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A Look at the Causes of Gender Identity with the Help of Four Core Genotype Mice

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A Look at the Causes of Gender Identity with the help of Four Core Genotype Mice

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CONTENTS

Abstract

The purpose of this project is to better understand the influences underlying gender differences in the brain using Four Core Genotype mice. Four Core Genotype mice are transgenic mice in which the SRY gene has been translocated from the Y chromosome to another location. This enables separation of the genetic sex and gonadal sex. For example, there are female mice based on sexual organs but their chromosomes are XY(UY- in mice). This allows us to determine whether sexual differentiation in the brain is due to genes or hormones. In this project, I looked at a sexually dimorphic area of the brain, the Bed Nucleus of the Stria Terminalis (BNST), which is twice as large in males than in females. I hypothesize that both chromosomes and gonadal hormones play a part in sexually differentiating the brain including the BNST and thus I predict that the size of the BNST will be the same in XX males (UUSRY) and XY females (UY-). I measured the BNST from five XX female (UU) and five XY male(UY-SRY) four core genotype mouse brains and confirmed that the BNST is larger in males than in females, as it is in normal mice (p= .057). I processed and measured the size of the BNST in ten brains of XX males and XY females to see if the size of the BNST matches the chromosomes or the gonads. The results had a trend in the data that suggested chromosomes play more of an effect on sexual differentiation of the BNST. The overall goal of this project is to contribute to research examining the causes of gender identity in humans by relating this work to other works in the field.

Introduction

Gender is a term that seems simple at first. When you are born, you are assigned male or female. As you grow, society treats you as someone of that gender by fitting you into certain categories or stereotypes. For example, girls should wear pink as children, and boys should wear blue. However, what a lot of our society fails to see is that biological sex is assigned at birth, and gender is not. Biological sex is a "term used to refer both to the two groups distinguished as males and females, and to the anatomical and physiological characteristics associated with maleness and femaleness" (The Free Dictionary). The more obvious differences between the sexes are the sexual organs and the effects of testosterone on appearance. For example, males tend to develop muscle mass easier than females, and have more facial hair. Gender is defined, however, "as the result of socially constructed ideas about the behavior, actions, and roles a particular sex performs" (World health organization)[1]. Gender identity, therefore, is how someone chooses to label or not label themselves based on the gender binary. This label, or identity, can match the person's biological sex but does not have to. Society assumes that our gender identity, however, matches our biological sex and that people should be treated as such. What happens when someone's gender does not match up to their biological sex? This leads to people identifying as transgender, or other terms similar such as genderqueer and transsexual. The term transgender was first used by Virginia Prince and is defined as "the state of one's gender identity (self-identification as woman, man, neither or both) not matching one's assigned sex" (GLAAD). Transsexual refers to someone that is transgender and has undergone sex reassignment surgeries or hormone therapies. The term genderqueer has multiple meanings. It

[1] World Health Organization: "Who | what do we mean by "sex" and "gender"?" *world health organization*. 2011. web. 19 nov. 2011. <http://www.who.int/gender/whatisgender/en/index.html>.

can refer to someone who identifies as a third gender, a gender that is somewhere in the middle of male and female, someone who is trying to break gender norms, and anyone else that doesn't identify as male or female. With people not identifying as male or female, and those who identify as transgender, the structures of our society such as this gender binary become a little clearer. From childhood, people are put into either of two categories, male or female, with no in between or variation. The sex a person is born into is the gender they should accept as their own and it should match perfectly. However, it is difficult to conclude that every person fits perfectly into the picture of what should be "male" or "female" because of emerging knowledge of transgender people. Because of this binary, anyone who feels that they don't completely belong to the gender assigned to them at birth, become outliers. These outliers, or transgender, genderqueer and transsexual people, have to find ways to live in society when their gender does not match what society sees them as. People in the transgender community therefore face numerous difficulties that cis-gender, or people who identify with their biological sex, do not have to face. This includes discrimination not only in the work place but in health care and social violence (Lombardi et al, 2001). "Federal hate crimes' legislation does not document attacks based upon one's gender identity or presentation, and only a few cities currently have employment protection and/or hate crime legislation protecting people from discrimination and violence resulting from their gender nonconformity" (Lombardi et al, 2001). Violence against transgender people is also an unnoticed and unspoken problem: "The National Coalition of Anti-Violence Programs (NCAVP) began to collect data concerning attacks upon trans-people in 1995. While NCAVP documented only 69 such attacks in 1995 (2% of their entire sample), they believe that violence against trans-people is pervasive and grossly underreported (NCAVP, 1995). Although the NCAVP could not draw any definite conclusions because of the small sample size however, they

did find that while trans-people made up only 2% of their entire sample, trans-people made up 16% of all murder victims. For the most part, these incidents either go unreported or are misreported as anti-gay/lesbian incidents" (Lombardi et al, 2001). In general, transgender people are more likely to experience some form of discrimination in their life due to their gender identity than those who are cis-gender. "A study of transgender individuals within the United States showed that approximately 60% had experienced some form of harassment or violence and that 37% had experienced some form of economic discrimination" (Lombardi et al, 2001). It is common for health care professionals to not respect transgendered people's identities and tends to use the wrong pronouns on purpose (Lombardi, 2001). Often, transgender people will have two health care providers. One that is based entirely on their transgender identity and transitioning if they choose to do so, while the other is based on average health visits. Most health insurance companies will not pay for anything associated with transitioning (Lombardi, 2001). The only medical group that has become large enough to help "treat" transgender people is the International Gender Dysphoria Association (Lombardi, 2001). This group has given rise to people who consider themselves experts in the medical field dealing with transgender individuals and advertise themselves as the place for transgender individuals to use for transitioning. However, if a transgender person is not near one of these experts they may turn to other unsafe forms of transitioning such as hormones bought off the street or traveling to a different country to illegally change their sex (Lombardi, 2001). A large problem within health care is that most providers do not understand what it means to be transgender. Discrimination occurs because of the lack of research and knowledge on the subject. What social knowledge there is available is based on specific transgender people's accounts of how they feel and what they want changed about their body. For example, transgender people are said to have gender

dysphoria which is the innate feeling that your body does not match up to your gender identity (Israel et al, 2001). Gender Dysphoria causes a disconnect between how the mind perceives the body should look and the physical appearance of the body. Gender dysphoria is in the Diagnostic and Statistical Manual of Mental Disorders (DSM) and can be treated with hormone therapy and sex reassignment surgeries. According to the DSM 4th edition[2], the criteria for Gender Identity Disorder or gender dysphoria are as follows:

- "Long-standing and strong identification with another gender
- Long-standing disquiet about the sex assigned or a sense of incongruity in the gender-assigned role of that sex
- The diagnosis is *not* made if the individual also has physical intersex characteristics.
- Significant clinical discomfort or impairment at work, social situations, or other important life areas."

The Diagnosis for "Transsexual" people in the DSM are the following:

- "The desire to live and be accepted as a member of the opposite sex, usually accompanied by the wish to make his or her body as congruent as possible with the preferred sex through surgery and hormone treatment
- The transsexual identity has been present persistently for at least two years
- The disorder is not a symptom of another mental disorder or a chromosomal abnormality"

[2] Definition of transgender: Gay and Lesbian Alliance Against Defamation. ''GLAAD Media Reference Guide, 7th Edition", ''GLAAD'', USA, May 2010.

Because of this medicalization, most of the documented transgender people are diagnosed with either of these. But in reality, the majority of transgender people are those that don't try to alter their body in any way are left uncounted and unheard (Rosser et al, 2007). This does not mean that these transgender people don't have some form of gender dysphoria, but it does point to whether gender identity disorder should be medicalized or not. If there are many transgender people that don't seek transition or cannot afford it, perhaps considering these people to have a mental disorder that needs treatment is incorrect.

What exactly causes an individual to "feel" that they belong to a gender different than their biological sex? The goal of this thesis is to bring together other research, as well as my own, on the neurobiological processes of gender identity. To do so, I gathered research on different topics in the field and based on these, I was able to perform my own research on sexual differentiation of the Four Core Genotype Mouse brain.

There are a surprising number of studies that try to understand the neural basis of gender identity. Many of these studies have been done on animals. However, animals cannot tell us how they "feel" in regards to their bodies and therefore cannot identify with a category of male or female. What can be studied in animals are gender behaviors that are usually specific to a certain sex. For example, there was a study done on Rhesus Monkeys in which certain "transgender" behaviors were observed based on testosterone given to the female either early or late in gestation (Wallen et al., 2009). The monkeys born after receiving testosterone earlier in gestation had ambiguous sexual organs or what humans would consider a form of intersex. The monkeys had sexual organs that appeared to be either male or female and therefore, their sex could not be determined. The monkeys given testosterone later in gestation, however, had gender behaviors similar to those of the opposite sex. For example, the toys that the monkeys chose to play with

matched what the opposite sex's preferences were. That is, the monkeys with female sexual organs had male like behaviors although they were not as prominent as normal biological male monkeys. Both behaviors were present in female monkeys with the male like behaviors being dominant. This is similar in transgender people in that socially, a person is raised to fit into and think as their biological sex, but their gender identity is that of the opposite sex. So both the feminine and the masculine behaviors can occur with transgender people just as it showed with the Rhesus Monkeys. There are other studies that look at prenatal hormones and their effect on gender identity (Swabb, 2010, Byne, 2006, Colapinto, 2001, Auyeung et al, 2009, Hines et al, 2002, Bakker et al, 2003). Along with those there are studies that also look at postnatal hormones, genetics, birth order and effects from the mother, sex specific brain regions, intersex people, and any combination of the above.

Prenatal Hormones:

Prenatal hormones are thought to have a very large effect on development, specifically on sexual differentiation of the brain. According to Swabb (2004-2010), "Once the differentiation of these sexual organs is settled, sexual differentiation of the brain happens, mainly under the organizing effects on the brain of sex hormones that are permanent." (Swabb, 2010). More specifically, "human testes begin to secrete androgens by the seventh or eighth week of gestation, a process that is initially regulated by human chorionic gonadotropin secreted by the placenta. By the fifteenth week of gestation, the regulation of androgen secretion is taken over by gonadotropin from the fetal pituitary, which is in turn regulated by the fetal hypothalamus" (Byne, 2006). Towards the end of gestation, Gonadotropin secretion decreases. This causes the fetal androgen in males to elevate between the weeks of 8-24 during gestation, with peak levels

at 14-16 weeks (Byne, 2006). Males have increased levels of testosterone at birth until the third month when it decreases. In females, the ovaries secrete large levels of estradiol during the first 6-12 months after birth (Byne, 2006). “A sharp reduction of gonadal activity then occurs in both sexes until 10 or 12 years of age, when sex characteristic adult hormonal profiles occur” (Byne, 2006).

In boys, there are two critical periods of high levels of testosterone; one being at mid-pregnancy and the other being within the first three months after birth. “These fetal and neonatal risings of testosterone, together with the functional steroid receptor activity, are thought to fix, to a major degree, the development of structures and circuits in the brain for the rest of a boy’s life, and are called “programming” or “organizing” effects. The “activating” effects of rising hormone levels during puberty stimulates circuits and behavioral patterns that have been set up during development, in a masculinized and defeminized direction for male brains, or in a feminized and demasculinized direction for female brains” (Swaab 2004). Because this sexual differentiation of the brain takes place later in pregnancy than the formation of the sexual organs, a person’s gender identity or gender behaviors does not have to match up to their biological sex. An extreme, but very real, case of how this holds true is the story of David Reimer who at eight months of age lost his penis due to a surgical mistake. His testicles were also removed at the age of seventeen months. He was raised as a girl and given estrogen in puberty. However, despite what leaders in gender research at the time stated about the boy living happily as a woman, he started living as a man when he was fourteen years old. He lived an unhappy life and committed suicide in 2004 (Colapinto, 2001). In opposition to this study, another male bodied person whose penis was lost at 7 months of age, was raised as a girl and continues to identify as female into adulthood. Because this person does not identify as male, and the gender identity appears to be

due to complete social factors, the study suggested that there are social factors that can overcome biological factors of gender identity (Bradley et al, 1998). However, the study does not discuss the possibility that this person's gender identity could have been female regardless of biological sex as seen in transgender people.

A different type of longitudinal study was done to see how much effect prenatal testosterone had on gender behavior in children (Auyeung et al, 2009). While gender behavior does not directly equate to gender identity, the study was important in showing the effects of prenatal hormones. Known from previous studies, testosterone in the amniotic fluid is usually higher in males than in females. The goal of this study, however, was to have a "direct measurement of fetal testosterone and of childhood sex-typed behavior in a large sample of girls and boys, using a sensitive, reliable, standardized measure, (which) could clarify the role of testosterone in human sexual differentiation" (Auyeung et al, 2009). Four hundred and fifty two mothers had amniotic fluid samples taken between weeks eleven and twenty-one of gestation which is thought to be the time of sexual differentiation in the brain. A Pre-school Activities Inventory was given to the mothers when the children were at a mean age of 8.59 years that has been valid and reliable in measuring gender behavior in children, with a higher score being that of male behavior. The study found a significant relationship between fetal testosterone and sexually differentiated play behavior in both boys and girls (Auyeung et al, 2009). "Because children in the current study were developing typically, and because measures of testosterone were taken directly from the fetal environment, our results strengthen the evidence that testosterone plays a role in sexual differentiation of human behavior" (Auyeung et al, 2009). This data was the first documentation that prenatal androgen exposure relates to sexually differentiated play behavior in boys and girls. "In addition, the current results support an

organizational, as opposed to current, activational role of testosterone, because play behavior is measured in childhood, when concurrent testosterone levels are low" (Auyeung et al, 2009). This study allows us to see how prenatal testosterone levels at the time of sexual differentiation in the brain affect gender behavior later on. Another study done showed the linear correlation between prenatal testosterone and gender behavior in pre-school girls, but did not find a significant correlation with boys (Hines et al, 2002). This included no significant effects of older brothers and sisters in the home, parental adherence to traditional sex roles, the presence of a male partner in the home, and the mother's education. Although these studies did not directly examine gender identity, they do show that prenatal hormones have effects on gender behavior after birth.

It has been a common thought among many researching in this field that the male brain forms with testicular secretions, and the female brain forms without these secretions (Bakker et al, 2003). However, a study was done where estrogen was seen as a possible necessity for developing the female brain (Bakker et al, 2003). Prenatal estrogen showed effects on exhibiting female sexual behaviors in the adulthood of guinea pigs. However, this study didn't have unequivocal evidence for the necessity of estrogen in forming the female brain. This is because the feminization of the brain seen in the study could not be specifically linked to estrogen but potentially a drug called Tamoxifen that acted on the neural estrogen receptors (Bakker et al, 2003). Aromatase knockout mice were used in this new study, and unlike estrogen receptor knockout mice, these mice have functioning estrogen receptors. ArKO mice can be administered estradiol as adults allowing the consequences of the absence of the estradiol biosynthesis and cellular action earlier in life to be accessed. Because of this, the ArKO mouse is an ideal model in which to understand the necessity of estradiol for the development of the female brain (Bakker et al, 2003). When estradiol was administered to adult female ArKO mice that had been

ovariectomized, female sexual behaviors were greatly impaired. These data suggest that the behaviors were due to prenatal deprivation of estradiol in formation of the brain. In summary of the study, Bakker et al found that because estrogen later in life did not fix the impairment of female sexual behavior, the lack of prenatal estrogen was the root cause for the impairment. This brings the conclusion that estrogen is necessary in forming pathways in the brain that allow for female sexual behavior. While sexual behavior is not gender identity, it is one of the few gender specific behaviors in mice that can be tested for.

Birth Order and Effects from the Mother:

In addition to hormonal contributions to sexual differentiation of the brain, birth order has also shown to play a role in the neural basis of gender identity. There have been many studies done since the 1930's on effect of birth order and amount of siblings, male and female, on sexual orientation (Green, 2000). A study looking at effects on transgender identity found very similar results to those on sexual orientation (Green, 2000). These results show that the more older brothers a male bodied person has, the more likely the person will be attracted to males and/or be transgender. The authors hypothesize that there is a maternal immune response during pregnancy to a group of transplantation antigens called H-Y antigens. These are found in the developing male on the Y chromosome. "Analogy is drawn to Rh blood factor incompatibility where an Rh+ mother, with an Rh- fetus, develops antibodies to the Rh factor. The child of the pregnancy does not suffer but subsequent pregnancies have a blood dyscrasia consequent to maternal anti-bodies attacking fetal blood protein" (Green, 2000). Another hypothesis for effects of birth order on 'feminization' of male bodied people is the testosterone levels in the umbilical cord. After each birth of a male bodied child, the amount of testosterone in the umbilical cord decreases (Green,

2000). To know whether this is significant or not, a study would have to be done on whether the testosterone levels in the umbilical cord matched the levels in gestation, and more specifically at the time of sexual differentiation of the brain.

Hormones during gestation can also be affected by drugs such as DES. Between the 1940's and 70's the drug diethylstilbestrol (DES) was administered to pregnant women in order to prevent miscarriages. The drug is "a potent estrogenic chemical…first developed in 1938 and initially became available in the U.S. for treating a range of gynecologic conditions in 1941" (Kerlin, 2004). A study examined the effects this drug had on male bodied people born to mothers who were administered DES. A website forum was constructed to keep track of these male born people and allow them to discuss their identities and lives. Over the five years that the website existed, at least a quarter to a third of the population identified with some kind of gender dysphoria or transgender identity. Out of the one hundred members on the forum, sixty-three stated how they identified. The largest group, at twenty-three people, said they identified as transsexual. The second largest group at eleven people said they were straight. The third largest group at ten people stated that they were transgendered. And the remaining two groups that had the same amount of people at eight identified as intersex (androgynous) and gay or bisexual. The study suggests that gender dysphoria and transgender people may be the most beneficial way to examine the effects of endocrine disruptors on human health (Kerlin, 2004). Endocrine disruptors have been seen to cause multiple problems including cancer when exposure is at critical periods of development. "an endocrine disruptor is defined as an exogenous agent that interferes with the synthesis, storage/release, transport, metabolism, binding, action or elimination of natural blood-borne hormones responsible for the regulation of homeostasis and the regulation of developmental processes" (Kavlock et al, 1997). Overall, these endocrine

disrupters interfere with the normal functioning of estrogen and its receptors. There are many well documented studies that show the effects of endocrine disruptors on sexual differentiation (Kerlin, 2004). These studies include animals other than humans such as turtles, rats, alligators and fish.

Postnatal Hormones:

As evidenced by the prenatal hormone section, sexual differentiation of the brain seems to occur in gestation. The effects of postnatal hormones are that of "feminizing" and "masculinizing" transgender bodies. However, a study on the effects of hormone treatment on the adult brain showed significant changes in the size of structures in the brain. This study suggests that transgender people have the brain structures of their biological sex, and changes after hormone therapy (Pol et al, 2006). For example, the third and lateral ventricles either decreased or increased in size based on hormone treatment. This does not explain how then transgender identity occurs in the brain if not by sexual differentiation prenatally. (An explanation could be that transgender people have an 'on switch' of sorts that causes their brains to be altered by sex hormones in ways that those who are not transgender. Perhaps this possible 'on switch' is part of genetics or prenatal hormones.) In order to understand how postnatal hormones can have an effect on the brain, the specific drugs that transgender people take should be looked at. The most common hormones for changing the appearance of male to female transgender people are the gonadotropin-releasing hormone agonist (GnRHa) and oral oestradiol-17beta valerate (Dittrich et al, 2005). With this 'treatment' a study found that, "there was a significant decline in gonadotropins, total testosterone and calculated free testosterone… an equal increase in breast size was achieved compared to common hormone therapy…

significantly increased estradiol and SHBG serum levels were detectable…We conclude that cross-sex hormone treatment of male-to-female transsexuals using GnRHa and oestradiol-17beta valerate is effective, and side effects and complication rates can be reduced using the treatment regimen presented here" (Dittrich et al, 2005). The treatment for female to male transgender people can be administered through intramuscularly or transdermally. The transdermal administration has the advantage of avoiding peak ups and downs in testosterone levels, thus delivering a constant dose of hormone (Protocols, 2006). This form can be an effective alternative in patients who are more sensitive to variable testosterone levels.

A study that examined the effects of these postnatal hormone treatments on cognition tested for verbal fluency and mental rotation (Sommer et al, 2007). They found that "Language activation increased after sex steroid treatment in both groups and total language activity was correlated to post-treatment estradiol levels. Lateralization was not affected by the reversal of sex steroids (and) activation during mental rotation did not increase during treatment but post-treatment testosterone levels correlated to total activation during mental rotation" (Sommer et al, 2007). This is interesting because there have been studies that previously show that males have better skills in certain areas and that females have better skills in others. For example, a study showed that there is a sex difference in mental rotation seen early in human infancy (Moore et al, 2008). These studies have also debated on whether this is due to sexual differentiation or the environment in which someone grows up in or that it's both. Sommer et al. (2007) shows the effect of hormones on these skills and their ability to change when the brain is already sexually differentiated and gender identity remains constant. This suggests that gender identity is separate from stereotypical skills associated with being male or female.

Another study directly looked at the effects of postnatal hormones in rats. Postnatal hormones can affect connections, size, and function of the substantia nigra more than prenatal hormones (Veliskova and Moshe, 2001). Male rats just after birth that had their gonads removed neonataly showed a more female like version of the SN. Female rats given testosterone just after birth showed a more male like version of the SN. This experiment shows signs that postnatal hormones have an effect on sexual differentiation of the brain as well as show that lack of testosterone prenatally has an effect of differentiating the brain as seen in the male rats.

Sexual differentiation in specific brain regions:

The sexual differentiation of the brain can be seen in multiple regions. There are differences between sexes in the Corpus Callosum, a large fiber tract that connects the two cerebral hemispheres. In a study that attempts to allow a biological approach to diagnosing gender identity disorder, the Corpus Callosum was measured in 211 males, 211 females, 22 Male to Female and 28 Female to Male transgender individuals (it is unclear as to whether these transgender individuals underwent hormone therapy but they were diagnosed with gender identity disorder). The area had significant differences in shape and size between males and females in that, for example, parts of this tract are more bulbous in females than in males, and some parts are thinner in males than in females (Yokota, et al. 2005). Using this data, the same measurements were taken for FTM's and MTF transgender individuals. The results showed that the area matched more to their gender identity and not that of their assigned sex at birth. This is because the areas measured were similar to those of their gender identity in shape and size and not of their biological sex. This study, along with others on areas of the brain that are sexually differentiated, can help lead to diagnosing gender identity disorder.

The Bed Nucleus of the Stria Terminalis (BNST) is an essential brain region for sexual behavior and is now considered an important area in gender research. “The BNST plays an essential role in masculine sexual behavior and in the regulation of gonadotrophin release in rats. There has been no direct evidence that the BNST has such a role in human sexual behavior but our demonstration of a sexually dimorphic pattern in the size of the human BNSTc, which is in agreement with the previously described sex difference in a more caudal part of the BNST (BNST-dspm), indicates that this nucleus may also be involved in human sexual or reproductive functions” (Zhou, 1995). In humans the BNST is 44% larger in heterosexual males than in heterosexual females, and 62% larger in homosexual males than in heterosexual females (Zhou, 1995). This area of the brain has been extensively studied in rodent sexual behavior for the following reasons: The BNST is a major aromatization center in the developing rat brain and androgen and estrogen receptors have been found there as well. The rat brain receives projections from the amygdala to the BNST and provides strong input in the preotypic-hypothalamic region. There are also reciprocal connections between the hypothalamus, amygdala and the BNST which are well documented in experimental animals. In addition, Gonadal steroids influence sex differences in the size and cell number of the BNST during development. In humans, a particular caudal part of the BNST (BNST-dspm) is 2.5 times as large in men than in women (Zhou, 1995). A study looking at the BNST in relation to Male to Female transsexuals found a female sized BNST suggesting that the area matched the gender identity of that individual and not their biological sex. In an article previously discussed in the postnatal hormone section, changes in sizes of brain regions were due to cross sex hormone therapies. This study, however, showed that the brain region size was due to developmental factors: “The BNSTc volume of a 46-year-old woman who had suffered for at least 1 year from a tumor of the

adrenal cortex that produced very high blood levels of androstenedione and testosterone, was within the range of that of other women. Furthermore, two postmenopausal women (aged over 70 years) showed a completely normal female-sized BNSTc. As all the transsexuals had been treated with estrogens, the reduced size of the BNSTc could possibly have been due to the presence of high levels of estrogen in the blood. Evidence against this comes from the fact that two of the transsexuals both showed a small, female-like BNSTc, although one stopped taking estrogen about 15 months before death, since her prolactin levels were too high and the other stopped hormone treatment since a sarcoma was found about three months before death; also a 31-year-old man who suffered from a feminizing adrenal tumor which induced high blood levels of estrogen, nevertheless had a very large BNSTc" (Zhou, 1995). Overall, the findings of the study showed that the size of the BNST was not influenced by adult hormone factors, but instead by developmental factors. This is supported through the fact "that neonatal gonadectomy of male rats and androgenization of the female rats indeed induced significant changes in the number of neurons of the BNST and suppressed its sexual dimorphism" (Zhou, 1995). Taken together, these studies suggest that gender identity is caused by sex hormones and potentially also caused by genetics on the brain during development. This continues to be a very interesting area in gender identity research because there failed to be differences seen in other hypothalamic areas such as the PVN, SDN, and SCN. That is, those areas did not have female sized structures as seen in the BNST for the same transsexual subjects. "This might be due to the fact that these nuclei do not all develop at the same time, or to a difference between these nuclei and the BNST with respect to the presence of sex hormone receptors or aromatase" (Zhou, 1995). With this correlation of gender identity to the BNST, more research on sexual differentiation of this area can help lead to understanding the causes of gender identity.

Genetic influence on gender identity:

In terms of development, there are two ways in which genes can affect the differentiation of the brain. The first is the genes act on brain cells and alter brain phenotype and the other is the genes act on other tissues which then sexually differentiate the brain. The latter would most obviously be seen in the gonads. For example, the genes would act on the gonads to form testes which produce testosterone which would then act on the brain. This can form permanent masculinization or differentiation of neural circuits. These hormones seem to have the most profound effect on sexually differentiating the brain. For example, by giving XX females testosterone the brain seems to masculinize and by blocking testosterone in a XY male, the brain is demasculinized. However, in the studies that look at gonadal hormone effects, there are still possibilities of other factors playing a role on sexual differentiation. The sex chromosomes are differentiated and therefore, the X versus the Y chromosome is more adaptive in one sex than the other (Arnold, 2004). There are selective pressures on each of these chromosomes, and because of this, certain mutations form. For example, the Sry gene is found only on the Y chromosome and it differentiates the gonads to testes. Without this gene on the Y chromosome, the gonads will differentiate to ovaries. Testes produce high levels of testosterone which is thought to be the cause of permanent masculinization of the brain during development. "Male mice that differ only in the strain origin of their Y chromosome have different levels of testosterone in their blood…suggesting that Y alleles (*Sry* or others) also modulate testosterone levels" (Arnold, 2004). While there are specific Y genes expressed in the male brain, including the Sry gene, many of these effects are reduced by a partner X gene. In fact, the small number of Y genes "reduces the number of possible male-specific effects of Y genes and gene effects on sexual differentiation of the brain may disappear if there was an inactivation of an X chromosome.

Second, the mosaicism of expression of inactivated X-linked genes leads to sex differences in organ function if the X gene is polymorphic. In that case, the female's tissues, including the brain, are typically a mosaic of cells in which different X alleles are expressed, whereas the male's tissues express a single variant at each locus" (Arnold, 2004). Further, the X chromosome has many genes related to function and brain development, so this mosaicism could contribute to sex differences in brain function.

In understanding how chromosomes affect gender identity, studies that focus on the condition of intersex have been increasingly helpful. There are multiple forms of intersex such as Androgen Insensitivity syndrome, Congenital Adrenal Hyperplasia, Klinefelter syndrome, Turner Syndrome and others (intersex website)[3]. An example of these types of studies is one on Androgen Insensitivity Syndrome (AIS) in terms of the "correct" way to raise these children as male or female. These children have the X and Y chromosomes, however they appear to be female to doctors at birth. This syndrome is "an X-linked disorder of genetic male differentiation that is the result of an absent or defective androgen receptor (AR) gene. In this syndrome, the fetal testes function normally, but the utilization of androgens is impaired" (Mazur, 2005). From birth, these children are raised as girls and are most likely not diagnosed with the condition until later. These children do not have internal feminine organs such as a uterus due to hormones like testosterone and estradiol released by the testes. There are two forms of this condition, one being Complete (CAIS) and the other being Partial (PAIS). In the study, cases of people with either complete or partial androgen insensitivity syndrome were asked about their assigned sex at birth, and whether they had any forms of gender dysphoria. Those with CAIS were all raised as female

[3] Mathias, A., Dreger A., Lippert A., Herndon A., et al. "Intersex Conditions | Intersex Society of North America." *Intersex Society of North America | A World Free of Shame, Secrecy, and Unwanted Genital Surgery*. ISNA, 2003. Web. 19 Nov. 2011. <http://www.isna.org/faq/conditions>.

and there were no reports of gender dysphoria in their research. Those with PAIS were raised as males or female due to the condition of "micropenis". There were multiple accounts of gender dysphoria with male to female and female to male. The conclusions of this study were somewhat ambiguous in that there weren't clear answers on how children with PAIS would identify in terms of gender. Many children raised as female continued to live as female without gender dysphoria. However, the author of the study recommends that these children be raised as male for the following three reasons: "(a) no major medical interventions (e.g., feminizing surgery and later creation of a vagina) are necessary; (b) lifelong hormone (testosterone) therapy starting in adolescence to induce a male puberty may not be necessary although this depends on the diagnosis associated with the micropenis (e.g., hypogonadotropic hypogonadism); and (c) preservation of possible fertility" (Mazur, 2005). The suggestions given by Mazur seem to focus more on difficulty level of transitioning. If children with PAIS were raised as female and wanted to transition to male it would be a more difficult process than being raised as male and transitioning to female.

A case study that looked at two "brothers" with Complete Androgen Insensitivity Syndrome showed the first evidence linking the condition to an Androgen Receptor mutation. These "brothers" had the genotypes XY but showed female genetalia and breasts. However, they also had testes and showed normal levels of testosterone. Their gonads were removed and hormone substitution therapy was started during the time of the study when one patient was fifteen and the other was eighteen. Genetic and hormonal tests showed that they had normal hormonal levels of men of their age which means this form of CAIS is a new mutation inherited from the mother on the X-chromosome. A transversion mutation was found in the "brothers" located in the region of receptor functioning of androgens causing the androgen insensitivity.

"The result of the mutation is a truncated nonfunctional protein, which causes CAIS phenotype in the 2 studied patients" (Radpour, et al. 2008). The study did not discuss how the "brothers" identified in terms of gender, but it did mention that they underwent gonadectomy and estradiol hormone therapy which suggests that they chose to live their lives as female. While making this assumption and not relating the gonadectomy to the fact that they had female genetalia, there seems to be a link between mutations of the Androgen receptors and gender identity.

In terms of the possible effects chromosomes have on sexually differentiating the brain there is evidence that genes have a specific effect on brain cells instead of just gonadal tissue. "To isolate the direct actions of sex chromosome genes on the brain from the indirect actions that are mediated by gonadal secretions, the strategy is either to measure neural phenotype before gonadal hormones become effective, or to make gonadal secretions identical in animals that differ in their complement of sex chromosomes" (Arnold, 2004). There are multiple studies that show genes affecting other tissues before the gonads including a detection of gene expression in sexual differentiation of the mouse brain.

Separating genetic and hormonal sex using Four Core Genotype Mice:

Another way to look at the effects of genetics on sexual differentiation in the brain before gonadal hormones is through the Four Core Genotype mouse model. This model of mice takes the Sry gene off of the Y chromosome causing the mouse to form ovaries. The Sry gene can be placed back onto the chromosomes of that XY mouse causing testes to form but the gene is no longer Y chromosome linked. If this male is mated with an XX female, there are four new genotypes created. These include two males by gonads that are XY^{-}Sry and XXSry, and two females that are XY^{-} and XX (Fig. 1).

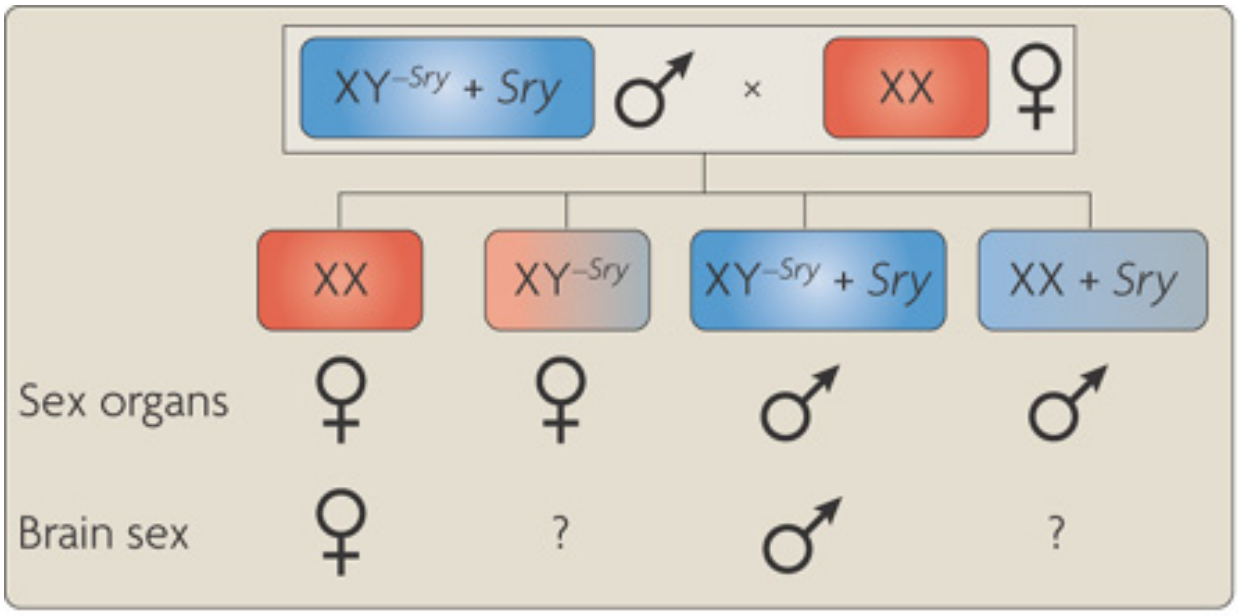

Figure 1: The Four Core Genotype mouse model (Cahill et al, 2010).

"This makes it possible to assess the effects of the sex chromosomes in mice with either ovarian or testicular hormones" (Arnold, 2004). In order to assess these mice without the sexually dimorphic affects of gonadal secretions in adults, the four genotypes can be gonadectomized and be treated equally with testosterone. In terms of behavior, this model of mice show more male specific behavior (copulatory behavior, social exploration) when they have testes instead of ovaries despite their sex chromosomes. This then suggests that gonadal secretions are inducing masculinization of the brain. In contradiction to this however, in a brain region of the male mice, there was a difference in the numbers of vasopressin-containing fibers and a similar difference was found in the females. This difference was found due to the chromosome compliment in the animals. "The results do not exclude the possibility that the sex chromosome effect is mediated by differences in gonadal secretions between XX and XY animals of either sex. However, such a

gonadal explanation seems unlikely because higher androgen levels, for example, in XY animals would have caused a group difference in several of the hormone-sensitive sexual dimorphisms measured" (Arnold, 2004). XX and XY brain cells in vitro are similar in that XY cells form more dopamine receptors than XX cells. This is not explained through gonadal secretions because when the XY^- female cells had more dopamine receptors than the XXSry males, it showed that the masculinizing effect on dopamine neurons was due to the Y chromosome, not the testes from the Sry gene. A case of an animal that also looks to determine the effects of chromosomes on the brain is the gyandromorphic finch. The animal had male plumage and testes on the right side of its body and female plumage and an ovary on the left side suggesting that the gonadal development was lateralized. In finches, the males are ZZ and the females are ZW. This bird showed W-linked genes on the left side more than the right. The song circuit was more masculinized on the right side, which is a difference that can be attributed to the lateral genetic difference in sex chromosomes. It is not due to levels of gonadal hormones because both sides of the brain would have been influenced equally (Arnold, 2004).

The four core genotype mouse model also allows the ability to see the different effects of chromosomes versus gonadal secretions on sexual differentiation of the brain. In order to look at these individual effects, I measured the Bed Nucleus of the Stria Terminalis which is a sexually dimorphic area. My hypothesis was that both chromosomes and gonadal secretions would play an equal role in sexually differentiating the BNST.

Methods

Twenty Four Core Genotype mouse brains were obtained from William Grisham in the Arnold Laboratory at University of California Los Angeles.

Histology

I was blind to the identity of the brains throughout the experiment. Brains were cryroprotected in 30% sucrose in 4% paraformaldehyde overnight. The brain was sliced coronally from anterior to posterior in 40 μm sections using a freezing microtome (Micron). Sections were mounted onto slides subbed in gelatin and dried overnight. The sections were stained with Cresyl violet and cover slipped. The Bed Nucleus of the Stria Terminalis, located using the Allen brain atlas, was photographed (model: Nikon E400 Eclipse, software: NIS-Elements). The size of the BNST was measured using Image J. The Bed Nucleus area was calculated for both sides of the brain. After all the measurements had been made the genotypes of the brains were revealed.

Statistics

An t-test was performed to see if there were any significant differences between XX females and XY^-Sry males in the Bed Nucleus. This was to ensure that the measurements showed a significant difference between the two as seen in normal mice. This entire process was repeated with ten new brains. Five of them were XXSry and the other five were XY^-. A two way ANOVA was done on all four genotypes as well to find any differences in the Bed Nucleus with respect to the chromosomal sex versus the gonadal sex.

Results

The BNST has been shown to be sexually dimorphic, being larger in males than females. In addition, the size of this nucleus is more 'female-like' in MTF transgender people suggesting that the BNST is a marker for gender identity. To begin to understand the contribution of gonadal sex and chromosomal sex on the size of the BNST, I measured the Bed Nuclei in all four genotypes (Table 1).

Table 1. The Four Core Genotypes measurement data.

	XX Female (in mm^2)	XY^-Sry Male (in mm^2)	XY^- Female (in mm^2)	XXSry Male (in mm^2)
	2037.7	2530.5	1999.7	2412.3
	1726	2597.3	2507	1594.8
	2410.5	2333.2	2136.9	1947
	2694.3	2734.5	2179.9	1463.9
	1471.8	2225	1612.9	1717.8
AVG	2068	2484	2087.2	1827.1

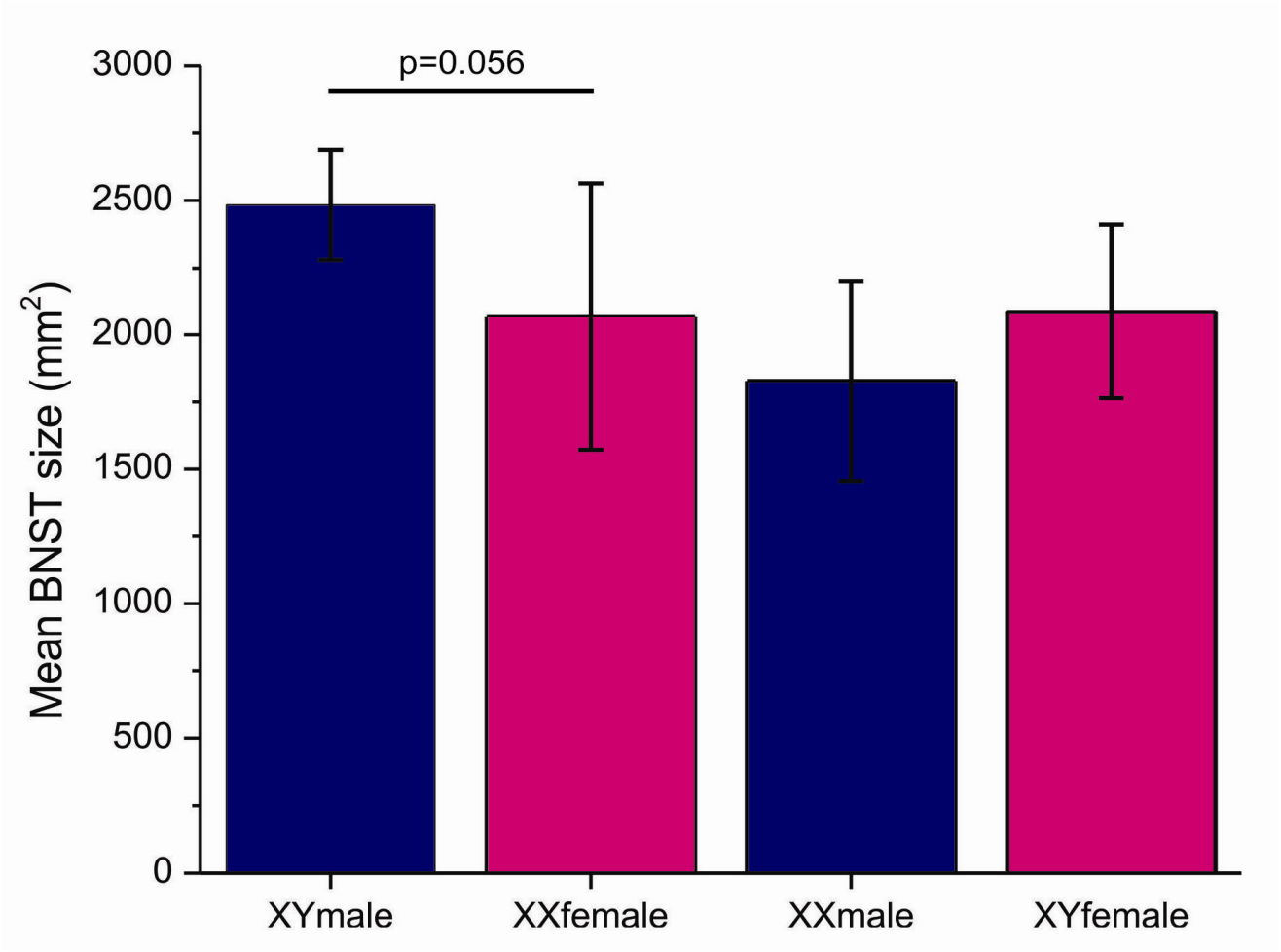

Figure 2. Area of BNST in the Four Core Genotype. Data are mean +/- SD.

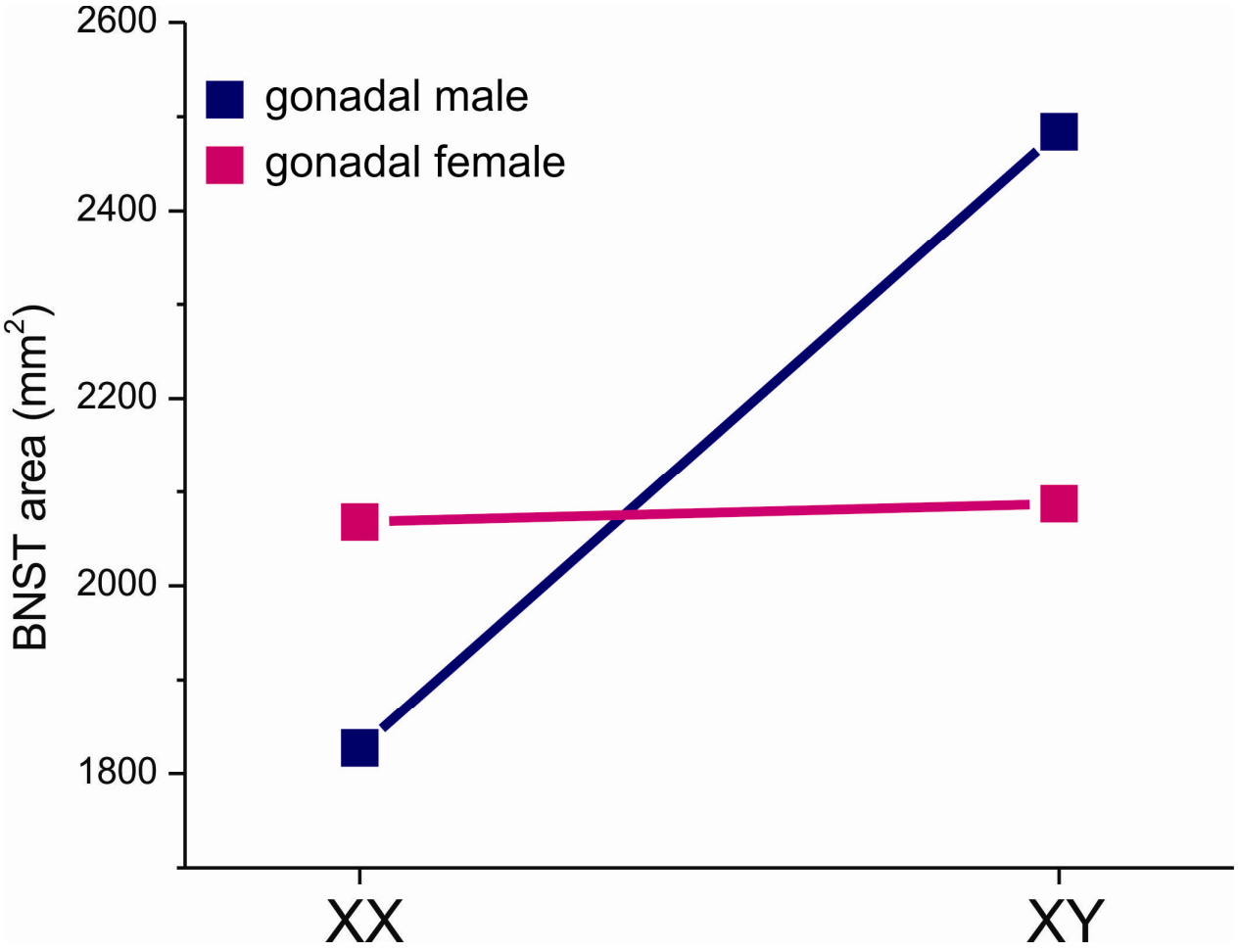

Figure 3. There is an interaction effect of chromosomes versus gonadal sex.

A two way ANOVA was conducted on all four of the data as well as a T-test on the XY male and XX female. There was a significant difference between the area of the BNST in XY male and the XX female, as seen in previous data (t-test, P=0.056, Fig 2) (Forger, 2009). A trend in the data also suggests that there is a difference in the effect of chromosomes versus gonadal sex on sexual differentiation of the BNST, with chromosomes having more of an effect seen in the interaction effect (P=0.068, Figure 3). Another trend in the data suggests that mice need male gonads and a Y chromosome to have a fully masculinized brain as seen by the difference between XX male and XY male (P=0.07). The interaction effect of chromosomes highlights that chromosomes are causing a significant difference where gonadal sex is not (Fig 3). The significant difference in the BNST size of the XX chromosomes and the XY chromosomes is P=0.054. These data suggests that chromosomes are playing a larger part in sexually differentiating the BNST than gonadal sex.

Discussion

The purpose of the project was to look at the different effects of chromosomes and hormones on sexual differentiation in the brain. My results showed a few significant findings as well as trends in the data that suggest chromosomes have more of an effect on sexual differentiation of the Bed Nucleus of the Stria Terminalis than gonadal sex alone. This is shown by the effect chromosomes have on the differences between the genotypes (fig. 3), and by the difference between XY male and XX male. While these trends are not significant, they are approaching significance with a small sample size of five brains for each genotype. Increasing the number of samples may reveal statistical significance. Along with chromosomes seeming to play a larger part in sexual differentiation, it seems that the Y chromosome and male gonads are necessary to have a statistically fully masculinized brain (fig.1). Because of these findings, my hypothesis was not correct in that both chromosomes and gonadal sex would play an equal role in sexual differentiation. It seems to be that chromosomes and gonadal sex both do play a role, but chromosomes have more of an effect.

Relating results to previous research

In my study, the Bed Nucleus was measured in order to look at the sexual differentiation. Zhou (1995) discussed the Bed Nucleus as a marker for gender identity when the area was found to be the size matching the perceived gender and not the biological sex assigned at birth. This was found to be true due to developmental effects and not to postnatal "treatment" hormones based on other studies of postnatal hormones having no effect on the size of the Bed Nucleus. Even though this study had a small sample size of six humans, it was consistent in its findings

and compelling in its evidence for the Bed Nucleus to be a marker of gender identity. Sexual differentiation of that area could relate to the differentiation of gender identity in the brain. If this is so, chromosomes may have more of an effect on gender identity than gonadal sex.

As discussed in the introduction, not everyone fits into male or female in terms of how they identify with gender. Some are in the middle, or identify as genderqueer. Further, an argument can be made that even those who fully identify with male or female, may still have both masculine and feminine traits due to potential social pressures. My results may also give light to this discussion in terms of the mice needing both the Y chromosome and male gonads to have a fully masculinized brain. For example, if someone was assigned female at birth and is genetically female but identifies as male or gender queer, their brain may still have feminine traits not just due to social pressures but due to the lack of a Y chromosome or lack of male gonads. Therefore, the Bed Nucleus, for example, may resemble that of an XY male, but it may not be as fully masculinized. This was seen also in the Zhou et al. study in that the male to female transsexuals had a Bed Nucleus size that was 52% the size of the average male size, which statistically would still be bigger than the average female sized Bed Nucleus. Therefore both Zhou et al. study and my data suggest that perceived gender identity will show a matching Bed Nucleus size, but statistically it will not be fully masculinized or feminized without the matching chromosomes and gonadal sex.

Arnold's paper in 2004 also saw this extra effect of chromosomes on sexual differentiation instead of gonadal sex. While gonadal sex seems to have an effect as seen by the numerous studies done on prenatal hormones, chromosomes can have a larger effect specifically on the brain as seen in the gonadomorphic finch. That finch, whose body was half female and half male, had lateralized sexual differentiation of the brain due to chromosomes, not gonadal

hormones. If the brain had been differentiated due to gonadal hormones, both of the hormones secreted by the gonads would equally affect the brain, and not just specifically the side of the brain corresponding to the gonads.

Limitations

The first limitation of my study is that it's difficult to match my findings to gender behaviors. As stated in the introduction, gender behaviors are not the same thing as gender identity, but they are the only way to behaviorally study gender identity without looking at the biological processes in the brain. However, mice do not have very many sex specific behaviors. There is the difference of sexual aggression which, in the Four Core Genotype Mice, show more aggression with male gonads regardless of the chromosomes. While this may suggest that gonadal hormones would then have more of an effect on behavior, there is also plenty of evidence against this and for chromosomes playing more of an effect (Arnold, 2004). This lack of specific gender behaviors is a limitation in that it is difficult to match my data to gender behaviors.

Future studies

A possible future study that would improve my study would be to look at the BNST in intersex people, specifically those with XXY chromosomes and those who are gonadally female with XY chromosomes. Because my data showed effects of chromosomes on sexually differentiating this area, it could be beneficial to see the effects these chromosomes have on the area and relate these findings to their gender identity. This study could allow us to see the effect

of a Y chromosome in a biological female human on the BNST and compare that to their gender identity.

A second future study would be to examine the effects of endocrine disruptors, such as DES, on gender identity. Endocrine disruptors affect developmental factors, therefore, it may be beneficial to see which hormones or genes are altered due to a drug such as DES (Kavlock et al, 1997). In order to examine these effects, a study could be performed on Rhesus monkeys where mothers are exposed to endocrine disruptors during gestation. Both behavior of the offspring and the size of the BNST could be evaluated in determining what effects the drug had on chromosomes and hormones.

A possible third study would be to look at imaging of brain regions in transgender people who have not started postnatal hormone "treatment." By looking at the brain before adding hormones, the regions would be unaffected by any outside influences. This imaging could be done at different points in the person's life, including childhood, puberty, teenage years, and adulthood. Perhaps measuring areas of the brain at these different time periods would give more insight onto developmental factors after birth and how those play an effect on gender identity.

In relating prenatal hormones to my conclusions, there still seems to be evidence that these hormones do have some sort of effect on gender identity. In the introduction, there were studies that showed the effects of testosterone later in gestation having an effect on brain region size (Veliskova and Moshe, 2001) and gender behaviors (Wallen et al., 2009) after birth. I hypothesize that specific levels of testosterone later in gestation acts as an 'on switch' of sorts for gender identity in the chromosomes. That is, perhaps certain genes are turned on during this critical period in gestation due to certain levels of testosterone. A study that could help look at this hypothesis would be to take measurements of fetal environmental testosterone during the

late critical period of gestation. These measurements would then be analyzed with regards to how those children identify later on in terms of gender. While this doesn't show testosterone as an 'on switch,' it does offer a model in which to see the effects of prenatal testosterone on gender identity.

Conclusion

Overall, this study adds to the amount of knowledge we have on the causes of gender identity. This study allows us to realize the complexity of gender identity, and how there seems to be multiple factors involved. For example, chromosomes seem to have more of an effect than gonadal sex, but both seem to play a part. Further, gender identity is not clear black and white, but instead seems to be a spectrum biologically as well as socially in terms of those identifying as genderqueer. What this study does tell us is that there does seem to be a biological aspect to gender identity and therefore, those identifying as transgender are able to relate the innate feeling of gender to something biological. In terms of discrimination and lack of understanding of the transgender community, this can hopefully push our society to realize that we are much more biologically complex than the two categories of 'male' and 'female'.

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