.013$ $\eta_p^2 = .105$).

The above results are in accordance with the expected findings in relation to the effect of the 'hormonal profile' on free recall scores. In order to further explore the effects of hormonal profile within each level of the free recall paradigm, the parameter estimates of three regressions with the dependent variable being each of the three types of free recall scores are presented in Table 12.

Table 12. Parameter Estimates of Model 4 (Dependent Variables: FR1, FR2 and FR3 / Independent Variables: Age, Backward Digit Span, Tower of Hanoi Moves, Hormonal Profile)

Dependent Variable	**FR1**			**FR2**			**FR3**		
Independent Variables	**Coefficient**	**p - value**	**η^2_p**	**Coefficient**	**p - value**	**η^2_p**	**Coefficient**	**p - value**	**η^2_p**
Constant	8.767	0.000	0.356	8.650	0.000	0.376	3.073	0.116	0.058
Age	-0.013	0.687	0.004	-0.034	0.289	0.027	-0.027	0.444	0.014
Backward Digit Span	0.061	0.383	0.018	0.079	0.232	0.034	0.132	0.079	0.072
Tower of Hanoi Moves	-0.005	0.224	0.035	-0.006	0.105	0.061	0.004	0.344	0.021
Hormonal Profile	0.939	0.019	0.124	0.493	0.183	0.042	1.838	0.000	0.328

According to Table 12, the variable hormonal profile appears to have a statistically significant positive effect on both FR1 free recall of List 1 (b_4 = .939, p<0.05) and FR3 free recall of List 1 after distraction (b_4 = 1.838, p<0.05). Specifically, an increase of the hormonal profile value by one unit increases the FR1 by .939 units and FR3 by 1.838 units. Moreover, the effect size indicates that this specific variable explains 12.4% of the variability of FR1 and 32.8% of the variability of FR3. As a result, the parameter estimates present adequate evidence that the variable hormone profile has a greater impact on FR3 than in FR1 ($\eta^2_{p,FR1}$ = .124 < .328 = $\eta^2_{p,FR3}$).

Part 2: *Classification of 'cognitive brain sex type' based on measures of productive vocabulary and free recall, and consideration of whether it is associated with degrees of masculinization / feminization based on (a) males/females (b) sex and 2D:4D, and (c) e/t ratio and 2D:4D classifications.*

(a) Males / Females Classification

Following the above findings that indicated that free recall after distraction (FR3) was more strongly related to hormonal profile than FR1 was, and given the hypothesis that free recall after distraction (FR3) may serve as an index of hippocampal performance relatively isolated from the synergistic action of amygdala, FR3 was used in the classification of cognitive brain sex along with productive vocabulary, as was done in Chapter 2. According to these findings, $D_{pv\text{-}fr}$ and $C_{pv\text{-}fr}$ are created (see Data analysis section for the exact process of creation and use of $D_{pv\text{-}fr}$ and $C_{pv\text{-}fr}$; <PV>=43.73; <FR3> = 4.96). Table 13 shows the mean scores for the two variables as well as the results of the comparisons of the created variables between males and females. As it can be seen, both $D_{pv\text{-}fr}$ and $C_{pv\text{-}fr}$ variables appear to be significantly differentiated by sex.

Table 13. Descriptive statistics and results of t-tests for the difference between standardized productive vocabulary test and the standardized free recall after distracter (D) and the combined scores (C) by sex

Variable	Group				95% CI for Mean Difference	t	p	df	Hedge's gs
	Male		Female						
	M	SD	M	SD					
$D_{pv\text{-}fr}$	0.058	0.113	-0.005	0.069	-7.7, 0.17	3.368*	0.001	23.436	1.16
$C_{pv\text{-}fr}$	-0.059	0.144	0.511	0.143	-7.7, -0.013	2.319*	0.026	46	0.75

As seen in Figure 4, there is an observable differentiation of the data along the D axis, but not along the C axis. Specifically, in Figure 4 in which the D axis runs from the bottom right to the top left corner, it can be seen that the majority of male population (marked as cycles) lies within the positive side of the D axis (that is, top left corner), whilst the majority of the female population (marked as squares) lies within the negative side of the D axis (that is, bottom right corner).

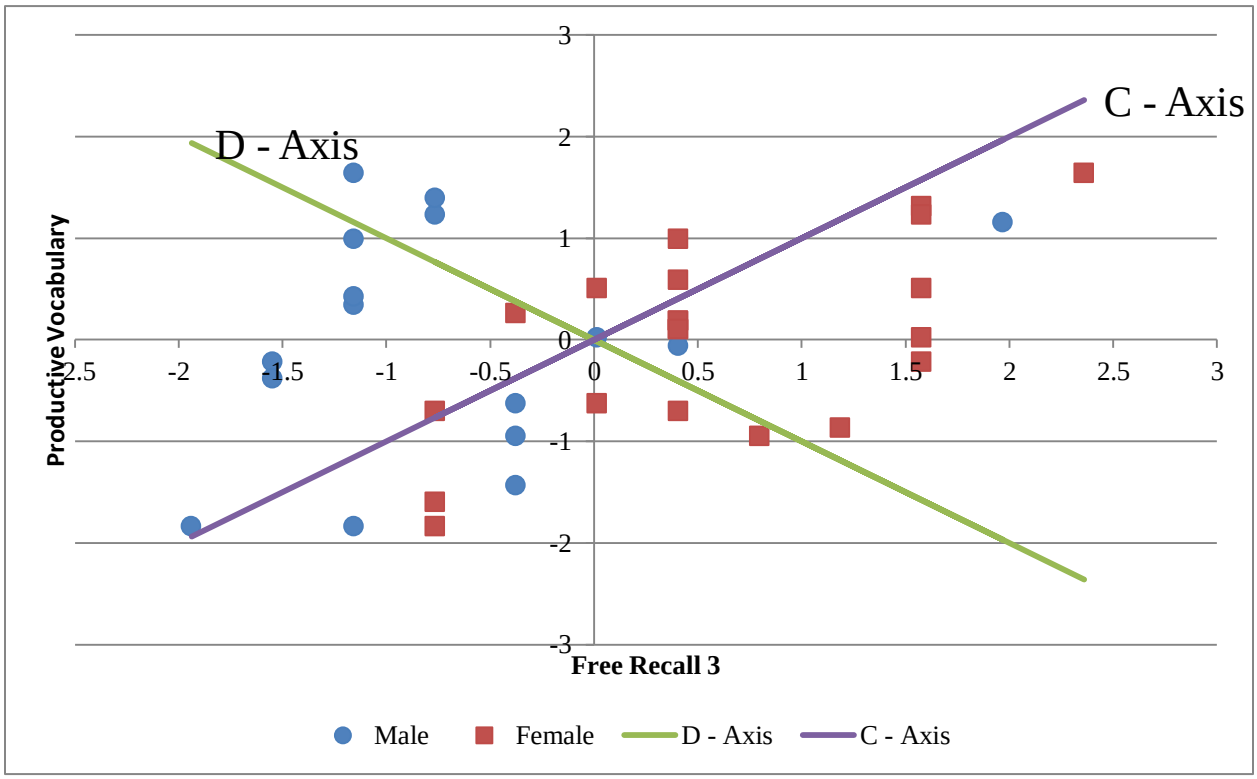

Figure 4. Scatter plot of the standardized PV and FR3 scores. D and C axes are also displayed on the figure. Males and females categorization

Additionally, the cumulative distribution of the $D_{pv\text{-}fr}$ and $C_{pv\text{-}fr}$ factors was plotted (Figures 5 and 6). Figure 5 shows that there is a significant difference in the cumulative distribution between males and females; indicated by the spacing between the cumulative distributions of the two groups. This indication allows us to argue that these groups represent two distinct populations defined by their $D_{pv\text{-}fr}$ value. A one-way ANOVA was used in order to quantify the above observation, indicating a significant effect of group ($F(1, 36)= 12.866$, $p=.001$).

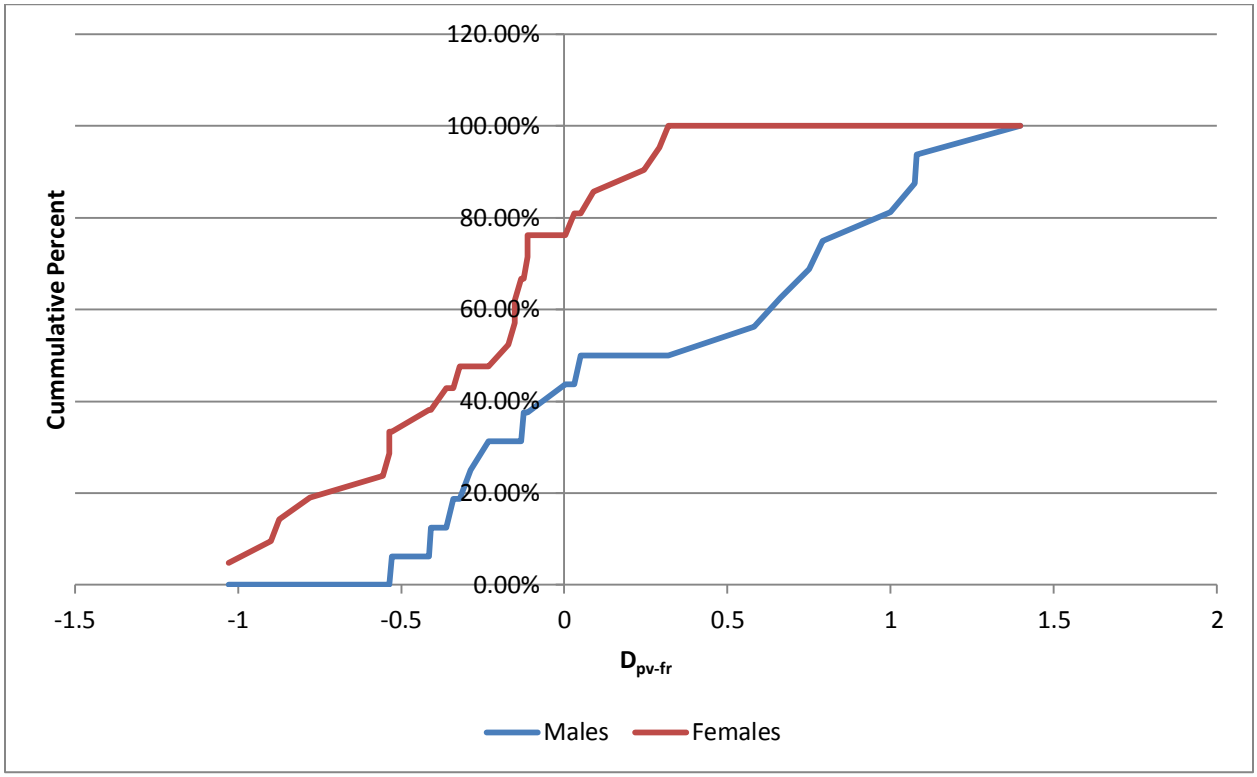

Figure 5. Cumulative distribution functions of $D_{pv\text{-}fr}$. Categorization between males and females

Observing our groups based on the $C_{pv\text{-}fr}$ value (Figure 6), it seems that there is a segregation based on sex. A one-way ANOVA was used in order to quantify the above observation, indicating a significant effect of group ($F(1, 36)= 5.378$, $p=.026$). Nevertheless, there are still males and females present at the extreme $C_{pv\text{-}fr}$ values. If there was an actual differentiation it

would be expected that one of the extreme sides (positive or negative) would contain only males, and the other extreme side only females - as occurs in D distribution. Thus we argue that C value cannot act as a categorization variable.

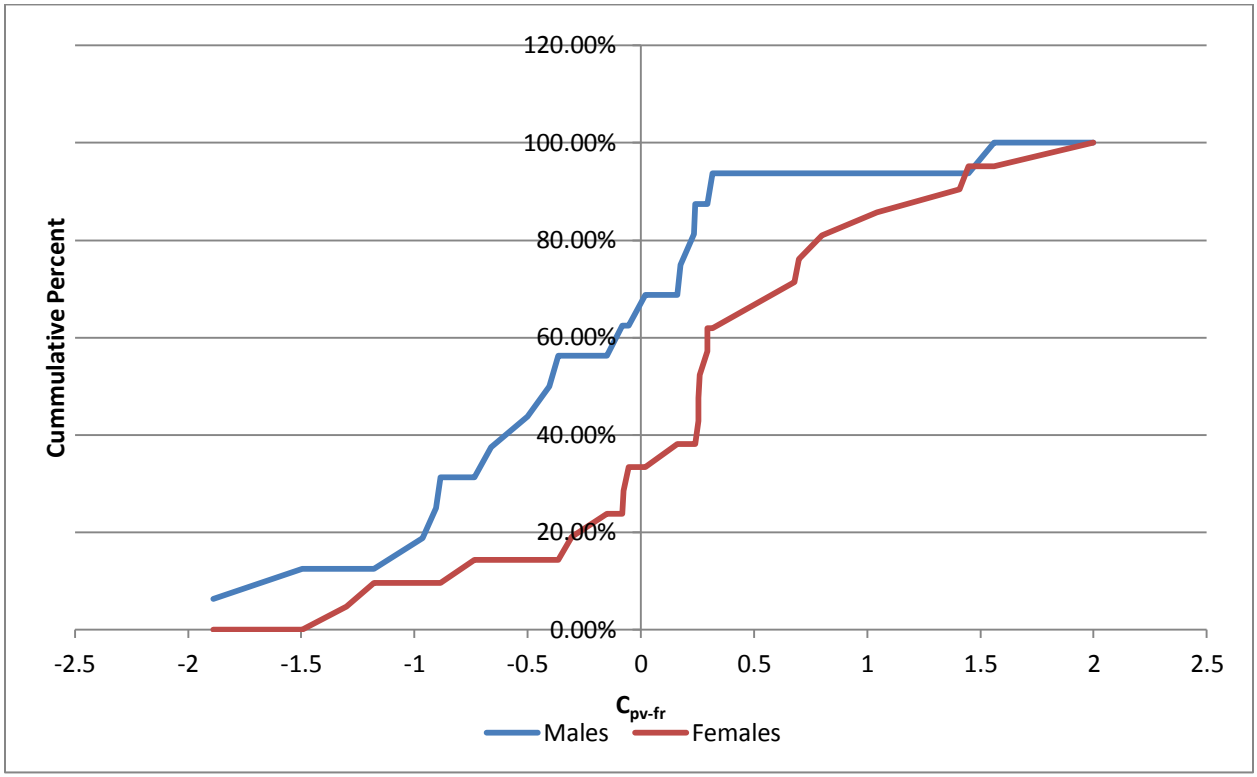

Figure 6. Cumulative distribution functions of C. Categorization between males and females

Categorization based on D values is shown in Table 14, where both groups are classified according to brain sex. In the 'PV>>FR' and the 'PV>FR' types, only males are present. Moreover, males and females are almost equal in the 'PV≈FR' brain type, whilst moving towards 'PV<FR' brain type and 'PV<<FR' brain types, the percentage of males is almost zero. In order to quantify the above observation, that is to determine if there are significant associations between sex and the five brain sex categories, a Fisher's exact test of independence

was performed. The results indicated that male and female presence in the five brain sex categories associate significantly (p=.002, Fisher's exact test).

Table 14. Classification of brain sex based upon median positions of males and females

Defining Characteristic	**PV<<FR3**	**PV<FR3**	**PV≈FR3**	**PV>FR3**	**PV>>FR3**
Brain types based on median positions of the two sub-populations Male, Female					
Brain Boundary (median)	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
Females (N) Males (N)	3 0	5 1	13 7	0 4	0 4
Brain Types Based on Percentiles of the two sub-groups Males, Females					
Brain Boundary Percentile	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
Percentile (Per)	Per<8.1	8.1<per<24.3	24.3<per<78.3	78.3<per<89.2	Per>89.2
Females Males	14% 0%	24% 6%	62% 44%	0% 25%	0% 25%

(b) Sex and 2D:4D Classification

Classifying brain sex based on sex and 2D:4D, descriptive statistics indicated that participants whose masculinization/feminization level was classified as 'extreme masculinized' scored higher in $D_{pv\text{-}fr}$ (M =0.108, SD = 0.102) than those classified as 'masculinized' (M =-0.005, SD = 0.097). In the same line, those whose masculinization/feminization level classified them as 'feminized' (M=-0.004, SD= 0.058) scored higher than those classified as 'extreme feminized' (M=-0.66, SD=0.066).

As seen in Figure 7, there is an observable differentiation of the data along the D axis (which runs from the bottom right to the top left corner), while this is not the case regarding the C axis (which runs from the bottom left to the top right corner). Specifically, it shows that the majority of the males within the 2D<4D group (referred as extreme masculinized) population (marked as cycles) lies within the positive side of the D axis (that is the top left corner), whilst the majority

of the females within 2D>4D group (referred as extreme feminized) population (marked as triangles) lies within the negative side of the D axis (that is the bottom right corner). A Kruskal-Wallis test was used in order to quantify the above observation regarding the $D_{pv\text{-}fr}$ value, indicating a significant effect of group (H(3)= 13.968, p=.003). Mann–Whitney tests were used to follow up this finding. A Bonferroni correction was applied and consequently all effects are reported at the .0166 level of significance. It appeared that $D_{pv\text{-}fr}$ scores were no different when the extreme masculinized group (male and 2D<4D) was compared to the masculinized group (male and 2D>4D) (U = 11.000, z = −2.170, p=.031) or to the feminized group (females and 2D<4D), (U = 11.000, z = −1.886, p=.066). However, the extreme masculinized group (male and 2D<4D) compared to the extreme feminized group (females and 2D>4D) a significant difference appeared (U = 10.000, z = −3.429, p<.001). Jonckheere's test revealed a significant trend in the data: indicating that as we proceed from extreme feminized (females and 2D>4D) to extreme masculinized (males and 2D<4D) the median $D_{pv\text{-}fr}$ value decreased, T_{JT}*=114.500, z=-3.581, p<.001*.

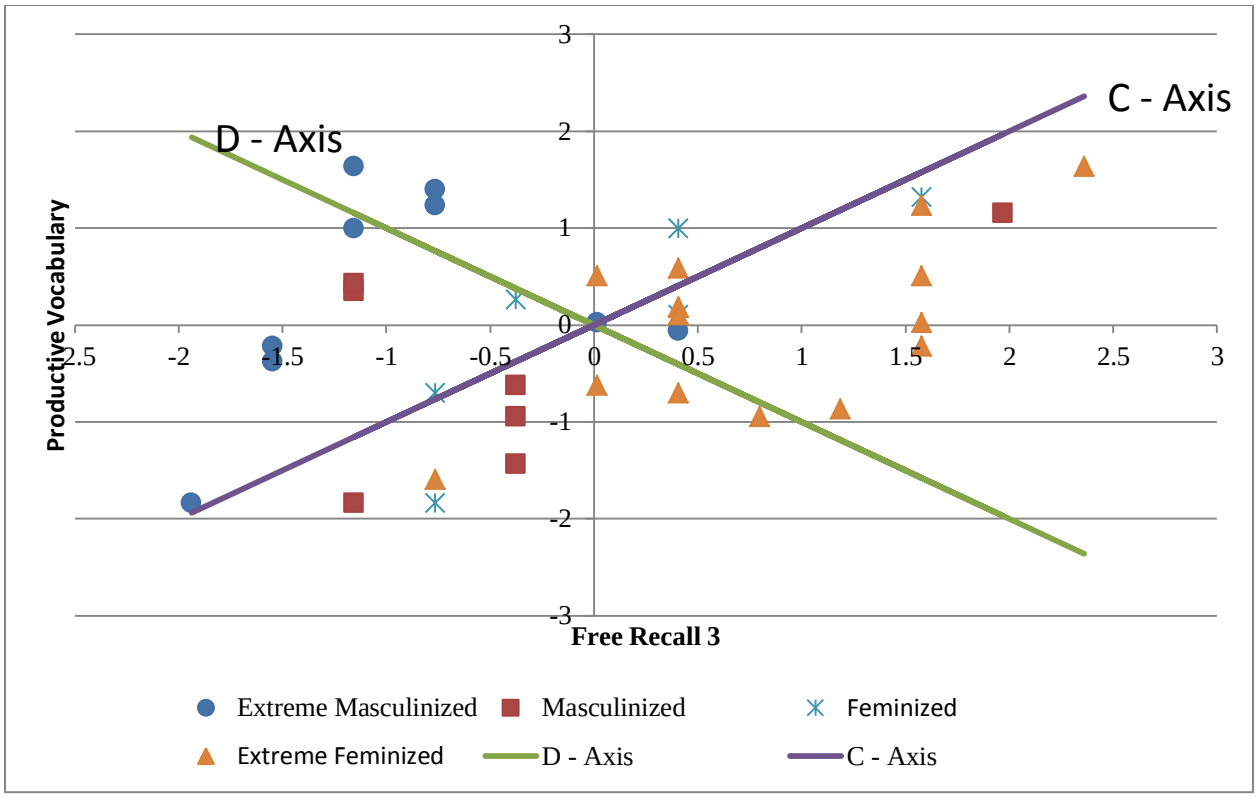

Figure 7. Scatter plot of the standardized PV and FR3 scores. D and C axes are also displayed on the figure. Extreme masculinized (MM), masculinized (M), feminized (F) and extreme feminized (FF) categorization.

The difference between extreme masculinized (MM), masculinized (M) feminized (F), extreme feminized (FF) can be seen in the cumulative distribution of $D_{pv\text{-}fr}$ (Figure 8). It worth mentioning that extreme masculinized (MM), masculinized (M), feminized (F), extreme feminized (FF) may represent three populations indicated by the spacing between the cumulative distributions of the four groups. The distribution of masculinized (M) seems to become entangled with the feminized (F) distribution, with an exception in the middle of the distribution.

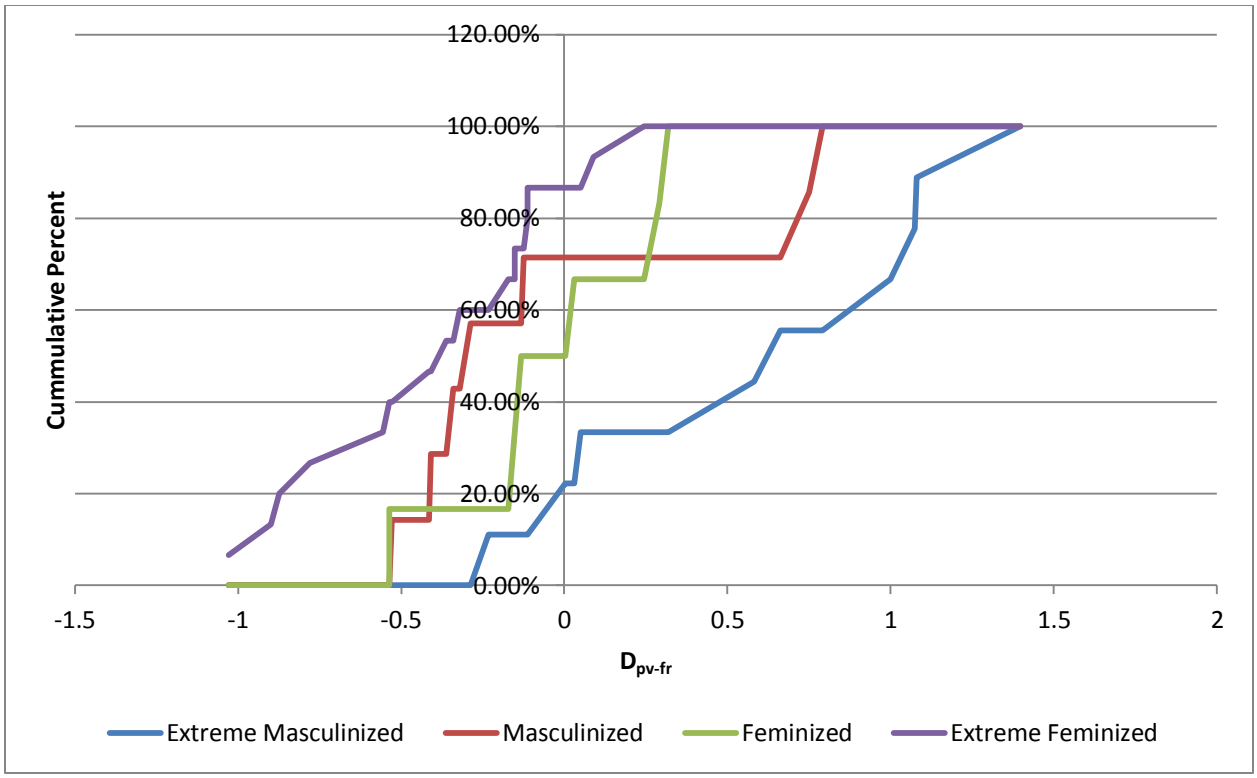

Figure 8. Cumulative distribution function of $D_{pr\text{-}fr}$. Categorization among extreme masculinized (MM), masculinized (M), feminized (F) and extreme feminized (FF) categorization.

Observing our groups based on the C value in Figure 9, it seems that there is no clear pattern that distinguishes the four groups in any way. Thus it is argued that C value cannot act as a categorization variable.

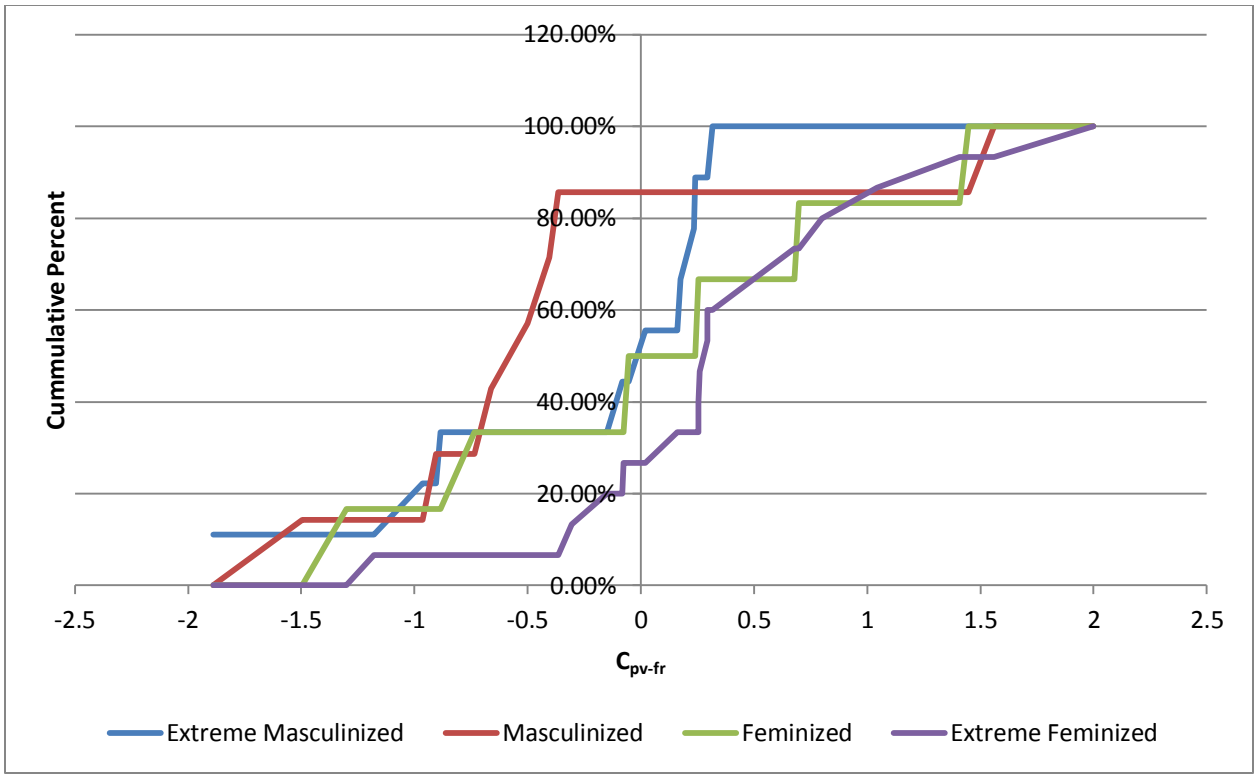

Figure 9. Cumulative distribution function of C. Categorization among extreme masculinized (MM), masculinized (M), feminized (F) and extreme feminized (FF) categorization.

Categorization based on $D_{pv\text{-}fr}$ values is shown in Table 15, where all four groups are classified regarding their brain type. In the 'PV>>FR' types group there are only extreme masculinized group members (MM), while in the 'PV>FR' type group, there are masculinized (M) and extreme masculinized group members (MM) present. Moreover, the 'PV≈FR' brain type includes higher percentages of masculinized (M) and feminized (F) group members. Moving towards 'PV<FR' brain types we see only a few masculinized group members (M) while the 'PV<<FR' brain category consists of only extreme feminized group members (FF). In order to quantify the above observation, that is to determine whether there are significant associations between brain sex type group membership (this refers to the five brain sex type categories defined by PV-FR) and masculinization / feminization levels (that is, sex and 2D:4D

categorization), a Fisher's exact test of independence was performed. The results indicated that the distribution of individuals classified according to sex and 2D:4D and their presence in the five brain sex type categories are significantly associated with each other (p=.014, Fisher's exact test).

Table 15. Classification of brain types based upon median positions of Extreme masculinized (MM), Masculinized (M), Feminized (F) and Extreme feminized (FF) categorization

Defining Characteristic	**PV<<FR3**	**PV<FR3**	**PV≈FR3**	**PV>FR3**	**PV>>FR3**
Brain types based on median positions of the four sub-populations mm, m, G, GG					
Brain Boundary (median)	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
FF (N) F (N) M (N) MM (N)	3 0 0 0	4 1 1 0	8 5 4 3	0 0 2 2	0 0 0 4
Brain Types Based on Percentiles of the four sub-groups					
Brain Boundary Percentile	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
Percentile (Per)	Per<8.1	8.1<per<24.3	24.3<per<78.3	78.3<per<89.2	Per>89.2
FF F M MM	20% 0% 0% 0%	27% 17% 14% 0%	53% 83% 57% 33%	0% 0% 29% 22%	0% 0% 0% 45%

(c) e/t ratio and 2D:4D classification

When masculinization / feminization was based on e/t ratio and 2D:4D, descriptive statistics indicated that participants whose masculinization/feminization level was classified as 'extreme masculinized' scored higher in $D_{pv\text{-}fr}$ (M =.095, SD = 0.103) than those classified as 'masculinized' (M =-0.03, SD = 0.11). In the same line, those whose masculinization/feminization level classified them as 'feminized' (M=-0.002, SD= 0.065) scored higher than those classified as 'extreme feminized' (M=-0.05, SD=0.061).

The difference between extreme masculinized (MM), masculinized (M) feminized (F), extreme feminized (FF) groups can be seen in the cumulative distribution of $D_{pv\text{-}fr}$ (Figure 10). It is observed that extreme masculinized (MM), masculinized (M), feminized (F), and extreme feminized (FF) represent four distinct populations indicated by the spacing between the cumulative distributions of the four groups. A Kruskal-Wallis test was used in order to quantify the above observation regarding the $D_{pv\text{-}fr}$ value, indicating a significant effect of group ($H(3)= 12.566$, $p=.006$). Mann–Whitney tests were used to follow up this finding. A Bonferroni correction was applied and consequently all effects are reported at the .016 level of significance. There were no significant differences in D scores when the extreme masculinized group (low e/t ratio and 2D<4D) were compared to the masculinized group (low e/t ratio and 2D>4D) ($U = 13.000$, $z = -2.399$, $p=.0164$) and the feminized group (high e/t ratio and 2D<4D) ($U = 13.000$, $z = -1.470$, $p=.165$). However, there was a significant difference between the extreme masculinized group (low e/t ratio and 2D<4D) and the extreme feminized group (high e/t ratio and 2D>4D) ($U = 12.000$, $z = -3.397$, $p<.001$). Jonckheere's test revealed a significant trend in the data: as we move from extreme feminized to extreme masculinized the median $D_{pv\text{-}fr}$ value decreased, $T_{JT}=136.000$, $z=-3.007$, $p=.001$.

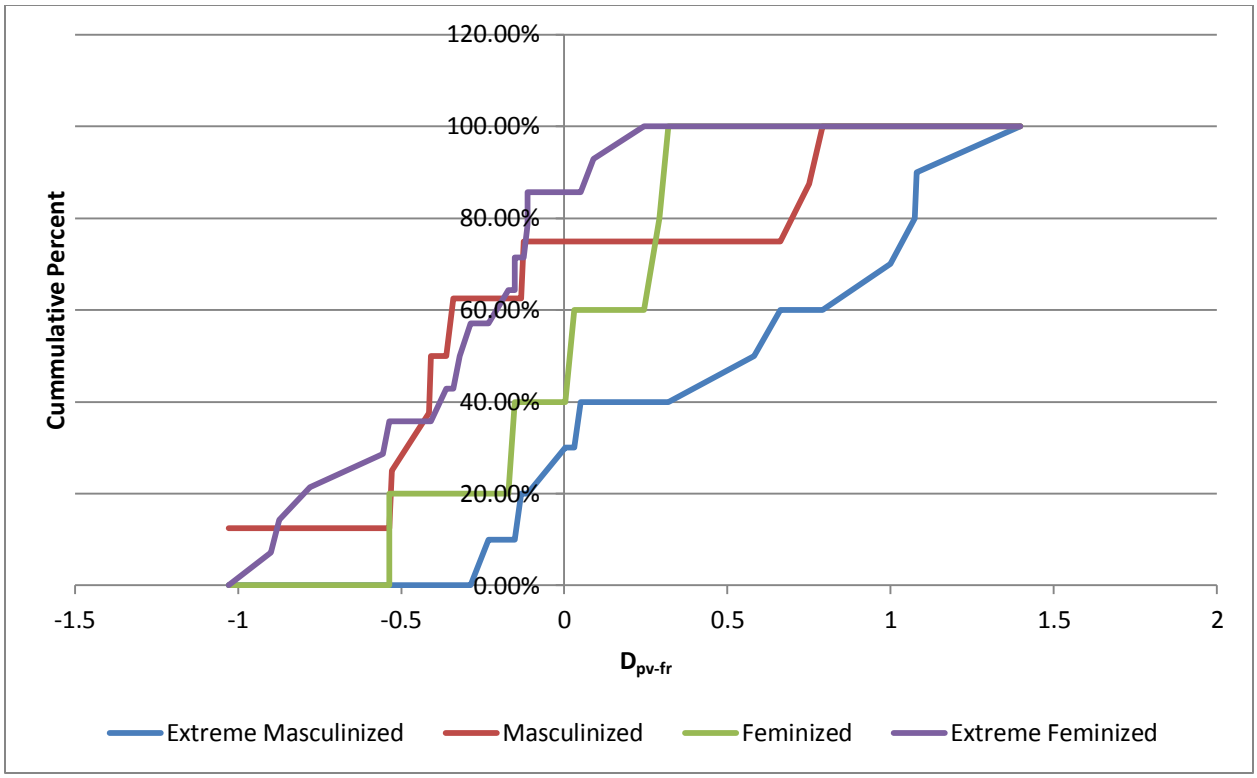

Figure 10. Cumulative distribution function of D. Categorization among extreme masculinized (MM), masculinized (M), feminized (F) and extreme feminized (FF) categorization.

Categorization based on D values is shown in Table 16, where all four groups were classified according to their cognitive profile. In the 'PV>>FR' types group there are only extreme masculinized individuals (MM), while in the 'PV>FR' type group, there are masculinized (M) and extreme masculinized group members (MM) present. Moreover, the 'PV≈FR' brain sex type is dominated by feminized (F) individuals while includes percentages of masculinized (M) feminized (F) and extreme masculinized individuals. Moving towards 'PV<FR' brain types we see only a few masculinized (M) individuals while the 'PV<<FR' brain sex category consists mainly extreme feminized (FF) individuals and one masculinized (M). In order to quantify the above observation, that is to determine if there are significant associations between cognitive profile (this refers to the five brain sex type categories) and masculinization / feminization levels

(this time defined by e/t ratio and 2D:4D), a Fisher's exact test of independence was performed. The results indicated that masculinized and feminized presence in the five brain sex categories associate significantly (p=.042, Fisher's exact test).

Table 16. Classification of brain types based upon median positions of Extreme masculinized (MM), Masculinized (M), Feminized (F) and Extreme feminized (FF) categorization

Defining Characteristic	**PV<<FR3**	**PV<FR3**	**PV≈FR3**	**PV>FR3**	**PV>>FR3**
Brain types based on median positions of the four sub-populations MM, M, F, FF					
Brain Boundary (median)	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
FF (N) F (N) M (N) MM (N)	2 0 1 0	3 1 2 0	9 4 3 4	0 0 2 2	0 0 0 4
Brain Types Based on Percentiles of the four sub-groups					
Brain Boundary Percentile	D<-0.14	-0.14<D<-0.075	-0.075<D<0.075	0.075<D<0.15	D>0.15
Percentile (Per)	Per<8.1	8.1<per<24.3	24.3<per<78.3	78.3<per<89.2	Per>89.2
FF F M MM	14.3% 0% 12.5% 0%	21.4% 20% 25% 0%	64.3% 80% 37.5% 40%	0% 0% 25% 20%	0% 0% 0% 40%

Part 3: Exploring systemizing / empathizing scores in relation to degrees of masculinization / feminization based on (a) males/females (b) sex and 2D:4D, and (c) e/t ratio and 2D:4D classifications.

Descriptive statistics indicated that males scored higher in systemizing (M=58.06, SD=17.00) and lower in empathizing (M=43.50, SD=11.153) compared to females, who scored lower in systemizing (M=55.54, SD=15.624) and higher in empathizing (M=51.58, SD=11.964). Moreover, descriptive statistics in the full sample indicated a mean score of 56.57 (SD= 16.05)

for systemizing and 48.27 (SD= 12.18) for empathizing[60]. Although the observed numerical differences between males and females in systemizing ($t(42)=.507$, $p=.615$, Hedges's $g_s=0.09$) were not statistically significant, sex differences in empathizing scores were ($t(42)=2.263$, $p=.029$, Hedges's $g_s=0.71$).

(a) Males / Females Classification

Following the procedure described in Goldenfeld et al. (2005), systemizing scores and empathizing scores were standardised and D_{s-e} variable was created. An independent samples t-test indicated that there were statistically significant differences in D_{s-e} variable between males (M=0.034, SD=0.084) and females (M=-0.24, SD= 0.088; ($t(42)=2.211$, $p=.033$, Hedges's $g_s=0.66$); with males presenting higher values of D_{s-e} than females.

(b) Sex and 2D:4D classification.

Initially the factor '2D:4D' was added to the factor 'sex', and the sample was grouped based on the levels of masculinization / feminization defined by 'sex and 2D:4D' (extreme masculinized, masculinized, feminized, and extreme feminized). A Jonckheere-Terpstra test indicated a statistically significant trend of D_{s-e} with sex and 2D:4D ($T_{JT}= 239.500$, $z=-2.183$, $p=.029$); indicating an association between sex and 2D:4D and D_{s-e}. That is, more masculinized individuals (as indicated by sex and 2D:4D) were associated with extreme systemizing (extreme Type S type; as indicated by D_{s-e}) and lower empathizing scores; while the opposite is true for feminized individuals, who were associated with extreme empathizing (extreme type E type; indicated by D_{s-e}) and lower systemizing scores. In addition, the direction of the association

[60] These means are similar to means from a larger scale study that used 3906 participants (Baron-Cohen et al., 2014). In Baron-Cohen et al.'s (2014) study, descriptive statistics from the full sample indicated a mean score of 59.66 (SD= 22.15) for systemizing and 44.87 (SD=14.58) for empathizing.

between the sex and 2D:4D grouping and scores on systemizing and empathizing was considered. A Jonckheere-Terpstra test did not indicate a significant trend for systemizing. However, there was a statistically significant trend indicating a progressive association between sex and 2D:4D category and participants' empathizing scores (T_{JT}= 441.000, z=2.142, p=.032).

(c) e/t ratio and 2D:4D classification

The next grouping was based on the estradiol to testosterone ratio (e/t ratio) and 2D:4D. More specifically, the levels of e/t ratio were grouped as high / low based on the sample's median values. Then, four groups were formed based on e/t ratio grouping and 2D:4D; featuring low e/t ratio and 2D<4D (extreme masculinized), low e/t ratio and 2D>4D (masculinized), high e/t ratio and 2D<4D (feminized) and high e/t ratio and 2D<4D (extreme feminized). The results from a Jonckheere-Terpstra test failed to reach statistical significance for D_{s-e} (T_{JT}= 248.000, z=-1.746, p=.081) and systemizing (T_{JT}= 320.000, z=-.155, p=.877). However, regarding e/t ratio and 2D:4D and empathizing, the results indicated the existence of a significant trend (T_{JT}= 418.000, z=2.024, p=.043)

Part 4: Associations between systemizing-empathizing and 'cognitive' brain sex classification

The final step was to test for an association between the systemizing-empathizing 'D_{s-e}' grouping and the productive vocabulary-free recall 'D_{pv-fr}' for the whole sample. Specifically, five groups were created for D_{s-e} featuring extreme empathizers (extreme type E; S<<E), empathizers (type E; S<E), balanced (S≈E), systemizers (type S; S>E) and extreme systemizers (extreme type S; S>>E). A Jonckheere-Terpstra test indicated a statistically significant trend of 'D_{pv-fr}' in the 'D_{s-e}' groups (T_{JT}= 244.500, z=2.518, p=.012); indicating a progressive association between 'D_{s-e}' categorization and participants' 'D_{pv-fr}' scores.

3.4 Discussion

3.4.1 Verbal free recall and sex

This study aimed to explore sex differences in verbal free recall in order to explore the cognitive mechanisms that are implicated in this task. In addition to the rationale from Chapter 2, biological factors (and stricter inclusion / exclusion criteria) were included in order to better explore a more direct association between hormones and aspects of cognitive performance as well as indirect connections between cognition and specific brain structures[61]. According to the theoretical background, gonadal hormones affect specific brain areas that determine information-collection rate. This collection rate, or as it was referred in this thesis, 'basic information processing ability' creates structural differences in the neuronal connections of the brain. Moreover, these structural differences are potentially responsible for creating observed cognitive differences between the two sexes. Stated differently, these neuronal structural differences may influence the expressed cognitive profile of an individual; that is, cognitive brain sex. Thus, an estimation of cognitive brain sex via the measurement of the deviation in cognitive performance between two behavioural measures that appear to be female-biased (free recall) and neutral / not-female-biased (productive vocabulary) was conducted.

In the first part of this study, Experiment's 1 (Chapter 2) version of a word free recall task was employed, in order to establish the exact conditions under which sex-differences in verbal word free recall appear. The results here are in line with previous studies that have indicated a significant female advantage in verbal free recall (Krueger & Salthouse, 2010; Bolla-Wilson &

[61]This refers to hippocampus and amygdala, which appear to be significantly affected by pre- and post- natal gonadal hormones; while they are both engaged in cognitive functions that include the collection of information (such as free recall).

Blicker, 1986; Geffen et al., 1990; for a review see Andreano & Cahill, 2012) as well as the results from Experiment 1 in Chapter 2. That is, females appear to outperform males in every level of the free recall paradigm, with the first free recall as well as the free recall after distraction showing a statistically significant difference. Moreover, females appear to outperform males when each level of the free recall task was compared to each other. This difference remained significant even after controlling for the influence of executive function and working memory.

The results also indicated a significant contribution of executive function (measured by the 'Tower of Hanoi moves' variable) on inter-individual free recall performance differences in every model that was explored. In addition, individual hormonal profiles appear to affect free recall and account for group differences; that is, group differences created by the hormonal profile appear to have a larger effect (η_p^2 = .206) compared to sex-based group differences (η_p^2 = .191; see adjusted Model 2, Table 10). Again, the interaction between hormonal profile and free recall was stronger (η_p^2 = .159) compared to the interaction between sex and free recall (η_p^2 = .116). Thus, it can be argued that hormonal profile provides more information regarding free recall performance compared to sex; and executive function appears to be a significant influence on free recall performance.

Executive function and working memory appear to be significant contributors to free recall performance, since both factors relate to frontal lobe function (Moscovitch, 1992; Moscovitch & Winocur, 1992). Moscovitch and Winocur, (1992) argued that free recall performance depends on the synergistic action of hippocampus (which it is argued to create associations and retrieving them through a rather automatic process) and frontal-lobe (responsible for strategic, self-initiated retrieval). Previous research has indicated that sex differences in free recall remain significant

even after controlling for general differences in other cognitive abilities that are known to be related to free recall performance (i.e. episodic verbal memory ability measured by tests of paired associates and story recall tests; Krueger & Salthouse, 2010); while an impaired executive function has been linked to impaired free recall performance (Taconnat et al., 2010). Thus, based on the above it can be suggested that the current results support previous studies regarding the contribution of frontal lobe function on free recall performance and extend them by introducing the variable of hormonal profile as a significant determinant of free recall performance.

Based on the findings of the current study as well as those of Experiment 1 (Chapter 2), it can be argued that sex differences in free recall cannot be simply attributed to differences in executive function and/or working memory; Thus, following Moscovitch and Winocur's (1992) rationale, the hippocampus is left as a main candidate for affecting the observed sex differences.

As was argued in previous chapters, the hippocampus (and amygdala) is significantly affected by pre-natal hormones as well as post-natal hormones. More specifically, it was argued that pre-natal and post-natal hormones determine the hippocampus-amygdala system's functionality. Thus, the finding that 'hormonal profile' (a variable that combines both pre-natal hormone effects as well as post-natal hormone levels) creates stronger between subject differences as well as stronger interaction with free recall performance provides a good support for this hypothesis.

Contrary to Experiment 1 (Chapter 2), a significant interaction between free recall condition and sex was found. The analysis of that interaction indicated that free recall in males differed significantly only between the first free recall and the free recall after distraction; while in females every level of free recall appear to differ significantly from each other. In other words, males and females displayed different levels of performance loss as they proceeded from the first

free recall condition to the recall of the second (distracter) list. To interpret this finding, it needs to be considered within the hippocampus-amygdala hypothesis.

It can be argued that in the first and second free recall (distracter), performance cannot be attributed to either the hippocampus[62] or amygdala[63] only. Both structures may contribute and performance is achieved through a synergistic action. However, in free recall three (that is, free recall of the first list after the distraction) the effects of amygdala are (theoretically) reduced due to the distracter. This occurs because the distracter leads to the amygdala-based activation that was caused by list A to switch off in order to be replaced by amygdala-based activation caused by list B (distracter). Thus, in a male (performance dominated by amygdala) versus a female cognitive system (performance dominated by hippocampus) this 'switch off' is expected to have stronger effects in males compared to females. Based on this rationale, the observed sex difference on the levels of performance loss within the three free recall conditions can be attributed to sex-related differences that may be related to differences in hippocampal functionality.

Following the same rationale, free recall after distraction becomes the part of the free recall task that is theoretically related to hippocampal function relatively isolated from the contribution of the amygdala. This argument is supported by the apparent sex differences within each level of the free recall paradigm that was used. More specifically, the results indicated that males and females differed significantly in the first free recall (FR1) and free recall after distraction (FR3) only. Adjusting for executive function and working memory (i.e. frontal lobe covariates) these sex differences became stronger as observed through the effect sizes, which appeared to be larger

[62] That is, recall is due to a formation of association between the words-stimuli (see Chapter 1, Section 1.3.2, page 16)

[63] That is, recall is due to a longer-lasting stimulus-based activation of the related sensory networks (see Chapter 1, Section 1.3.2, page 16)

compared to that shown in the unadjusted model. Moreover, based on the effect sizes it was observed that free recall after distraction (FR3) was the part of the free recall task where sex differences were more prominent. That is, looking at sex differences in the first free recall (FR1), it can be observed that the effect size (η_p^2= .118; Model 2) was smaller compared to free recall after distraction (FR3; η_p^2=.281; Model 2). These findings allow the argument that the free recall after distraction (FR3) is the part of the free recall task that is mostly affected by sex and can produce sex differences even in a moderate sample size. Thus, performance on free recall after distraction is left to be based on the hippocampal-based associations that were formed/achieved during free recall one (FR1); that is, free recall after distraction (FR3) can be considered as a potential test / proxy of hippocampal functionality.

As it was mentioned above, free recall after distraction presented the highest sex differences, even after controlling for factors that previous studies have reported as significant mediators of / contributors to task performance. According to the hippocampus-amygdala hypothesis, this part of the free recall task (i.e. FR3) is hypothesised to be a potential proxy for hippocampal function, which is a brain structure heavily affected by both pre- and post- natal gonadal hormones. Based on these findings it can be argued that the combined effects of pre-natal hormones (2D:4D) and post-natal hormones (estradiol and testosterone) can replace the factor 'sex' as a dissociating factor in sex-biased tasks. Expanding this argument, the term 'sex-biased task' can be considered as the following: 'a task that present consistent and significant sex differences, which differences are significantly related to the hormonal profile of each individual (the combination of pre- and post-natal gonadal hormone levels), and not due to individual differences in cognitive abilities that are not related to an individual's hormonal profile'. Thus, free recall after distraction may be

considered as a sex-biased task and its relationship to sex can potentially be attributed to hormone-affected cognitive mechanisms.

The relationships between hippocampus, memory, learning and gonadal hormones have been discussed by Cherrier, Craft and Matsumoto (2003) who suggested estradiol as well as testosterone (through the process of aromatization to estradiol) as possible factors that affect hippocampal function. According to Cherrier et al. (2003), higher testosterone levels as well as higher estradiol levels are linked to higher hippocampal function. The exact relationship between gonadal hormones and hippocampal function may be related to Di-hydro-testosterone (DHT). DHT is a metabolic product of testosterone, but unlike testosterone, cannot be aromatized to estradiol (Burger, 2002). Therefore, the more testosterone that is turned to DHT, the less there is to be aromatized to estradiol. In addition, high DHT levels seem to have a negative effect on testosterone production, while DHT supplementation also leads to a significant drop of testosterone levels (Cherrier et al., 2003). Interestingly, testosterone levels have been consistently related to Insulin-like Growth Factor-I (IGF-I) in healthy males and females (Jorgensen, Vahl, Hansen, Skjaerbaek, Fisker, Orskov, Haqen & Christiansen, 1998; Hobbs, Plymate, Rosen & Adler, 1993) and hypo-gonadal females (Cristiansen et al., 2005). IGF-I is also linked to cognitive performance (Cherrier et al., 2004) while animal studies have linked IGF-I function to hippocampal endogenous acetylcholine (ACh) release (Seto et al., 2002). More specifically, higher IGF-I levels were linked to lower ACh release in the hippocampus (Seto et al., 2002); while IGF-I levels seem to be reduced by oestrogen or estradiol administration (Moe, Prinz, Larsen, Vitiello, Reed & Merriam, 1998). According to Ryan et al. (2012), the molar ratio of free estradiol to free testosterone (e/t ratio) is almost equal to the molar ratio of total estradiol to total testosterone. Thus, the e/t ratio may serve as an indirect index of the transformation

capability of testosterone to DHT; where this capability is also linked to pre-natal masculinisation (Sharpe, Taylor, Gist & Baskin, 2013). In conclusion, it can be argued that estradiol may increase hippocampal ACh, and since IGF-I is positively related to free testosterone, while all the above factors are related with pre-natal masculinization, then the combined effects of pre-natal and post-natal hormones (i.e. hormonal profile) may provide some information regarding the effects of estradiol on hippocampal ACh.

At this point it is important to add some information regarding the indirect relationship between hippocampal function and the free recall task. Indeed, the relationship between the hippocampus and free recall performance has been indicated before. Except for studies that have indicated a significant impairment in word-list free recall performance in patients with hippocampal trauma (Moscovitch & Winocur, 1992), further support for the engagement of hippocampus in word-list free recall comes from research on Down syndrome. Impairment in verbal free recall of word lists is observed in Down syndrome cases (Carlesimo, Marotta & Vicari, 1997), and such impairment is also linked to hippocampal dysfunction (for a review see Jarrold et al., 2008). According to studies that explored the neurobiological underpinnings of the observed memory dysfunction in Down syndrome, there seems to be a close relationship between the development and function of hippocampal formations with the observed memory-related impairments (Jarrold et al., 2008). That is, reduced hippocampal cholinergic innervations (Hyde & Crnic, 2001) as well as reduced hippocampal volume appear to be significant correlates with Down syndrome memory deficits (Jarrold et al., 2008). Consequently, enhanced word-list free recall performance is linked to higher function of cognitive system(s) that include the hippocampus; while based on the results of the current study, individual hormonal profile is a potential indicator of the functionality of cognitive systems where the hippocampus is actively engaged.

3.4.2 Cognitive phenotype and its relationship to systemizing / empathizing scores.

Similar to Experiment 1 (Chapter 2), Experiment 2 addressed and expanded the method of identification of individual cognitive phenotypes through score-deviation between verbal free recall (female-biased) and productive vocabulary (non-female biased; $D_{pv\text{-}fr3}$). The results indicated a good fit for the behavioural data on the brain sex type scale. Once again a gradual reduction in the number of male participants was noted from the extreme masculinized brain type category (indicated from higher productive vocabulary and lower free recall; PV>>FR) towards the extreme feminized brain type category (indicated by a lower productive vocabulary and higher free recall; PV<<FR) while the opposite was true for females. However, the addition of the 2D:4D factor (i.e. the index of pre-natal hormone effects) to the factor sex and finally the grouping solely based on hormone levels (estradiol to testosterone ratio and 2D:4D) indicated a significant link between gonadal hormone effects and brain type. More specifically, this study indicated the existence of a significant association between levels of masculinization / feminization (defined by sex and 2D:4D, estradiol to testosterone ratio and 2D:4D) with 'cognitive brain type'. That is, higher levels of masculinization were associated with allocation on higher levels on the (cognitive) brain type scale; while the opposite was true for levels of feminization and feminized brain types. According to these results, brain type may not be sex specific, but it seems to be closely related to gonadal hormones (expressed by an individual's hormonal profile). These results are in line with other researchers, such as Manning et al. (2010) and Goldenfeld et al. (2005), who classified brain sex by the use of the measures of systemizing and empathizing and concluded that brain sex type deviates from the strict boundaries of sex.

Finally, participants were also categorised by the psychological indices of systemizing and empathizing, as has been done with previous studies (e.g. Goldenfeld et al., 2005). The results

indicated a sex-based differentiation in systemizing-empathizing. Moreover, a similar association between levels of masculinization / feminization and systemizing-empathizing was observed, suggesting that higher levels of masculinization are associated with extreme systemizing (and higher levels of feminization were associated with extreme empathizing). However, the association between participants' systemizing-empathizing category and their level of masculinization / feminization failed to reach significance when masculinization / feminization was measured by solely hormonal indices.

There is one possible explanation for this finding, which concerns the attributes of the brain type measure defined by systemizing and empathizing. Previous studies have indicated a clear relationship between brain type when measured by systemizing-empathizing and masculinized / feminized brain type, pointing to a relationship between extreme systemizing and brain masculinization indicated by allocation of populations that are linked to extreme masculinization (i.e. Autism) on the extreme Type S brain type category (Goldenfeld et al., 2005; Baron-Cohen et al., 2014; Wheelwright et al., 2006). More specifically, neuro-typical individuals were allocated to either extreme empathizers, empathizers, balanced or systemizers categories while the extreme systemizers (extreme Type S) category was dominated by individuals who were on the Autistic spectrum[64] (Goldenfeld et al., 2005; Baron-Cohen et al., 2014; Wheelwight, et al., 2006). In the current study, a specific sample of individuals on the Autistic spectrum was not collected; thus, extreme systemizers were expected to be few (if not completely absent). Consequently, it can be argued that there are differences in the sensitivity of each 'brain sex' measure (systemizing-empathizing, productive vocabulary- free recall) to the effects of masculinization / feminization. However, in order for this argument to be valid further research

[64] In a sample of 723 neuro-typical males and 1038 neuro-typical females, 5% of typical males and 0.9% of typical females fall in the extreme systemizers (extreme type S) category (as reported in Wheelwright et al., 2006)

is needed where a measure of Autistic traits is also taken into account (e.g. the Autism Spectrum Quotient; A.P.A., 1994) in order to identify and relate accurately individuals that fall within the autistic spectrum with both measures of brain sex as well as masculinization / feminization levels.

Last but not least, the association between the two measures of brain sex (empathizing-systemizing; productive vocabulary-free recall) was found to be significant. This finding supports the argument for the existence of a common cognitive base underlying the two measures of brain sex. Taking also in mind the association of each brain sex measure with masculinization / feminization levels, this argument may be extended to suggest a common neural background for these two measures, although further research is needed in order to support such a suggestion.

There are several factors that must be taken into account when evaluating the current results. Although there is a theoretical background that supports a link between hippocampal function, gonadal hormones and free recall, in this study only gonadal hormones and free recall were directly tested. Thus, caution should be exercised when considering the argument that is raised based on the current study's results regarding the underlying brain structures. Further study is necessary to establishing the link between the hippocampus and free recall or the hippocampus and levels of masculinization / feminization (as indicated by gonadal hormones).

Moreover, the current sample size did not allow the use of parametric analysis for the association between cognitive brain sex groups and psychological brain sex groups with masculinization/ feminization (expressed by direct hormone levels and 2D:4D). Thus, further research that will

be proportional (in terms of sample sizes) to systemizing-empathizing existing studies is needed in order to be able to draw conclusive results.

Another significant limitation of the current study (except from the moderate sample size, which for statistical reasons limits the interpretations and generalizability of the results) is the measure of handedness. In this study handedness was only measured by participants' self-report. This type of handedness measurement has been found not to be as accurate as other measures (such as grip control; Siengthai et al., 2008). Since handedness has been reported as a significant mediator of performance in multi-trial free recall paradigms (Siengthai et al., 2008), it is considered essential for future studies to include a more accurate measure of this factor (in larger samples) in order to enable more accurate results in terms of interpretation and generalizability.

3.4.3 Summary

This experiment supported and expanded Experiment's 1 (Chapter 2) findings regarding the memory paradigm that can best highlight the female-advantage on verbal free recall. These findings support the argument that performance on verbal free recall after a word-based distraction is a female-biased, sex-sensitive task. Experiment's 2 results allow a step further towards the understanding of sex-sensitive tasks and the cognitive mechanisms involved. In particular, current findings highlight the importance of pre-natal configuration and post-natal gonadal hormone effects with regard to free recall performance. Based on these results it can be argued that the importance of the factor 'sex' is focused on the information it provides regarding pre-natal and post-natal hormone-based, brain configuration. Simply stated, sex dissociation on sex-biased tasks serves only as a broad index of pre-natal and post-natal hormonal organizational / activational effects on the brain. It is argued that further research is needed in order to locate the

exact tasks that can be considered as genuinely sex-biased. That is, tasks that present consistent and significant sex differences, which are significantly related to the hormonal profile of each individual (the combination of pre- and post-natal gonadal hormone levels), and not due to individual differences in cognitive skills that are not related to gonadal hormones or even socially (related to gender)-biased acquired skills. This classification will potentially help our general understanding behind the factors that create cognitive sex differences.

Considering cognitive phenotypes (based on the difference between productive vocabulary and free recall) and their classification, it can be argued that Experiment 1's (Chapter 2) findings regarding a behavioural brain-sex type classification are supported and extended. In particular, a similar sex-based classification of brain sex types based on the behavioural data was observed. However, the addition of the 2D:4D factor (i.e. the index of pre-natal hormone effects) to the factor sex and finally the grouping of participants solely based on hormone levels (estradiol to testosterone ratio and 2D:4D) enabled a similar classification, always in the same theoretical line, indicating a significant association between gonadal hormone effects and cognitive 'type'.

This link was further supported by the appearance of an association between systemizing and empathizing and the brain sex classification created from the cognitive-behavioural data. This finding is of major importance since the psychological index of brain sex is already linked to the male-brain theory of Autism (see Section 1.9.2); a theory that highlights the relationship of (gonadal) sexual hormones and this condition.

Bearing in mind the positive relationship between hormonal profile and free recall performance, the engagement of the hippocampus in cognitive tasks (Chapter 1, Section 1.3.) as well as the significant effect that pre-natal hormones have on this area (Chapter 1, Section 1.2) it can be

argued that hippocampus may be a potential candidate for being a biological factor underlying cognitive sex differences and a potential influence on brain type. As it was stated in Chapter 2, the use of systemizing and empathizing for assessing brain type is useful because it directly relates specific conditions (i.e. Autism) with brain masculinization. However, the use of Systemizing-Empathizing brain type does not allow a direct link with the brain structures that may support/determine the cognitive phenotype of the brain. That is, brain type measured by systemizing and empathizing provides consistent cues regarding the existence of extreme masculinization in, for example Autism, but there is less evidence available which can direct the research towards the brain structures / functions that are impacted by extreme masculinization, and consequently cognition. Consequently, measuring brain type through cognitive assessments can potentially address this gap in the literature and supplement our understanding of how masculinization impacts cognition and enhance our understanding of the factors that may contribute to the appearance and development of conditions such as Autism. Moreover, given that current findings indicate that brain type can be measured through the second to forth finger ratio (2D:4D) and a simple saliva analysis (in order to obtain estradiol to testosterone ratio) it can be easily utilized in future research studies that might relate to the effects of hormones on the brain.

Further research is needed to explore the link between the hippocampus and free recall or hippocampus and masculinization / feminization (as indicated by gonadal hormones/ hormonal profile). Since the direct manipulation of hippocampal function (or amygdala) is difficult for ethical reasons (or it is restricted only to populations that are not neuro-typical), research into a link between observed hippocampal function and masculinization / feminization is considered to be a more feasible option.

Chapter 4: The effects of sex and individual levels of masculinization / feminization on intrinsic brain activity (Experiment 3)

4.1 Introduction

In Chapter 2, a cognitive brain sex typology was constructed from performance on two cognitive tasks (a non-female biased and a female-biased task; see Chapter 2 for more details). Using this typology of cognitive brain sex, it was observed that sex was largely distributed in line with theoretical expectations. The next step was to identify biological factors that could help to account for the appearance of the above association between biological sex and cognitive performance. Following the hippocampus-amygdala hypothesis, it was argued that the potential factors that could affect the above-mentioned association were gonadal hormone effects. Consequently, gonadal hormone effects on the sex-biased task as well as cognitive brain sex were explored in Chapter 3. The results indicated that individual differences in masculinization / feminization levels were also associated with cognitive brain sex type. This finding provided support for the hippocampus-amygdala hypothesis and encouraged further research. Subsequently, the next step was to explore the relationship between individual masculinization / feminization levels and observed brain function that relates to hippocampal activity. More specifically, it is argued that since cognitive brain sex type was significantly associated with levels of masculinization / feminization then, if the hippocampus-amygdala hypothesis stands true, individual levels of masculinization / feminization will be associated with observable brain activity that is related to hippocampal function.

According to the hippocampus-amygdala hypothesis, hippocampal formations appear to be significantly affected by sexual hormones, especially during pre-natal life (Zaidi, 2010; Lombardo et al., 2012; Heany, van Honk, Stein, Brooks, 2016). Moreover, hippocampal formations appear to be related to information-encoding ability; which also appears to be sexually differentiated, with females having an advantage over males on acquiring information. This advantage is observed (behaviourally) in new-borns, primary school children and adults (Nagy et al., 2007; Ozcaliskan & Goldin-Meadow, 2010; Iverson & Goldin-Meadow, 2005; Kaushanskaya et al., 2011; Krueger & Salthouse, 2010). Thus, it can be argued that the ability to acquire information is sex-sensitive, with a female advantage being observed throughout development (see Chapter 1, Section 1.8.1).

Based on the idea that the ability to acquire information may not only affect the amount of information that an individual can collect and process but also may affect the interconnectivity in the related memory networks, which in turn may affect cognitive performance, the concept of cognitive brain sex was elaborated. In order to further explore this idea, the existence of a similar association to that which was found between individual masculinization / feminization levels and cognitive brain sex type (Chapter 3) was explored via intrinsic brain activity[65]. However, in order to narrow down the research as well as to enable conclusions that can be linked to the hippocampus-amygdala hypothesis, the research reported in this chapter focused on brain rhythm(s) and brain areas that can be linked to information encoding, gonadal hormones and / or

[65] Raichle and Snyder (2007) suggested that intrinsic brain activity is an index of brain functional organization. More specific, Raichle and Snyder (2007) argued that since brain intrinsic activity (brain activity measured at a resting state; that is, either eyes-closed or eyes-open fixating on a specific stable target) is significantly more energy-consuming (energy consumption for brain intrinsic activity reaches up to 80% of brain's energy budget; while that energy is utilized for neuronal communization) than evoked brain activity (energy consumption for evoked brain activity corresponds to 1.0% of brain's energy budget) then brain intrinsic activity must be linked to a default mode of brain functioning; that is a default mode of brain functional organization.

the hippocampus. Electroencephalograph oscillations in the Theta and in the Alpha band appear to associate with memory and cognitive performance (Klimesch, Doppelmayr, Wimmer, Schwaiger, Rohm, Gruber & Hutzler, 2001). More specifically, synchronization[66] of Theta and de-synchronization of Alpha are associated with good performance in memory tasks as well as cognitive tasks (Klimesch et al., 2001; for a review Klimesch, 1999). Moreover, Klimesch (1999), using EEG / fMRI, stressed the importance of hippocampal-cortical communication for encoding novel information (see also Bastiaansen & Hagoort, 2003). Klimesch (1999) suggested that the communication between hippocampal and cortical structures is the main generator of Theta rhythm in humans. In particular, Klimesch (1999) argued that Theta rhythm acts as a binding mechanism for topographically separated cortical cell assemblies; an activity related to information-encoding. Thus, according to Klimesch (1999), hippocampal structures are related to the encoding of information and this relationship is reflected in Theta rhythm.

According to Bastiaansen and Hagoort (2003), the presence / increase of Alpha rhythm (7.5-12.5Hrz) is indicative of poor / obstructed communication between the cortex and sensory information, while the opposite (i.e. absence / reduced α-rhythm) indicates active / unobstructed communication between the cortex and sensory information. In a "rest" versus "test" condition, α-rhythm appears to be reduced during testing, indicating a positive relationship between cognitive performance and Alpha de-synchronization (Klimesch, 1999). However, a series of studies by Barry and colleagues (Barry, Clarke, McCarthy, Selikowitz, Rushby, Ploskova, 2004; Barry, Clarke, Johnstone, Magee, Rushby, 2007; Barry & De Blasio, 2017) it was argued that Alpha de-synchronization is more of an index of central nervous system arousal caused by the

[66] Synchronization is the increase of e.g. Theta rhythm from a resting state (e.g. eyes-closed) to a testing state (e.g. eyes-open); on the same line, de-synchronization is the decrease of e.g. Theta rhythm from a resting state compared to a testing state (Klimesch, 1999).

input of visual information in the 'test' conditions, specifically when testing EEG at resting states; that is, eyes-closed (rest / baseline condition) versus eyes-open (test condition). More specifically, Barry and colleagues have indicated a significant interaction between Alpha reactivity (synchronization / de-synchronization of Alpha rhythm between eyes-closed and eyes-open conditions) and measures of arousal (such as skin conductance levels; Barry et al., 2009). Moreover, Barry and colleagues indicated that Alpha rhythm appears to be globally reduced from the eyes-closed to the eyes-open condition without presenting any significant place-specific changes. The absence of place-specific changes in Alpha reactivity along with the observed (negative) relationship between Alpha reactivity and measures of arousal, led Barry and colleagues to the conclusion that Alpha reactivity is just a reflection of tonic energy levels, triggered by basic sensory input. Thus, Alpha reactivity can be seen as an index of arousal caused by visual input (Barry et al., 2007; 2017). Consequently, it can be argued that Alpha reactivity and its relationship to cognitive performance is based on the individual's arousal levels and not due to a relationship between Alpha reactivity and a specific cognitive function; while this is not the case for other EEG rhythms including Theta reactivity (Barry et al., 2017).

Measuring EEG Theta band reactivity at a base-line level (that is, at a resting states; eyes-closed and eyes-open), Barry et al. (2007; Barry & De Blasio, 2017) argued that a decrease of Theta band oscillations from eyes-closed to eyes-open conditions is translated as an increase in activation of the specific area where these oscillations occur. Thus, the importance of topography in regard to Theta reactivity (and all other rhythms except Alpha rhythm) is stressed, since topographical differences in Theta reactivity may yield differences in underlying cognitive mechanisms that are related to the specific areas that Theta reactivity is located. Consequently, it is important to have a general understanding of the topographical brain areas that Theta rhythm

is being observed from and which cognitive function is being related to. Gabrieli, Poldrack, and Desmond (1998) concluded that left pre-frontal cortical activity is related to semantic working memory; arguing that generating verbs (or making a judgment regarding a novel picture; or choosing among many different options[67]) places higher demands on simultaneous semantic processing. Khader and Rosler (2004) indicated differences in Theta de-synchronization between noun and verb processing, and reported that verbs cause significantly higher Theta de-synchronization. Gentner (2006) argued that verbs are linguistically more difficult to learn (in many languages), which is reflected in a greater requirement to collect and combine information[68], compared to nouns. Gentner argued that *"...many concrete nouns refer to naturally individuated referents. In contrast, even fairly concrete verb meanings (such as those of motion verbs) make a selection from the available relational information..."* (p.544, Gentner, 2006), indicating verbs as a linguistic unit of collective semantic information. This argument supports and extends Gabrieli et al.'s (1998) suggestion regarding the functionality of the left frontal cortex and its relation to the ability to collect, combine and retrieve information; an ability that is related to hippocampal function (Moscovitch & Winocur, 1992). Thus, left frontal cortical activity is related to simultaneous (semantic) information processing; while Theta rhythm synchronization / de-synchronization is an index of processing of this type of information, which is also related to hippocampal functionality.

In summary, Theta and Alpha band EEG oscillations appear to be associated with performance on memory and cognitive tasks. Moreover, hippocampal formations appear to be engaged in information-encoding, a female-biased ability (that is, females seem to have an advantage which is observed behaviourally through the life-span). In addition, information encoding appears to be

[67] All these functions are related to hippocampal function
[68] Associations between stimuli is a function related to hippocampal function

related to Theta rhythm and left frontal cortex, while all the above factors are closely related to sexual (gonadal) hormones.

Based on the above it is hypothesised that Theta reactivity (measured at resting eyes-closed and eyes-open states) will appear to be sexually differentiated. Following the hippocampus-amygdala hypothesis and the above-presented research, it is argued that sex differences in Theta reactivity will be linked to masculinization / feminization levels. Thus, it is predicted that individual masculinization / feminization levels as indicated by sex, sex and prenatal hormone effects (indicated indirectly through 2D:4D), and estradiol to testosterone ratio and prenatal hormone effects (hormonal profile) will be associated with Theta reactivity. The reason why all these three levels of masculinization / feminization levels were used is the fact that they incorporate different information. Specifically:

- sex incorporates general information regarding genetic background of each individual and pre-natal and post-natal hormone effects[69],
- sex and 2D:4D incorporates information regarding the effects of pre-natal hormones including the mediating effect of sex which it was argued to be a significant factor (Zhengui & Cohn, 2011), and
- hormonal profile incorporates specific information regarding post-natal hormone levels as well as indirect information regarding pre-natal hormone effects. [70]

Summarizing, Theta reactivity is expected to be associated with the level of masculinization as indicated by sex, sex and 2D:4D, and estradiol to testosterone ratio and 2D:4D; while this

[69] According to Ropers and Hamel (2005) sex is considered as the major differentiating factor in biology (see also Chapter 1, Section 1.2)
[70] However, the potential mediation effects of sex are being excluded since the factor sex is not taken into account.

association is expected to appear in the left frontal cortex, since this area is linked to information encoding and consequently to hippocampal function.

4.2 Method

4.2.1 Participants

For this experiment an opportunity sample from Experiment's 2 sample was used. This sample consisted of 41 participants. However, the recordings of 16 participants (participant numbers 1, 3, 7, 9, 11, 12, 14, 15, 16, 22, 24, 25, 29, 30, 31, and 42) were excluded due to enhanced background noise (excessive EEG artefacts), which made data extraction impossible. Thus, the final sample consisted of 25 participants, of which 14 were male (mean age 35.40) and 11 were female (mean age 35.60).

4.2.1.1 Inclusion / Exclusion criteria

This sample was a sub-sample of the participants who took part in Experiment 2. Thus, the same inclusion/ exclusion criteria apply here. Specifically, only right handed individuals, with no prior experience to tower of Hanoi, over twenty-five years old and native English speakers were recruited.

4.2.2 Materials

Performance, biological and somatometric data from the following measures taken during Experiment 2 was used in the analyses for this study: *Free recall*; *Productive vocabulary*; *Digit*

Span; *Tower of Hanoi*; *Second and Forth digit length (2D:4D)*; *Cognitive Brain sex type (productive vocabulary-free recall)*; *Systemizing-Empathizing Typology*.

Physiological recordings: EEG activity for Eyes-Open and Eyes-Closed conditions was recorded in two separate 2-minute sessions from 32 sites, placed according to the international 10-20 system. Moreover, eye movements were recorded by electrodes placed above and below the left eye (vertical eye movement; vertical electro-occulogram) and on the outer canthus of the right and left eye (horizontal eye movement; horizontal electro-occulogram). A Fast Fourier Transformation was used in order to compute the average power spectra in each electrode for Alpha (8-13Hz) and Theta (4-7Hz) bands.

4.2.3 Design

The design of this study was similar to that of the experiment described in Chapter 3, except that the dependent variables for this study were Alpha and Theta (re-)activity. Following the procedure described in Barry, Clarke, Johnstone, Magee, and Rushby (2007), EEG data were grouped in the following regions: Left Frontal (FP1, AF3, F3, F7), Midline Frontal (Fz, FC1, FC2), Right Frontal (FP2, AF4, F4, F8), Left Central (T7, C3, FC5, CP5), Midline Central (Cz), Right Central (T8, FC6, C4, CP6), Left Posterior (P7, P3, PO3, O1), Midline Posterior (Pz, Oz, CP1, CP2), Right Posterior (P4, P8, PO4, O2). The above grouping was used in order to create two topographic dimensions, a sagittal and a lateral dimension. To construct the sagittal dimension, regions were grouped into *Frontal* (Left Frontal, Midline Frontal, Right Frontal), *Central* (Left Central, Midline Central, Right Central) and *Posterior* (Right Posterior, Midline Posterior, Left Posterior). To construct the lateral dimension, regions were grouped into *Left*

hemisphere (Left Frontal, Left Posterior, Left Central), *Midline region* (Midline Frontal, Midline Central, Midline Posterior) and *Right hemisphere* (Right Frontal, Right Central, Right Posterior).

The indices of Theta reactivity and Alpha reactivity were calculated from the EEG values using the formulas suggested by Fonseca, Tedrus, Fondelo Reis and Fontoura (2011):

Theta reactivity index= Theta power, eyes-open / Theta power, eyes-closed[71]

Alpha reactivity index= Alpha power, eyes-open / Alpha power, eyes-closed[72]

The independent variables for this study were as follows:

1. Sex (with two levels: males, females).
2. Sex and 2D4D (with four levels: males with 2D<4D (extreme masculinized), males with 2D>4D (Masculinized), females with 2D<4D (Feminized) and females with 2D>4D (extreme feminized).
3. Estradiol to testosterone ratio and 2D:4D (with four levels: low e/t ratio and 2D<4D (extreme masculinized), low e/t ratio and 2D>4D (masculinized), high e/t ratio and 2D<4D (feminized) and high e/t ratio and 2D<4D (extreme feminized; for more details please see Chapter 3).

[71] A higher reduction in Theta activity in the eyes-open condition leads to a lower value of Theta reactivity index and vice-versa. Thus, high reactivity index equals to small activity reduction from eyes-closed to eyes-open.
[72] A higher reduction in Alpha activity in the eyes-open condition leads to a lower value of Alpha reactivity index and vice-versa.

4.2.4 Procedure

An extension of Experiment 2a's ethical approval in order to cover EEG experimentation was obtained from Coventry University's Ethics committee. A briefing was given to each participant prior to the experimental procedure and written consent was obtained (Appendix 5).

All participants were advised not to use any hair products at the day of the EEG recording. Each participant was initially asked to sit on a comfortable chair in a sound proof[73]room and was asked to close their eyes. After the EEG recording for the eyes-closed condition was finished (2 minutes), the participant was asked to open their eyes and fixate their gaze on a designated mark on the wall, which was placed directly in front of them (approximately on the same height of their gaze); EEG was again recorded for two minutes. During all EEG recordings, all participants were advised to reduce any body movements as much as possible. The quality of the recording of each EEG site was visually inspected before the initiation of the recording phase in order to check for issues regarding the conductance of each cable. Any issues with conductance were dealt with before the recording phase.

4.2.5 Data analysis

A Friedman's test was used to examine the equality of Theta and Alpha reactivity indices in both sagittal (frontal, central, posterior; Part 1) and lateral (left, midline, right; Part 2) dimensions and a Mann-Whitney test explored the sex differentiation in each dimension. In case of a statistically significant sex differentiation in a dimension, the exact region of it was also indicated by a Mann-Whitney test; Bonferroni corrections were applied (Part 3). In these exact regions, a trend-analysis was employed (Part 4) in order to explore the existence of an

[73]The EEG laboratory was under development during the testing period; thus, background noise was elevated at some days. This issue potentially resulted to limited capacity of data extraction.

association / trend of the sex-differentiated regions with the individual level of masculinization / feminization as defined by sex alone, by sex and 2D:4D, and by estradiol to testosterone ratio and 2D:4D). Finally, in cases where differences between males and females were observed, differences in baseline levels (among eyes-closed condition) were also investigated using a Mann-Whitney test (Part 5) in order to be assured that the observed differentiations were not a result of different baseline levels. A more detailed description of the data analysis procedure may be seen in the account of the results for Theta. Moreover, the above analyses were computed for Alpha separately.

4.3 Results

4.3.1 Descriptives

In Table 17 age, cognitive measures and hormone levels for this sample of participants are presented and compared between males and females. As can be seen there are no significant differences between the groups on age, forward digit span, backward digit span, free recall, productive vocabulary, tower of Hanoi moves, tower of Hanoi time, 2D:4D, cognitive brain sex and psychological brain sex. However, significant differences are indicated in estradiol to testosterone ratio (e/t ratio). These results indicate that the only differentiating factors in this sample are circulating (free) gonadal hormones.

Table 17. Sample description and age, cognitive and hormonal data for males and females (results from Mann-Whitney tests)

	Sex						
	Male		Female		Total		Difference (Male-Female) P Value
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	
Counts	15.00		10.00		25.00		
Age	35.4	7	35.6	10	36	8	0.84
Digit span Forward	25.36	4.81	23.82	3.28	24.68	4.20	0.40
Digit span Backward	16.64	5.09	17.55	4.25	17.04	4.67	0.72
Free Recall	4.23	2.31	4.82	2.52	4.50	2.38	0.61
Productive vocabulary	42.33	15.44	41.90	8.70	42.14	12.54	0.97
Tower of Hanoi (moves)	171.00	76.92	183.64	102.99	176.56	87.55	0.91
Tower of Hanoi (time)	435.31	411.04	444.04	347.40	439.15	376.59	0.94
2D:4D	1.00	0.04	1.01	0.05	1.00	0.051	0.29
Estradiol_to_Testosterone	0.02	0.01	0.05	0.02	0.03	0.02	0.00*
BrainSex (cognitive)	0.04	0.12	-0.03	0.07	0.01	0.11	0.35
BrainSex (psychological)	0.05	0.08	-0.02	0.10	0.02	0.09	0.07

Note. *Results from Mann-Whitney tests.*

In Table 18 the distribution of males and female participants in relation to their individual hormone levels are presented for the three hormone-based groupings that were used in this experiment; that is, sex and 2D:4D, and estradiol to testosterone ratio and 2D:4D. Looking at the sex and 2D:4D grouping, it can be seen that the distribution of males and females in each category is in line with what we might expect, with only men represented in the 'masculinized' categories, and only females represented in the 'feminized' categories. Looking at the e/t ratio and 2D:4D grouping, it can be observed that in the extreme masculinized category, only males are present; while females are increasingly present as we proceed towards the extreme feminized category. Additionally, there were two male individuals allocated in the extreme feminized category.

Table 18. Sample grouping depending on individual hormone levels as defined by Sex and 2D:4D (four-level grouping), estradiol to testosterone ratio and 2D:4D

	Gender					
	Male		Female		Total	
	N	%	N	%	N	%
Sexand2D4D						
Extreme masculinized	6	42.9%	0	0.0%	**6**	**24.0%**
Masculinized	8	57.1%	0	0.0%	**8**	**32.0%**
Feminized	0	0.0%	4	36.4%	**4**	**16.0%**
Extreme feminized	0	0.0%	7	63.6%	**7**	**28.0%**
E/T Ratio and 2D:4D						
Extreme masculinized	6	42.9%	0	0.0%	**6**	**24.0%**
Masculinized	6	42.9%	1	14.3%	**7**	**29.2%**
Feminized	0	0.0%	3	30.0%	**3**	**12.5%**
Extreme feminized	2	14.3%	6	60.0%	**8**	**33.3%**
Total	**15**	**58.3%**	**10**	**41.7%**	**25**	**100.0%**

4.3.2 Theta reactivity index

Part 1: Regions of interest: Frontal, Central, Posterior (Sagittal factor)

Differences between the regions of the Sagittal factor in the whole sample.

The effects of topography on the whole sample's Theta reactivity were explored using a Friedman test, which is a non-parametric test for repeated measures (three levels: frontal, central, posterior). In Table 19 a summary of the results from the Friedman test is presented. As it can be seen in Table 19 there were no significant topographical differences between the regions of interest (χ^2=5.429, p>0.05); that is, Theta activity does not present any significant topographical differences across eyes-closed and eye-open conditions within the whole sample. Looking at the values of Theta reactivity in each topographical region, a Wilcoxon test indicated a greater Theta posterior decrease from eyes-closed to eyes-open condition compared to frontal (p=.009) and

central areas (albeit not significantly; p=.244)[74]. This finding is in line with previous studies that have reported a similar decrease in Theta power in these areas across eyes-closed / eyes-open conditions (Barry et al., 2007; Barry & De-Blasio, 2017).

Table 19. EEG theta reactivity index comparison for the Sagittal factor (whole sample)

	Sagittal Frontal	Sagittal Central	Sagittal Posterior
Descriptive			
Mean	1.209	0.970	0.907
Std. Deviation	0.653	0.211	0.314
Minimum	0.305	0.578	0.435
Percentile 25	0.763	0.842	0.642
Median	1.041	0.973	0.924
Percentile 75	1.447	1.134	1.069
Maximum	2.662	1.333	1.762
Friedman Test	**Chi - Squared**	5.429	
	p-value	0.066	

Sex differences in relation to the Sagittal factor.

Sex differences in Theta reactivity for Sagittal factors were explored using a Mann-Whitney test. More specifically, within the Sagittal factor, a Mann-Whitney test was used to compare the frontal, central and posterior areas between males and females. In Table 20 a summary of the results from the Mann-Whitney test is presented. As can be seen in Table 20, Theta reactivity shows a significant difference between the two groups only on the Sagittal frontal (z=-2.132, p<0.05) and on the Sagittal posterior (z=-1.990, p<0.05) areas; indicating that males had a greater reduction in Theta activity from eyes-closed to eyes-open compared females in these areas.

[74] Bonferroni correction was applied; alpha level set at 0.016

Table 20. Theta reactivity index on the EEG for the male and female groups (Sagittal factor)

	Sagittal Frontal		Sagittal Central		Sagittal Posterior	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	1.455	0.882	0.982	0.954	1.017	0.759
Standard Deviation	0.718	0.380	0.195	0.243	0.353	0.181
Minimum	0.500	0.305	0.578	0.622	0.435	0.478
Percentile 25	1.011	0.653	0.894	0.852	0.784	0.615
Median	1.120	0.815	0.992	0.877	1.001	0.764
Percentile 75	2.078	1.005	1.103	1.148	1.245	0.900
Maximum	2.662	1.565	1.333	1.307	1.762	1.042
Mann - Whitney Test						
Mann - Whitney U	24.000		47.000		26.000	
Z - Statistic	-2.132		-0.497		-1.990	
p-value	0.033		0.619		0.047	

Part 2: Regions of interest: Left Hemisphere, Midline region, Right hemisphere (Lateral factor).

Differences between the regions of the Lateral factor in the whole sample.

The effects of topography in the whole sample's Theta reactivity were explored using a Friedman test, (three levels: left hemisphere, midline regions, right hemisphere). In Table 21 a summary of the results from the Friedman test is presented.

Table 21. EEG theta reactivity index comparison for the Lateral factor (whole sample)

		Left Hemisphere	Midline region	Right Hemisphere
Descriptives				
Mean		1.085	0.981	1.020
Std. Deviation		0.488	0.233	0.430
Minimum		0.441	0.559	0.405
Percentile 25		0.793	0.814	0.752
Median		0.972	1.013	0.997
Percentile 75		1.300	1.177	1.178
Maximum		2.535	1.375	2.136
Friedman Test	**Chi - Squared**		2.571	
	p-value		0.276	

As it can be seen in Table 21 there are no significant topographical differences between the regions of interest; that is, Theta activity does not present any significant topographical differences across eyes-closed and eye-open conditions.

Sex differences on the Lateral factor

Sex differences in Theta reactivity for Lateral factors were explored using a Mann-Whitney test. More specifically, within the Lateral factor, a Mann-Whitney test was used to compare the left hemisphere, midline regions, right hemisphere areas between males and females. In Table 22 a summary of the results from the Mann-Whitney tests is presented. As it can be seen in Table 22, Theta reactivity presents a significant difference between the two groups only on the left hemisphere (z=-2.203, p<0.05).

Table 22. Theta reactivity index on the EEG for the male and female groups (Lateral factor)

	Left Hemisphere		**Midline Region**		**Right Hemisphere**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	1.267	0.842	1.046	0.894	1.142	0.858
Standard Deviation	0.553	0.244	0.231	0.218	0.484	0.300
Minimum	0.479	0.441	0.611	0.559	0.424	0.405
Percentile 25	0.929	0.723	0.895	0.748	0.835	0.650
Median	1.122	0.826	1.044	0.909	1.039	0.876
Percentile 75	1.573	0.912	1.224	1.036	1.376	0.997
Maximum	2.535	1.351	1.375	1.185	2.136	1.325
Mann - Whitney Test						
Mann - Whitney U	23.000		33.000		33.000	
Z - Statistic	-2.203		-1.492		-1.492	
p-value	0.028		0.136		0.136	

Part 3: Exploration of the sub-areas most affected by sex

Based on the above findings (from both sagittal and lateral observed sex differences), the areas where sex-differences are observed are the sub-areas that constitute frontal[75] and posterior[76] following the observed differences in the sagittal factor; and the areas that constitute the left hemisphere[77] following the observed differences in the lateral factor. Thus, the next step was to explore sex differences using a Mann-Whitney test across these areas in order to locate the exact region(s) that produce sex differences.

Starting with sex differences in the frontal areas, the summary of the results for sex differences in the frontal areas are presented in Table 23. As it can be observed in Table 23, sex differences are significant only on the left frontal area (z=-2.772, p<0.016).

Table 23. Theta reactivity index on the EEG for the male and female groups on the frontal areas (Left frontal, midline frontal, right frontal)

	Left frontal		**Midline frontal**		**Right frontal**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	1.66	0.81	1.09	0.94	1.62	0.90
Standard Deviation	1.03	0.43	0.34	0.30	1.08	0.54
Minimum	0.38	0.09	0.64	0.63	0.48	0.11
Percentile 25	1.08	0.67	0.85	0.71	1.05	0.63
Median	1.15	0.73	1.04	0.79	1.12	0.70
Percentile 75	2.34	0.94	1.22	1.26	2.25	1.12
Maximum	3.77	1.72	1.93	1.39	3.94	1.78
Mann - Whitney Test						
Mann - Whitney U	15.000		41.000		30.000	
Z - Statistic	-2.772		-0.924		-1.706	
p-value	0.006		0.356		0.088	

Note: Bonferroni correction applied. Alpha level was set at 0.016.

[75] Left frontal, midline frontal and right frontal
[76] Left posterior, midline posterior and right posterior
[77] Left frontal, central midline, right posterior

Continuing with sex differences in the posterior areas, the summary of the results for sex differences in the posterior areas are presented in Table 24. As it can be observed in Table 24, no sex differences are significant in either of the posterior areas.

Table 24. Theta reactivity index on the EEG for the male and female groups on the posterior areas (Left posterior, midline posterior, right posterior)

	Left posterior		**Midline posterior**		**Right posterior**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	1.11	0.78	1.06	0.85	0.88	0.65
Standard Deviation	0.57	0.16	0.25	0.25	0.33	0.24
Minimum	0.46	0.53	0.62	0.41	0.22	0.35
Percentile 25	0.77	0.69	0.85	0.75	0.62	0.48
Median	1.01	0.80	1.10	0.86	0.92	0.64
Percentile 75	1.26	0.85	1.30	1.05	1.13	0.79
Maximum	2.71	1.00	1.35	1.22	1.29	1.10
Mann - Whitney Test						
Mann - Whitney U	27.000		29.000		29.000	
Z - Statistic	-1.919		-1.777		-1.777	
p-value	0.055		0.076		0.076	

Note: Bonferroni correction applied. Alpha level was set at 0.016.

Next, sex differences in the left hemisphere[78] were explored. A summary of the results is presented in Table 25. As it can be seen in Table 25, sex differences are significant only on the left frontal areas. (z=-2.772, p<0.016). Based on these results it can be concluded that there is a higher reduction of Theta activity in males from eyes-open to eyes-closed condition in the left frontal area compared to females.

[78] Left frontal, left central, left posterior

Table 25. Theta reactivity index on the EEG for the male and female groups on the left hemisphere (Left frontal, left central, left posterior)

	Left frontal		Left central		Left posterior	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	1.66	0.81	1.03	0.94	1.11	0.78
Standard Deviation	1.03	0.43	0.27	0.22	0.57	0.16
Minimum	0.38	0.09	0.60	0.65	0.46	0.53
Percentile 25	1.08	0.67	0.87	0.76	0.77	0.69
Median	1.15	0.73	1.01	0.95	1.01	0.80
Percentile 75	2.34	0.94	1.15	1.12	1.26	0.85
Maximum	3.77	1.72	1.61	1.33	2.71	1.00
Mann - Whitney Test						
Mann - Whitney U	15.000		41.000		27.000	
Z - Statistic	-2.772		-0.924		-1.919	
p-value	0.006		0.356		0.055	

Note: Bonferroni correction applied. Alpha level was set at 0.016.

Part 4: Individual masculinization / feminization levels and left frontal Theta reactivity.

Following the observation of sex differences in the left frontal area, the next step was to explore whether the indices of masculinization / feminization levels are associated with the observed difference in the left frontal area. Thus, a Mann-Whitney U test and a Jonckheere-Terpstra test were employed, examining first the masculinization / feminization as it was measured by sex alone, by sex and 2D:4D, and by estradiol to testosterone ratio and 2D:4D). .

As it can be seen in Table 26, there is a significant association (trend) between levels of masculinization/ feminization as defined only by sex and Theta reactivity in the frontal areas (z=-2.465, p<0.05). Specifically, the descriptive statistics of the distributions of the two masculinization / feminization level groups show that Theta reactivity index tends to be lower in female than in male participants.

Table 26. Summary of the results from a Mann-Whitney U test. Association between levels of masculinization/feminization defined by sex in the left frontal area

	Sex	
	Male	**Female**
Descriptives		
Mean	1.66	0.81
Standard Deviation	1.03	0.43
Minimum	0.38	0.09
Percentile 25	1.08	0.67
Median	1.15	0.73
Percentile 75	2.34	0.94
Maximum	3.77	1.72
Jonckheere - Terprsta Test		
Mann - Whitney U	20	
Z - Statistic	-2.465	
p-value	0.013	

In the same context, a Jonckheere-Terpstra test was employed, examining the masculinization / feminization as it was measured by sex and 2D:4D. As it can be seen in Table 27, there is a significant association (trend) between levels of masculinization / feminization and Theta reactivity in the frontal areas (*St. J-T*=-3.024, $p<0.05$). Specifically, the descriptive statistics of the distributions of the four masculinization / feminization level groups show that Theta reactivity index tends to reduce as the feminization level increases (masculinization level decreases).

Table 27. Summary of the results from Jonckheere-Terpstra. Association between levels of masculinization/feminization defined by sex and 2D:4D in the left frontal area

	Masculinization/ feminization level			
	Extreme masculinized	**Masculinized**	**Feminized**	**Extreme feminized**
Descriptives				
Mean	2.10	1.22	0.83	0.80
Standard Deviation	1.21	0.64	0.11	0.53
Minimum	0.96	0.38	0.73	0.09
Percentile 25	1.11	1.06	0.73	0.65
Median	1.74	1.14	0.81	0.68
Percentile 75	3.25	1.24	0.94	0.95
Maximum	3.77	2.36	0.94	1.72
Jonckheere - Terprsta Test				
J-T Statistic		33		
Standard J-T Statistic		-3.024		
p-value		0.002		

Finally, the index of masculinization / feminization based on the individual hormone profile (based on estradiol to testosterone ratio and 2D:4D[79]) was used. As can be seen in Table 28, there is a significant association (trend) between levels of masculinization / feminization (defined by e/t ratio and 2D:4D) and Theta reactivity in the frontal areas (*St. J-T*=-1.968, $p<0.05$). Specifically, the descriptive statistics of the distributions of the four masculinization / feminization level groups show that Theta reactivity index tends to reduce as the feminization level increases (masculinization level decreases).

[79] Four groups were formed featuring extreme masculinized (low e/t ratio and 2D<4D), masculinized (low e/t ratio and 2D>4D), feminized (high e/t ratio and 2D:4D) and extreme feminized (high e/t ratio and 2D>4D); please refer to design section for more information.

Table 28. Summary of the results from Jonckheere-Terpstra. Association between levels of masculinization/feminization defined by e/t ratio and 2D:4D in the left frontal area

	Masculinization/feminization levels (e/t ratio and 2D:4D)			
	Extreme masculinized	**Masculinized**	**Feminized**	**Extreme feminized**
Descriptives				
Mean	2.28	1.15	0.90	0.90
Standard Deviation	1.25	0.76	0.23	0.50
Minimum	0.96	0.38	0.73	0.09
Percentile 25	1.11	0.65	0.73	0.67
Median	2.33	1.14	0.81	0.95
Percentile 75	3.25	1.24	1.16	1.14
Maximum	3.77	2.36	1.16	1.72
Jonckheere - Terprsta Test				
J-T Statistic		44		
Standard J-T Statistic		-1.968		
p-value		0.049		

Summarizing the above findings it can be observed that masculinization / feminization levels are associated with left frontal Theta reactivity. More specifically, as we move from more masculinized towards more feminized individuals left frontal Theta activity appears to be increased from eyes-closed to eyes-open. It is noteworthy that the observed trend is more intense and clear when the masculinization / feminization levels take into account both the sex of the participants and 2D:4D (p-value = 0.002), while it becomes weaker in cases when sex alone is taken into account (p-value = 0.014) and is marginally statistically significant when the sex of the participants is ignored, which is the case of e/t ratio and 2D4D variable (p-value 0.049).

Part 5: Theta activity at Eyes-closed condition (baseline)

Finally, since sex-differences were observed, analysis of EEG activity for the eyes-closed condition only was performed in order to specify whether the observed sex differences in reactivity can be attributed to the baseline (eyes-closed) condition. Exploring differences in the baseline level using a Mann-Whitney or a Kruskal-Wallis test (depending on the levels of the

masculinization / feminization categorization) enables the interpretation of reactivity; that is, the absence or presence of sex differences at the baseline level (eyes-closed) enables a better understanding of the main cause of any observed sex differences in reactivity (as argued by Kober & Neuper, 2011).

Regions of interest: Frontal, Central, Posterior (Sagittal factor)

Sex differences on the Sagittal factor.

In Table 29 a summary of the Mann-Whitney results is presented. As can be seen in Table 29, Theta activity does not show any significant difference between the two groups indicating that males and females do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas. These results are in line with Kober and Neuper (2011) who also indicated non-significant sex differences in the eyes-closed condition.

Table 29. Theta activity during eyes-closed condition for the male and female groups (Sagittal factor)

	Sagittal Frontal		**Sagittal Central**		**Sagittal Posterior**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	15.640	18.873	12.727	28.370	17.618	19.957
Standard Deviation	11.559	11.668	7.774	49.528	14.024	8.034
Minimum	6.117	7.292	3.925	8.675	5.175	9.625
Percentile 25	8.492	9.275	8.275	10.000	8.600	14.250
Median	10.800	16.033	10.500	11.975	12.413	19.975
Percentile 75	22.492	25.433	15.425	23.375	19.575	26.100
Maximum	43.242	46.392	29.475	176.800	47.550	36.500
Mann - Whitney Test						
Mann - Whitney U	62.000		57.000		50.000	
Z - Statistic	-0.821		-1.095		-1.478	
p-value	0.434		0.291		0.149	

Masculinization / feminization (based on sex and 2D:4D grouping) differences on the Sagittal factor.

In Table 30 a summary of the results from the Kruskal-Wallis test is presented. As it can be seen in Table 30, the four groups do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas.

Table 30. Theta activity during eyes-closed condition for the sex and 2D:4D groups (Sagittal factor).

Masculinization/feminization	Extreme masculinized	Masculinized	Feminized	Extreme feminized
		Sagittal Frontal		
Descriptives				
Mean	16.663	14.874	14.992	21.090
Standard Deviation	10.944	12.689	9.354	12.937
Minimum	8.450	6.117	7.292	8.033
Percentile 25	10.008	7.563	8.496	9.275
Median	11.250	9.892	12.225	19.050
Percentile 75	22.492	17.363	21.488	25.433
Maximum	36.525	43.242	28.225	46.392
Kruskal - Wallis Test				
Chi Squared		1.778		
p-value		0.620		
		Sagittal Central		
Descriptives				
Mean	12.792	12.678	51.900	14.925
Standard Deviation	8.991	7.381	83.270	6.430
Minimum	3.925	6.350	9.400	8.675
Percentile 25	8.275	7.963	9.738	10.000
Median	9.825	10.500	10.700	12.625
Percentile 75	15.425	14.575	94.063	23.375
Maximum	29.475	29.000	176.800	24.725
Kruskal - Wallis Test				
Chi Squared		1.308		
p-value		0.727		
		Sagittal Posterior		
Descriptives				
Mean	12.792	12.678	51.900	14.925
Standard Deviation	8.991	7.381	83.270	6.430
Minimum	3.925	6.350	9.400	8.675
Percentile 25	8.275	7.963	9.738	10.000
Median	9.825	10.500	10.700	12.625
Percentile 75	15.425	14.575	94.063	23.375
Maximum	29.475	29.000	176.800	24.725
Kruskal - Wallis Test				
Chi Squared		2.861		
p-value		0.414		

Masculinization / feminization (based on e/t ratio and 2D:4D grouping) differences on the Sagittal factor.

In Table 31 a summary of the results from the Kruskal-Wallis test is presented. As it can be seen in Table 31, Theta activity does not present any significant difference between the four groups indicating that the four groups do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas.

Table 31. Theta activity during eyes-closed condition for the e/t ratio and 2D:4D groups (Sagittal factor).

Masculinization/feminization	Extreme masculinized	Masculinized	Feminized	Extreme feminized
		Sagittal Frontal		
Descriptives				
Mean	16.663	18.130	17.558	17.465
Standard Deviation	10.944	13.032	9.576	13.389
Minimum	8.450	6.633	9.700	6.117
Percentile 25	10.008	8.492	9.700	8.654
Median	11.250	11.258	14.750	12.708
Percentile 75	22.492	23.467	28.225	22.242
Maximum	36.525	43.242	28.225	46.392
Kruskal - Wallis Test				
Chi Squared		0.371		
p-value		0.946		
		Sagittal Central		
Descriptives				
Mean	12.792	15.325	65.842	12.328
Standard Deviation	8.991	8.547	96.098	5.021
Minimum	3.925	6.350	9.400	6.425
Percentile 25	8.275	9.500	9.400	9.338
Median	9.825	10.600	11.325	12.213
Percentile 75	15.425	24.725	176.800	12.863
Maximum	29.475	29.000	176.800	23.375
Kruskal - Wallis Test				
Chi Squared		0.774		
p-value		0.856		
		Sagittal Posterior		
Descriptives				
Mean	17.092	22.154	22.033	16.578
Standard Deviation	11.611	16.316	13.555	7.070
Minimum	5.175	7.375	9.625	5.400
Percentile 25	9.425	8.600	9.625	11.988
Median	14.950	13.775	19.975	16.188
Percentile 75	19.575	40.625	36.500	21.938
Maximum	38.475	47.550	36.500	27.000
Kruskal - Wallis Test				
Chi Squared		0.433		
p-value		0.933		

Regions of interest: Left Hemisphere, Midline region, Right hemisphere (Lateral factor).

Sex differences on the Lateral factor

In Table 32 summary of the results from the Kruskal-Wallis test is presented. As it can be seen in Table 32, Theta activity does not present any significant difference between the two groups indicating that males and females do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas.

Table 32. Theta activity index on the EEG for the male and female groups (Lateral factor)

	Left Hemisphere		**Midline Region**		**Right Hemisphere**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	16.557	19.702	13.914	28.455	15.514	19.043
Standard Deviation	12.431	8.538	9.011	47.239	11.463	10.223
Minimum	5.975	9.650	4.383	8.225	6.200	6.950
Percentile 25	8.925	10.425	9.575	10.592	8.650	10.775
Median	11.150	20.350	10.888	13.700	10.188	17.675
Percentile 75	19.700	27.550	18.017	21.967	19.775	24.725
Maximum	43.900	31.100	33.467	170.125	42.425	40.825
Mann - Whitney Test						
Mann - Whitney U	56.000		53.000		49.000	
Z - Statistic	-1.150		-1.314		-1.533	
p-value	0.267		0.202		0.134	

Masculinization / feminization (based on sex and 2D:4D grouping) differences on the Lateral factor.

As it can be seen in Table 33, Theta activity does not present any significant difference between the four groups indicating that the four groups do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas.

Table 33. Theta activity during eyes-closed condition for the sex and 2D:4D groups (Lateral factor)

Masculinization/feminization	Extreme masculinized	Masculinized	Feminized	Extreme feminized
		Left Hemisphere		
Descriptives				
Mean	16.404	16.672	17.575	20.918
Standard Deviation	11.412	13.926	10.019	8.159
Minimum	7.000	5.975	9.650	10.425
Percentile 25	8.925	8.113	9.688	11.500
Median	12.438	10.650	15.038	22.850
Percentile 75	19.700	22.988	25.463	27.550
Maximum	37.925	43.900	30.575	31.100
Kruskal - Wallis Test				
Chi Squared		1.911		
p-value		0.591		
		Midline regions		
Descriptives				
Mean	14.488	13.483	50.467	15.876
Standard Deviation	9.937	8.931	79.795	5.293
Minimum	4.383	5.617	8.225	9.983
Percentile 25	9.575	8.421	9.408	11.708
Median	11.150	10.888	11.758	14.700
Percentile 75	18.017	15.083	91.525	21.967
Maximum	32.650	33.467	170.125	24.333
Kruskal - Wallis Test				
Chi Squared		2.160		
p-value		0.540		
		Right Hemisphere		
Descriptives				
Mean	15.654	15.409	17.831	19.736
Standard Deviation	9.848	13.219	15.518	7.228
Minimum	7.725	6.200	6.950	10.350
Percentile 25	9.975	7.500	8.863	12.400
Median	11.275	9.425	11.775	20.300
Percentile 75	19.775	20.400	26.800	24.725
Maximum	33.900	42.425	40.825	31.575
Kruskal - Wallis Test				
Chi Squared		3.397		
p-value		0.334		

Masculinization / feminization (based on e/t ratio and 2D:4D grouping) differences on the Lateral factor.

As it can be seen in Table 34, Theta activity does not present any significant difference between the four groups indicating that the four groups do not have significant differences in Theta activity at the baseline level (eyes-closed) in these areas.

Table 34. Theta activity during eyes-closed condition for the e/t ratio and 2D:4D groups (Lateral factor)

Masculinization/feminization	Extreme masculinized	Masculinized	Feminized	Extreme feminized
		Left Hemisphere		
Descriptives				
Mean	16.404	20.286	20.217	17.225
Standard Deviation	11.412	14.469	10.426	8.727
Minimum	7.000	7.275	9.725	5.975
Percentile 25	8.925	8.950	9.725	10.963
Median	12.438	10.675	20.350	14.763
Percentile 75	19.700	33.025	30.575	24.638
Maximum	37.925	43.900	30.575	31.100
Kruskal - Wallis Test				
Chi Squared		0.316		
p-value		0.957		
		Midline Regions		
Descriptives				
Mean	14.488	16.323	63.758	13.093
Standard Deviation	9.937	9.125	92.146	5.468
Minimum	4.383	6.883	8.225	5.617
Percentile 25	9.575	10.767	8.225	9.971
Median	11.150	11.725	12.925	12.704
Percentile 75	18.017	21.967	170.125	14.721
Maximum	32.650	33.467	170.125	24.333
Kruskal - Wallis Test				
Chi Squared		0.697		
p-value		0.874		
		Right Hemisphere		
Descriptives				
Mean	15.654	19.000	21.458	16.053
Standard Deviation	9.848	13.490	16.802	8.299
Minimum	7.725	6.200	10.775	6.350
Percentile 25	9.975	9.400	10.775	9.500
Median	11.275	11.475	12.775	15.038
Percentile 75	19.775	29.325	40.825	20.713
Maximum	33.900	42.425	40.825	31.575
Kruskal - Wallis Test				
Chi Squared		0.738		
p-value		0.864		

Based on these results and in line with Kober and Neuper's findings and interpretation, it can be argued that the above sex-differences in Theta reactivity refer to the eyes-open condition, especially on the active processing of unstructured visual information.

4.3.3 Alpha reactivity index

Part 1: Regions of interest: Frontal, Central, Posterior (Sagittal factor)

Differences between the regions of the Sagittal factor in the whole sample.

The effects of topography in the whole sample's Alpha reactivity were explored using a Friedman test (three levels: frontal, central, posterior). In Table 35 a summary of the results from the Friedman test is presented. As it can be seen in Table 35 there are significant topographical differences between the regions of interest (χ^2=35,524, p<0.05); that is, Alpha activity present significant topographical differences across eyes-closed and eye-open conditions. Specifically, the Alpha in eyes open condition is about 1/3 of the Alpha in eyes closed condition in the posterior region, while in the frontal and the central regions it is about 1/2. Thus, the Alpha reduction is larger in the posterior region in comparison to the Alpha reduction in the frontal and the central regions. Again, these results are in line with previous studies that have indicated a greater posterior reduction compared to frontal and central areas due to a higher activation of the occipital lobe (Barry & De Blasio, 2017).

Table 35. EEG Alpha reactivity index comparison for the Sagittal factor (whole sample)

	Sagittal Frontal	Sagittal Central	Sagittal Posterior
Descriptives			
Mean	0.500	0.565	0.336
Std. Deviation	0.304	0.255	0.248
Minimum	0.115	0.194	0.050
Percentile 25	0.247	0.326	0.123
Median	0.451	0.531	0.247
Percentile 75	0.753	0.798	0.597
Maximum	1.204	1.005	0.798
Friedman Test	**Chi - Squared**	35.524	
	p-value	0.000	

Sex differences on the Sagittal factor.

Sex differences in Alpha reactivity for Sagittal factors were explored using Mann-Whitney tests. Table 36 provides a summary of these results. As it can be seen, Alpha reactivity does not present any significant difference between the two groups on the Sagittal factor.

Table 36. Alpha reactivity index on the EEG for the male and female groups (Sagittal factor)

	Sagittal Frontal		Sagittal central		Sagittal posterior	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	0.459	0.554	0.516	0.629	0.305	0.378
Standard Deviation	0.263	0.361	0.238	0.277	0.240	0.266
Minimum	0.119	0.115	0.194	0.284	0.050	0.114
Percentile 25	0.241	0.276	0.301	0.427	0.126	0.124
Median	0.428	0.455	0.527	0.543	0.225	0.267
Percentile 75	0.706	0.794	0.667	0.804	0.483	0.599
Maximum	0.860	1.204	0.950	1.005	0.798	0.774
Mann - Whitney Test						
Mann - Whitney U	46.000		41.000		43.000	
Z - Statistic	-0.569		-0.924		-0.782	
p-value	0.570		0.356		0.434	

Part 2: Regions of interest: Left Hemisphere, Midline region, Right hemisphere (Lateral factor).

Differences between the regions of the Lateral factor in the whole sample.

The effects of topography in the whole sample's Alpha reactivity were explored using a Friedman test, which is a non-parametric analysis test for repeated measures (with three levels: left hemisphere, midline regions, right hemisphere). In Table 37 a summary of the results from the Friedman test is presented. As it can be seen in Table 37 Alpha activity does not present any significant topographical differences across eyes-closed and eye-open conditions.

Table 37. EEG Alpha reactivity index comparison for the Lateral factor (whole sample)

	Left Hemisphere	**Midline region**	**Right Hemisphere**
Descriptives			
Mean	0.497	0.453	0.450
Std. Deviation	0.257	0.279	0.267
Minimum	0.189	0.075	0.129
Percentile 25	0.315	0.167	0.215
Median	0.414	0.435	0.391
Percentile 75	0.812	0.735	0.674
Maximum	0.954	0.929	0.979
Friedman Test	**Chi - Squared**	4.667	
	p-value	0.097	

Sex differences on the Lateral factor.

Within the Lateral factor, a Mann-Whitney test was used to compare the left hemisphere, midline regions, right hemisphere areas between males and females. As it can be seen in Table 38, Alpha reactivity does not present any significant difference between the two groups on the Lateral factor.

Table 38. Alpha reactivity index on the EEG for the male and female groups (Lateral factor)

	Left Hemisphere		Midline region		Right Hemisphere	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Descriptives						
Mean	0.472	0.531	0.401	0.522	0.406	0.509
Standard Deviation	0.247	0.280	0.253	0.311	0.236	0.309
Minimum	0.189	0.215	0.075	0.149	0.129	0.149
Percentile 25	0.266	0.338	0.167	0.299	0.213	0.312
Median	0.402	0.449	0.411	0.465	0.362	0.404
Percentile 75	0.723	0.826	0.546	0.794	0.578	0.770
Maximum	0.847	0.954	0.896	0.929	0.865	0.979
Mann - Whitney Test						
Mann - Whitney U	45.000		41.000		41.000	
Z - Statistic	-0.640		-0.924		-0.924	
p-value	0.554		0.382		0.382	

The above results showed a total absence of sex differentiations in Alpha reactivity.

4.4 Discussion

Following the rationale and findings from Chapter 3, this Chapter was designed to search for a sex differentiated rhythm of intrinsic brain activity related to information encoding / hippocampal activity. Following Barry et al.'s (2004) procedure for EEG data analysis, the results indicated that the difference between eyes-open and eyes-closed left frontal Theta activity (that is, Theta reactivity) is differentiated by sex. More specifically, these results indicated that there is a higher reduction (de-synchronization) of Theta activity in males from eyes-open to eyes-closed condition in the left frontal area compared to females. According to the work of Barry and colleagues a decrease in localized theta activation from eyes-closed to eyes-open is an index of an increased activation of the specific area; and since this activation is not related to a specific task it is related to 'unstructured visual processing of the immediate environment' (Barry

& De Blasio, 2017, p. 302). Based on the current results it can be argued that opening the eyes caused higher activation of left frontal areas on males compared to females. Since there were no topographical sex differences in Alpha reactivity (in line with Barry, Clarke, Johnstone, Brown, 2009) it can be argued that no sex differences in the central nervous system arousal levels between the two sexes were present in the current sample and consequently did not affect current findings regarding Theta reactivity. Thus, it can be concluded that in both sexes visual stimulation (that is the eyes-open condition) resulted in an equal response in the visual cortex. The observed change in Alpha reactivity can be translated as an increase in tonic energy levels; while this increase is related to an increase in arousal levels driven by basic sensory input (Barry et al., 2009). Looking at the observed sex differences in localized Theta along with the absence of sex differences in Alpha reactivity as well as the absence of sex differences in Theta activity at the baseline level (eyes-closed condition), it can be argued that sex differences in Theta reactivity occurred due to differences in localized activation and not due to just a general difference in arousal (Barry et al., 2009).

High Theta de-synchronization in left frontal areas has been related to the processing of information that is cognitively demanding (Khader & Rosler, 2004). In other words, processing of information that has a high demand on collecting, combining and retrieving information is associated with high left frontal Theta de-synchronization (Khader & Rosler, 2004). As it was stated above, in the absence of a specific task, Theta synchronization / de-synchronization is related to unstructured visual processing of the immediate environment (Barry & De Basio, 2017). Thus, it can be argued that the observed sex difference in Theta synchronization / de-synchronization in the left frontal areas indicate a baseline sex difference in the processing of unstructured visual information. More specifically, males appear to use more effort when

processing information than females and this is reflected on a higher Theta de-synchronization on the left frontal areas; while this is further supported from the observed higher activation on the left frontal areas of males compared to females. In fact, current results indicated that in males only, Theta was de-synchronized, while in females Theta was synchronized from eyes-closed to eyes-open condition. Consequently, in the absence of a specific task, these findings allow one to argue for the existence of a sex-specific baseline difference in information processing ability.

Similar results were reported by Kober and Neuper (2011), who indicated a higher female Theta synchronization in a rest versus test (spatial navigation task) condition. In their results, Kober and Neuper (2011) indicated a significant sex difference in Theta synchronization, concluding that in a task that did not produced any sex differences in performance, sex differences in Theta synchronization appeared to be significant. Based on the results from Kober and Neuper (2011) and current study results it can be argued that Theta reactivity is related to sex; while this sex-related difference is also observable at a baseline level (measured at eyes-closed versus eyes-open resting states). Thus, females, by default, appear to have an advantage in processing information; an advantage that has been observed at a behavioural level from early ages (see Chapter 1, Section 1.8.1).

Theta rhythm and hippocampal function have been previously reported to be related (Klimesch, 1999; Bastiaansen & Hagoort, 2003). In fact, existing literature suggests that Theta rhythm (in humans) is generated from the communication between cortical structures and hippocampal structures (Klimesch, 1999). Moreover, hippocampal structure and function appears to be significantly affected by gonadal hormones (pre-natal and post-natal; see Chapter 1, Section 1.2). Consequently it would be informative to explore whether individual masculinization / feminization levels are associated to Theta rhythm; since this association will enable the

exploration of a potential link between masculinization / feminization and information processing.

Consequently, the next step was to look for a statistical trend of left frontal Theta reactivity associated with the individual level of masculinization / feminization as indicated by sex alone, sex and 2D:4D factor and estradiol to testosterone ratio and 2D:4D factor. Results indicated that there was an association between masculinization / feminization levels and left frontal Theta reactivity as measured in terms of synchronization / de-synchronization (eyes-open / eyes-closed). More specifically, Theta reactivity in the left frontal region was found to be associated with masculinization / feminization when masculinization / feminization was measured by sex, by sex and 2D:4D variable as well as estradiol to testosterone ratio and 2D:4D categorization. Based on these results it can be argued that information processing ability at a base-line level (Theta reactivity in eyes-closed versus eyes-open condition) is linked to gonadal hormones; while this link implicates the hippocampus as the main candidate for affecting sex differences in information processing.

Individual masculinization / feminization levels measured by sex and 2D:4D appear to be better associated with left frontal reactivity ($p=.002$) compared to the levels defined by sex alone ($p=.014$), while the levels measured both by sex and 2D:4D variable and sex alone appear to be better associated to left frontal reactivity compared to e/t ratio and 2D:4D variable[80] ($p=.049$).

Based on the idea that the sex of the foetus mediates the effects of pre-natal hormones on the brain (see Zhengui & Cohn, 2011; also Chapter 1, Section 1.2) it is suggested that sex as a factor should be included in future research exploring individual hormonal profile. The association

[80] From now on referred as hormonal profile

between sex and 2D:4D-based level of individual masculinization / feminization and Theta reactivity possibly indicates a closer relationship between pre-natal masculinization / feminization and information processing ability; a finding that relates to the hippocampus-amygdala hypothesis, since this hypothesis stresses the importance of pre-natal configuration of the hormone-affected brain structures with regard to cognitive development. Consequently, the finding of a weaker association between individual levels of masculinization / feminization measured by hormonal profile only and Theta reactivity may stress the importance of the factor of sex in the pre-natal masculinization / feminization effects as well as sex linked (XX-XY) chromosomal information. However, due to the small sample size of the current research, these inferences cannot be conclusive and may be regarded more as an indication for the potential relationship between individual levels of masculinization / feminization and Theta reactivity (i.e. information processing ability).

At this point it is important to stress the fact that this study suffered significantly from data loss. EEG recordings from almost 50% of the sample were not eligible for further analysis due to enhanced signal noise, which made data extraction impossible. This problem can be attributed to work undertaken on the EEG laboratory and specifically on the sound-proof and electromagnetic-proof repair that took place during a part of the testing period. This issue probably led to a significant reduction of the tested sample (41 individuals to 25 individuals that their EEG recordings were readable). However, in the remaining sample the proportion of males and females were equal, while there were no cognitive differences observed in this sample. This allows the argument that the observed EEG differences cannot be attributed to sample-differences on cognitive mechanisms that might be related to EEG activity. Since current findings are in line with previous studies in terms of Alpha and Theta topography as well as sex

differences in frontal Theta reactivity, it can be concluded that current study is in line with existing research. In addition, the apparent relationship with hormonal profile and left frontal reactivity supports the idea that individual hormonal profile may be related to information processing ability. In general, these findings support the notion that emerged from the previous experiments, that sex adds information regarding pre-natal brain masculinization / feminization and consequently the qualities of sex as a hormonal regulating factor are those worthy of research. Thus, it can be argued that pre-natal hormones affect specific brain structures that are related to basic information processing ability (such as hippocampus) and this is reflected on left frontal Theta reactivity.

As was indicated by studies with rodents and humans (Chapter 1, Section 1.3.1) the hippocampus appears to belong to a system responsible for collecting and creating 'units' of information and incorporating them to the memory system. According to the findings and suggestions of Gentner (2006) and Gabrieli et al. (1998) this activity is related to the left-frontal cortex while Theta rhythm synchronization / de-synchronization[81] is a potential index of this activity / ability.

Summarizing, sex differences were observed in left frontal Theta reactivity; while no sex differences appeared in the Alpha reactivity index. These findings allow for the argument that the observed sex differences in the Theta reactivity index are not caused / affected by sex differences of the central nervous system arousal levels. Moreover, left frontal Theta reactivity index was inversely related to individual levels of masculinization / feminization; that is, higher feminization levels were related with lower Theta reactivity and vice-versa. Based on the existing literature regarding the attributes of left frontal Theta reactivity, it was argued that the

[81] That is, Theta reactivity.

observed difference in Theta reactivity is related to a baseline sex-difference in information processing ability. Since sex differences in information processing ability are observed behaviourally from a young age, the results from the current study support and extend this behavioural observation. Based on the current study it can be argued that there might be a sex difference in information processing ability and this sex difference is related to individual levels of masculinization / feminization. The observed relationship between levels of masculinization / feminization and information processing ability takes us beyond the previously-observed behavioural sex difference in information processing, to argue that the difference is not between sexes but between different hormonal profiles. That is, different hormonal profiles affect the organization and activation of the brain, resulting in differences in information processing ability, which leads to the development of different cognitive profiles. In Chapter 3 it was suggested that individual hormonal profile is related to individual cognitive phenotype (that is, brain sex type). Consequently, based on the current research findings it can be argued that the link between hormonal profile and brain sex type may be information processing ability. However, a direct relationship between information processing ability and brain sex type might not be possible due to the developmental effect that information processing ability has on the brain. That is, a different level of information processing ability leads to different developmental cognitive pathways, resulting in the development of an individual's brain sex type. However, this argument still remains a hypothesis and further research is needed.

Chapter 5: General Discussion

5.1 Introduction

In this chapter, the key findings from the earlier chapters will be summarized, and the main findings and implications will be discussed. Next, all results will be considered in the context of a theoretical framework to connect and rationalize existing findings and theories from developmental, behavioural and neuro-cognitive psychology.

5.2 Summary of findings

5.2.1 Experiment 1 (pilot study)

The main hypothesis of this experiment addressed a common and widely researched female advantage on verbal free recall tasks. Previous research has argued that verbal free recall is based on the functionality of the hippocampus and the functionality of the frontal lobe (Moscovitch & Winocur, 1992). Considering these findings alongside the hippocampus-amygdala hypothesis it was argued that sex differences in free recall may be linked to brain masculinization / feminization levels. Specifically, it was argued that 'sex', as a factor, provides relative information regarding three indices that appear to affect hippocampal and amygdala masculinization / feminization levels; that is, X-Y related chromosomal background, pre-natal hormone effects (including the function of alpha-pheto-protein) and post-natal hormone effects. Consequently, since free recall is sexually differentiated and also related to the hippocampus, it was argued that the reason behind the observed sex-bias in this task may be due to individual hippocampal masculinization / feminization levels. Initially, sex differences in free recall were

examined controlling for factors that appear to engage frontal lobe function in an attempt to examine whether sex differences will remain in such an analysis. Moreover, based on the hippocampus-amygdala hypothesis, it was argued that the effect of sex would be stronger in a condition where free recall was required after distraction. The results indicated that sex differences in free recall task remained significant even after controlling for variables that engage frontal lobe function[82]. However, the prediction regarding the strength of the observed sex differences in free recall after distraction was not supported. However, a factor that was not taken into consideration was the handedness of participants, which has also been reported by previous studies to be related to free recall performance (Siengthai et al., 2008) and pre-natal hormone effects (Kelso et al., 2000). Thus, based on the finding that free recall after distraction did produced the stronger effect at baseline level as well as the potential effects of handedness on both brain masculinization / feminization and free recall performance the initial hypothesis was retained for further consideration.

At this point it is useful to analyse in more in-depth the exact function of the verbal recall task. 'Verbal free recall' is an umbrella term that is used to describe a group of tests that require the verbal recall of any type of information (this might be words, letters, numbers, pictures etc.). However, the use of different stimuli may result in different mechanisms of the cognitive system or intervening factors being tapped; for example, using free recall of letters and numbers is accepted as a verbal working memory task (see Rossenbaum & Park, 2002; Poromaa & Gingnell, 2014). Thus, the identification of the exact paradigm that produces the most prominent sex differences was considered important in order to enable the identification of the factors that lie beneath those differences.

[82] Executive function and working memory are cognitive function related to frontal lobe (Welsh et al., 1999). Note: it is not implied that the whole range of frontal lobe function can be covered by the tasks that were used.

Working memory is a theoretical construct proposed by Baddeley (1998; 2000) and is based on the idea that cognitive performance is the result of an orchestration of different components (I.e. the *phonological loop*, the *visuospatial sketchpad*, the *episodic buffer* and the *central executive*) alongside long-term memory. This model is considered to be the most successful account of memory processes to-date, since it can explain most of the experimental findings on cognition (see: Repovs & Baddeley, 2006). However, Baddeley's model has not been unchallenged. Several researchers have challenged Baddeley's model based on experimental findings that either do not fit in the unitary nature of Baddeley's model[83] or violate some basic assumptions[84] of it. Such findings initiated a new line of research towards a non-unitary model of cognition (Collette & Linden 2002; Chein, Ravizza, & Fiez, 2003; Zimmer, 2008). Consequently, in a non-unitary cognitive system the engagement of different 'components' during a task translates to an engagement of different factors that affect performance. Thus, based on this rationale, when a task is thought to tap on working memory, such as specific versions of verbal free recall do, it can be argued that several different factors intervene and affect performance. In these cases such tasks are not useful for isolating the factor(s) behind sex-biased tasks.

Exploring the effects of the verbal, word-based free recall task through Baddeley's working memory model, it can be seen that only a few components are engaged during this task (episodic

[83]For example Smith, Jonides and Koeppe *(*1996) indicated an activation of left parietal cortex during tasks using non-verbal stimuli. However, this area should only be activated by phonological input, meaning that the results stand in contrast with the notion that every component of working memory model is unitary. Similarly, Collette and Linden (2002) based on neurological studies assessing *central executive*, indicated that lesions on the *frontal lobe* do not always evoke executive problems, which raises the issue that *central executive* may also not be a unitary component.

[84]For example, Baddeley and Levy (1971 cited in: Repovs & Baddeley 2006) reported that in 'immediate recall' experiments a higher recall performance can be observed in cases where given words can be paired in such way as to have a common meaning. This observation stands in contrast with Baddeley´s assumption that verbal input is stored only by its sound. Furthermore, Baddeley (2000) stated that a continuous sub-vocal rehearsal of a certain word should interrupt the processing of verbal information completely. In practice experiments indicate only a reduction of processed input.

buffer and phonological loop). Equally, if verbal word free recall task is explored through a more unitary model (such as the one proposed on within this thesis) again there are few factors left that are capable of mediating / affecting performance[85]. As can be seen, the free recall paradigm used in this thesis limits the number of potential mechanisms / factors that can affect performance compared to multi-trial free recall paradigms (such as the one used in Krueger & Salthouse, 2010) and provides a reasonable basis for analysis and interpretation.

The next step was to use verbal free recall after distraction as a reference point in order to define an individuals' cognitive phenotype. That was done by introducing another task that appears in the literature as non-female-biased; that is a productive vocabulary task[86]. Thus, measuring an individual's free recall after a distraction in relation to his / her productive vocabulary scores, his / her cognitive phenotype was characterised, resulting in a brain type scale featuring the following groups: productive vocabulary (PV) >> free recall (FR), PV > FR, PV ≈ FR, PV > FR, PV >> FR. Results indicated a good fit for cognitive phenotypes on the brain type scale, as observed by the proportion of males and females falling into each category / brain type; that is, more males were allocated on the PV >> FR category (where no females were allocated in this category) while more females were allocated on the PV << FR category (where no males were allocated in this category). These results constituted a promising starting point regarding the exploration of brain type. The next step was to investigate the biological factors that may be related to these behavioural patterns.

[85] According to the rationale of this thesis these factors are basic information processing ability and memory structure which cause sex differences in this task as well as to every sex-biased task

[86]It was taken under consideration that both tasks needed to utilize common cognitive mechanisms (insofar of permissible) in order to produce results that could be analysed safely; for this reason both tasks were language-based and consequently both required the engagement of existing memory.

5.2.2 Experiment 2

Following up Experiment 1's findings, Experiment 2 was designed to address the hypothesized effects of gonadal hormones on free recall performance. In addition, stricter inclusion / exclusion criteria were applied in order to exclude the potential impact of handedness in free recall performance; an impact that it was suspected to be responsible for reducing within task sex differences in the previous study. The results supported and extended the findings from the first experiment. That is, a female advantage in free recall performance remained significant even after controlling for executive function, working memory and age. In addition, the observed female advantage in free recall was found to be significant in the first part of the free recall task and the free recall after distraction (where the greatest effect size was observed). These findings were interpreted to suggest that free recall after distraction is the aspect of free recall that drives the observed effect of sex differences. In terms of the hippocampus-amygdala hypothesis, this finding is related to the hypothesis that free recall after distraction is a free recall task that is more related to hippocampal function, relatively isolated from amygdala contribution.

The results also indicated that the combination of pre-natal and post-natal hormone indices (i.e. individual's hormonal profile) eliminated the effects of sex on free recall performance. Moreover, the factor 'hormonal profile' was found to create stronger differentiations in free recall performance compared to the factor 'sex'. These findings were interpreted to suggest that the factor 'sex' is less informative regarding free recall performance compared to the factor 'hormonal profile'; leading to the argument that sex differences in free recall performance stem from individual masculinization / feminization differences.

Based on studies that have indicated the hippocampus and amygdala as two brain structures that are significantly affected by gonadal hormones (both pre- and post-natal) as well as studies that are related to free recall performance and hippocampal function, it was argued that the relationship between individual masculinization / feminization levels and free recall performance may stem from the relationship between individual masculinization / feminization effects on the hippocampus. In short, it was argued that hippocampal activity, individual masculinization / feminization levels and free recall may be related. This argument was further supported by the finding of a higher explanatory capacity of the hormonal profile variable on free recall after distraction. Based on the hippocampus-amygdala hypothesis, free recall after distraction is the part of the free recall task that is relatively isolated from the contribution of amygdala and consequently more strongly related to hippocampal function. Thus, this finding was in line with the hippocampus-amygdala hypothesis supporting the idea of a relationship between hippocampal function and individual masculinization / feminization levels.

Further extending the hypothesis and findings from Experiment 1, an individual's cognitive phenotype was calculated from performance on free recall and productive vocabulary scores, and its relationship to all masculinisation / feminization factors (i.e. sex; sex and 2D:4D; hormonal profile) was explored. The results supported and expanded findings from Experiment 1 since the addition of 2D:4D indicated that brain type as measured via the behavioural data was closely associated with pre-natal masculinisation / feminization. Moreover, grouping participants based on pre- and post-natal hormone levels also indicated an association between pre- and post- natal masculinization / feminization and cognitive brain type. Based on these results it was suggested that brain type is not confined to the limits of sex (i.e. a 'female' brain type can be observed in

males also; an observation in line with previous studies that have also addressed brain type (Goldenfeld et al., 2005)

Next, in order to explore the relationship between the behaviourally measured brain type and Baron-Cohen's (2014) proposed systemizing-empathizing typology (S-E brain type), measures of systemizing and empathizing were added. The results regarding S-E brain type and male-female allocation on the brain type scale appeared to be in line with previous studies, indicating a male prevalence in type S brain type category and a female prevalence in type E brain type category. Moreover, the relationship between individual masculinization / feminization levels and S-E was explored. The results indicated a significant association between masculinization / feminization levels (measured by sex and 2D:4D) and S-E brain type. That is, higher masculinization levels were associated with brain types towards the type S and extreme type S categories, while higher feminization levels were associated with brain types towards the type E and extreme type E categories. However, the same association was not significant when masculinization / feminization levels were measured by hormone indices solely. Finally, the association between brain type measured by systemizing-empathizing and brain type measured by productive vocabulary-free recall performance was tested. The results indicated a significant association between the two typologies; allowing the possibility of a common neuro-biological basis to the two groupings.

Based on the results from Experiment 2 it was concluded that the factor 'sex' may be seen as an index that provides relative information regarding an individual's pre-natal hormone effects as well as post-natal hormone effects on the brain structure and function. Consequently, when addressing sex-biased tasks, the use of a more accurate index of pre-natal and post-natal hormone effects (e.g. hormonal profile) may enable a better understanding of the underlying

cognitive mechanisms that are related to cognitive sex differences. Moreover, the apparent association between individual masculinization / feminization levels and brain type led to the conclusion that the hippocampus-amygdala system is a good potential candidate for explaining the appearance of this association. Consequently, the next step would be to address directly either the argued link between hippocampus-amygdala function and free recall (and / or brain type), or the link between masculinization / feminization levels and hippocampal/amygdala functionality.

5.2.3 Experiment 3

As stated above, there were two possible ways to approach the argued engagement of hippocampus in the development of brain type and / or on free recall performance. The first would be to directly manipulate hippocampal function during free recall, while the second approach would be by exploring the argued link between masculinization / feminization levels and hippocampal functionality. Since the first approach had a significantly higher difficulty level for ethical reasons, the second approach was adopted.

Directed by the existing literature on EEG brain rhythms, Theta and Alpha rhythms were chosen in order explore individual masculinization / feminization levels and hippocampal functionality. Specifically, existing literature (Klimesch, 1999) directly implicates hippocampal formations with the production of Theta rhythm in humans; while Alpha rhythm is seen as a measure of the level of central nervous system arousal (Barry, 2004; 2007). Following this literature it was hypothesized that Theta rhythm (reactivity) will be sexually differentiated. In addition, it was argued that this sexual differentiation would be due to the information that the factor ‘sex’ provides regarding individual masculinization / feminization levels. That is, individual

masculinization / feminization levels were expected to be significantly associated to Theta reactivity.

The results indicated that Theta reactivity[87] was sexually differentiated in left frontal areas; with males indicating higher reactivity compared to females. Following existing literature (see the work of Barry et al., 2004; 2009; 2017 as well as Khader & Rosler, 2004), these results were interpreted to suggest for the existence of a reduced ability in males in processing unstructured visual information. Since similar findings have been observed by other researchers (see Kober & Neuper, 2011) where a female advantage in processing information was linked to Theta synchronization, then current findings were seen as an indication of a reduced baseline level of basic information processing ability in males. Consequently, the next step would be to investigate whether basic information processing ability, as indicated by left frontal Theta reactivity, is linked to individual masculinization / feminization levels. The results indicated that there was a significant association between left frontal Theta reactivity and individual levels of masculinization / feminization as indicated by the sex and 2D:4D factor, and the hormonal profile factor. That is, higher masculinization levels were associated with lower information processing ability while higher feminization levels were associated with higher information processing ability. These results supported the proposed relationship between individual masculinization / feminization levels and basic information processing ability, providing a link between hippocampus, gonadal hormones and cognition.

[87] That is, the difference between eyes-open and eyes-closed Theta activity

5.3 Gonadal hormone-affected biological factors that relate to cognition

In Section 1.2 the way that sexual (gonadal) hormones affect the structure of the brain in the pre-natal and post-natal life was discussed; tuning the brain either towards the masculine spectrum or towards the feminine spectrum. It was argued that the main brain structures that are significantly affected by sexual hormones and are actively engaged with cognitive functions, as well as being sexually differentiated, are the hippocampus and amygdala.

The hippocampus appears to be involved in functions that require the collection of multiple cues (Micheau & Marighetto, 2011). Moreover, the hippocampus appears to be a significant mechanism for the creation of associations between these cues; that is, creating informational 'units' (see Section 1.3). On the other hand, the amygdala appears to be engaged in situations where few (or even a single) stimuli are available and useful for a task. In particular, the amygdala appears to be a significant mechanism that enhances the strength of a sensory input in order for the collected stimulus to be integrated into memory structures. In addition, hippocampal function appears to act antagonistically to amygdala function, while amygdala function appears to act synergistically to hippocampal function. Thus, a functional differentiation on these two mechanisms is expected to have a direct, short-term effect on the way that information is processed. That is, higher hippocampal functionality will enable more processing in the presence of multiple cues; while high amygdala functionality will enable higher acuity and performance on tasks where novelty is of high importance and available cues are limited. In addition, bearing in mind Besnard et al.'s (2012) memory formation theory (discussed in Section 1.5.2), this functional differentiation is also expected to have a long-term effect on memory structure. In particular, the enhanced ability to process and collect information (i.e. high hippocampal function) will result in the construction of multiple, albeit confined, memory

networks while the opposite will be true for a high performing amygdala system; that is, the construction of a limited number of extended memory networks.

5.4 Amygdala-hippocampus function and male and female cognitive phenotypes

As discussed in Section 1.4, sex differences that appear in cognitive tasks are mainly concerned with two major factors; these are cue-availability and time exposure to stimuli. These factors relate directly to the above argued direct / short term functions of the hippocampus and amygdala; but what about the long-term effects? More specifically, what are the effects of the above described hippocampus-amygdala differences on cognitive development?

As discussed in Section 1.8, sex differences are observed in the development of the memory system. In particular, females appear to build their memory system faster than males and this is observable throughout the lifespan (Ozcaliskan & Goldin-Meadow, 2010; Nagy et al., 2007; Iverson & Goldin-Meadow, 2005). Thus, a significant female advantage is observed regarding the development of motor skills and the subsequent building and expansion of linguistic skills. Consequently, the above observations support the idea of the female cognitive system being more capable of collecting information and consequently developing faster.

However, considering Besnard et al.'s (2012) theory (see Section 1.5) a difference in the speed by which a cognitive system is developed is also expected to cause structural differences to the memory system. That is, high capability for collecting information will lead to a faster

developing memory system, albeit composed by many separate and short-ranged[88] memory networks. On the other hand, reduced ability to collect information will lead to a slower developing memory system, composed by fewer separate but wide-ranging memory networks. As discussed in Section 1.4.3, males appear to have more functionally interconnected brains than females from a neurological point of view; while these differences appear to be stabilised by late childhood. Thus, males and females do present differences in the way that their memory networks are structured and these differences can be linked to hippocampus and amygdala functions; both of which appear to be affected by gonadal hormones.

5.5 Determining the extremes: Extreme masculinized brain versus extreme feminized brain

As it was discussed in Chapter 1, individual differences in gonadal hormone effects may vary to such an extent that the formation of a memory system surpasses the more commonly observed boundaries of sex. That is, due to factors reported in Chapter 1 the hardwiring of the brain towards a 'female' range or towards a 'male' range may not, in fact, be sex specific; resulting in some males having cognitive systems that lie within a so-called 'female cognitive spectrum' and some females having cognitive systems that lie within a so-called 'male cognitive spectrum'. However, in their extreme forms, these structures would be either composed of a few different memory networks (extreme masculinized brain), or composed of many different memory

[88] The range of a memory network refers to the spectrum of information that each network incorporates. According to Besnard et al. (2012), the memory system is built by attaching novel information on networks that contain similar information to the novel one. Consequently if a memory network is kept open to alterations for a short time then, all the attached information is expected to be similar to each other. On the other hand if a memory network is kept open to alterations for a long time then, the spectrum of the attached information is expected to increase.

networks (extreme feminized brain). The appearance or not of these extremities depends mostly on a mixture of chromosomal differences, gonadal hormones and the sex of the foetus. In particular, depending on the sex of the foetus, the effects of maternal gonadal hormones may vary; thus, a male foetus is more susceptible to defeminisation compared to a female foetus and vice-versa. Consequently, depending on maternal circulating oestrogen levels, a male foetus can be 'hardwired' towards the extreme side of 'male range'; while the opposite can occur for a female foetus, 'hardwiring' the brain towards the extreme side of 'female range'.

5.6 Application of the theoretical model to developmental sex differences

The existence of individual developmental differences, especially among males, is well documented by studies that have explored sex differences in general intelligence (Lynn & Kanazawa, 2011). More specifically, Lynn and Kanazawa argued that there are significant maturation-rate differences between the two sexes, with females having an advantage between the ages of 7 and 11 years old, with males demonstrate advantages at 16 years of age. Interestingly, males present greater variance in intelligence in all the above age groups (Lynn & Kanazawa, 2011). Thus, it can be argued that individual differences in development are much more prominent in males rather than females. These differences may be linked to the existence of significant variations between individual levels of foetal testosterone among males, while these levels are relatively stable between females (Lutchmaya et al., 2004). According to the current thesis, these hormone levels set the dynamics for neural development. This thesis also proposes that a memory system with limited basic information processing ability, which is more likely for males, will be more affected by environmental fluctuations in informational availability. That is,

more factors are capable of affecting the (cognitive) developmental course of males (or individuals within the male cognitive range) compared to females (or individuals within the female cognitive range). Thus, observed inconsistencies in general intelligence may be due to sex differences in basic information processing ability and the consequent effect that this factor has on the development (in terms of structure) and functionality of the memory base/system.

5.7 Application of the theoretical model to neuro-development disorders

As was described in Section 1.7.1, Baron-Cohen and Hammer (1997) argued for the existence of a continuum of brain types that extend from the neuro-typical to the autistic spectrum. According to Baron-Cohen and Hammer (1997), this continuum is created by differences in pre-natal masculinization; while Autism is the result of extreme pre-natal masculinization i.e. an extreme male brain. Goldenfeld et al. (2005; Baron-Cohen et al., 2014) expanded the idea of a brain type continuum through the formation of two psychological measures (systemizing and empathizing) by which they classified brain type in populations within the autistic spectrum as well as within the neuro-typical range. In addition, the psychological measures of systemizing and empathizing were also related to 2D:4D and consequently to pre-natal hormone effects (Manning et al., 2010); however, there was no link made between the above factors and a biological factor with specific effects on specific brain structure(s) that might be related to brain type.

A potential link between brain type and a biological factor may be provided through the theoretical and experimental findings of this thesis. According to Experiment's 2 (Chapter 3) findings, where it was indicated an association between productive vocabulary-free recall brain types and S-E brain types, it was argued that both brain type measures potentially depend on the

same underlying brain structures. Based on the arguments raised within this thesis, an individual's cognitive brain type is the result of the effect(s) of basic information processing ability on the memory system throughout development. According to previous research information processing ability appears to be related to Theta reactivity (see Chapter 4, Section 4.1; Kober & Neuper, 2011); which Theta reactivity was also found to be associated with individual masculinization / feminization levels (Experiment 3, Chapter 4). Since individual masculinization / feminization levels were associated with both brain type and Theta reactivity it can be concluded that basic information processing ability is potentially related to hippocampal function. Consequently, the hippocampus becomes the main candidate for being the brain structure that links gonadal hormone effects and brain type.

Connecting the theory proposed in this thesis to findings related to the extreme male brain theory of Autism, it can be proposed that extreme masculinization leads to the development of an extreme male brain type, which according to Baron-Cohen and Hammer (1997) underlies Autism. Thus, it can be suggested that Autism may be related to the functionality of hippocampus and amygdala system. However, this claim requires direct assessment in future research (see Section 5.11.).

The potential connection between the extreme male brain theory of Autism and hippocampus and amygdala function is in line with the amygdala theory of Autism too. Baron Cohen et al. (2000) argued that the amygdala may constitute the biological factor that relates to Autism. However, the appearance of inconsistencies between males and females in regard to amygdala size and disabilities related to autism indicates a theoretical (and practical) gap (Schumann et al., 2009; a review at Lai et al., 2015; see also Section 1.7.1). Based on the hypothesis of the current thesis and its findings, it can be argued that the observed sex differences in the level of

impairment in relation to amygdala size are explained by the hippocampus-amygdala interaction rather than simply by the amygdala alone. As it was described in Section 1.2, hippocampus-amygdala interaction is not bi-directional. In particular, the hippocampus reduces amygdala function, while the amygdala enhances hippocampal function. Based on the theory proposed in this thesis, basic information processing ability is determined by the relationship between the hippocampus and amygdala. Moreover, this relationship is defined by the size and functionality of each structure, and in turn the size and functionality of each structure are both affected by gonadal hormones. This translates into a male cognitive system with high dependence on amygdala function and a female cognitive system with high dependence on hippocampal function (see Section 1.7.1). Based on this rationale, the size of the amygdala and the severity of autistic symptomatology are expected to be closely related in males but not in females. That is, an abnormal amygdala would be expected to significantly reduce basic information processing ability in males and subsequently slow down the development of the memory system. However, in females basic information processing ability is not expected to be significantly reduced and consequently their memory system development is not expected to be as compromised as that of males.

As was argued above through studies that explored the effects of sexual hormones in the human brain, it was suggested that the male brain goes through de-feminization by maternal circulating oestrogens (MCE) as well as through masculinisation by foetal sexual hormones. The masculinisation process is mainly driven by the hormone testosterone (Rosselli et al., 2009; Lombardo et al., 2012), which is the main cause of high levels of 'insulin-like Growth factor-I' (IGF-I); a factor responsible for reducing the levels of acetylcholine (ACh) deriving specifically from the hippocampus, without affecting ACh deriving from other areas including the amygdala

(Cherrier et al., 2004; Seto et al., 2002). The neurotransmitter Acetylcholine (ACh) is linked to the functionality of both the hippocampus and amygdala (McIntyre et al., 2002; Gold, 2004). Thus, it can be argued that the hippocampus-amygdala competition is affected by both MCE and foetal testosterone in males; however, in females only MCE is left to affect hippocampus-amygdala competition. Consequently, the cognitive phenotype of males with hippocampus < amygdala and females with hippocampus > amygdala functionality may be retained even within the autistic spectrum. This rationale provides an explanation regarding the inconsistencies between amygdala size and autistic severity between males and females indicated by Schumann et al. (2009) and provides support for the amygdala theory of Autism as stated by Baron-Cohen et al. (2002). In addition, this theoretical model allows for the existence of cognitive sex differences within the autistic spectrum although their appearance is currently considered to be a theoretical flaw of the extreme male brain theory of Autism by Baron-Cohen et al. (2014).

In conclusion, based on this thesis it can be argued that Autism and disorders that belong on the Autistic spectrum may be related to basic information processing ability. In particular, it is argued that a potential disruption in basic information processing ability potentially creates an impairment in the normal development of the memory system, which is vital for its functionality and expansion. The relationship between Theta reactivity under resting-state conditions in the left frontal areas and individual masculinization / feminization levels constitute a positive indication for a future use of this measure as an index of impaired basic information processing ability and potentially also an estimation of Autistic severity.

5.8 Application of the theoretical model to neuro-degenerative disorders

In Section 1.5.3, the significance of new memory formation for the viability of the memory system was highlighted. In other words, it was argued that in order for the memory system to retain its functionality, the ability to form new memories is required. When we consider the case of Alzheimer's disease, memory loss appears to start from latest memories and proceed towards the earliest. In terms of memory-types, degeneration initially affects language-based memory and proceeds towards visuo-spatial memory and finally motor-memory. From a neurological point of view, the main impairment in Alzheimer's patients is detected on the cholinergic system and especially in the reduction of Acetylcholine (ACh). As it was discussed in Section1.2, ACh and especially hippocampal ACh is necessary for the unobstructed function of hippocampus. According to the current thesis, reduced ACh would mean reduced basic information processing ability, which in turn would lead to reduced ability to collect and incorporate new information into the memory system. Collecting and incorporating new information into the memory system has dual benefits. The first and most obvious is the expansion of the memory system and the second and probably the most important is the re-activation of existing memory networks (Deianna et al., 2010; see Section 1.7.3, page 41). Re-activation of existing networks appears to have a conservative role of the engaged networks since it strengthens all the involved synapses. In other words, re-activation of existing networks preserves them and subsequently the functionality of the memory system is preserved also. Consequently, losing the ability to collect new information and activate the memory system (that is, losing basic information processing ability) can potentially lead to network inactivity and potential neuronal death.

The above argument is partially supported by the case study of the agnostic patient HJA who lost his memory for items that he could not recognize and consequently stopped activating his

memory system. However, the idea that basic information processing ability underlies Alzheimer's disease can be supported by existing sex differences in the rate of appearance, rate of progress and cognitive reserve that each sex has. In particular, although females are more susceptible to Alzheimer's disease (Alzheimer's Association, 2014), after the onset of A.D. the disease progression is significantly more rapid in males (Spampinato et al., 2012). These differences can be explained by the model proposed in this thesis and particularly with the suggested structural (in terms of neuronal connections) sex differences.

As has been argued, basic information processing ability has a developmental effect on memory structure. In particular, high basic information processing ability leads to a memory system composed of many different memory networks which contain specialized information; that is a memory system within the 'female' range. On the other hand, low basic information processing ability leads to a memory system composed of fewer memory networks on which all available information is built; that is a memory system within the 'male' range.

If it is assumed that inability to incorporate and consequently re-activate existing networks is compromised (i.e. compromised basic information processing ability) and neuronal death is imminent, then based on the above model it would be expected that individuals within the female range will be more susceptible to neuronal deactivation. That would be due to the structure of their memory system being composed of many different networks, which require a plethora of different information to be activated. Consequently, reduced basic information processing ability would lead more quickly to the initiation of neuronal death; at least compared to individuals within the male range. On the other hand individuals within the male range are not going to be significantly affected by a reduction in their basic information processing ability since their memory system's need for activation is already reduced. In particular, fewer memory networks

mean that each network is related to a wide variety of information and consequently has more opportunities to be activated by seemingly irrelevant information. However, the cost of losing one network is expected to be greater compared to the cost of losing an individual network within the female range. That is because one network is related to a greater amount of information in individuals within the male range compared to individuals within the female range. Thus, enhanced cognitive reserve which is observed in females can be attributed to a higher number of different memory networks; which is the result of higher basic information processing ability.

Although the proposed explanation is at an early stage of development and evidence, post mortem studies have indicated a significant reduction of hippocampal volume in Alzheimer's patients; while this reduction is more prominent in male patients (Spampinato et al., 2012). Due to the role that the hippocampus has in basic information processing ability and consequently in memory structure and activation according to this theory, these observations may be considered potentially supportive of the proposed model.

5.9 Summary and conclusions for the application of the current theoretical model on neurodevelopmental and neurodegenerative diseases and disorders

It has been argued that our cognitive system functions based on a small number of very simple mechanisms. These mechanisms are basic information processing ability and memory structures, which are strictly interrelated. Complexity is created by the incorporation of information which creates neural connections. From the point that the cognitive system is fully developed, individual differences become irrelevant and even sex differences can only be traced in very

specific tasks under very specific conditions. This general balance that the cognitive system presents is evidence for the ability of the cognitive system to compensate for its limitations[89] and retain performance at optimum levels.

According to the proposed model, disorders that fall within the Autistic spectrum may be linked to differences in basic information processing impairment, which in turn create differences in the structure of the memory base. Along the same lines, Alzheimer's disease and cognitive impairments that are neurologically induced may be linked to a progressive impairment of basic information processing ability and, most importantly, on the existing memory structure. The importance of the memory structure in terms of how expanded it is can be seen from studies that have indicated the importance of constant learning through lifespan as a neuro-protective action (Bornebroek & Breteler, 2004). That is, an expanded memory structure can potentially raise an individual's resilience to neuro-degeneration, delaying the symptoms and consequently retaining higher living standards.

5.10 Overall Conclusions

The main aim of this thesis was to explore sex-related differences in cognition, in order to understand the aspects of these differences that were interrelated in order to propose a unifying cognitive model. Current literature has highlighted differences between males and females in aspects of their brain structure and function, psychology and cognitive function. Thus, the formation of a unifying cognitive model ought to incorporate all the above differences and provide a rational explanation for the appearance and functional importance of those differences.

[89] See Jausovec and Jausovec (2005;2009;2010) where it is indicated how each sex activate more or less of its brain in tasks that has an advantage or a disadvantage in order to retain optimum performance

Within this framework, factors that may have consequent biological, psychological and cognitive effects on the human brain were sought and explored. This research indicated sexual (gonadal) hormones as the basic differentiating factor with both structural and functional effects on the brain. The idea that gonadal hormones have both structural and functional effects on the brain is an old idea known by the name 'the organizational-activational' hypothesis (Sculz, Molenda-Figueira & Sisk, 2009). Although the 'organizational-activational' hypothesis provides a solid ground regarding the effects of gonadal hormones on specific brain areas, such as the hippocampus, that are seen as responsible for the apparent sex-difference in spatial cognitive performance, it does not provide a direct link between gonadal hormones and cognition.

Based on studies that have explored human brain sex differences, it was concluded that the basic brain structures that are significantly engaged in cognitive performance are the hippocampus and amygdala. Based on experiments with rodents as well as experiments that explored sex-related cognitive differences in humans, it was argued that the hippocampus and amygdala have a very specific function. This function was argued to relate to the ability to collect information, creating 'informational units' and activating the memory system; a function referred to as basic information processing ability. Moreover, based on developmental sex differences as well as sex differences in neuro-developmental and neurodegenerative disorders, it was argued that basic information processing ability potentially has a significant effect on an individual's memory system.

Existing studies that have explored sex differences in neural connectivity supported this idea and subsequently it was hypothesised that basic information processing ability interacts with the environment (in terms of informational availability) and in the long-term (through development) causes differences in the neuronal structure of male and female brain. In order to test this

hypothesis it was argued that the long-term effects of basic information processing ability (that is memory structure) would be stabilized (more or less) from the age of 25 and onwards and consequently it would be possible to see their effects behaviourally. Based on the theoretical framework that this thesis was built on, it was argued that in terms of cognitive performance, basic information processing ability and memory structure are inseparable and interrelated. Consequently, a measure that can access their combined effects was needed.

Based on developmental studies it was argued that language-based memory networks are developed based on visuo-spatial memory networks, which in turn are based on motor-memory networks. That is, memory is developed in a strictly hierarchical way. Consequently, engaging language-based memory networks in a task can potentially provide us with a general view of an individual's memory structure. However, in order for this measurement to be as accurate as possible, it is necessary that the task used did not engage other aspects of cognition that could affect performance (if the cognitive system is viewed via a unitary-based model such as Baddeley's) or that the task's performance is not affected by other factors (if the cognitive system is viewed via a non-unitary cognitive model such as the one argued in this thesis).

The solution to this problem was to utilize tasks that engage language and appear to be sex-biased. Using a sex-biased task reduces the possibility that a task is affected by exogenous individual differences and increases the possibility that this task is related to a biological and / or cognitive-hormone related-factor(s). Thus, a word-based verbal free recall task was used, which appears in the current literature to demonstrate a significant female advantage.

It was proposed by this thesis that the observed female advantage occurs due to sex-differences in the mechanism(s) engaged in stimuli collection; a function assisted by their memory structure

since it engages existing memory. However, an individual's cognitive phenotype could not be measured just from this task since this task confirms only that there is this sex-related difference in cognitive performance, which is also related to pre- and post-natal hormones. This task does not provide information regarding 'how much' difference there is between males and females or more correctly, this task provides information of 'how much' difference there is within the examined samples only; where taken the relatively small sample sizes, it would not be safe to draw a solid conclusion. In order to overcome this obstacle the idea of Goldenfeld et al. (2005) was used. Goldenfeld et al. measured an individual's psychologically-based cognitive phenotype via the measurement of a female-biased psychological trait in relation to a male-biased psychological trait. The key idea in Goldenfeld et al.'s (2005) paper was the personalization of an individual's cognitive performance by actually comparing how good someone was in the one extreme of a cognitive continuum compared to how good someone was on the other extreme of the same continuum. Thus, a more accurate measurement of an individual's cognitive status was developed.

Following the same rationale, the main argument of this thesis was that an individual's cognitive phenotype can be measured by a female-biased task (that was free recall) and a non female-biased task. However, both tasks needed to be based on language and memory base access. Thus, a productive vocabulary task was also utilized since current literature reports it as a non female-biased task. Consequently it was argued that a productive vocabulary task could be used as a reference point by which to measure an individual's cognitive phenotype or as it was stated in this thesis, an individual's brain type.

The appearance of a relationship between pre-natal hormones and brain type as well as the association between Goldenfeld et al.'s (2005) brain type measurement based on systemizing and

empathizing, strengthened the argument regarding a common biological basis of brain type; either measured by cognitive-based or psychologically-based measures. In order to specify (insofar as permissible) the underlying biological factors, an EEG study was conducted. Following previous literature that has indicated brain's intrinsic activity as the most energy consuming (Raichle & Snyder, 2007), eyes-open and eyes-closed EEG measurements of Alpha and Theta rhythms were taken. The appearance of a relationship between Theta reactivity and individual masculinization / feminization levels strengthened the argument of this thesis regarding the role of hippocampus as a fundamental biological factor that may lie beneath basic information processing ability and subsequently memory structure.

5.11 Limitations

This thesis attempted to address and expand a question that has been addressed by previous researchers exploring cognitive sex differences; that is, the exact nature of cognitive sex differences. In order to achieve that, the hypothetical index of cognitive brain type, the indirect index of pre-natal gonadal hormone effects, the direct levels of circulating gonadal hormone levels and intrinsic brain activity measures were utilized. The main hypothesis was based on the idea that the factor 'sex' serves only as a general index of brain masculinization / feminization; while brain masculinization / feminization is responsible for observed sex differences. In order to explore this hypothesis the relationship between indices of brain masculinization / feminization and a female-biased task[90], brain type and brain intrinsic activity was explored. The results indicated that indices of brain masculinization / feminization were associated with a female-

[90] A sex-biased task was used in order to make sure that any observed performance differences cannot be attributed to factors not related to brain masculinization / feminization levels.

biased task, brain type and intrinsic brain activity. These results provided some support for the theoretical model developed within this thesis, arguing for a potential relationship between the hippocampus-amygdala system and cognitive function. However, there are certain limitations that must be taken under consideration when evaluating current findings. To begin with, all studies were based on a relative moderate sample size[91], which fact by itself limits the statistical generalizability of the results. That is, despite the fact that the results were in line with the theoretical model that was proposed within this thesis, the size of the explored sample does not allow for accurate predictions for the general population. Moreover, small studies that show significant effects tend to overestimate effect sizes; thus, the first step would be to replicate these findings using a larger sample size in order to have sufficient power for generalizing findings.

Looking at the first experiment (Chapter 2), although the expected female advantage in free recall was observed, the prediction regarding an interaction between free recall after distraction and sex did not reach significance. The lack of this interaction creates doubts regarding the experiment, suggesting significant consideration when interpreting the results. In Chapter 2 it was argued that this lack of significant interaction could be potentially attributed to the factor handedness; a factor that was not controlled in Experiment 1. Since previous research has indicated that handedness is significantly related to multi-trial free recall performance (Siengthai et al., 2008), pre-natal gonadal hormones (Kelso et al., 2000) and cognition (Johnston et al., 2013), then it was argued that not controlling for this factor may be the reason behind the lack of the expected effect.

[91] The sample size is evaluated moderate compared to previous studies that have explored sex differences in verbal free recall (for more details see Chapter 2, Table 1)

Consequently in Experiment 2 (Chapter 3), the factor handedness was controlled and the expected interaction between sex and free recall after distraction was observed. However, handedness was measured by a method which previous studies have pointed out may be insufficient (Siengthai et al., 2008). Thus, it was argued that handedness and subsequently brain laterality was somewhat controlled, but it might not have been done as accurately as it could have been. Consequently, it must be noted that the conclusions drawn, especially regarding underlying brain structures may apply only to a homogenous sample in terms of brain lateralization; which might not be the case in the current experiments. In the same way, it must be noted that in Experiment 2, the argued relationship between gonadal hormones, free recall, brain type and hippocampus-amygdala is based on a purely theoretical approach. Indeed, previous studies may have indicated a link between hippocampal function and free recall performance (e.g. Moscovitch & Winocur 1992; Jarrold et al., 2008) but in the current experiment the relationship between free recall and hippocampal function was not directly assessed. Thus, all the links between underlying brain structures and individual masculinization / feminization or cognition are made based on previous studies and not directly from the experiments performed. Consequently, caution must be taken when evaluating current findings regarding underlying brain structures. Similarly arguments regarding underlying brain structures resulting from Experiments 1 and 2 must be viewed more as suggestions / potential links that need further research in order to be validated.

Moving on to the final experiment (Experiment 3, Chapter 4) the most profound issue was the significant data loss in the EEG data extraction process. As mentioned in Chapter 4 (Section 4.4), this data loss may have occurred due to repairs of the EEG lab that took place; resulting to a

further reduction of the existing sample size. Although that the remaining sample was not small[92] for an EEG study, sample sizes of this magnitude in EEG have been accused of producing results that are not easily generalizable (Barry & De Blasio, 2017). Thus, caution must be exercised when interpreting current results.

At this point some considerations that arose regarding the indices that were used for defining individual levels of masculinization / feminization must be discussed. There were two different indices of masculinization / feminization used in Experiments 1 and 2. The first used the factor 'sex' as a base[93] and 2D:4D was added in order to add more information regarding the pre-natal masculinization / feminization background of each individual. Thus, males with 2D<4D were seen as individuals undergone high pre-natal masculinization, while females with 2D>4D were seen as individuals undergone high pre-natal feminization. The second index of masculinization / feminization that was used, was a combination of circulating -saliva measured- gonadal hormones and pre-natal gonadal hormone effect index (2D:4D). The rationale behind this approach was based on previous studies that have pointed out that the effects of post-natal hormones on the brain are related to the pre-natal masculinization / feminization of each individual (see Chapter 1, Section 1.2, or alternatively see Gillies & McArthur, 2010). Consequently, it was argued that higher pre-natal masculinization and high post-natal circulating gonadal hormone levels will be indicative of a masculinized brain and vice-versa. However, this approach has not been used before and although the results were supportive of the argued attributes of the factor 'hormonal profile', further research is needed in order to validate this measure of individual hormonal profile.

[92] Kober and Neuper (2011): 27 participants; Chen et al. (2007): 15 participants.
[93] In Chapter 1 it was argued that sex as a factor carries general information regarding XX-XY chromosomes, AFP effects and post-natal hormonal effects and production.

As a final issue that might be of significance is the method of 2D:4D measurement. The protocol that was followed was a standardized procedure of 2D:4D measurement and included two consecutive direct measures of the second and forth finger of the right hand of each participant using an electronic calliper, conducted by one researcher. A potential issue with this process is reported to be the time interval that elapses between the two measurements. That is, Sanders, Bereczkei, Csathó, Manning (2005) have reported that small time intervals between the two measurements may bias the measurements, since the first measurement may be remembered by the researcher. In the current research time intervals were not controlled and this might have affected the results. Consequently, although the meta-analysis indicated that current study's 2D:4D data do not deviate from findings of previous studies, some caution must be taken when evaluating current study's results.

5.12 Future studies

Future studies are necessary to test the above argument in both neuro-typical as well as individuals that fall within the Autistic spectrum and individuals with Alzheimer's disease. For example, based on the proposed memory model it is argued that sex differences in Alzheimer's disease phenotype and progression are based on sex differences on the memory base structure. Thus, a personalization of medical treatment based on the memory system structure (i.e. brain type) may potentially enhance the effectiveness of the existing drugs that are mostly ACh regulators (e.g. Exelon, Donepezil).

For example, studies have argued against the use of drugs that raise the amount of time of acetylcholine activity in the neuronal synapses for short-lived positive effects as well as

detrimental adverse effects that eventually cause the death of memory neurons (Jang et al., 2010; Francis, Nordberg, & Arnold, 2005). According to the present thesis the observed adverse effects of ACh medicines may just reflect the different reaction of different memory structures to this medical treatment; an adverse effect that based on the above reasoning can be reduced and potentially reversed. That is, it is suggested that the effectiveness and / or adverse effects of ACh medication should be explored in association to individual's cognitive brain type or individual's masculinization / feminization levels. Bearing in mind that the above suggestion is addressing non-neurotypical individuals, the measurement of an individual's brain type via cognitive tests may not be appropriate due to the effects of neuro-degeneration on cognition. Consequently, future studies exploring the effects of ACh based medication can utilize the measure of hormonal profile (as presented in this thesis) in order to explore the hypothesis regarding an association between individual masculinization / feminization levels and ACh medication effectiveness. In fact, sex differences in the effectiveness of ACh related medication have started to draw the attention of researchers (Canevelli, Quarata, Remiddi, Lucchine, Lacorte, Vanacore, Bruno, Cesari, 2017; Davis & Barrett, 2009). For example Davis and Barrett (2009) reported that there are significant sex differences in the effectiveness of Donepezil; with males being more benefited than females. Based on their findings, Davis and Barrett argued that the observed sex differentiation could not be attributed to sex differences in the severity of the condition. Animal studies relate the effectiveness of this type of medication with testosterone and acetylcholine levels (a review in Haywood and Mukaetova-Ladinska, 2006); which as it was argued in this thesis are two interrelated factors significantly linked to sex. Thus, an exploration of ACh medication effectiveness in relation to individual masculinization / feminization levels can potentially enable a personalization of medical treatment.

At the moment the most utilized method for detecting the appearance and progression of Alzheimer's disease is through cognitive tasks (for a review of the most effective and utilized tasks see: Rentz, Rodriguez, Amariglio, Stern, Sperling & Ferris 2013). However, the effectiveness of these tasks, especially for relating cognitive status to biological markers of cognitive impairment, appears to be low (Rentz et al., 2013). Interestingly, the most effective behavioural tasks for detecting early A.D. development are tasks that assess associative memory and most of them are found (through fMRI / MRI studies) to be directly related to hippocampal (dys)function (Rentz et al., 2013). Based on the current findings, hippocampal baseline function is associated with individual masculinization / feminization levels (see Chapter 4). Thus, controlling for individual masculinization / feminization levels may enable a more accurate assessment of task performance and consequently lead to a more accurate assessment of A.D. progression, even from a pre-clinical stage.

Regarding neurodevelopmental disorders such as Autism, future studies looking for biological factors that may lead to these conditions may consider the relative size and functionality of hippocampus in relation to amygdala size and functionality. Previous researches have highlighted the role of the amygdala in autism; however, inconsistencies in findings between males and females weakened the impact of these studies (see Section 1.7.1). According to the theoretical base of this thesis, these inconsistencies may be resolved if the relative size and function of hippocampus is considered in the 'autistic severity-amygdala size' equation. That is, assuming that amygdala is the main biological factor affected in autism, the severity of Autistic symptomatology in males should be related to amygdala size. This is expected because according to this thesis the male cognitive system depends heavily on amygdala function. Thus, a potential disruption of amygdala function will affect cognitive function directly; an effect that is expected

to be proportional to amygdala alteration (see Section 1.7.1). In contrast, the female cognitive system depends mostly on hippocampal function. Thus, the relationship between amygdala and autistic severity is expected not to be proportional since hippocampal function weakens the relationship between them. Consequently, it would be interesting to re-examine the amygdala theory of autism by incorporating the size and functionality of hippocampus in the 'autistic severity-amygdala size' equation. That is, future studies should explore the relationship between Autistic severity and amygdala in relation to hippocampal volume / function.

Looking at neuro-typical populations it would be interesting to re-examine cognitive sex differences within the context of this thesis. That would be to look at cognitive differences with hormone profile as the main dissociating factor instead of sex. As shown in Chapter 1, there are specific types of cognitive tasks that appear to provide an advantage to either males or females. These are tasks of navigation, spatial rotation, object location, and verbal memory (see Andreano & Cahill, 2012). The current thesis addressed performance on free recall, a test of verbal memory that appears a significant and consistent female advantage. In the same way it would be interesting to examine the effectiveness of hormonal profile (or any other index of brain masculinization / feminization that incorporates both pre-natal and post-natal hormone levels / effects) compared to the factor sex in other tasks that are sexually differentiated. That would provide a better understanding regarding the underlying factors that create cognitive sex differences; which according to the current hypothesis these factors are the hippocampus-amygdala system and its effects on memory development. Providing support for this hypothesis will enable to diverge from the logic of a strictly dichotomous cognitive sexual differentiation and direct towards a biochemically induced cognitive differentiation that may surpass the strict boundaries of sex.

5.13 Summary

1. Verbal word-list free recall with a word-list distracter is a sex-biased task that demonstrates females as having a significant advantage over males.

2. In a right-handed, 25 years and older, English speaking sample, verbal (word-based) free recall after distraction appears to be more affected by sex and hormonal profile compared to the first free recall and the second (distracter) free recall. In the same way, hormonal profile appears to be a stronger dissociating factor for free recall performance than sex is. Based on the above it is argued that hormonal profile may potentially replace the factor 'sex' as a dissociating factor in sex-biased tasks.

3. Indices of masculinization / feminization as measured by sex only, sex and 2D:4D and estradiol to testosterone ratio and 2D:4D (hormonal profile) appear to be associated with an individual's brain type classification as defined behaviourally by calculating the deviation in performance between a female-biased task and a non-female biased task. That is, higher levels of masculinization appear to be associated with a brain type defined by high productive vocabulary (PV) and low free recall (FR). In contrast, high levels of feminization appear to be associated with a brain type defined by low PV and high FR. Moreover, a trend was observed between levels of masculinization / feminization and allocation on the brain type scale. Based on the above, it was argued that brain type is linked to individual masculinization / feminization levels of the brain.

4. Empathizing-systemizing brain type classification was associated with individual masculinization / feminization levels as defined by sex (only) and sex and 2D:4D; but not with hormonal profile. Empathizing-systemizing brain type classification showed a positive

association with productive vocabulary-free recall brain type. Based on the above it was argued that both measures of brain type possibly rely on the same underlying cognitive factors.

5. Theta reactivity appears to be sexually differentiated in the left frontal lobe. Moreover, individual masculinization levels as defined by sex only, sex and 2D:4D and estradiol to testosterone ratio and 2D:4D (hormonal profile) appear to be associated with Theta de-synchronization, while individual feminization levels were associated with Theta synchronization. These results were interpreted as support for the existence of sex differences as well as individual masculinization / feminization level differences in information processing at a baseline level. Following these findings, it is argued that individual masculinization / feminization levels (and consequently information processing ability) are linked to hippocampal function.

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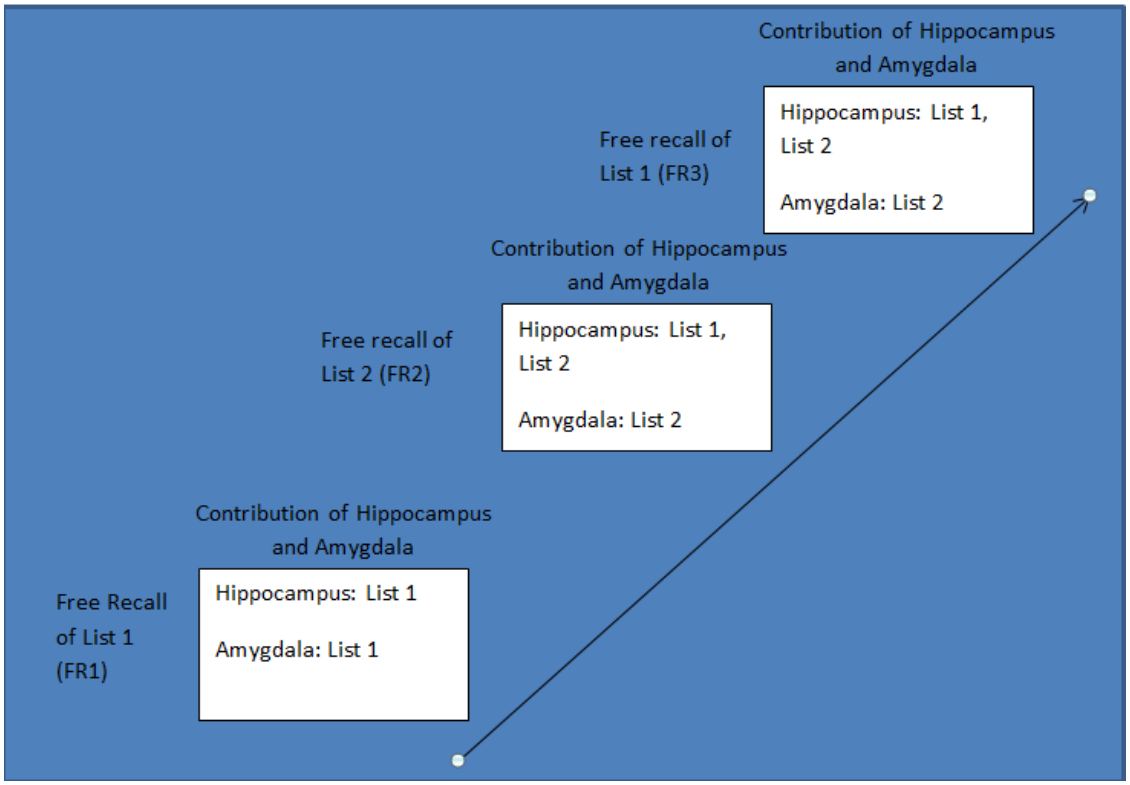
Appendix 1: Schematic representation

The schematic representation of the hippocampus and amygdala engagement / contribution through the three stages of the free recall paradigm appears below.



Appendix 2: Free recall lists of Experiment 1

Free recall list A and B with word-lengths and word-frequencies[94] used for experiment 1.

Word	Length	Frequency
List A		
Τύμπανο (drum)	7	3,54
Κουρτίνα (curtain)	8	4,53
Κουδούνι (bell)	8	2,45
Καφές (coffee)	5	2,69
Σχολείο (school)	7	3,67
Γονέας (parent)	6	1,39
Φεγγάρι (moon)	7	2,89
Κήπος (garden)	5	2,35
Καπέλο (hat)	6	3,15
Γεωργός (farmer)	7	0,9542
Μύτη (nose)	4	3,24
Γαλοπούλα (turkey)	9	2,40
Χρώμα (colour)	5	3,48
Σπίτι (house)	5	4,4641
Ποτάμι (river)	6	3,1082
List B		
Θρανίο (desk)	6	1,56
Δασοφύλακας (ranger)	11	1,53
Πουλί (bird)	5	3,1629
Παπούτσι (shoe)	8	2,6010
Φούρνος (oven)	7	1,9031
Βουνό (mountain)	5	2,9079
Γυαλιά (glasses)	6	3,0191
Πύργος (tower)	6	2,3424
Σύννεφο (cloud)	7	2,2923
Καράβι (ship)	6	2,5453
Αρνάκι (lamp)	6	1,9685
Πιστόλι (pistol)	7	2,8338
Μολύβι (pencil)	6	2,4031
Εκκλησία (church)	8	3,2524
Ψάρι (fish)	4	2,9961

[3]Frequencies and word-lengths were extracted from the SUBTLEX-GR for word frequencies based on Greek subtitles (Dimitropoulou, M., Duñabeitia, J., Avilés, A., Corral, J.& Carreiras, M. (2010) Subtitle-based word frequencies as the best estimate of reading behaviour: the case of Greek. *Frontiers in Psychology,* 1:218, 1-12)

Translation of the vocabulary subtest items of the experiment 2a.-lengths and word-frequencies sound localization at that was used in experiment 1.

Items	
9. Πουλί (bird)	32.Παρόρμηση (impulse)
10. Ημερολόγιο (calendar)	33.Επιπολαιότητα (haste)
11. Αριθμός (number)	34.Τάση (trend)
12. Κουδούνι (bell)	35.Διαλείπων (intermittent)
13. Δείπνο (dinner)	36.Ευλαβής (devout)
14. Αστυνομία (police)	37.Ανάρμοστος (impertinent)
15. Διακοπές (vacation)	38. Εξειδικευμένος (niche)
16. Κατοικίδιο (pet)	39.Αλαζόνας (presumptuous)
17. Μπαλόνι (balloon)	40.Δεινός (formidable)
18. Μεταμορφώνω (transform)	41.Μηρυκάζω (ruminate)
19. Αλιγάτορας (alligator)	42.Πανάκεια (panacea)
20. Καρότσα (cart)	
21. Κατηγορώ (blame)	
22. Χορός (dance)	
23. Σκοπός (purpose)	
24. Διασκεδάζω (entertain)	
25. Διάσημος (famous)	
26. Αποκαλύπτω (reveal)	
27. Δεκαετία (decade)	
28. Έθιμο (tradition)	
29. Αγάλλομαι (rejoice)	
30.Ενθουσιώδης (enthusiastic)	
31. Αυτοσχεδιάζω (improvise)	

Appendix 3: Consent Statement and Participant Information Sheet (Greek Version)

<u>Δήλωση συγκατάθεσης</u>

Η σχέση της γλώσσας με την μνήμη

1. Επιβεβαιώνω ότι έχω διαβάσει και καταλάβει το φύλλο πληροφοριών συμμετεχόντων για την παραπάνω μελέτη και είχα την ευκαιρία να θέσω ερωτήσεις. ☐

2. Καταλαβαίνω ότι η συμμετοχή μου είναι εθελοντική και ότι είμαι ελεύθερος / η να αποσυρθώ απο την έρευνα ανά πάσα στιγμή χωρίς να δώσω λόγο. ☐

3. Κατανοώ ότι όλες οι πληροφορίες που παρέχω θα αντιμετωπίζονται με εχεμύθεια. ☐

4. Κατανοώ ότι έχω επίσης το δικαίωμα να αλλάξω γνώμη για την συμμετοχή μου στη μελέτη για μια σύντομη περίοδο μετά την ολοκλήρωση της μελέτης (περίπου δεκαπέντε ημέρες μετά το πέρας της πειραματικής διαδικασίας). ☐

5. Συμφωνώ να συμμετάσχω στο ερευνητικό πρόγραμμα. ☐

Όνομα συμμετέχοντος:
Υπογραφή του συμμετέχοντος:
Ημερομηνία:

Όνομα ερευνητή:
Υπογραφή ερευνητή:
Ημερομηνία:

Φύλλο πληροφοριών συμμετεχόντων : Η σχέση της γλώσσας με την μνήμη

1. Πληροφορίες για την έρευνα / Σκοπός της έρευνας.

Αυτή η έρευνα διερευνά τις διαφορές ανδρών και γυναικών σε συγκεκριμένα γνωστικά τεστ με απώτερο σκοπό τον υπολογισμό / προσδιορισμό του γνωστικού φύλου του εγκεφάλου.

2. Γιατί επιλέχθηκα να συμμετέχω;

Επιλέχθήκατε γιατί για αυτό το πείραμα αναζητούμε υγιή άτομα ηλικίας μεταξύ 25 και 60 ετών με μητρική γλώσσα τα ελληνικά.

3. Πρέπει να λάβω μέρος;

Όχι. Η συμμετοχή είναι προαιρετική.

4. Τι πρέπει να κάνω?

Η μελέτη αυτή περιλαμβάνει τέσερις διαφορετικές μετρήσεις. Αρχικά θα δοκιμάσουμε την κατανόηση της γλώσσας σας μέσω μιας απλής τυποποιημένης μέτρησης, η οποία προέρχται απο την Wechsler Abbreviated Scale of Intelligence (WASI) (1999), όπου θα σας ζητηθεί να περιγράψετε 33 λέξεις. Στη συνέχεια, η λειτουργία της μνήμης σας θα μετρηθεί με ενα απλό τέστ μνήμης, όπου θα πρέπει να απομνημονεύσετε συγκεκριμένες λέξεις και να τις ανακαλέσετε όταν σας ζητηθεί. Επιπλέον, θα σας ζητηθεί να ολοκληρώσετε ένα παιχνίδι παζλ σε ένα tablet. Τέλος, θα σας ζητηθεί να απομνημονεύσετε και να ανακαλέσετε ακολουθίες αριθμών, οι οποίες θα αυξάνονται σταδιακά.

5. Ποιοι είναι οι κίνδυνοι που συνδέονται με αυτό το έργο;

Δεν υπάρχουν γνωστοί φυσικοί ή ψυχολογικοί κίνδυνοι που σχετίζονται με αυτή την έρευνα. Αν και η πειραματική διαδικασία είναι μεγάλη (περίπου 25 λεπτά) και μπορεί να βαρεθείτε, το ερευνητικό προσωπικό θα κάνει ό, τι είναι δυνατόν για να το αποτρέψει αυτό.

6. Ποια είναι τα οφέλη από τη συμμετοχή σε αυτή την έρευνα;

Η συμμετοχή σας θα προσφέρει στην επιστημονική κοινότητα πολύτιμες πληροφορίες σχετικά με τη σχέση της μνήμης και της γνωστικής λειτουργίας, η οποία μπορεί τελικά να οδηγήσει σε περεταίρω κατανόηση τηςλειτουργίας του εγκεφάλου. Επιπλέον, αυτή η συμμετοχή θα σας δώσει μια εικόνα για τον τρόπο διεξαγωγής έρευνας σε αυτόν τον τομέα της ψυχολογίας.

7. Επιλογές απόσυρσης απο την έρευνα.

Θα σας δοθεί ημερομηνία μέχρι την οποία θα μπορείτε να αποσύρετε τα δεδομένα σας από την έρευνα (15 ημέρες περίπου από την ημέρα της συμμετοχής σας). Το πιο σημαντικό είναι ότι μπορείτε να σταματήσετε την πειραματική διαδικασία και να αποσύρετε οποιαδήποτε καταγεγραμμένα δεδομένα ανά πάσα στιγμή, ακόμα και κατά τη διάρκεια της ερευνητικής διαδικασίας.

8. Προστασία δεδομένων & εμπιστευτικότητα.

Στην αρχή της ερευνητικής διαδικασίας θα σας δοθεί ένας προσωπικός αναγνωριστικός αριθμός. Μετά από 15 ημέρες, το όνομά σας θα διαγραφεί από τα αρχεία των δεδομένων σας και θα διατηρηθεί μόνο ο αριθμός αναγνώρισης και η φόρμα συναίνεσής σας. Μόνο ο επικεφαλής ερευνητής θα έχει πρόσβαση στα δεδομένα σας, μόνο μέσω του αριθμού αναγνώρισης. Επιπλέον, η φόρμα συναίνεσής σας θα αποθηκευτεί με ασφάλεια σε προστατευόμενο χώρο, ενώ τα δεδομένα σας θα φυλαχθούν σε προσωπικό υπολογιστή που προστατεύεται με κωδικό πρόσβασης, τοποθετημένο σε ένα γραφείο με περιορισμένη πρόσβαση και δεν θα είναι διαθέσιμα σε κανέναν άλλο εκτός από τον επικεφαλή ερευνητή. Το αρχείο της συγκατάθεσης σας θα αποθηκευτεί με ασφάλεια μέχρι την ολοκλήρωση του έργου, μέχρις ότου δηλαδή εξετάστεί το διδακτορικό δίπλωμα του Αθανάσιου Ρίζου, μετά το οποίο θα καταστραφεί.

9. Τι γίνεται αν τα πράγματα πάνε στραβά;

Παρόλο που η πιθανότητα ενός προβλήματος είναι ελάχιστη, ο επικεφαλής ερευνητής θα είναι παρόν σε κάθε φάση της έρευνας προκειμένου να σας βοηθήσει με οποιονδήποτε τρόπο Εάν υπάρχουν οποιαδήποτε παράπονα σχετικά με τη έρευνα, μπορείτε να τα εκφράσετε άμεσα ή να επικοινωνήσετε μέσω e-mail με τον επικεφαλής ερευνητή στο τέλος της πειραματικής

διαδικασίας. Εναλλακτικά, μπορείτε να επικοινωνήσετε με τον πρόεδρο της επιτροπής δεοντολογίας, τον καθηγητή Neil Forbes (n.forbes@coventry.ac.uk).

10. Τι θα συμβεί με τα αποτελέσματα της έρευνας;

Τα αποτελέσματα αυτής της μελέτης θα αποτελέσουν μέρος μιας διδακτορικής διατριβής και όπως απαιτεί ο πανεπιστημιακός κανονισμός, θα είναι διαθέσιμα στο κοινό. Επιπλέον, τμήματα αυτών των δεδομένων μπορούν να καταχωρηθούν προς ακαδημαϊκή δημοσίευση, παρόλο που δεν θα είναι δυνατή η αναγνώριση των δεδομένων από τα τελικά αποτελέσματα.

11. Ποιος αξιολόγησε τη μελέτη αυτή;

Η μελέτη αυτή έχει αξιολογηθεί από την επιτροπή δεοντολογίας του Πανεπιστημίου του Coventry.

Περισσότερες πληροφορίες / Βασικά στοιχεία επικοινωνίας.

Εάν χρειάζεστε πρόσθετες πληροφορίες για την έρευνα ή τη συμμετοχή σας, μπορείτε να επικοινωνήσετε με τον επικεφαλή ερευνητή μέσω ηλεκτρονικού ταχυδρομείου.

E-mail: rizosa@uni.coventry.ac.uk

Επικεφαλής ερευνητής: Αθανάσιος Ρίζος

Appendix 4: Free recall lists of Experiment 2

Free recall list A and B with word-lengths and word-frequencies[95] used for experiment 2a.

Word	length	frequency
List A		
dollar	6	4.14
ocean	5	4.49
college	7	4.65
shoes	5	4.65
butter	6	4.68
hotel	5	4.76
machine	7	4.83
letter	6	4.85
river	5	4.93
paper	5	4.94
tree	4	4.95
blood	5	4.98
child	5	5.14
market	6	5.22
house	5	5.83
List B		
Word	length	frequency
baby	4	5.29
church	6	5.02
corner	6	4.96
doctor	6	5.02
engine	6	4.5
fire	4	4.42
flag	4	4.42
garden	6	5.28
palace	6	4.55
rock	3	5.01
sky	3	4.79
table	5	5.1
village	7	4.92
water	5	5.53
wife	4	5.15

[95]Frequencies and word-lengths were extracted from the SUBTLEX-UK for word frequencies based on British English subtitles.

Appendix 5: Consent Statement and Participant Information Sheet (English Version)

Consent statement

The relationship between language and cognition.

1. I confirm that I have read and understood the participant information sheet for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. ☐

3. I understand that all the information I provide will be treated in confidence.

4. I understand that I also have the right to change my mind about participating in the study for a short period after the study has concluded (approximately fifteen days after the end of the experimental procedure). ☐

5. I agree to take part in the research project. ☐

6. I consent to provide a sample of my saliva for analysis in order to define the levels of Testosterone and Estradiol in my circulatory system only. ☐

7. I understand that the hormonal analysis does not have any clinical or diagnostic validity and that any attempt to offer clinical interpretations could be misleading. ☐

Name of participant: .....

Signature of participant: .....

Date: .....

Name of Researcher: .....

Signature of researcher: .....

Date: .....

Participant Information Sheet

The effects of sexual hormones on cognition.

1. Information about the project / Purpose of the project.

This study explores the potential relationship between an individual's language comprehension and cognitive performance in accordance to brain electrical activity, in order to explore the exact effects of sexual hormones on memory formation, structure and activation.

2. Why have I been chosen?

You have been chosen because for this experiment we seek healthy, native English speaking individuals between 25 to 60 years old.

3. Do I have to take part?

No. Participation is voluntary.

4. What do I have to do?

This study involves three different measurements. Initially we will test your language comprehension through a simple standardized measurement, the "Wechsler Abbreviated Scale of Intelligence" battery (WASI) (1999), where you will be asked to describe 33 words. Then, your memory function will be measured with a simple memory task, where you will have to memorize three lists of words and recall them on demand. Moreover, you will be asked to complete a puzzle game on a tablet. Then, you will be asked to memorize and recall sequences of numbers of escalating length (digit span

task). Next, your brain's electrical activity will be recorded (E.E.G.). The E.E.G. procedure involves wearing an E.E.G. cap on to which the researchers will attach sensors. A small amount of a special gel will be applied through holes in the cap to bridge the gap between each sensor and the part of your scalp below the sensor. The E.E.G process is completely painless and will not feel any different from just wearing a regular swimming cap. There is no sensation from the recording itself. Finally, a researcher will measure the length of your 2^{nd} and 4^{th} finger as it is considered to be an indirect measurement of sexual hormone levels, and you will be asked to provide a sample of your saliva in a plastic tube in order to measure directly the level of testosterone and estradiol in your system. Female participants will be asked to provide additional information for their average length of their menstrual cycle, their current day of their menstrual cycle as well as for the use of contraceptives; as these factors may produce short-term hormonal imbalances, which in turn can potentially affect cognitive performance and produce inconsistencies in the expected relationships between hormonal measurements and measurements of cognitive performance.

5. What are the risks associated with this project?

There are no known physical or psychological risks associated with this research. Although the experimental procedure is quite long (approximately 60 minutes) and you might get bored, the research personnel will do their best to prevent that. Moreover, the application of the EEG cap uses a water based gel that may temporarily affect your hairstyle. However, the University offers shower facilities if you decide to wash it out directly after the end of the experiment. Additionally some individuals might find questions about menstruation and use of contraception sensitive. However, these

questions will be asked by a female researcher in order to reduce any embarrassment or discomfort that may otherwise occur.

6. What are the benefits of taking part?

Your participation will provide the scientific community with valuable information on the relationship of hormones and cognitive function, which may ultimately lead towards new therapies and preventive methods for neurodevelopmental and neurodegenerative diseases. Moreover, this participation will provide you with an insight on how research is conducted in this subject area.

7. Withdrawal options.

You will be given a date (15 days starting at the day of your participation) up to which you can withdraw your data from the research. Most importantly, you are able to stop the experimental procedure and withdraw any recorded data at any time during your participation.

8. Data protection & confidentiality.

At the beginning of the study you will be given a participant identification number. After 15 days your name will be erased from your data files and only your identification number and consent form will be retained. Only the researcher will have access to your data through the identification number. Moreover, your consent form will be stored securely in a locked draw while your raw data will be secured on a password protected personal computer in an office with restricted access, and will not be available to anyone else other than the lead researcher. Your record of consent will be stored

securely until the completion of the project, when Athanasios Rizos' PhD has been examined and the degree awarded, after which it will be destroyed

9. What if things go wrong?

Although the possibility of a problem is minimal, two researchers will be present during every part of the study in order to assist you in any way. If there are any complaints about the study you may directly express them or contact via e-mail the head researcher at the end of the experimental procedure. Alternatively you can contact the chair of Ethics committee, Professor Neil Forbes **(n.forbes@coventry.ac.uk).**

10. What will happen with the results of the study?

The results of this study will be part of a PhD thesis and as the university's regulations require, will be available to the public. Additionally, parts of these data may be written up for academic publication, although no data will be identifiable from the collated results.

11. Who has reviewed this study?

This study has been reviewed by the Ethics committee of Coventry University.

12. Further information / Key contact details.

If you require additional information for the project or your participation you may contact the researcher by e-mail.

E-mail: rizosa@uni.coventry.ac.uk

Researcher's name: Athanasios Rizos

Appendix 6: Validation of 2D:4D findings

A one-way ANOVA was used in order to further assess sex differences in 2D:4D. The results indicated a non-significant difference between male 2D:4D (M=.999, SD=.045) and female 2D:4D (M=1.021, SD=.045), F(1.47)= 2.792, p=.101, η_p^2=.057.

In order to evaluate the current study's 2D:4D findings, a meta-analysis of seven studies that used the same (direct) method of (right-hand) 2D:4D measurement in the United Kingdom was performed. A homogeneity test of effect sizes revealed that the study by Burriss et al. (2007) was an outlier in terms of both magnitude and direction (d=.514). Therefore, this study was removed from the meta-analysis. Over the six remaining studies, males (*N* = 945) had a mean 2D:4D digit ratio of .98 (SD = .04) whereas females (*N* = 1033) had a mean 2D:4D ratio of .99 (SD = .04; Table 38). When weighted by study sample size, the computed effect size for this group of studies was *d* = .28.

Table 39. Descriptive data and computed effect sizes for the six studies

	Males			Females			*d*
	N	**M**	**SD**	**N**	**M**	**SD**	
Manning et al. (2006)	340	0.99	0.05	340	1	0.05	0.27
Fink et al. (2006)	127	0.98	0.03	117	0.99	0.03	0.34
Rizwan et al. (2007)	215	0.97	0.04	219	0.98	0.03	0.32
Manning et al. (2000)	117	0.98	0.03	183	0.99	0.04	0.28
Manning et al. (2002)	98	0.98	0.04	127	0.99	0.04	0.25
Kempe (2009)	48	0.97	0.03	47	0.99	0.03	0.22

The effect size of the current study appears to be larger (d=.49) than those from the studies that were used for the meta-analysis. However, current study's 2D:4D ratios fall within the range of those for the six studies that were included in the meta-analysis. Thus, it can be argued that there

are 2D:4D means for each sex within the current study that follow the trend found in similar studies. The direction of the difference between means also follows this trend. However, current study's effect size is larger but failed to reach significance because of the moderate sample size. Therefore, it is legitimate to argue that 2D:4D ratios for males and females within the current study are typical of what has been found in similar previous research.

A larger meta-analysis performed by Honecopp and Watson (2010) on 23 studies that used a direct method of 2D:4D measurement including populations from various countries concluded that the general population's effect size of the right-hand 2D:4D was d=.35. Honecopp and Watson concluded that regarding right hand direct measurement, observed 2D:4D sex-related differences that fall between d=.09 and d=.61 can be seen as normal. Summarizing, current study results appear to fall within the normal 2D:4D ratios for males and females, whereas the effect size of the 2D:4D sex-difference is also within the typical population values.