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Sex differences in the brain: the role of testosterone

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Abstract

On average, there are sex differences in the brain, but none of these differences are so large that there is no overlap between male and female brain structures, so the typical “male brain” and “female brain” does not exist. This led to the development of the mosaic hypothesis, which suggests that various factors transform different subregions of the brain independently, resulting in brains that are composed of both male-like and female-like features. One of these factors that differentiates brain areas is testosterone, which will be the main focus of this essay. During early life, the hormone acts as a transcription factor and organises multiple different parts of the brain by epigenetic editing, more effectively in males than in females. This neural organization results in slight behavioural changes between neonate boys and girls. Testosterone also temporarily activates tissues through its activational effects, which occur when testosterone levels in the body are high. However, there is often an interaction between activational and organizational effects, where a certain organization of neural structures is required for activational effects to occur. So, when effects of testosterone occur, divergences in the neural structures arise, where one type of structure is overall, when considering large groups of men and women, more prevalent in one gender than in the other. However, every person’s brain has both structures that differentiated into a more masculine form, and into a more feminine form.

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Introduction

"One is not born, but rather becomes, a woman" (Beauvoir, 1949). Feminists often strive for a world in which women and men are treated equally, because, they say, 'men' and 'women' are in essence indifferent to each other. The differences regarding gender arise from the expectations that society imposes on them; women are thought to be the more empathetic gender because they are expected to be so (Moi, 1999). Research shows that these cultural assumptions are reflected in the differences in emotional skills between men and women; females have superior social skills such as social understanding, and they are more sensitive to emotional expressions (Baron-Cohen, 2000). Some feminists would thus say that this difference is a consequence solely of societal expectations moulding women into their female form. However, generally, biologists occupy a different point of view in this discussion. They think there are good reasons to believe that behavioural differences in women and men have a genetic, natural cause. One of the arguments in favour of this belief is that there are neurological diseases with high heritability where a skewed sex ratio is seen, such as autism spectrum disorder (ASD), where the ratio is 4:1 in favour of males, or Alzheimer's disease or social anxiety disorder (SAD), which are more prevalent in females (Pavlova, 2017). In addition to these observations, tests with neonates that are less than 48 hours old have been done and these show that on average, girls perform differently than boys on tasks testing for social perception (Baron-Cohen, 2000). These two factors point to a more natural cause of sex differences. However, the feminists could say, this does not prove that none of the sex differences are the result of societal factors, because of a phenomenon called neuroplasticity: changes in the organization of the brain happen for about 90% after birth (Zerilli, 2021). So even if genetically determined factors regulate sexual differences before there is a chance for cultural influences to do so, these genetic components determine only a small part of the brain. The consequence hereof is that it is impossible to find out the congenital cause of the sex differences by assessing the brain as it is later in life. However, since sex differences already occur during foetal development, it is possible to look at the underlying mechanisms of the congenital variations. Three main mechanisms can be focused on: gonadal hormones (*e.g.,* testosterone, oestrogen, progesterone), sex chromosomes (and for a much smaller part autosomes), and responsiveness to the environment that is sex-specific (Bale, 2016). The latter factors' effectiveness is seen in that males are more responsive to stress during gestation, which results in a higher likeliness for males to develop neurological diseases in that period (Sutherland & Brunwasser, 2018). Sex-related genes are thought to have a much smaller effect on the differentiation of the human brain than hormonal sexual differentiation (Arnold & Chen, 2009), so the focus of this paper will lay primarily on one of the hormones secreted by the gonad; the effect of testosterone. Testosterone has two ways to affect the brain, namely via organizational and activational pathways, which will both be assessed in this paper. Organizational effects include effects that occur before (or just after) birth when the brain is very sensitive to factors from the outside (such as hormones), and these organizational effects are permanent and sexually differential (Phoenix, Goy, Gerall, & Young, 1959). Activational effects on the other hand, are temporal; they occur in periods where levels of the hormone are high and cause short-term behavioural changes. Before discussing the effects of testosterone, an overview will be given of the main differences between the sexes regarding the brain (Crewther, Pastuszak, Sadowska, Górski, & Cook, 2022).

Sex differences in the brain

To investigate the sexually differentiated structures in the brain that are influenced by testosterone, it is necessary to first gain an understanding of the main sex differences in the brain. In 2018, the largest study on this topic was conducted, including 5216 participants of all ages (Kurth, Cherbuin, & Luders, 2019). This study, which used data from the UK Biobank (Ritchie, 2018), significantly enhanced our understanding of sex differences in the brain due to its large sample size. The results indicated that, on average, females have a thicker brain cortex, while males have a larger brain volume and more surface area. In fact, 92.1% of males have a total brain volume that is larger than the average brain volume of females. In addition, males tend to have larger ventricles and a higher volume in brain regions involved in emotion, such as the left isthmus of the cingulate gyrus (a subregion of the amygdala) and the insula (part of the limbic system). They also have larger subregions involved in decision-making, such as the orbitofrontal cortex and the right fusiform gyrus. However, in some regions, such as the right fusiform gyrus and the insula, females have a larger surface area despite having a smaller volume. Regions that tend to be larger in females include the nucleus accumbent (part of the reward system) and the hippocampus. Other differences include that males generally have more intra-hemispheric activity, while females have more interhemispheric activity (Toledo, Feltrin, Barbosa, Tahira, & Brentani, 2022). Additionally, females tend to have thicker cortices, particularly in the bilateral inferior parietal regions. These findings establish the existence of significant sex differences in the brain.

In addition to differences in subregional size, and intra- and interhemispheric activity, males and females also differed in the fractional anisotropy of their white matter, which is a measure of the directional diffusion of water. Males tended to have a higher value, indicating that their diffusion was more restricted to one axis, compared to females. Females generally scored higher on orientation dispersion, which indicates that their white matter tracts are more complex compared to those of males (Ritchie, 2018).

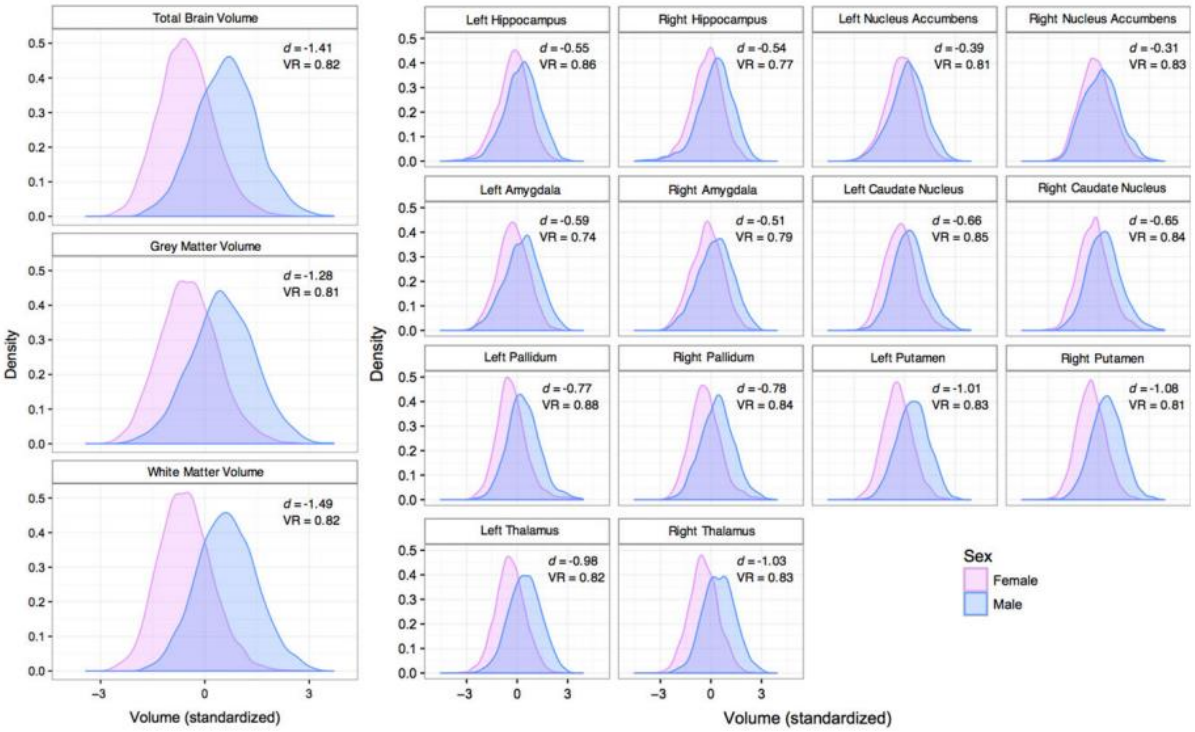

***Figure 1**. Density plots of male-female differences in brain volume and in the volume of subcortical structures. Males on average have both a larger total brain volume and subcortical volume, however, there is an overlap between males and females for each plot. (Ritchie, 2018)*

In the previous subsection it was discussed that research shows that some sexual differences in the brain, such as subregional brain areas and white matter structure differences, exist between male and female brains. However, none of these differences were so great that there was no overlap between male and female brains (Figure 1). On the contrary, even for the brain regions that showed the most significant differences, there was a large overlap between the sexes. Also, the amount of fractional anisotropy and complexity of white matter structures showed a big overlap between male and female neuronal tracts (Ritchie, 2018). So, in the following subsection, a deeper analysis of the divergences in neural structures will be presented.

The overlap between differences in male and female brains, that is for instance seen in both subregional structures and fractional anisotropy, suggests that the previously held belief, that testosterone causes male brain development to diverge from the female form, is incorrect. According to the widely accepted 'mosaic hypothesis,' a female can have a 'typical male' brain due to her brain's subregions containing a male-like form (Joel, Garcia-Falgueras, & Swaab, 2020). This means that a 'male brain' does not necessarily belong to a male body, but rather that the brain more closely resembles a typically male brain.

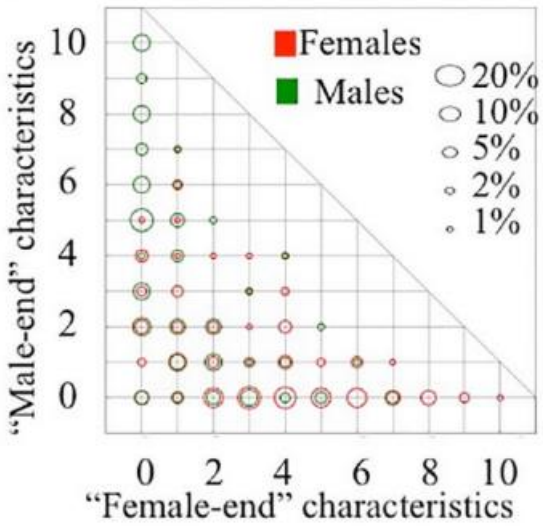

***Figure 2**. Group level differences in brain structure. Most brains are composed of male and female-like structures. There are more brains of males in the male-end zone and more brains of females in the female-end zone. (Joel, Garcia-Falgueras, & Swaab, 2020)*

This indicates that sexual differentiation in the brain might be a more complex process than was previously thought. It appears that during this differentiation process, subregions are transformed into a male or female form by factors such as hormones, genes, and environmental factors. These factors do not affect the entire brain at once, but rather influence different subregions through independent pathways. This can explain the findings of this study, namely that there are some neural structures that, on average, differ between males and females, but in most cases, there is overlap, and the individual differences between brains are greater than the sex differences. In other words, there are female brains with both subregions that have a typical female form and subregions that have a typical male form. Moreover, it appears that very few people have 'consistent brains,' meaning that they have all masculine or all feminine brains. The mosaic hypothesis is illustrated in figure 2 (Joel, et al., 2015), which shows that in most cases, at least one brain region was more masculine and at least one other was more feminine.

So, even though it has been demonstrated that there are average group differences between the brains of males and females, the 'male brain' or the 'female brain' does not exist. Rather, different neural structures are independently differentiated into different forms. Some of these forms appear to be more prevalent in male brains, and others in female brains, which is observed when performing group studies. The type of structure that is more prevalent in female brains will be further addressed as a feminine structure and the type that is more prevalent in male brains as a masculine structure.

Organizational effects of testosterone on the brain

The mosaic theory raises the question of how these brain structures become more masculine or more feminine; what are the various factors that independently transform subareas of the brain? This question will be tackled in the coming section.

As previously mentioned, sex-related genes and sex-specific responses to the environment play a role in this differentiation of neural subareas, but the primary cause of sexual differentiation is hormones (Arnold & Chen, 2009). In this paper, the focus will lay primarily on the role that the gonadal hormone testosterone plays in this. Due to it being impossible, for ethical reasons, to do experiments on human foetal brains with different levels of hormones, it is difficult to get information about mechanisms of the microstructure of the human brain, which is affected and changed by these hormones. By using fMRI, the macrostructure of human brains can be assessed, but the information that comes out is already violated by social/ cultural influences. However, by combining theoretical knowledge with ethically acceptable practical experiences studying the effects of testosterone on humans, it is possible to gain a better understanding of the organizational effects of testosterone on the foetal brain.

To assess the effect that testosterone has on the independent differentiation of neural structures, it firstly is useful to learn more about the production pathway and the differences in testosterone levels between the sexes. During gestation, a bipotential gonad differentiates into testes when the Sry gene, located on the Y chromosome, is present. If not, the gonad develops into ovaries. When it develops into testes, the endoplasmic reticulum in the Leydig cells (located in the testes) produces a wave of cholesterol. This cholesterol is transported by the main regulator, steroidogenic acute regulatory protein, to the inner mitochondrial membrane of cells that will subsequently produce testosterone. Here, it is converted into pregnenolone, which is the main substrate for testosterone. In early pregnancy, testosterone is aromatized, meaning it is converted into oestradiol. During this same period, the ovaries produce 7 to 8 times less testosterone than the testes (Celec, Ostatnı´kova´, & Hodosy, 2015), so the female brain does not develop in the same way as the male brain.

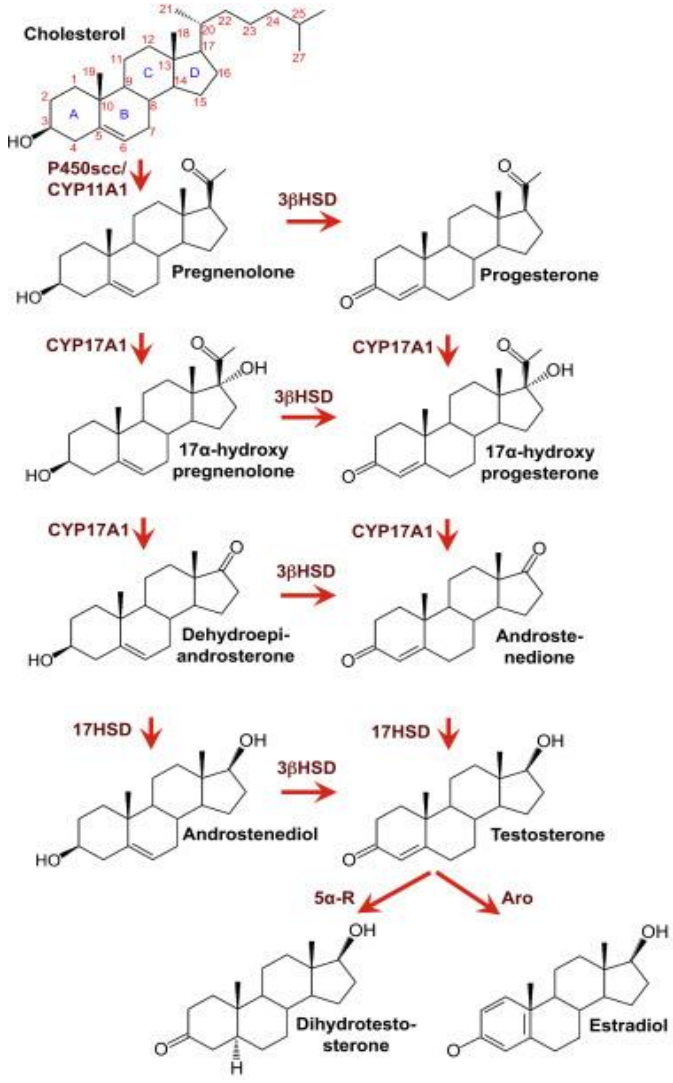

***Figure 3**. Metabolic pathway of testosterone. From cholesterol to oestradiol. The hormones are presented in black, and the enzymes are depicted in red (Zakharian, 2020).*

Although the female brain does not undergo the changes induced by oestradiol early in pregnancy, it may be 'masculinized' by hormones through different mechanisms. The adrenal glands produce testosterone in small amounts, but for women, these small amounts are significant; 67% of all their testosterone is made from dehydroepiandrosterone (DHEA), which is secreted by the adrenal glands (Filová, Ostatníková, Celec, & Hodosy, 2013). DHEA is converted into androstenedione by 17alpha-hydroxysteroid dehydrogenase (HSD) and 3 alpha-HSD. This process occurs in the adrenal cortex, but for women, it may also occur in the ovaries, which produce small amounts of testosterone. Androstenedione is then converted into testosterone.

Part of the testosterone produced by the gonads and the adrenal glands is transferred to the brain and utilized there. However, the enzymes aromatase,

17alpha-HSD, 3alpha-HSD, and 5alpha - reductase are also found in the brain, which suggests that the described process of testosterone biosynthesis also occurs in the brain; the brain itself synthesizes androgens and oestrogens (Schmidt, et al., 2008). This occurs, for instance, in the hippocampus. These steroids produced by the brain are called neurosteroids (Corpechot, Robel, Axelson, Sjovall, & Baulieu, 1981), which function as ligands for oestrogen receptors, which are found throughout the brain. There are different types of oestrogen receptors present. Oestradiol binds to some of them, and this hormone is mainly active during early foetal periods. In later life, testosterone is more often converted into 5 alpha-dihydrotestosterone (DHT) by 5alpha - reductase. DHT can be further metabolised into 5alpha-androstane3alpha,17beta-diol (3alpha-diol) and 5alpha-androstane-3beta,17beta-diol (3beta-diol). These two metabolites also function as ligands for an oestrogen receptor called ERbeta (Edinger & Frye, 2007). In this way, testosterone is present in and therefore organizes the human brain, but it does so in higher quantities in the male brain compared to the female brain.

This testosterone, that is active in the brain, interacts with and organises the neural structures. It does so by acting as a ligand and by binding to steroid receptors on the nucleus of a cell. A cascade of reactions occurs, and the chromatin, in the nucleus of the cell, is modified into a more open or closed state. So, the steroids here act as a transcription factor (Knoedler & Shah, 2018). Foetal development is a period in which chromatin remodelling due to testosterone occurs frequently, and this has an organisational effect on the brain. It has been shown that the factors that interact with sex-biased genes, such as transcription factors, are more responsible for creating differences in the foetal brain than the differentially present genes themselves (Werling, 2016), so even when genes are not differentially present between males and females, there can be differences in the regulation and thus the expression of genes, which causes sex differences. This is why transcription factors (among which, testosterone) and more regulatory proteins lie on the basis of sex differences (Lopes-Ramos, et al., 2020).

The genes on the autosomal chromosomes that are differentially expressed due to transcription factors, demonstrate the differences that these factors can make. To further assess these differences, the study by Victor Hugo Calegari de Toledo et al., 2022 took samples from foetal brains by collecting them from abortions. The genes on the sex chromosomes were removed to focus fully on the autosomal genes, and to see the effect of gene regulators rather than the effects of differentially present genes. The genes were then investigated to see which were co-expressed between different samples of different foetal brains, since this indicates that these genes regulate the same pathway or have the same function. These co-expressed genes were then clustered into co-expression modules, which are thus based on gene expression correlation between different samples. The co-expression modules created from this study provide an overview of the sex differences in gene expression in foetal brains. Two modules were found to be more active in female brains and related to the specification of synapses and differentiation of neurons, as well as energy production in mitochondria. Also, modules were created that are more active in male brains. One of these relates to the biological process of the proliferation of cells in response to stress, and another to cell cycle regulation (Toledo, Feltrin, Barbosa, Tahira, & Brentani, 2022). These differentially active modules imply the difference in gene expression that transcription factors can make.

To connect this information about the difference that transcription factors can make to the development of the human brain, it is convenient to zoom in on how the foetal brain develops over time, so it becomes visible in which period the brain is most affected by the sex differences due to the differentially expressed genes. In the third month of conception or the early foetal period, the neurons start to migrate to their designated places and proliferate. Once there, they specialize. They also cumulate in the establishment of the cortical plate. One month later, the proliferation and specialization continue, while cells migrate away from the cortical plate, causing these cells to disperse and eventually form the cortical subplate. In the fourth month or the mid-foetal period, the most profound happenings are the changes in the cytoarchitecture of the neurons and their aggregation, the in- and outgrowth of axons, the specification of neurons, and the differentiation of dendrites (Kostović, Sedmak, & Judaš, 2019). If these processes of early and mid-foetal brain development are compared to the biological processes that the sexually differentiated genes are associated with, it

shows that the latter are partly the same processes as the former. For example, there is the module that is expressed in males, but not so much in females, which is associated with cell cycle regulation. It is shown that the brains of males grow more prenatally than the brains of females, which might (partly) be the cause of this sexually different module. Apart from the foetal brain, the proliferation of cancer cells is also higher in males; the incidence of and mortality rate due to cancer is higher in males (Kim, Lim, & Moon, 2018). The same goes for neurological diseases such as ASD, which is also associated with abnormal differentiation of neuronal cells (Marchetto, et al., 2017). More differentially expressed autosomal genes and regulators result in differences affecting the development of the foetal brain, which mainly involve proliferation, metabolism and delamination. In conclusion, some of the biological processes associated with the genes that are differentially expressed between males and females can be linked to processes in foetal brain development. This linkage implies the period in which the brain is affected by regulating factors.

The study of Toledo et al., 2022 did not specify exactly which regulators took care of the sex differences in the organization of the brain, however, they mentioned that two of the male-biased modules and one of the female-biased modules were enriched for oestrogen receptor targets, which points to the direction that in this case oestrogen (or 3alpha-diol or 3beta-diol) might have acted as a transcription factor.

A study by Chura et al., 2010 did, in contrast to the study described in the previous subsection, not just look at the effect of any regulatory factor, but at exactly the organisational effect of testosterone. They zoomed in on this effect on the corpus callosum, the greatest white matter tract, consistent of 200 million neuronal fibres. Already back in 1906, it was noticed that the male corpus callosum size was larger than the females' (Bean, 1906). and that it is also more asymmetrical in certain subregions. The researchers measured the levels of foetal testosterone in boys during the 13th and 20th weeks of gestation and later used fMRI to measure the size of their corpus callosum at ages 8-11. They found that higher levels of foetal testosterone were correlated with a more asymmetrical corpus callosum, with the right side being larger than the left, particularly in the Isthmus and Hofer-Frahm IV subregions (figure 4). However, no association was found between foetal testosterone levels and the overall size

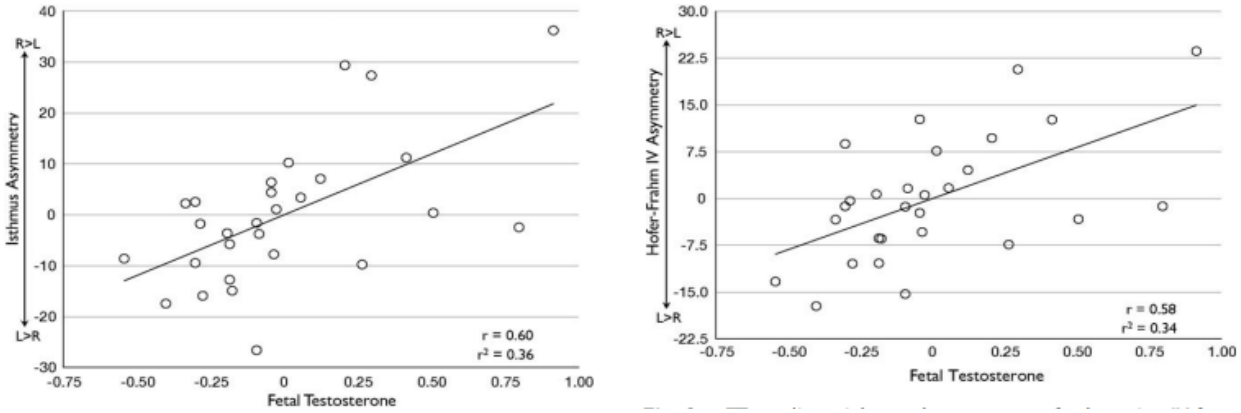

Figure 4. *Asymmetry in Isthmus (Y-axis, left graph) and Hofer-Frahm IV (Y-axis, right graph) subregions of the corpus callosum, due to the amount of foetal testosterone (X-axis) (Chura, et al., 2010).*

of the corpus callosum. This suggests that testosterone may function as a transcription factor in the isthmus, stimulating neuroprotective processes such as the proliferation of cells in the white matter, but that the size of the corpus callosum is not influenced by foetal testosterone levels (Chura, et al., 2010).

In addition to the corpus callosum, there are many more areas in the brain where the perinatal organizational effects of testosterone have changed the human brain by the mechanisms described above. Examples of other brain areas where this happened are the sexual dimorphic nucleus (SDN), the amygdala and the hippocampus (Filová, Ostatníková, Celec, & Hodosy, 2013), however, there are

many more, but due to the brain being a very complex organ, by far not all organizational effects of testosterone are known.

The previously discussed organisational effects of testosterone on the brain may have behavioural consequences. However, assessing these consequences may be challenging, as social influences begin to shape the brain immediately from birth. One way to study these effects is to experiment with neonates, allowing for the examination of differences in male and female behaviour without the influence of social factors. Not many experiments of this sort have yet been done, since working with neonates can be challenging. Simon Baron-Cohen et al. have performed such an experiment by measuring the time a neonate looks at a face. they presented 102 neonates under two days old with a moving head or a moving mobile and recorded their eye movements. It appeared that 25.0% of males and 36.2% of the females had a preference for looking at the face, while 43.2% of the males and 17.2% of the females had a preference for looking at the mobile. The rest (31.8% of males and 46.6% of females) had no preference. So, males preferred looking at the mobile, and females had no preference or preferred the face. This suggests that neonates as young as one day old show differences in social and mechanical perception, with females tending to prefer faces and males tending to prefer mechanics (Baron-Cohen, 2000).

This shows that testosterone has perinatal organizational effects on the brain that influence behaviour, with males generally receiving more testosterone than females. These differences in neural structure and function contribute to the observed differences in male and female behaviour.

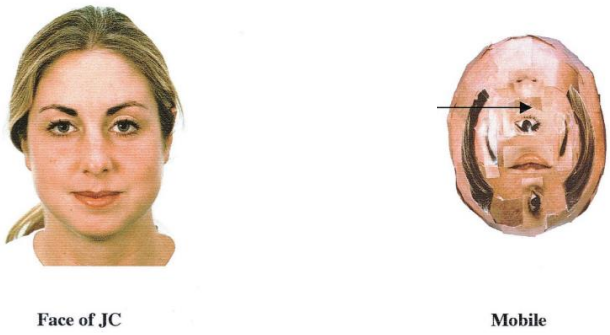

Figure 5. *The image of the face and of the mobile that was shown to the neonates (Baron-Cohen, 2000).*

Activational effects of testosterone

In the previous section, the organizing effects of testosterone, where the hormone alters neural structures by epigenetic editing, were discussed. In the next section, the focus will lay on the other effect that testosterone has on the brain, which is the activational effect, where it activates specific tissues. This latter effect starts to show predominantly during puberty, where the level of steroids expressed rises, and when the level of testosterone gets much higher in males than in females. This is because these effects only occur when the steroid is present in large quantities in the target tissue. It is thus a transient, short-term effect (Filová, Ostatníková, Celec, & Hodosy, 2013).

To get a clearer idea of what the activational effects of testosterone in humans are, transgender men who are treated with testosterone to get more male-like traits can be studied. It appears that they, after testosterone treatment, experience reduced anxiety and depression and improved sexual desire, compared to before the treatment. These changes may be due to changes in the levels of testosterone in the brain. To assess the activational effects of testosterone, or in other words, to see what the differences in the brain between before and after treatment are, it is necessary to first note that the brains of transgender men before treatment are slightly different from that of cisgender females. On average, transgender men have a larger right cerebellum and putamen than cisgender females (Hoekzema, Schagen, Kreukels, & al., 2015). Their prefrontal orbital cortex was equally thick as that of cisgender males, so slightly thinner than that of cisgender females (Zubiaurre-Elorza, Junque, Go´mez-Gil, & al., 2013). As discussed earlier, females have lower levels of white matter fractional anisotropy than men, and transgender men have fractional anisotropy levels similar to that

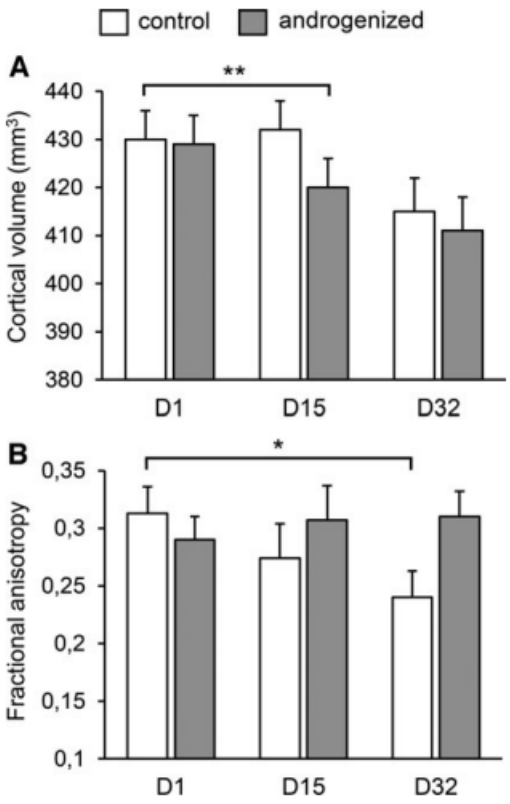

Figure 6*. Differences in cortical volume (upper graph) and fractional anisotropy (bottom graph) between control (white) and androgenized (grey) rats, for day 1 (D1), day 15 (D15) and day 30 (D30) (Zubiaurre-Elorza, et al., 2021) .*

of cisgender males in some parts of the brain, like the forceps minor. Their anisotropy levels differ from both cisgender sexes in the corticospinal tract.

Now that it is clear what the brain of transgender males looked like before treatment, their brains during testosterone treatment can be assessed. The study implies that there was an increase in the brain volume, the volume of several regions in the cortex, e.g., the fusiform and occipital areas, and in subcortical structures. Also, the fractional anisotropy in the white matter increased. The way testosterone alters these brain areas is the same as described before: it will circulate from the peripheries to the brain and there, due to aromatase or reductase it will transform to oestradiol, or 3beta-diol or 17beta-diol respectively, and they act on androgen- or oestrogen receptors, in a genomic (organizational) or non-genomic (activational) way.

This increase of fractional anisotropy might be a hint towards the reason for the increase of the brain/ cortex volume since this means that the directionality of the diffusion of water is more restricted to one axis. Testosterone is known for its anabolic function in muscles (increased testosterone levels mean an increase in muscle mass (Bhasin, Storer, Berman, & al., 1996), so the enhanced fractional anisotropy hints at the hypothesis that testosterone also has anabolic effects in the brain. The extra proteins that are synthesized due to the effects of testosterone call for the diffusion of water towards the area of these proteins to maintain osmotic homeostasis. The result of this process is an increase in the volume of the concerned region. This hypothesis was tested in rats: a group of female Wistar rats that were 70 days old, was given a shot of testosterone (that is comparable to the amount that transgender men can get) each week, for one month. There also was a control group that was injected with saline. Brain measurements were performed. It appeared that the values of fractional anisotropy of the control group went down, due to the increasing age of the rats, while that of the androgenized group did not change (figure 6). The cortical volume of control rats also declined with age, but the decline of the androgenized rats was steeper. Also, the proportions of some metabolites were different than in control rats. For example, the androgenized rats contained more proteins in the astrocytes, but less mI (Myo-inositol) and Gln (glutamine) which are major osmolytes, due to the increase of testosterone. This explains the decrease in cortical volume.

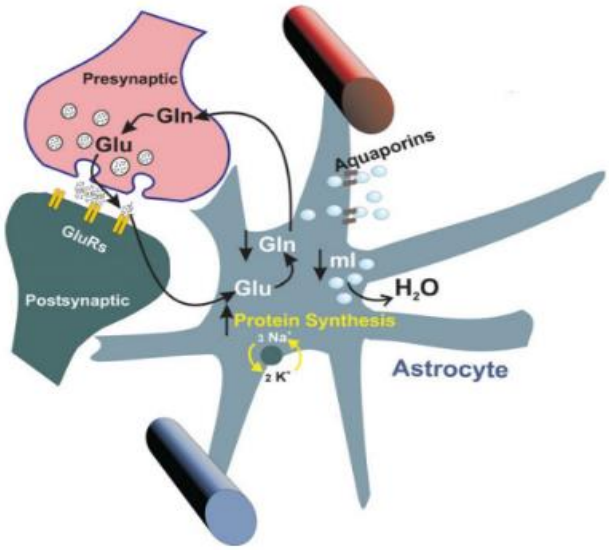

Figure 7. *The anabolic effect of testosterone. Testosterone increase leads to a decrease of mI and Gln in astrocytes, which in turn induces an osmotic stream that causes water to leave the astrocyte (Zubiaurre-Elorza, et al., 2021).*

Since mI is usually present in astrocytes, it indicates that the reduced cortical volume is mainly a consequence of reduced astrocyte volume. So, the experiment shows that in presence of testosterone, water diffuses towards the astrocytes due to the anabolic properties of testosterone in these cells. To prevent the astrocytes from exploding as a consequence of too much water, mI and Gln leave the astrocytes, which creates another osmotic stream, which causes water to leave and brain volume to decrease (figure 7) (Zubiaurre-Elorza, et al., 2021).

The increased cortical volume measured in transgender men treated with testosterone is contradictory to the decrease in the rat's cortical volume. However, brain measurements of bodybuilders who have been under testosterone treatment for longer than one year do show signs of a decrease in cortical volume (Bjørnebekk, et al., 2017). It might thus be that in humans, the increase in brain volume is a short-term effect of testosterone treatment, while a decrease in brain volume is a long-term effect.

In transgender men, who are born with female sex chromosomes, less organizational effects of testosterone have occurred due to the lack of the *Sry* gene. However, in most other cases, there is an interplay between the organizational and activational effects. An example that makes this clear is the gene *Cckar*: this gene is required for female sexual behaviour in mice. It codes for a receptor that is present in the ventrolateral region of the ventromedial hypothalamus, and it is highly expressed in wildtype (WT) females and only slightly in WT males. When females are castrated at adult age, the expression of the receptor decreases to male levels. However, when these female mice are treated with oestrogen, the expression increases back to wildtype female levels. When castrated males are treated with oestrogen, the number of receptors expressed does not increase. This shows that an organizational effect is necessary to open up the chromatin earlier in life, and that an activational effect is required to activate the gene and initiate transcription (Xu, Coast, Yang, & Shah, 2012).

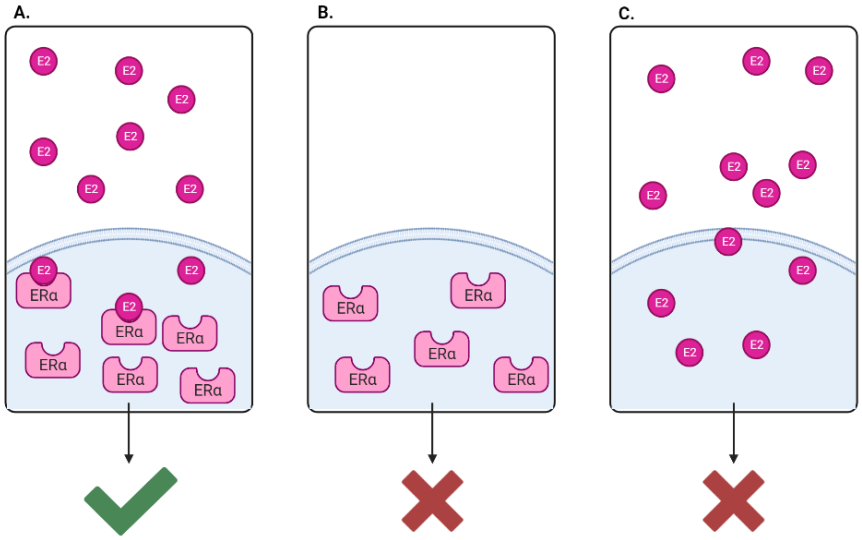

***Figure 8**. Interaction between organizational and activational effects. A: both effects are present, B: only organizational effects have occurred, C: only activational effects are present.*

A similar interaction can be observed in the hippocampus of rats. In females, the CA1 pyramidal cells have a higher dendritic spine density when oestrogen levels are higher, during the proestrus phase. In addition to oestrogen, androgens such as testosterone have been shown to increase spine density. Testosterone and other male hormones also increase the size of brain structures in rats by encouraging cell proliferation and extending their longevity. This growth of tissue is also observed in the dentate gyrus (of which the hippocampus is a part) of castrated male rats treated with dihydrotestosterone, but no differences were seen when adding oestradiol. More sex differences due to administered oestradiol are seen when looking at levels of potentiation of neurons in the hippocampus. Decreased oestradiol results in impaired long-term potentiation in female rats, which is an indicator of impaired memory. Their hippocampal neurons also contained fewer spines. In males, a decrease in oestradiol had no effect. The fact that oestradiol ensures tissue growth and neuronal

potentiation only in female rats, shows that these activational effects need a certain type of organizational effect to be effective (Garelick & Swann, 2014) (Smith, Vedder, & McMahon, 2009).

As established previously, the organisational effects due to testosterone, that are interfered with by activational effects, happen during the perinatal period. However, multiple studies suggest that organization also happens during adolescence (Schulz & Sisk, 2016). It was tested whether there are modifications of the activational effects of testosterone in adult life due to the levels of testosterone received during puberty. This was done by measuring testosterones' effect on social cognition, by mapping the response of the brain of male adults to faces that were shown to them (Liao, Tilley, Mouraviev, Khairullah, & Paus, 2021).

To do this, the first step was measuring the testosterone levels during adolescence (age nine to seventeen) in 507 men. When these men reached the age of nineteen, it was assessed whether the levels of testosterone they had when in puberty, moderated the relation between their current testosterone levels and their response to faces, using fMRI, focussing on 25 brain regions. The faces they observed were either angry or ambiguous looking.

Based on the results hereof, the participants were divided into three groups: those with low-, medium- or high pubertal hormone levels. In all three groups, there was a different relationship between the levels of testosterone they received during adulthood (AT) and the brain response to faces. This means that the correlation between these two factors is modified by the levels of testosterone during adolescence. The correlation between AT and brain response was the strongest for the low pubertal testosterone (PT) group, for both responses to angry and ambiguous-looking faces. Especially in the right and left amygdala of the low PT group, there was a strong positive correlation between AT and brain response to angry-looking faces. This positive relation was not seen in men who received medium or high levels of testosterone during adolescence (figure 9). Also, in the group with low PT, there was a higher correlation between the level of AT and node strength across most of the 25 regions than in the medium and high

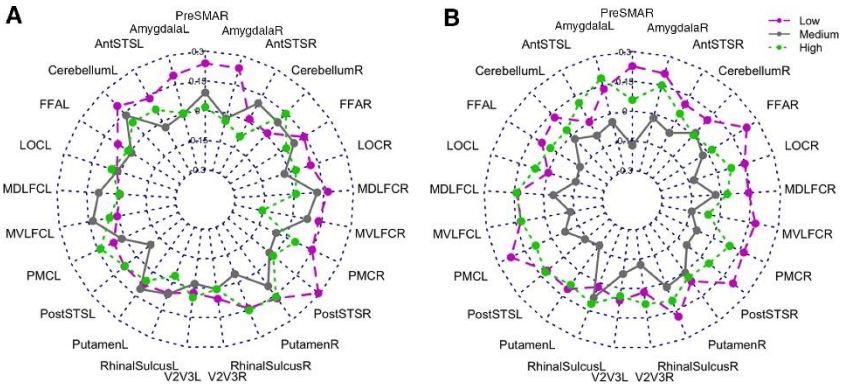

***Figure 9**. The correlation between neuronal activity and adult testosterone for the 3 pubertal testosterone groups (low PT: purple, medium PT: grey, high PT: green). In graph A, the participants looked at an angry face. In graph B, the participants looked at an ambiguous face (Liao, Tilley, Mouraviev, Khairullah, & Paus, 2021).*

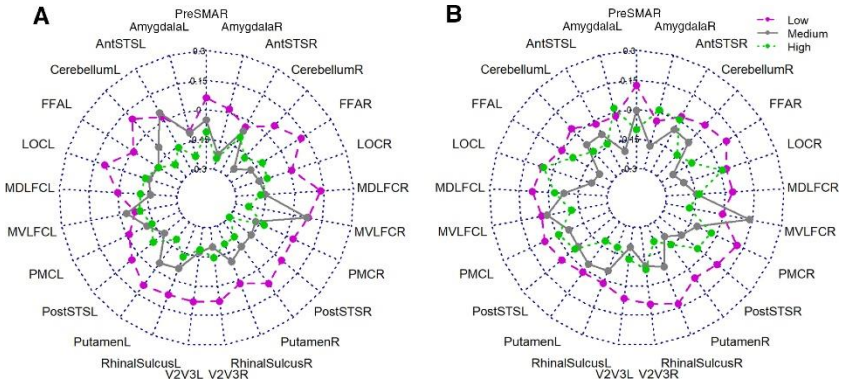

***Figure 10**. The correlation between node strength and adult testosterone for the 3 pubertal testosterone groups (low PT: purple, medium PT: grey, high PT: green). In graph A, the participants looked at an angry face. In graph B, the participants looked at an ambiguous face (Liao, Tilley, Mouraviev, Khairullah, & Paus, 2021).*

groups, especially when the participants looked at angry faces (figure 10).

These results show that during puberty, the amount of received testosterone does organize the brain since it moderates the relationship between the level of adult testosterone and the amount of brain activation and nodal strength in brain regions in response to faces. This positive correlation was especially strong when low testosterone levels were experienced during adolescence, so here, the brain was most strongly primed for later testosterone.

How these results should be interpreted is difficult to say, because not many studies have yet been done about the organizational effect of adolescent testosterone in humans. Animal studies about this topic point towards the other direction; high levels of adolescent testosterone prime for a stronger reaction to the activational effects of adult testosterone. An answer to this seemingly contradictory outcome may be that the outcome depends on the task that was examined. In animal studies, this was reproductive behaviour, while in this experiment social behaviour was studied (Eisenegger, Haushofer, & Fehr, 2011). The few other human experiments that have previously been done are in line with this experiment; a study about SAD (social anxiety disorder) concluded that testosterone treatment reduces symptoms of anxious women by for example increasing the time they fixate on the eyes of others. People who suffer from SAD often have low natural levels of testosterone. When the same dose of testosterone is given to healthy women, it results in the opposite effect: the time of fixation on the eyes decreases (Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2016).

The reason for those results may be due to the way the brain is formed by testosterone in adolescence. Certain cells proliferate and grow in volume, which causes brain structures to grow. In this case, according to studies done with hamsters, the posterior dorsal medial amygdala grew in volume. This is due to pubertal testosterone acting as a transcription factor that, among others, increases the amount of expressed androgen receptor genes (Lorme, Schulz, Salas-Ramirez, & Sisk, 2012).

So, here it is seen that even though the effects of testosterone on the brain are to be divided into two kinds, namely organizational- and activational effects, whereby the latter affects the brain temporarily when the hormone is present in high quantities, an interplay between the two effects occurs. The interplay exists of certain specific organizations of the neural substructures being activated by testosterone.

Discussion & conclusion

Studies found that there generally are several distinctions visible between the brains of males and females, but that these differences are so modest that many males have more female-like brains and *vice versa*. It appeared that not the whole brain at once, but subregions independently differentiate into more typically male-like or female-like variants, so as a result, the human brain is made up of a mosaic of more male and more female-like brain regions. Many factors differentiate brain structures, and one of those is testosterone; a steroid that is more occurrent in males than in females due to the Y chromosome in the genome of males that contains the *Sry* gene, which codes for the initiation of the creation of the testes. Testosterone can operate as an organizer, such that it, by acting as a transcription factor, remodels the chromatin and thus opens up or closes the DNA. The effect hereof is life-lasting and usually happens in the first period of one's life, but it may also take place in later life. The other way in which the hormone creates variation in the brain is by acting as a ligand and transiently activating brain regions. This happens when levels of testosterone surrounding certain neural tissues are high. Often, subregions have to be organized in a certain way for the activational effects to be successful, since the chromatin needs to be opened up for steroid receptors to be expressed at the cell surface so that testosterone can act on these receptors and carry out their activational effects. This means that an interplay between organizational and activational effects occurs and it has an impact on brain function.

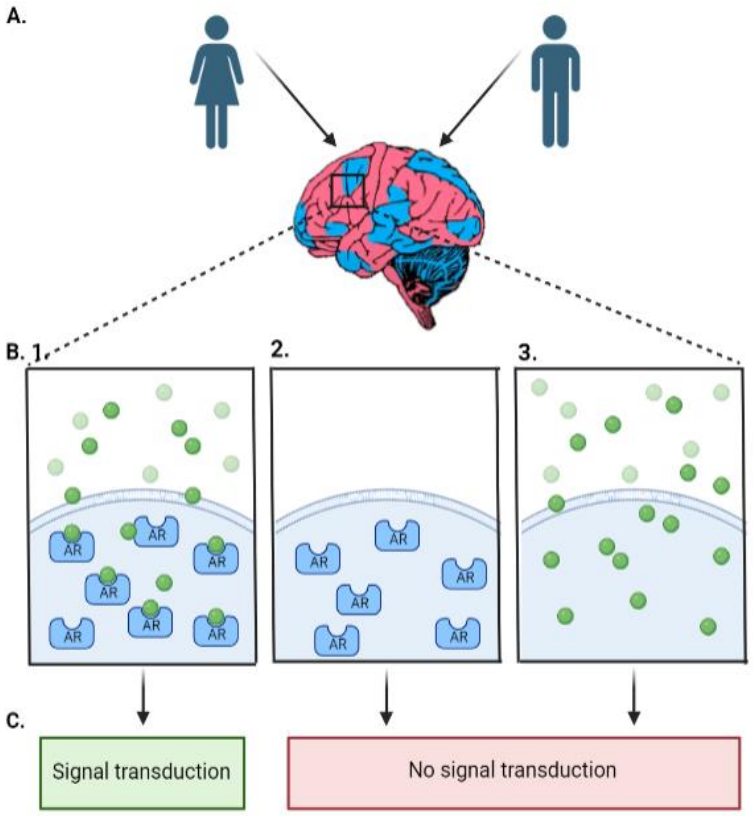

Figure 11. A*. the brains of males and females consist of a mosaic of more male-like and more female-like structures.* ***B****. There are personal differences between the presence or absence of organizing and activating effects of testosterone in these neural structures;* ***1****. Both organizing and activating effects are present,* ***2****. Only organizing effects are seen,* ***3****. Only activating effects are active.* ***C****. often an interplay between both effects of testosterone is necessary for a certain output.*

These findings have important implications for understanding the sex differences in behaviour and for the development of sex-specific treatments for neurological and psychiatric disorders. For the latter case, the recognition of these differences is essential, because they can help to identify different risk factors and prognoses for people with different neural networks. An example of a disease that is affected by the amount of organizing and activation of testosterone in the brain is social anxiety disorder. SAD is more prevalent in females than in males, which may be explained by the often-low levels of testosterone circulating in the brain, and the disorder presents itself differently in males than in females. An example of the different presentation is that women with social anxiety disorder may be more likely to avoid social situations, while men with the disorder may be more likely to engage in risky or impulsive behaviours as a way of coping with their anxiety (Asher & Aderka, Gender differences in social anxiety disorder, 2018). The diagnosis and prognosis of the disease also differ between individuals and understanding the sex differences can help to improve them (Asher, Hermesh, Gur, Marom, & Aderka, 2019).

Another disease for which knowledge of sex differences is important is Alzheimer's disease, since men and women are affected differently by it. Research has shown that women are more likely to develop Alzheimer's disease than men and that the disease tends to progress more quickly in women. Additionally, there may be differences in the way that the disease presents itself in men and in women, with men being more likely to experience more severe symptoms such as delusions and aggression. Awareness of these differences can help to inform the development of treatment and care strategies for individuals with Alzheimer's disease and it can help to identify risk factors, which could ultimately lead to the development of more effective prevention strategies (Laws, Irvine, & Gale, 2018).

This divergent representation of a disease in men and women is also seen in Autism spectrum disorder. It is thought that ASD is four times more prevalent in males than in females, however, since the disorder presents itself differently in males than in females, it is likely that males are more probable to be diagnosed. Understanding sex differences can help improve the accuracy of diagnosis, also for females, and the effectiveness of treatment for individuals with autism (Ferri, Abel, & Brodkin, 2018).

In addition to the improvement of the diagnosis and prognosis of neurological and psychiatric disorders, the findings of this literature also have implications for understanding sex differences in behaviour, which in turn may give rise to social and cultural implications. Using the information acquired about the role that testosterone plays, it can be said that when considering large groups of males and females, some essential average differences in neural structures and volume sizes of subregions of the brain, between the groups do exist. This is supported by the skewed sex ratios that occur in some congenital diseases. However, as suggested by Joel's research (Joel, et al., 2015) (Joel, Garcia-Falgueras, & Swaab, 2020), the average difference between the brains of the sexes (on group level) is considerably smaller than the average difference between the brains of two random people; there are some differences in some brains between some men and some women. This means that according to this information, in many cultures the divergence between man and woman is too great. On individual level, no differences in intelligence, performance, behaviour or even personality can be attributed to being male or female, and overall, on group level, only slight differences in abilities like social perception can be associated with gender.

So, in conclusion, it can be said that group studies do reveal average differences between the brains of the sexes, but that these differences are almost negligible when comparing two random brains. However, these studies have used brains of participants who have been living in a certain kind of culture for all their lives. Since cultural gender differences are omnipresent, these differences have influenced the brains of these participants. In the human brain, about 10% of the neuronal connections are made before birth, which means that the remaining 90% of the connections are created under influence of social gender norms. This means that the outcome of the studies researching sex differences in the brain are influenced by the occurrent sex differences in society. Further experiments need to be done with e.g., neonates in order to isolate and study the congenital effects of sex differences in the brain.

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Epilogue

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