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Will There Be Changes in Sexually Differentiated Behaviors in Mice Manipulated For the SRY Gene As They Mature Into Adultood?

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Will there be changes in sexually differentiated behaviors in mice manipulated for the *SRY* gene as they mature into adulthood?

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Abstract

Sexually differentiated behavior has been shown to be affected by both genes and hormones. The discovery of the SRY gene, which codes for the development of testes, lead to the development of the Four Core Genotypes model of mice, and the separation of sex chromosomes and its resulting gonadal hormones. Using the FCG model, this study aims to look at the development of sexually differentiated behavior in mice, and track how it changes throughout their life. FCG mice will be divided into individual and social housing, and repeated experiments carried out to test their reaction to both intact female and male stimulus mice. The expected outcome is that the initial effect of the SRY gene on will fade with time and repeated experiments, and that it will fade quicker in socially housed mice.

Introduction

Sexually differentiated behavior is not a cut and dry binary divide. (Arnold, 2020). Phoenix et al (1959) was the first to note the organizing effects of hormones - by introducing prenatal guinea pigs to testosterone by injecting their mothers with the hormone, they discovered T-affected females displayed 'male-like' behavior at sexual maturity such as mounting their partners. However, there was only a set period shortly after birth where the rodent's behavior could be changed with hormone injections. Since then, multiple studies including Arnold (2009) proposed an updated model for sexual differentiation: XX and XY cells are different prior to production of gonadal hormones, and gonadal hormones may affect XX and XY cells unequally. There are also genes that activate before and independently of any effect of hormones, and sometimes the effect of genes and hormones cancel each other out (Arnold, 2020). What researchers learned over the years is that chromosomes and hormones both impact sexually differentiated behavior in different ways, necessitating the development of methods to separate the effects of the two.

For years, the main way to test sexual differentiation was to manipulate their hormones. They gave testosterone to XX mice, castrated XY mice, and looked at how their behavior changed. The discovery of SRY gene gave scientists another method to separate the effects of genes and hormones. The SRY (Sex-determining region Y) gene, first discovered on the Y chromosome, was a gene that coded for the development of testes - an SRY gene would masculinize gonads regardless of the existence of a full Y chromosome (Sinclair et al, 1990). The SRY gene could then be knocked out from the Y chromosome and inserted back into an autosome, creating XY* mice. Using this, De Vries et al (2002) developed what would be later called the Four Core Genotype (FCG)(Arnold & Chen, 2009) model by breeding XY* and XX mice together. FCG consists of four different genotypes, XX mice with ovaries and SRY (XX+), XX mice with testes without SRY (XX-), XY mice with testes and SRY (XY+), and XY mice with ovaries without SRY (XY-) (Table 1). These genotypes allowed for the recombination of hormones and genes in mice, and gave researchers another tool to work with.

Table 1. The Four Core Genotypes, explained.

Four core genotypes				
Genotype	**XX+**	**XX(-)**	**XY(+)**	**XY-**
SRY	O	X	O	X
gonads	testes	ovaries	testes	ovaries
in-utero hormone exposure (T)	O	X	O	X

While Jordan-Young & Rumiati (2012) suggested that the effects of early hormone manipulation such as these may fade over time (Hendricks et al, 1982), but there hasn't been any direct research done to test this idea. There has been past research looking at juvenile behavior (Cox & Rissman, 2011) and adult behavior (De Vries et al, 2002) in FCG mice, but there hasn't been one that tracked these mice throughout their growth. There also haven't been much research that focused on non-sexual behavior in adult mice, and those designed to look at social behavior had only compared between one sex of mice. (S. S. Moy et al, 2004; Sheryl S. Moy et al, 2007; Rein et al, 2020).

Using the FCG model of mice, this study aims to look at how **behavior of FCG mice emerge and change throughout its life,** and how genes, hormones, and experience could affect its development. My hypothesis is that **repeated social experience will have an impact on sex differentiated behaviors,** where I expect that the difference between each genotypic group will change over time. There had been studies that some hormonal effects were reversed through repeated experiments, so I expected that the difference caused by the SRY gene will decrease over time. Additionally, past studies found that mice housed in a more enriched environment had better memory and neural plasticity than mice housed in dividually (Wang et al, 2020). Therefore, I expect housing to make an impact on changes in social behavior as well.

Methods

The mice would be procured through the Jackson Laboratory. A total of 32 mice will be raised, with 8 mice in each genotype. They will be marked with tattoos on their forepaws, and ear notches once it becomes difficult to tell ink color (Armstrong et al, 2020).

Following Cox and Rissman (2011), these mice will be left largely unhandled until PN20 (postnatal 20, or 20 days after birth). From PN21, they are weaned and grouped per their genotypes. Each group is then each divided into two additional groups - individual housing, and group housing. 4 mice from each group will be housed individually, with one mice in one cage. The remaining 4 mice will be housed together, allowed to freely interact with each other.

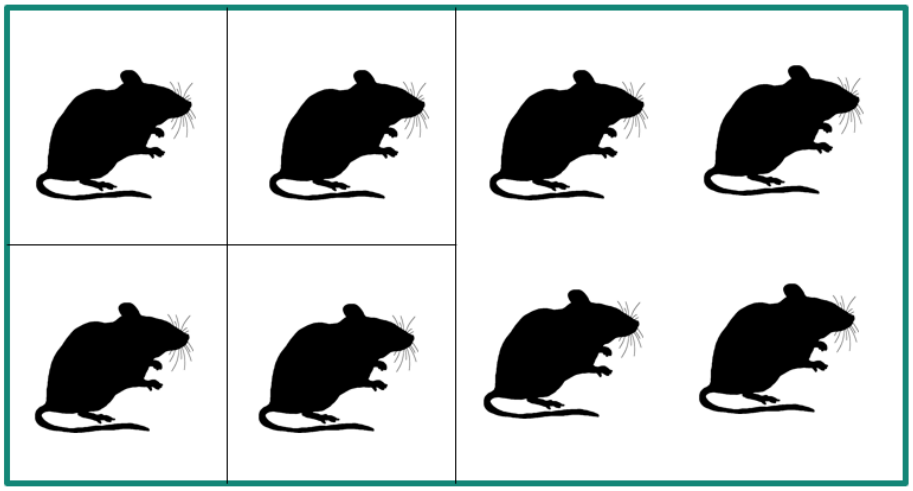

Figure 1. Example housing setup for XX+ mice. All other genotypes will be housed similarly.

Each mouse will be observed twice weekly, once with an intact male and once with an intact female stimulus mouse of the same age. Following Cox & Rissman (2011), the test mouse and the stimulus mouse are first placed in an empty cage with bedding similar to their home cages, without food or water for an hour to let them habituate to the testing room (Figure 2). The stimulus mouse will be marked with sharpie on the base of its tail to tell it apart from the test mouse. After the hour, they are then moved to the test cage and observed for 30 minutes, recording the frequency and duration of their social behavior.

After the experiment, they are moved back to their home cages. Examples of observed juvenile social behavior will include sniffing, sitting side-by-side, and grooming. (Table 2)

Table 2. Behaviors observed and noted in test mice throughout their growth. Both social behavior and sexual behavior will be observed and tracked. List taken and adapted from Cox and Rissman (2011), De Vries et al. (2002)

social behavior	**sexual behavior**
allogrooming side-by-side sitting arogenital sniffing nose sniffing following crawling pushing approaching	lordosis mounting intromission thrusting ejaculation

Once the mice reach adulthood, the stimulus mice will also be sexually competent adult mice. Observed behavior will include sexual mating behavior, which will be categorized as either 'female-like' (lordosis), and 'male-like' (mounting, intromission, ejaculation). Observed behavior will be divided and scored according to Cox & Rissman (2011) and attached to each individual mouse. The change in each individual mouse's data as well as the genotypic group data be analyzed for effects of social housing and core genotypes.

Figure 2. The above diagram shows an example experiment design for XX+ mice. Both experimental and stimulus mice placed in an empty cage without food or water for 1 hour to habituate in test room. They are then moved to the test cage and observed. This is repeated twice every week, once with an unaffected female stimulus mouse, and once with an unaffected male mouse. All other genotypes will be observed similarly.

Expected Results

The data gathered from experiments will be analyzed in two parts - grouped according to genotype, and grouped according to housing. Additionally, each individual mice data will be analyzed to see if there were any outliers in our group, and further studied to see what might have been the causes of this.

Effects of repeated testing

Previous studies have observed that the effect of neonatal injections to XX mice fade over time with repeated experience (Hendricks et al, 1982). Based on this, I expect that the effect of SRY to fade over time as well. The behavior of XX mice with testes as well as XY mice with ovaries will shift to display 'sex-typical' behavior more frequently (Figure 3). XX mice with ovaries and XY mice with testes will also display more 'typical' behavior due to repeated experience strengthening that behavior.

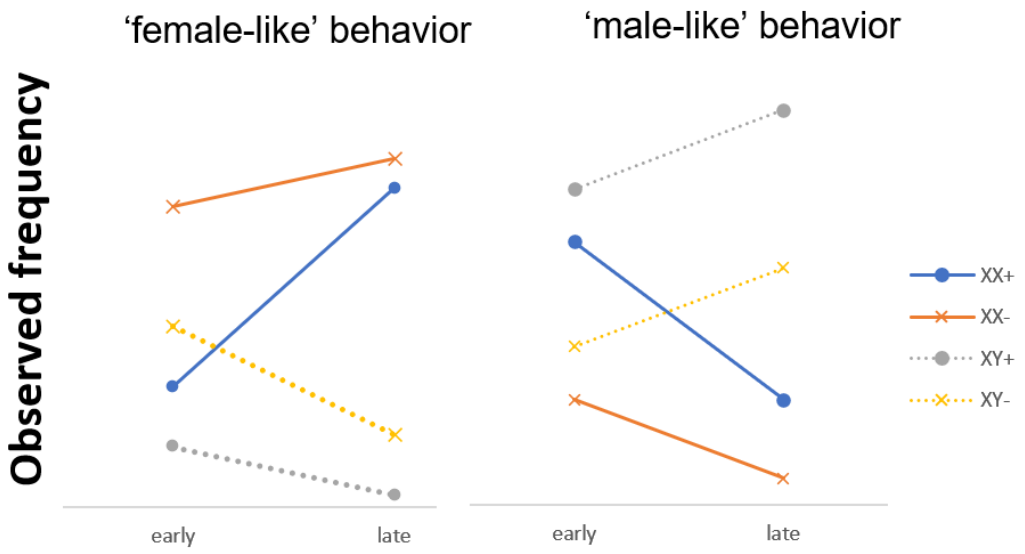

Figure 3. A graph summarizing the expected results. A solid line denotes mice with an XX chromosome, while the dotted line denotes mice with an XY chromosome. A circle means denotes mice with an SRY gene, while an X denotes mice without an SRY gene. I expect the initial effect of the SRY gene will decrease over time and experience. Thus, both graphs show the dots and X's start out closer to each other, while the solid and dotted lines end with more similar results.

Social Housing vs Individual

Mice placed in social housing are expected to show weaker initial effects of SRY, as well as a quicker change in behavior over time than those in individual housing. Past studies that had looked at socially housed vs individual housed mice found that social housing has a positive impact on memory and neural plasticity (Kempermann et al, 1997), (Wang et al, 2020). Based on this, I expect to see social mouse influence each other in how they act, and that a change in one mouse might expedite changes in other mice housed together.

Discussion

This study will track the difference between each possible genotype and housing groups. It focuses on not only the presence of a difference, but how the differences will change over the course of our experiment.

If there is an observed change: this study will function as a roadmap and emphasize the importance of 'experience' as a variable in observing the development of behavior. This result also suggests that previous studies linked to sexually differentiated behavior should be reviewed with a renewed perspective. Differences or changes between phenotypes could be less meaningful or potent than previously suggested, for it might be a result of natural variation in 'innately coded' behavior. Additionally, inclusion of experience as an important factor would make more areas of study available to scholars.

If there is no change: this study will provide grounding work for future studies in development of behavior, and suggest that past studies that discovered a difference in behavior were more significant than previously assumed. This will also strengthen future

results from studies on sexually differentiated mice behavior by acting as a background study. This study will also show their research will still be valid without needing to observe the animals throughout their entire lives.

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