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Mouse models for evaluating sex chromosome effects that cause sex differences in non-gonadal tissues

Arthur P. Arnold
Department of Physiological Science and Laboratory of Neuroendocrinology of the Brain Research Institute University of California, Los Angeles, California 90095, USA

Abstract

XX and XY cells have a different number of X and Y genes. These differences in their genomes cause sex differences in the functions of cells, both in the gonads and in non-gonadal tissues. This review discusses mouse models that have shed light on these direct genetic effects of sex chromosomes that cause sex differences in physiology. Because many sex differences in tissues are caused by different effects of male and female gonadal hormones, it is important to attempt to discriminate between direct genetic and hormonal effects. Numerous mouse models exist in which the number of X or Y genes is manipulated, to observe the effects on phenotype. In two models, the Afour core genotypes@ model and SF1 knockout gonadless mice, it has been possible to detect sex chromosome effects that are not explained by group differences in gonadal hormones. Moreover, mouse models are available to determine whether the sex chromosome effects are caused by X or Y genes.

I. Introduction: Are sex differences in phenotypes caused by non-gonadal effects of sex chromosome complement?

One goal of modern genetics is to determine how variations in the genome result in variations in phenotype. In mammals, the sex chromosomes attract special interest because among the chromosomes, they alone do not come as a matched pair, and therefore the representation of their genes in male and female cells is different. The sexual imbalance of X and Y genes in the genome has numerous interesting consequences. First, selection pressures on these genes are different than on autosomal genes, which has resulted in a concentration of specific types of genes on the two chromosomes (1-3). Second, all sex differences in phenotype must originate ultimately from sex differences in representation of the X and Y genes in the genome, since all other factors (autosomal and cytoplasmic) are thought to be (on average) equivalent in male and female zygotes. It is conceivable that this major conclusion is incomplete (for example if some autosomal alleles occur in different frequencies in populations of males and females), but it is widely accepted and in any case points to sex chromosome gene complement as a primary source of sex-specific signals.

The classic dogma of mammalian sexual differentiation, developed in the 20th Century (4-7), states that the Y-linked gene *Sry* is expressed in the undifferentiated gonad and commits that tissue to a testicular fate. The testes of males then secrete testosterone and Müllerian inhibiting hormone prenatally, two hormones that cause masculine differentiation of non-gonadal tissues, and inhibit feminine differentiation. The male brain is permanently masculinised and defeminised by the organisational effects of testosterone, which is secreted

Correspondence to: Arthur P. Arnold, Department of Physiological Science, UCLA 621 Charles E. Young Drive South, Room 4117, Los Angeles, CA 90095-1606, phone 310-825-2169, fax 310-825-8081, arnold@ucla.edu.

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by the testes perinatally and converted within brain cells to the active masculinising metabolite oestradiol. Later in life, gonadal secretions continue to produce less permanent sex differences via activational effects, which are sex-specific effects of ovarian and testicular secretions producing sex differences that disappear after gonadectomy. This dogma has been tested extensively and confirmed with regard to numerous reproductive phenotypes (differentiation of external genitalia, Müllerian and Wolffian ducts, and reproductive behavior) (8-10). However, we now believe that the dogma is incomplete because it does not include some sex differences caused by sex-specific signals other than gonadal hormones.

In the last 10-15 years, evidence has accumulated that some sex differences in non-gonadal tissues are caused by the differential effects of X and Y genes acting within non-gonadal cells themselves (11-16). Much of the evidence comes from the study of mice because the mouse is the most genetically tractable mammalian model species. Mice are available that differ in the number or type of X and Y chromosomes, sometimes independent of the gonadal sex of the animal, which therefore allow informative comparisons that illuminate the role of X and Y genes. The goal of this review is to compare several different mouse models that have been used for the study of brain or behavioral phenotypes. We focus on the relevance of each model to the following question: Do sex differences in phenotype arise because of differential representation of X and Y genes in non-gonadal cells of males and females?

The question at hand is not simply whether X and Y genes are involved in specific traits, but rather whether sex differences in traits can be attributed to the differential representation of sex chromosome genes within the cells that affect the trait. Thus, a traditional genetic analysis might identify an X locus that is linked to a trait, which means that genetic variation at that locus results in variation in phenotype. However, even when X genes play a role in a molecular network that determines a phenotype, that X gene probably does not cause sex differences. For example, most X genes are expressed at a similar level in males and females, because one X chromosome is inactivated in each XX female cell, so that only one X chromosome is active in cells of both sexes. On the other hand, some X genes escape X-inactivation, and are expressed higher in XX than XY cells (17,18). Such X escapees are candidates for genes that cause sex differences by virtue of their double representation in the genome of XX cells relative to XY cells. Besides dose of X genes, two other factors cause direct genetic sex differences. One is the difference in parental imprint of X genes, because XY male cells have a uniform maternal X imprint whereas XX female cells are mixed, with about 50% of cells in which the active X chromosome has a paternal imprint and 50% with a maternal X imprint. The other factor comprises male-specific effects of Y genes. Thus, mouse models that address the focal question of this review should optimally compare mice that differ along one or more of these three dimensions (one vs. two doses of X genes, XX vs. XY pattern of parental imprint on X genes, and zero vs. one copy of the Y chromosome). As is discussed below, other comparisons are informative but are less optimal for answering the question at hand.

The dominant sex-specific effects of gonadal secretions complicate the effort to determine whether sex chromosome complement causes sex differences via a non-gonadal mechanism. To study the differential effects of XX and XY genomes, a major goal is to compare the effects of these genomes on phenotypes under conditions in which the gonadal hormones do not themselves cause group differences, for example when the hormones are absent or are present at equivalent levels in mice that differ in sex chromosome complement. In practice it is usually difficult to control gonadal hormones perfectly, but in spite of this caveat some experiments convincingly show the direct effects of sex chromosome complement, as is explained further below.

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II. Mouse models that bear strongly on sex differences caused by sex chromosome complement

A. Direct manipulation of individual X or Y genes

The simplest approach to studying direct sex chromosome effects on non-gonadal phenotypes is to study mice in which the expression of an individual X or Y gene is manipulated in a manner that shows an effect relevant to sex differences in phenotype. For example, the role of *Sry* to cause differentiation of testes was proven by studying XY mice with deletions of *Sry* (which are gonadal females) or XX mice that received an *Sry* transgene (which are gonadal males) (6). Recently *Sry* expression has also been manipulated in the brain (19). *Sry* is expressed in the midbrain dopaminergic cells of the substantia nigra, and reduction in *Sry* expression alters the expression of tyrosine hydroxylase, and alters motor function. Thus, since *Sry* is expressed only in the male brain, it has a male-specific effect (19). This is the first identification of an X or Y gene that has direct effects on the brain (or any non-gonadal tissue) to cause sex differences in phenotype via non-hormonal mechanisms. To our knowledge, no individual X gene has been manipulated in mouse models to show that the gene dose or parent of origin leads to a sex difference. However, sex differences in incidence and severity of some phenotypes or diseases in humans has long been known to be caused by X-linkage of specific genes affecting the trait (e.g., Fragile X Syndrome, color blindness, Duchenne muscular dystrophy).

B. Four core genotypes (FCG) mice

One of the most widely applied models are FCG mice, in which sex chromosome complement (XX vs. XY) is independent of gonadal sex. The mice were produced by a two-step genetic manipulation. The *Sry* gene was deleted (a small spontaneous deletion that probably removed only the *Sry* gene, although deletion of other regulatory regions cannot be ruled out completely), and then a functional *Sry* transgene was inserted onto an autosome, driven by its own endogenous promoter (20-23). Thus, testis determination was transferred from the Y chromosome to an autosome; the Y chromosome no longer determines gonadal sex, and the number of X chromosomes is no longer correlated with gonadal sex. The four genotypes are XY gonadal males (XYM), XX gonadal females (XXF), XX gonadal males (XXM), and XY gonadal females (XYF). Comparisons of phenotypes in mice with different gonads (XXF vs. XXM, XYF vs. XYM) reveals the effects of gonadal secretions. More accurately, this comparison is between mice with and without the *Sry* transgene, which is the only gene that differs between the comparison groups. Most effects of *Sry* are indirect, the result of differences in gonadal secretions, which is why we can adopt the shorthand statement that these comparisons test for the effects of gonadal secretions and are called Asex effects@. It is important to remember, however, that some effects of *Sry* are not mediated by gonadal secretions (19). In contrast, comparisons of phenotypes of mice with the same type of gonad but different sex chromosome complement (XXF vs. XYF, XXM vs. XYM) yields information about the differential effects of an XX and XY genome. These are called Asex chromosome effects@. Note that gonadal hormones are not eliminated in this model. Instead, XX and XY mice are compared that developed with ovaries, and XX and XY mice are compared that developed with testes. Because FCG mice have been used so far mostly to determine if sex chromosome effects occur, they have typically been studied after gonadectomy in adulthood, so that at the time of testing there were no group differences induced by the acute (activational) effects of gonadal hormones. However, FCG mice gonadectomised as adults experienced the organisational effects of hormones, so that group differences in effects of gonadal secretions before the time of gonadectomy have not been controlled by this design. This issue is discussed further below. The removal of gonads of adult FCG mice prior to testing is critical, however, if the goal is to detect sex chromosome effects that are not mediated by differences in gonadal secretions. Many or most sex

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differences in phenotype are caused by activational effects of hormones. In a recent global study of gene expression in liver, for example, the majority of sex differences in gene expression were abolished by gonadectomy (24).

To date, FCG mice have been analyzed for numerous neural and non-neural phenotypes, including brain morphology, behavior, brain and liver gene expression, and phenotypes related to sex differences in disease (23). The earliest studies focused on brain and behavioral phenotypes representing some classic sexually dimorphic traits. These dimorphisms had previously been shown to be caused by organisational actions of testosterone or oestradiol. Among the dimorphic traits were male copulatory behavior, neuron number in the spinal nucleus of the bulbocavernosus (SNB), anteroventral periventricular nucleus of the hypothalamus (AVPV), thickness of the cerebral cortex, and progesterone receptor expression in the neonatal hypothalamus (22,25,26). These traits all were found to show sex effects (i.e., they differed in gonadal males vs. females) in adult FCG mice gonadectomised and treated equally with testosterone, but they showed no sex chromosome effects (i.e., they were not different in XX vs. XY). The results confirmed the long-standing conclusion that many sex differences are caused by organisational effects of gonadal hormones. In subsequent studies (27), FCG mice of the four groups were found not to differ on general measures of numerous traits (no sex or sex chromosome effects), including open field activity, elevated plus maze tests of anxiety, tests of olfaction, and threshold response to footshock. The lack of group differences suggests that the groups show no marked differences in physiology in the systems tested.

Studies of FCG have revealed several sex chromosome effects in which XX and XY mice differed, irrespective of gonadal sex. Among these are XX vs. XY differences in the number of tyrosine hydroxylase neurons in dissociated cell cultures of embryonic mouse mesencephalon (XY>XX) (28); the density of vasopressin fibers in the lateral septum of adult mice (XY>XX) (22,29); the response to thermal and chemical nociceptive stimuli in adult mice (XX>XY) (30); the response to thermal nociceptive stimuli in neonatal mice (XX>XY) (31); the learning of habits (XX>XY) (32); sniffing and grooming of an intruder mice (XX<XY) (27), and gene expression in several tissues (24,33-36). XX mice also show greater susceptibility to disease in mouse models of autoimmune diseases and neural tube closure defects (37-39). In other experiments, the XX vs. XY difference was found only in one sex. For example, when FCG were gonadectomised as adults and treated equally with testosterone, XXF showed fewer aggressive responses to an intruder mouse than the other three groups (29). XXF mice tested after gonadectomy without hormone treatment were also found to differ from the three other groups in their parental behavior (more pup retrieval) and they showed less asocial digging behavior in response to a cage intruder (27,29). Thus, sex chromosome effects have been found under a variety of conditions, involving different behavioral systems. A major conclusion from these studies and FCG studies discussed below is that XX and XY mice of the same gonadal sex differ in phenotype. These differences in XX and XY cells in the FCG model probably also operate in normal XY males and XX females, since the groups compared have XX and XY genomes that have the same three genetic differences (X dose, X imprint, Y dose) found in comparisons of normal XY males and XX females.

The study of Gioiosa, Chen and colleagues (30) on sex differences in nociception serves to illustrate the types of conclusions that can be drawn from studies of FCG mice. Pain is perceived and experienced in men and women differently (40-43), and sex differences are also found in mouse models of pain. FCG mice were tested in a standard test, in which the mouse is placed on a hot plate. The amount of nociception is inversely proportional to the latency with which the mouse licks its feet. In FCG mice gonadectomised as adults, XX mice responded more quickly to the thermal stimulus than XY mice, irrespective of their

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gonadal sex. In a test of acute nociception on naive mice, an injection of morphine increased latencies but did not differentially influence XX and XY mice. In a second test on the development of tolerance to morphine, mice were injected twice daily for six days with morphine or saline, with or without an N-methyl-D-aspartate (NMDA) antagonist which is thought to block the development of tolerance. On the seventh day, mice were tested on the hotplate, then injected with morphine and tested at various intervals after morphine injection. In this test, XX mice showed dramatically shorter latencies to respond relative to XY mice, before or after morphine, and the effect of morphine was greater in XY mice than XX mice (Figure 1). Thus, we conclude that thermal nociception differs in XX and XY mice. Because the sex chromosome complement of these mice mimics the difference in the genome between normal XY males and XX females, the results suggest that X- or Y-linked genes act to produce sex differences in the tissues that mediate the nociceptive response. The tests of FCG mice do not determine whether the genes are X- or Y-linked, nor do they resolve the sites of action of the sex chromosome effect. The chromosome of origin of the effect can be resolved in future studies that vary the number of X chromosome and Y chromosomes independent of each other (for examples see section III). Moreover, the mechanisms mediating the effect can be resolved once the responsible gene(s) is/are identified. Because of the dominant hormonal theory of sexual differentiation, an important question is whether the group differences in XX vs. XY mice could be mediated by group differences in gonadal secretions. Although a gonadal hormonal explanation remains possible, several considerations make it unlikely. Firstly, because the groups did not differ in their levels of gonadal secretions at the time of testing, the group differences were not caused by acute (activational) effects of gonadal hormones. The groups could have differed in the levels of gonadal hormones prior to gonadectomy, which could have had lasting effects. For example, it is conceivable that even though XXM and XYM both have testes, XXM might experience levels of testosterone different from those experienced by XYM during some perinatal critical period, which might influence the adult hotplate response. That idea also seems unlikely, because the hotplate response was no different in gonadal males and females under the conditions of testing (Figure 1), thus the response appears to be insensitive to the presence of testicular vs. ovarian secretions prior to gonadectomy. Under those conditions smaller within-sex differences in levels of gonadal hormones, prior to gonadectomy, would seem unlikely to account for XX vs. XY differences in the response. Moreover, XXM and XYM have been found both to be fully masculine on numerous traits, and to differ from XXF and XYF who are similar to each other and fully feminine on those same traits, a finding that argues that same-sex FCG mice generally experience similar levels of gonadal hormones during early critical periods (22,25,26). Thus, for some traits, studies of FCG mice have led to the confident conclusion that XX and XY genomes act directly on non-gonadal tissues to produce sizable differences in the trait.

Some of the largest sex chromosome effects detected by the FCG model are found in disease models. Many diseases differ in incidence or progression in men and women. Autoimmune diseases, such as multiple sclerosis (MS) and systemic lupus erthematosus (SLE), affect women more than men. The same sex differences can be detected in mouse models of these diseases. Experimental autoimmune encephalomyelitis (EAE) is a mouse model of MS that in some strains affects females more than males. Although androgens and oestrogens protect females from EAE, in the absence of gonadal hormones XX FCG mice show greater neural histopathology and faster progression of EAE than XY FCG mice, irrespective of their gonadal sex (37,38). In a model of SLE, XX mice show greater kidney pathology and die sooner than XY mice, again irrespective of gonadal sex (37). These sex chromosome effects are large and robust.

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C. Mice lacking gonads

In the FCG model, the effect of an XX vs. XY genome is compared both in a testicular hormonal environment, and in an ovarian hormonal environment. The value of the model stems in part from the power of comparing XX and XY under two different gonadal environments, because any differences between XX and XY are more likely to be direct sex chromosome effects (for example, as opposed to group differences caused by hormones) if they can be observed under different hormonal conditions. A disadvantage of the FCG model is that the effects of gonadal hormones have not been removed. Another mouse model solves this problem by observing mice that lack gonadal secretions altogether. This is possible in mice with a null mutation in the gene steroidogenic factor 1 (SF1, also called AD4BP)(44,45). SF1 has been shown to be critical for development of the gonads, adrenals, ventromedial nucleus of the hypothalamus (VMH), and anterior pituitary. Mice without SF1 lack gonads and adrenals, and die soon after birth because of the lack of adrenal secretions. These mice can be rescued by neonatal injections of glucocorticoids and then implantation of adrenal tissue. Sex differences found in such SF1 knockout mice clearly do not require gonadal secretions, and therefore are likely attributable to direct sex chromosome effects (XX vs. XY) on non-gonadal tissues (46,47). Gonad-independent sex differences are found in body weight of mice, and in the expression of nNOS (neuronal nitric oxide synthase) in several limbic brain regions (preoptic area, bed nucleus of the stria terminalis (BST), and AVPV). Moreover, sex differences are found in the expression of calbindin in the ventromedial nucleus of the hypothalamus (VMH). In other traits, mice without gonads did not show sex differences that are normally present in males and females that are gonadectomised before puberty (external genitalia, calbindin expression in the POA and BST, and aggressive behavior), indicating that the sex differences in these phenotypes require the presence of gonads before puberty and hence are likely the result of sex differences in gonadal secretions.

The use of mice without gonads is an important approach that helps tease apart direct genetic effects and hormonal effects that cause sex differences in phenotypes. One caveat in the use of SF1 knockout mice is that the SF1 mutation itself could create sex differences that are not found in normal mice. In adult SF1 knockout mice, the structural organisation of the VMH is disrupted (48-50). Thus, it is conceivable that the disruption of the VMH, or some other effect of the SF1 knockout, affects males and female differently. Still, such sex-specific effects do not require the gonads, and are evidence that XX and XY systems are not just differentiated by gonadal secretions (46). Moreover, a similar caveat applies in one form or another to all mice with disrupted sex chromosomes, since in each case one can imagine a situation in which the disruption in the genome could conceivably create differences between XX and XY that do not occur normally. As always, therefore, the conclusions based on studies of any one model should be confirmed using other approaches.

D. Mice with X chromosome monosomy (XO)

Recently several papers have examined the behavioral phenotype of gonadally female mice with one X chromosome. XO mice showed greater fear reactivity than XX mice, as measured by the time they spent in the open arms of an elevated plus maze (51), and also to show impaired discrimination in a demanding visual task requiring high levels of attention (52). In these tests, the parent of origin of the X chromosome of XO mice had no apparent effect. For fear reactivity, the difference between XO and XX was not caused by the different numbers of pseudoautosomal regions (PARs), since XY^{*X} mice (which have one X chromosome but two PARs; Figure 2 and Table 1) were similar in fear reactivity to XO (51). The authors concluded that the likely difference in fear reactivity between XO and XX was caused by difference in expressed dose of a non-PAR X gene that escapes inactivation. A different result was found in tests of visuospatial attention. In that case, the mice with one

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X chromosome plus two PARs differed from XO mice but were similar to XX mice, suggesting that haploinsufficiency of a PAR gene (probably steroid sulfatase) was responsible for the attention deficit of XO mice (52). In all of these tests of X monosomic mice, XO and XX mice were tested gonadally intact, so hormonal mediation of the X monosomy effect is possible although the authors argue against this idea (51,52). The X dosage effect found in fear reactivity should also operate in the comparison of XX and XY mice, so the results could be relevant to the main question of this review. However, FCG XX and XY mice tested after adult gonadectomy did not show a similar difference in fear responding (27), suggesting that the presence of the Y chromosome might mitigate the X dosage effect. In addition to its relevance to sex differences, the comparison of XO vs. XX difference is quite relevant as a model for understanding differences between XX and XO women.

Comparison of XO mice with a maternal vs. paternal X chromosome imprint has revealed that the parent of origin of the X chromosome influences cognitive functions (53). XmO mice (those inheriting their X chromosome from the mother) differed from XpO mice (with a paternal X imprint) in a test of reversal learning. Mice with a maternal X imprint were less able to alter their responses when the reward contingency changed. The X gene *Xlr3b* shows parent of origin effects on its expression and is expressed in brain regions important for reversal learning, and is thus a candidate gene accounting for the behavioral differences between XmO and XpO mice. These parent of origin effects are a model of behavioral differences caused by parent of origin in women with Turner=s Syndrome (XO) (54). It will be interesting to learn if the differences in imprinting of this gene contributes to sex differences in comparisons of XX and XY mice,

III. Attributing sex chromosome effects to X or Y genes

As discussed in section II, FCG mice and gonadless mice have provided the best information to date regarding sex differences that result from direct sex chromosome effects on non-gonadal tissues. Once such a sex chromosome effect is detected, other mouse strains are needed to resolve whether the effect is caused by X or Y genes. One approach is to vary the number of the X and Y chromosomes (or parts thereof) as independently as possible, to examine the separate effects of each chromosomal segment. For example, *Sxra* mice and sex chromosome aneuploids (XO, XXY, XYY, etc.), discussed further below, involve variations in the representation of X or Y segments. These variations have yielded insights into the effects of X or Y genes on embryonic growth, placental size, and spermatogenesis (55-60), but have yet to be used extensively to solve the chromosome of origin of sex chromosome effects on brain and behavioral phenotypes. Exceptions include the studies on X monosomy discussed in section IID (51-53), and the experiments discussed in the next paragraphs.

A. Variations on the Y chromosome cause differences in phenotypes

Male and female mouse embryos differ in their size from early stages of development, even before implantation of the blastocyst (55,56,61). The size differences obviously occur long before the gonads develop, and therefore this sex difference represents one of the first examples of a sex difference in non-gonadal tissues that could not be caused by gonadal secretions. Part of the sex chromosome effect on embryo weight may be caused by a Y chromosome effect. If mouse strains are compared that are genetically identical except for the strain of origin of the Y chromosome, the male embryos of the two strains differ in weight (55). The conclusion is that allelic differences in Y genes cause the difference in embryo weight, which in turn suggests but does not prove that the presence vs. absence of the same factor on the Y chromosome might contribute to an XX vs. XY difference in phenotype. Variation on the Y chromosome has also been reported to influence adult phenotypes. Adult mice or rats differing only in the strain origin of the Y chromosome are

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reported to show different levels of aggression, suggesting that factors on the Y chromosome contribute to sex differences (62,63). However, these studies comparing adult aggressive behavior have been conducted in mice that are gonadally intact, so that the difference in aggression could conceivably be attributed to differences in the level of androgens in the males (either during testing or at a previous time of life), or differences in the dynamic regulation of androgen levels during the behavioral testing. Strain differences in the Y chromosome are, in fact, also reported to influence the levels of androgen (63,64). Thus, the only strong evidence to date implicating the Y chromosome as the source of a male-specific signal that directly acts on the brain to cause sex differences in adult neural phenotypes is the action of the *Sry* gene on the substantia nigra reviewed in section IIA (19).

B. Variations in X chromosome number

Comparisons of mice with different numbers of X chromosomes, or different numbers of PARs, have shown that the dose of X genes influences some traits such as spermatogenesis, gonadal differentiation, birthweight, and fertility (59,65-67). With regard to brain or behavioral phenotypes, the dose or parental imprint of X genes has been implicated in the control of fear reactivity (51), visuospatial attention (52), and reversal learning (53) as reviewed above.

The X chromosome also appears to harbor factors that influence sex differences in neural tube closure (39). Failure of neural tube closure is a common developmental disorder in humans, and anterior neural tube closure defects (NTDs) affect females more than males. One mouse NTD model comprises mice with a null mutation of the tumor suppressor p53. Female p53-null mice have a high incidence of NTDs, and by the day of birth these mice usually die so that the only p53-null mice on the day of birth in C57BL/6 litters are males. Studies of FCG mice indicate that incidence of NTD is higher in XX mice than XY mice, irrespective of their gonadal sex (39). To shed light on the chromosome of origin of the sex chromosome effect, Chen et al. (39) used progeny of male XY* mice. The Y* chromosome is thought to be an abnormal recombination product of the X and Y chromosome (68,69). XY* fathers produce progeny with several kinds of abnormal chromosomes (Figure 2 and Table 1). Among these p53 deficient progeny of XY* mice, only mice that have two copies of the X chromosome showed NTD, and the presence or absence of the Y* chromosome appeared to have little effect (Table 1). Thus, the incidence of NTD in the p53 model seems to be attributable to difference in the dosage or imprint of X genes.

IV. Other manipulations of sex chromosome complement

A. Sex chromosome aneuploid mice: XXY and XYY

To understand the role of X or Y genes, it is informative to manipulate the number of X or Y chromosomes, both by increasing or decreasing their dose in the genome. In experiments that increase the dose of genes, for example in mice with two Y chromosomes, one caveat is that the expression of Y genes may be above the normal range and thus does not necessarily reflect the effects of the genes that occur when they are expressed in their normal physiological range. Nevertheless, testing the effect of increased Y dosage can suggest a functional relation that can be tested under more normal levels of expression.

One study compared the masculine copulatory of male mice with different numbers of X and Y chromosomes, in the presence or absence of an autosomal *Sry* transgene (70). Mice were gonadectomised as adults and given equal treatment with testosterone, then tested with receptive females. Comparing XY and XYY^- mice (where the Y^- chromosome is deleted for *Sry*, as in the FCG model), the mice with two Y chromosomes (XYY^-) showed faster latencies for copulatory behaviors, suggesting that a double dose of non-*Sry* Y genes affects copulation. However, similar comparisons in mice that also possessed the *Sry* autosomal

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transgene did not show a similar Y dosage effect (e.g., when comparing XX and XXY mice that both have an *Sry* transgene), suggesting that the non-Y genes affecting copulation might be sensitive to the dose of *Sry*. In other comparisons in mice with the *Sry* transgene (all males: XX vs. XXY^-, XY vs. XXY^-), the number of X chromosomes was inversely related to the amount of male copulatory behavior. Thus, an overdose of X and Y genes can both be found to influence male copulation. One possible factor that could mediate these genetic effects are differences in the levels of testicular hormones before the time of gonadectomy of these mice. Indeed, XXY mice are reported to have lower levels of plasma testosterone than XY mice (71). Therefore sex chromosome aneuploid mice, like XXY and XYY humans (72), have a different behavioral phenotype than XY males. In both species, there is the need to disentangle the effects of sex chromosome complement and the effects of gonadal hormones. This will be easier in mice than in humans. Thus, further work is needed to determine if the results on XXY and XYY mice bear strongly on the central question of this review.

B. Other sex-reversing mouse models

Three other mouse models have been used that allow comparison of XX and XY mice of the same gonadal type. One uses the Y^{POS} (poschiavinus Y chromosome), which when bred onto a C57BL/6 background yields XY^{POS} mice with ovaries (73). Some XY^{POS} mice possess exclusively ovarian tissue. Thus, this model allows the comparison of XX and XY gonadal females. In C57BL/6 mice, XY males and XX females differ in tests of open field activity, the Lashley maze, and active avoidance learning, and in Morris water maze learning. When gonadally intact XX and XY females were subjected to the same tests, small differences in Morris water maze learning were found, but not in the other behavioral tests (74). XY^{POS} females also did not differ from XX females in tests of aggression or male copulatory behavior (75). In all tests published to date using the XY^{POS} model, the animals were tested gonadally intact, and In the aggression tests the genotype of the intruder mouse co-varied with the genotype of the resident mouse. The presence of the gonads in these mice means that any group differences in behavioral traits might be mediated by group differences in the levels of gonadal hormones at the time of testing. Moreover, a lack of difference could potentially be explained by offsetting gonadal hormone effects and sex chromosome effects. Thus, these studies may not bear strongly on the issue of whether sex chromosome effects on non-gonadal tissues cause sex differences in behavioral phenotypes.

Another model is the Oddsex mouse, which was created by transgenic insertion of a coat color marker into a regulatory region of the Sox9 gene, which is required for testis determination (76). This model produces XX gonadal males carrying the transgene, which were compared to XY gonadal males that possess the transgene. Such XX and XY males were also found to show similar levels of aggression and male copulatory behavior. However, the tests were performed in gonadally intact mice (75) and thus do not yet bear strongly on the issue of possible sex chromosome effects on these behaviors.

In the *Sxra* model, a segment the Y chromosome (the *Sxra* locus) is carried as an addition to the normal Y chromosome by XY^{Sxra} males (77). When an XY^{Sxra} male is mated with an XX female, the *Sxra* locus can translocate to the X chromosome during male meiosis, producing an XX^{Sxra} mouse. The *Sxra* segment includes a small number (perhaps about nine) Y genes, including *Sry*, and thus XX^{Sxra} mice are gonadal males. Many of these Y genes are close homologues to X genes. Thus, four basic types of offspring are produced: normal XX females, XX^{Sxra} gonadal males, normal XY males, and XY^{Sxra} males. This cross therefore allows one to compare XX and XY gonadal males, but the comparison is complex. The XX^{Sxra} gonadal males have numerous Y genes plus two X chromosomes, and therefore differ from XY males along some dimensions that are unlike the comparison of XX and XY genomes. The comparison may be more akin to XXY vs. XY (except that the XX^{Sxra} has a

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only a partial Y) than it is to XX vs. XY. One study compared XX^{Sxra} males to XY males on measures of parental behavior (pup retrieval) and infanticide (78). This study did not discriminate XY from XY^{Sxra} males, which were grouped together into a genetically heterogeneous XY male group. The AXY@ group showed more infanticide that XX^{Sxra} mice. Mice were gonadally intact at the time of testing, thus group differences in the level of gonadal hormones could have contributed to group differences in behavior, although the authors argue against this idea. Moreover, the genetic differences between groups are complex and involve possible interactions of *Sxra* Y genes with two doses of X genes, so that it is difficult to relate the observations to an understanding of sex differences in the direct effects of XX vs. XY genomes.

V. The future of studies using mice with abnormal sex chromosomes

A strong thread running throughout this discussion is the importance of discriminating sex chromosome effects from those caused by gonadal hormones. This emphasis stems from the dominance of the formerly accepted theory that all sex differences in non-gonadal tissues are caused by gonadal hormones. That theoretical emphasis heightens interest in sex differences that are not caused by gonadal hormones but rather by the different effects of an XX vs. XY genome. We are now faced with a revised theory, which states that at least three main forces are the proximate causes of sex differences: organisational (permanent differentiating) effects of gonadal hormones, activational (reversible) effects of gonadal hormones, and direct genetic effects of X and Y genes. What experiments are now required to push the field of sex difference forward, using mouse models?

Although the interaction of organisational and activational hormonal effects has been studied for several decades, we need now to learn about how the hormonal and genetic factors interact. Do gonadal hormones swamp out, modify, or counteract the direct genetic effects? Are the effects of gonadal hormones the same in XX and XY cells? Do some sex chromosome effects occur only when gonadal hormones are present? It will be important to vary hormone levels or receptors in an experimentally controlled fashion, in some of the mouse models discussed here, to understand how they interact with sex chromosome effects.

A second focus for future studies is to expand the number of phenotypes that are tested for sex chromosome effects. To date only a restricted number of phenotypes have been tested, and we cannot yet discern much pattern. What kinds of phenotypes might be most affected by sex chromosome effects? Are some of the sex chromosome effects caused by the same sexual imbalance of X or Y gene expression in XX and XY cells?

A third important focus is to discover the genes responsible for sex chromosome effects. So far only one sex chromosome gene, *Sry*, is proven to have sex-specific effects by virtue of its differential representation in XX and XY genomes. We know that X genes mediate other sex chromosome effects. It is now time to identify those genes and study their mechanisms of action.

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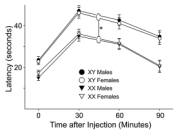

Figure 1.

Robust sex chromosome effect on nociception. FCG mice were gonadectomised as adults and then tested for the development of tolerance to morphine. Mice were injected twice daily with morphine or saline, plus an NMDA antagonist or saline. On the seventh day, mice were tested on a hotplate at time zero and then injected with morphine and tested after 30, 60 and 90 minutes. The graph shows the latency for the mice to lick or shake their paws. All data are averaged across drug treatment groups. XX mice of either gonadal sex showed much lower latencies than XY mice (*, $p<0.00001$), and the presence of testes or ovaries prior to gonadectomy had no effect. From (30).

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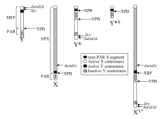

Figure 2.

Chromosome maps of variants of the X and Y chromosomes. Progeny of XY* males, mated with XX females, are as follows: XX, XY*, XO, XY*^{X}, XX^{Y}*. Illustrated are the normal X and Y chromosomes, and the recombinant sex chromosomes Y*, Y*^{X}, and X^{Y}*. The pseudoautosomal region (PAR) is separated into proximal to distal segments A,B,C. XPB, X-PAR boundary. YPB, Y-PAR boundary. NPX, non-PAR region of the X chromosome. MSY, male-specific (non-PAR) region of the Y chromosome. From (39) and based on (68,69).

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Table 1

Sex chromosomes of the progeny of XY* fathers

The progeny of XY* are useful for attributing sex chromosome effects to the X or Y chromosome.
The Y* chromosome shown in Figure 2 pairs with the X chromosome abnormally at male meiosis, producing five different types of sperm and hence five different progeny listed in the table. In tests of neural tube closure defects, mice with two X chromosomes (XX and XX^{Y*}) showed neural tube closure defects, but mice with one X chromosome (XY* and XY^{*X}) did not (39). This finding suggests that the number of X chromosomes (specifically NPX), not the presence/absence of the Y chromosome, causes the sex difference in this model.
Mice with *Sry* are gonadal males. NPX = non-pseudoautosomal region of the X chromosome. MSY = non-pseudoautosomal or male-specific region of the Y chromosome. PAR_{AB} = the regions of the proximal pseudoautosomal region (PAR) closest to the PAR boundary with NPX or MSY. PAR_C = the most distal (telomeric) portion of the PAR (see figure 2). Near-PAR X is a small portion of the NPX chromosome that is proximal to the PAR boundary. Xm or Xp = X chromosome with a maternal or paternal imprint, respectively. Based on (69).

	Sry	**NPX**	**MSY**	**PAR_{AB}**	**PAR_C**	**near-PAR**	**Xm**	**Xp**
XX	0	2	0	2	2	2	1	1
XX^{Y*}	1	2	1	3	1	2	1	1
XY^{*X}	0	1	0	2	2	2	1	0
XY*	1	1	1	3	1	2	1	0
XO	0	1	0	1	1	1	1	0