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# **Using mouse models to investigate sex-linked genetic effects on brain, behaviour and vulnerability to neuropsychiatric disorders**

William Davies

Behavioural Genetics Group, MRC Centre for Neuropsychiatric Genetics and Genomics, Neuroscience and Mental Health Research Institute, Schools of Medicine and Psychology, Cardiff University

Henry Wellcome Building,

Heath Park Campus,

Cardiff CF14 4XN

United Kingdom

Tel: +44-(0)29-2068-7047

Fax: +44-(0)29-2068-7068

e-mail: [daviesw4@cardiff.ac.uk](mailto:daviesw4@cardiff.ac.uk)

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## **Abstract**

Many brain and behavioural phenotypes in humans exhibit some degree of sexual dimorphism. Moreover, there are large and replicable differences in the vulnerability of the two sexes to a wide range of common brain disorders. Ultimately, sex differences in healthy individuals, or in pathological states, must arise as a consequence of the differential complement of sex-linked genes in males and females. These genes may act indirectly (for example through influencing gonadal hormone secretion), or directly, to influence brain development and function. In this review, I discuss how genetically tractable mouse models may be employed to inform our knowledge of the molecular basis of sexual differentiation of the mammalian brain, and how such models may therefore represent a useful tool through which to identify risk factors predisposing to sex-biased neuropsychiatric disorders.

## 1. The importance of sex

There is a substantial literature showing that mammalian males and females differ with respect to a number of neurobiological parameters. In humans, sex differences in brain function are evident from the earliest stages of postnatal life (Boatella-Costa et al., 2007), right through to old age (Petersen et al., 2010), and across a number of neurobehavioural domains including cognition and emotional function (Cahill, 2006). With regard to cognition, studies have consistently emphasised sex differences in the performance of tasks dependent upon visuospatial function, in perception and in some forms of memory (Halpern, 1997). Modern *in vivo* brain imaging techniques including (functional) magnetic resonance imaging (fMRI) and Diffusion Tensor Imaging (DTI) are now beginning to reveal fine details of sex-specific neuroanatomy and connectivity, to add to the existing data on sex differences in brain structure already gleaned from *post mortem* tissue samples (Luders and Toga, 2010). Many of the sexually dimorphic brain substrates described by these techniques are likely to be neural correlates of the sexually dimorphic behaviours listed above, but it is also possible that in some cases, brain structure differs between the sexes as a compensatory mechanism to ensure that the associated behavioural output is equivalent (Cahill, 2006; De Vries, 2004). The overall picture that is emerging is that many sex differences in human neurobiology are consistently observed and are of significant functional relevance (Cahill, 2006); nevertheless, there is still some degree of debate about the veracity, frequency and magnitude of male-female differences in the normal range (Fine, 2010; Jordan-Young, 2010).

In contrast, the existence of sex differences in many pathological conditions is indisputable (even allowing for possible ascertainment biases); indeed, Thomas Insel, Director of the National Institute of Mental Health, was once

quoted as saying that ‘it’s pretty difficult to find any single factor that’s more predictive for some of these (neuropsychiatric) disorders than gender’ (Holden, 2005). Sex differences may be observed in the incidence of these disorders, in their age-at-onset and clinical course, in their underlying neurobiology, or in their response to therapy (Bao and Swaab, 2010; Davies and Wilkinson, 2006). In terms of incidence, males are disproportionately vulnerable to neurodevelopmental disorders such as autism spectrum disorders and Attention Deficit Hyperactivity Disorder (ADHD), whereas females are more vulnerable to later-onset disorders of the affective systems such as unipolar depression, and anxiety-related illnesses (Holden, 2005). Many of the disorders showing a sex bias in their incidence also show sex biases in their presentation and associated co-morbidities; for example, males diagnosed with ADHD tend to exhibit the hyperactive-impulsive subtype and may present with externalising behaviours such as physical abuse, aggression and criminality whereas females tend to exhibit the inattentive subtype and be at increased risk of developing co-morbid eating and anxiety disorders (Biederman et al., 2002; Cumyn et al., 2009). We know that most, if not all, neuropsychiatric disorders have a substantial (epi)genetic basis, and much work is currently ongoing to identify risk variants (Cichon et al., 2009). However, whilst common genetic variants that explain just a small fraction of the risk of developing psychiatric disorders receive a disproportionately large amount of attention (and consequently funding), our understanding of how sex, the ‘most predictive factor’ for some of these disorders, mediates susceptibility remains deficient. Increasing our understanding regarding the molecular basis of sex differences in psychiatric disorders will be pivotal in identifying risk/protective factors and in being able to develop more effective therapies.

## **2. Sex-linked genetic effects on brain function**

Ultimately, sex differences in normal and abnormal brain function must arise as a consequence of the differential sex chromosome complements in male and female cells. In humans, females possess two X chromosomes in each cell, one inherited from either parent. In each cell of the female brain, one of these chromosomes is substantially silenced by the epigenetically-mediated process of X-inactivation; which of the two X chromosomes is silenced is initially randomly determined in early embryonic development, but the pattern of X-inactivation is then inherited clonally (Barakat et al., 2010). In humans, ~15-20% of all X-linked genes escape the process of X-inactivation and remain expressed from both of the X chromosomes (Carrel and Willard, 2005). Human males inherit a single X chromosome from their mother and a smaller Y chromosome from their father; in males, X-inactivation does not occur. The X chromosome is enriched for genes important in cognition (Zechner et al., 2001), whilst the Y chromosome also houses several genes expressed to a significant extent in the brain (Kopsida et al., 2009). Therefore, the combined role of these chromosomes in mediating sexually dimorphic neural functions is likely to be important.

There are three general genetic mechanisms via which may give rise to sexually dimorphic gene expression patterns, and hence sexually dimorphic brain phenotypes: expression of Y-linked genes, X-linked gene dosage, and X-linked genomic imprinting (Davies and Wilkinson, 2006). In the first mechanism, given that only males possess a Y chromosome, Y-linked genes can only be expressed in this sex. In the second mechanism, genes which escape the process of X-inactivation may theoretically be more highly expressed in female than male brain cells given that females possess two expressed alleles compared to the male's one. Importantly, due to incomplete escape on the inactivated chromosome, and possible mediating

hormonal factors, it is not necessarily the case that if a gene ‘escapes’ from X-inactivation it will be expressed significantly more highly in female than male cells. Therefore, the ability of the ‘escapees’ defined by Carrel and Willard (2005) to influence sexually dimorphic traits may be somewhat attenuated. In the third mechanism, X-linked ‘imprinted genes’ (i.e. genes which are monoallelically expressed in a parent-of-origin dependent manner) may exhibit female-limited expression if they are solely expressed from the paternally inherited X chromosome (specific to females), or male-biased expression if they are expressed predominantly from the maternally inherited X chromosome (common to both sexes), and are subject to X-inactivation (Davies et al., 2006; Davies, 2010). Imprinted genes are currently a hot topic in developmental neurobiology (Wilkinson et al., 2007), and are likely to influence the aetiology of a wide range of behavioural endophenotypes and psychiatric disorders (Kopsida et al., 2010); this class of genes is discussed further in Section 7. Here, it should be made clear that whilst many X-linked genes may be involved in, or be critical to, the expression of particular traits, their expression may not necessarily induce sex-specific phenotypes.

The three distinct genetic mechanisms outlined above may act to influence brain function directly (i.e. the sex-linked genes encode proteins which act within the brain in some capacity) or indirectly (i.e. the sex-linked genes encode proteins which influence the development of some other body tissue such as the gonads or the adrenal glands, which subsequently influence brain development and/or function via systemic compounds such as hormones).

The importance of the mechanisms listed above to brain and behavioural function (whether direct or indirect) is illustrated by the functional consequences associated with their dysregulation. Case studies of individuals with Y-linked chromosomal abnormalities (deletions or duplications

spanning multiple genes) have suggested that they may exhibit a number of psychological abnormalities and propensity to a variety of mental disorders (McConville et al., 1983; Mouaffak et al., 2007; Mulligan et al., 2008; Nanko et al., 1993; Olajossy et al., 2005; Yoshitsugu et al., 2003).

Individuals with abnormal dosage of X-linked genes as a consequence of reduced or increased numbers of X chromosomes also commonly show behavioural anomalies. Females with the developmental disorder Turner syndrome (TS), most frequently caused by complete loss of one X chromosome (45,X, X-monosomy), exhibit a subtle constellation of psychological problems including attention deficits (and heightened vulnerability to ADHD (Russell et al., 2006)), impairments in visuospatial skills (Nijhuis-van der Sanden et al., 2003), inability to recognise emotion in faces (Lawrence et al., 2003), and anxiety in certain situations (Schmidt et al., 2006). It has also been suggested that subjects with Turner syndrome may be significantly over-represented in samples of females with schizophrenia (Prior et al., 2000; Roser and Kawohl, 2010), although the data are not consistent (Mors et al., 2001). The behavioural abnormalities seen in individuals with Turner syndrome may be partially explained by altered systemic hormone levels, notably reduced estrogen and androgen levels (as a consequence of ovarian dysfunction) and reduced growth hormone levels (Gravholt, 2004). Indeed, there is limited evidence that estrogen, androgen and growth hormone replacement therapies may alleviate TS-associated cognitive deficits to some extent (Ross et al., 2000; Ross et al., 2003; Ross, 2005).

The severity of some behavioural and neurological phenotypes in TS may depend upon whether the remaining intact X chromosome is of paternal (45,X<sup>P</sup>, ~30% of 45,X cases) or maternal (45,X<sup>M</sup>, ~70% of 45,X cases) origin (Ergur et al., 2008; Hamelin et al., 2006; Loesch et al., 2005; Mullaney



and Murphy, 2009; Sagi et al., 2007); such parent-of-origin sensitive phenotypes may theoretically be modulated by the products of one or more X-linked imprinted genes.

Subjects with X-polysomy i.e. possessing more than the usual complement of X chromosomes (of which Klinefelter's syndrome 47,XXY is the most common), may present with developmental delay, language-based learning disabilities, executive dysfunction (including attention) and socio-emotional difficulties (Tartaglia et al., 2010; Geschwind et al., 2000); moreover, subjects with Klinefelter's syndrome display increased levels of autistic and schizotypal traits (van Rijn and Swaab, 2011). It is important to be aware that as the predicted incidence of Klinefelter's syndrome is approximately 1 in 500 males and only ~50% of individuals with Klinefelter syndrome are diagnosed (Herlihy et al., 2011), that the men who are diagnosed may exhibit physical and behavioural phenotypes at the extreme end of a spectrum. Individuals with Klinefelter's syndrome exhibit elevated Follicle-Stimulating Hormone (FSH) and Luteinising Hormone (LH) levels, and reduced testosterone levels from puberty onwards (Wikstrom and Dunkel, 2008); it is likely that these hormonal sequelae may underpin some of the psychosocial and behavioural features associated with the syndrome, as androgen replacement therapy seems to partially alleviate these (Simm and Zacharin, 2006).

Presumably, the TS phenotypes are a consequence of haploinsufficiency (reduced dosage) for products of one or more X-linked genes that escape X-inactivation, whereas phenotypes associated with X-polysomy result from over-expression of these genes. Dissociating whether these phenotypes are due to indirect effects of altered gene expression (mediated by altered systemic hormone levels) or whether they are due to direct effects on

neurobiology represents a major challenge, which may be best addressed using experimental systems.

Other sex chromosome abnormalities such as 47,XYY may also be associated with behavioural abnormalities (Bishop et al., 2010; Gotz et al., 1999), but given the restricted sample sizes employed in these studies, their conclusions should be interpreted cautiously.

Consistent with an abundance of 'cognition' genes on the X chromosome, X-linked copy number variants (CNVs) and mutations of X-linked genes are often associated with neurobehavioural sequelae. For example, deletions or duplications of one genetically unstable region of the short arm (Xp22.3) have been associated with vulnerability to a variety of developmental phenotypes (Liu et al., 2011) including autistic spectrum disorders (James et al., 1998; Shinawi et al., 2009; Thomas et al., 1999; Vorstman et al., 2006), ADHD (Doherty et al., 2003; Kent et al., 2008; Tobias et al., 2001), schizophrenia (Milunsky et al., 1999), mental retardation (Lonardo et al., 2007) and the Turner syndrome neurocognitive profile (Zinn et al., 2007). Genetic mutations causing inactivation or deletion of the X-linked genes *MAOA*, *FMR1* and *MECP2* have been associated with pathological impulsive aggression (Brunner et al., 1993), Fragile-X syndrome (Heulens and Kooy, 2011) and Rett syndrome (Adkins and Georgel, 2011) respectively. At the phenotypic level, Fragile-X resembles classical Kanner autism, and is characterised by mental retardation, by stereotypical movements, and by altered social behaviours (Boyle and Kaufmann, 2010); there may be some degree of sexual dimorphism in the presentation of the disorder, presumably due to the differential effects of the *FMR1* mutation in hemizygous males and heterozygous females (Reiss and Freund, 1990). Rett syndrome, a neuro-motor disorder, is also associated with behavioural features similar to autism including: mental retardation, stereotypical

behaviours such as hand-wringing and seizures (Mount et al., 2001). Male fetuses with Rett syndrome rarely survive to term, presumably as inactivating mutations in the single X-linked *MECP2* gene (which encodes a methyl binding protein critical for survival) result in complete loss of function.

### **3. The utility of mouse models**

On account of their idiosyncratic inheritance, recombination and expression patterns, linkage, association and CNV studies in humans often neglect the sex chromosomes. Hence, there is relatively little consistent data in humans regarding the influence of sex-linked genetic variants on brain and behavioural phenotypes and on vulnerability to psychiatric disorders. Model experimental systems may be of some use in helping to elucidate these effects. Mice are currently the *in vivo* model of choice for examining the effects of genetic manipulations on neurobiology, due to the extensive characterisation of their genome and their amenability to genetic manipulation (gene knockout/knockin, transgenesis or chromosomal mutation). Being mammals, rodents have an equivalent sex chromosome complement to humans (in contrast to other model organisms such as zebrafish, birds and nematode worms for example), and the genetic composition of these chromosomes is largely equivalent (albeit somewhat rearranged)(Davisson, 1987). In terms of analysis, rodents are relatively easy to maintain and breed in large numbers, and their neurobiology is well characterised and may be intimately examined *in vivo* and *post mortem*.

However, laboratory mice are not ideal experimental subjects for sex differences research, and it is important to be aware of caveats associated with their use. First, their X chromosome is more extensively silenced by X-inactivation than its human equivalent, possibly as a consequence of subtly

different species-specific inactivation systems (Okamoto et al., 2011); to date only a handful of genes have been shown to escape X-inactivation in mice (**Table 1**). This means that there are likely to be significant X-linked expression differences between humans and mouse, with potential implications for the mechanisms underlying sex-specific neurobiology in the two species. Second, the mouse pseudoautosomal region contains only one annotated gene (*Srs*) compared to 29 in the human pseudoautosomal regions (Flaquer et al., 2008) and the murine Y chromosome has fewer genes overall than its human counterpart. Third, complex human-specific phenotypes that may be influenced by sex-linked genes such as language and psychosis (Crow, 2008) are unlikely to be able to be accurately modelled in rodents, although measurement of ultrasonic vocalisations (Fischer and Hammerschmidt, 2011) and hippocampal activity as indexed by electrophysiological measurement (Gisabella et al., 2005) respectively may represent possible surrogate measures.

#### **4. Sex differences in mice: *caveat emptor***

Investigations into potential sex differences have been performed across a wide variety of neuroanatomical and behavioural measures in mice. Whilst some reported sex differences are large and robust (e.g. androgen receptor-positive and vasopressin cell numbers in the bed nucleus of the stria terminalis (Forger, 2009)), others (mainly behavioural measures) tend to be less consistently replicated. This lack of replicability could be due to the inherent variability in assay performance between laboratories, to inter-laboratory differences in the strain of mouse used, and to the fact that many studies do not explicitly take into account female oestrus status, a factor which is known to exert effects on emotional, social and cognitive behaviours (Sanchez-Andrade and Kendrick, 2011). Moreover, in the laboratory, mice are generally housed in artificially imposed single sex

groups post weaning; in nature, the sexes are free to interact in colonies typically consisting of a single dominant male, several females and their offspring. Hence, sex-specific behaviours in laboratory mice may not even be recapitulated in mice living outside the laboratory, much less in humans.

## 5. The 'Four Core Genotypes (FCG) Cross'

Even where a reliable sex difference in neurobiology is observed in laboratory mice, using wildtype mice alone, it is not possible to ascertain whether that difference is due to the direct actions of sex-linked genes, to downstream effects of sex-linked genes on gonadal hormone production and thereby on the measure of interest, or to a combination of the two. A recently developed model exploiting genetically-modified mice (the Four Core Genotypes (FCG) cross) enables a partial dissociation to be made between these two possibilities (reviewed comprehensively in Arnold and Chen, 2009)). In this model, genetically modified fathers are mated to wildtype females (40,XX) to produce offspring of four distinct genotypes; by comparing the phenotypes of mice of the four different genotypes, it is possible to obtain information regarding their underlying biological substrates. The fathers have the genotype 40,XY *Sry* i.e. they possess a Y chromosome deleted for the male-determining *Sry* gene (Y<sup>-</sup>, originally arising as a spontaneous mutation) and have the *Sry* gene reinserted as an autosomal transgene (*Sry*). The protein encoded by *Sry* acts early in embryonic development to direct the development of the initially bipotential gonad down a testis-specific trajectory (Kashimada and Koopman, 2010); the Leydig cells of the testes then secrete testosterone which masculinises the brain through developmental (organisational) and activational pathways (Arnold, 2009). It has recently been shown that Sry protein is also normally expressed into adulthood in the male rodent brain, where it can act as a transcriptional activator for genes of the monoaminergic system (tyrosine

hydroxylase *Tb* (Milsted et al., 2004), and monoamine oxidase *Maoa* (Wu et al., 2009)) and can mediate behavioural phenotypes (Dewing et al., 2006). The four genotypes produced by the cross are as follows: 40,XX (karyotypically and gonadally female), 40,XY<sup>-</sup> (karyotypically male, gonadally female due to loss of Sry activity), 40,XX*Sry* (karyotypically female, gonadally male due to Sry activity) and 40,XY<sup>-</sup>*Sry* (karyotypically male, gonadally male). For any given phenotype, if 40,XX mice differ from 40,XY<sup>-</sup> mice (both gonadally female), and 40,XX*Sry* mice differ from 40,XY<sup>-</sup>*Sry* mice (both gonadally male), the effect may be attributed to X or Y-linked genes other than *Sry* (i.e. a ‘sex chromosome effect’). Conversely, if 40,XX mice differ from 40,XX*Sry* mice (both karyotypically female), and 40,XY<sup>-</sup> mice differ from 40,XY<sup>-</sup>*Sry* mice (both karyotypically male), the effect may be attributed to the actions of Sry (i.e. an *Sry*-dependent effect). *Sry*-dependent effects may theoretically be direct (through regulating the activity of brain-expressed genes) or indirect (affecting brain function via influencing gonadal differentiation and ultimately hormonal secretion); in isolation, the model does not allow the two possibilities to be distinguished.

A multitude of *Sry*-dependent and sex chromosome effects have now been documented in mice. *Sry*-dependent effects have been shown on various behavioural indices and anatomical measures such as progesterone receptor immunoreactivity (Wagner et al., 2004) and cerebral cortical thickness (Markham et al., 2003). In contrast, sex chromosome effects have been described on measures as diverse as nociception (Gioiosa et al., 2008), vasopressin immunoreactivity in lateral septum (De Vries et al., 2002), number of tyrosine hydroxylase (TH)-ir neurons in mesencephalon from embryonic day 14.5 (Carruth et al., 2002), social interaction style (McPhie-Lalmansingh et al., 2008), aggression and pup retrieval (Gatewood et al., 2006), prodynorphin (Pdyn) expression in striatum (Chen et al., 2009) and habitual responding for food and alcohol reinforcers (Quinn et al., 2007;

Barker et al, 2010); these observations may have important implications for clarifying the molecular mechanisms underlying sex differences in vulnerability to disorders of sociality (e.g. autism), disorders of dopamine dysfunction such as pathological impulsivity in ADHD, and addiction behaviours. Both sex chromosome complement and gonadal hormone effects have been observed in immune response (Palaszynski et al., 2005), whilst sex chromosome complement and steroid hormones may act independently and/or in concert to influence the sexually dimorphic expression of key brain genes (notably outwith the hypothalamus) in a spatially-specific manner (Abel and Rissman, 2011). Although hormonal influences are probably the major contributor to gene expression differences between male and female tissues in mice (Arnold et al., 2009; Yang et al., 2006), the results above indicate that sex-linked genes are also likely to be having effects on a wide range of neurally-significant phenotypes.

## **6. Mouse models to identify sex-linked genetic mechanisms influencing brain function**

There are a number of chromosomally-mutant mouse models currently available that may be used to identify the precise genetic mechanism underlying ‘sex chromosome complement’ effects on phenotype; these models may be used subsequent to identifying such an effect using the Four Core Genotypes cross (as in Chen et al., 2009), or in isolation.

One such model is the 39,XO mouse (Lynn and Davies, 2007). Like the majority of individuals with Turner syndrome, these female mice possess a single X chromosome instead of the usual two. As such, they are haploinsufficient for genes that escape X-inactivation. As there appear to be a limited number of murine ‘X-escapees’ (see above), 39,XO mice do not show the gross anatomical and physiological abnormalities associated with

Turner syndrome i.e. short stature and ovarian dysfunction. However, they do appear to show subtle changes in physiology including diminished thyroid function (Deckers and van der Kroon, 1981), depressed activity of the estrogen-producing system/reduced fertility (Deckers et al., 1981) and behavioural abnormalities including heightened anxiety (Isles et al, 2004) and impaired attention (Davies et al, 2007). X-linked genes that escape X-inactivation in both mice and humans represent excellent candidates for behavioural and psychiatric phenotypes associated with Turner syndrome (Lopes et al., 2010; Raefski and O'Neill, 2005).

The value of the 39,XO mouse model for elucidating the genetic basis of psychiatric disorders can be illustrated with an example from our own work. We showed that the attentional deficit observed in 39,XO mice could be 'rescued' with the addition of a small so-called Y<sup>\*X</sup> chromosome containing approximately nine genes, thus implicating one or more of these genes in this particular cognitive function (Davies et al., 2007). On the basis of its expression pattern and associated protein function, we proposed that the human orthologue of the mouse *Sts* gene, encoding the enzyme steroid sulfatase (which cleaves sulfate groups from neuroactive steroids thereby altering their activity), might represent a candidate gene for attentional dysfunction in Turner syndrome and ADHD (Davies et al., 2007); both *Sts* and its human orthologue escape X-inactivation (Li et al., 1996; Salido et al., 1996). Subsequent work in humans has shown that subjects with deletions of *STS* are indeed at significantly greater risk of developing inattentive subtype ADHD (Kent et al, 2008) and that polymorphisms within *STS* may both modulate risk of developing the disorder (Brookes et al, 2008, Brookes et al, 2010) and influence inattentive symptomatology in individuals with ADHD (Stergiakouli et al, 2011). Ongoing investigations in a second mouse model, the 39,X<sup>Y\*</sup>O mouse, which lacks both copies of the *Sts* as a



consequence of an end-to-end fusion of the X and Y chromosomes (Trent et al., 2011; Davies et al., 2009; Odorisio et al., 1998), will permit us to identify and characterise the cellular and systemic mechanisms through which aberrant steroid sulfatase activity may influence vulnerability to disorders of attention.

In addition to providing insights into neurobiological processes sensitive to X-linked gene dosage, the 39,XO mouse may also be a useful tool for identifying processes sensitive to X-linked genomic imprinting, and for identifying and characterising the underlying X-linked imprinted genes. To identify effects dependent upon the latter process, 39,XO mice whose X chromosome is of paternal (39,X<sup>P</sup>O) or maternal (39,X<sup>M</sup>O) origin (generated through two separate crosses) may be compared at the molecular, cellular, systems and behavioural levels. Using such an approach, it has previously been shown that 39,X<sup>M</sup>O mice exhibit a greater degree of behavioural rigidity than 39,X<sup>P</sup>O (or 40,XX) mice (Davies et al., 2005), recapitulating human data whereby impaired social cognition (notably behavioural inhibition) and autism was more frequently observed in 45,X<sup>M</sup> than 45,X<sup>P</sup> Turner syndrome individuals (Skuse et al, 1997). Given that behavioural inflexibility, stereotyped behaviour patterns and restricted interests are hallmarks of autism, the 39,XO mouse may provide insights into the molecular pathogenesis of aspects of this disorder. A candidate maternally expressed X-linked imprinted gene (*Xlr3b*) for the cognitive effect was proposed (Davies et al, 2005; Raefski et al, 2005); however, this gene does not appear to have a human orthologue. Other X-linked imprinted genes have since been identified in mice through comparing gene expression in developing female and male embryos: *Rbox5* (Kobayashi et al., 2006) and the *Fthl17* family (Kobayashi et al., 2010). Whilst *Rbox5* has no close human orthologue, the *Fthl17* family does, although its brain expression and imprinted status remains to be determined. Therefore, to

date, whilst a number of neuroanatomical and psychological phenotypes sensitive to parental origin of the X chromosome have been documented in TS subjects (Section 2), no underlying X-linked imprinted genes have been identified to date in humans; however, new research in animal models may provide exciting new avenues for further investigation (see Section 7).

The 39,XO mouse may be useful for ascertaining the neurobiological effects of X-linked gene haploinsufficiency. Alternative mouse models may be used to examine the phenotypic effects of X-linked gene overdosage. Mice polysomic for the X chromosome (41,XXY) can be readily produced in the laboratory either through generating chimeric mice, or through mating sex chromosome mutant mice (Swerdlhoff et al., 2011). Adult 41,XXY mice recapitulate key endocrinological aspects of Klinefelter's syndrome (notably small testes, decreased plasma testosterone levels, and elevated plasma follicle-stimulating hormone (FSH) levels). Moreover, these mice show impairments in learning (as indexed by a simple Pavlovian conditioning paradigm) (Lue et al., 2005), and differences in gender preference and sociability (Liu et al., 2010); the first two behavioural features are somewhat reminiscent of those seen in individuals with Klinefelter's syndrome (Ross et al., 2008; Schiavi et al., 1988; Bancroft et al., 1982). A second mouse model for Klinefelter syndrome, the 41,XX<sup>Y\*</sup> mouse (possessing a normal X chromosome, together with an additional X chromosome fused end-to-end with a Y chromosome (X<sup>Y\*</sup>)) has been reported as exhibiting normal locomotor, anxiety-related and exploratory behaviours, but aberrant recognition memory (i.e. an impaired ability to recall whether or not an object had been investigated previously and to explore it accordingly) relative to control mice (Lewejohann et al., 2009); these mice, exhibit elevated FSH and luteinising hormone levels and hyperactive Leydig cells (Wistuba et al., 2010), hormonal abnormalities which may partially account for some of the observed behavioural phenotypes.

Together data from 39,XO and X-polysomic mice suggest that X-linked gene dosage is likely to influence a multitude of behavioural endophenotypes in mice. The effects of gene dosage on behaviour reported above may be due to direct effects of X-linked gene overdosage on brain development and/or function, or to intermediary effects on hormonal levels, and further work examining brain gene expression and systemic hormone levels in mouse models will be needed to dissociate between these two possibilities. Such studies may shed light on the molecular basis of neurocognitive abnormalities and psychiatric vulnerabilities in Turner syndrome and Klinefelter syndrome patients, and possibly also into sexually dimorphic phenotypes in healthy individuals.

## **7. Sex-linked effects on autosomal gene function**

Sex-linked genes may influence brain function through the actions of their own gene products, or, alternatively, their gene products may regulate the actions of downstream autosomal genes (or gene products). For example, the action of the enzyme catechol-O-methyltransferase (COMT), an effector of metabolic catecholamine degradation and a molecular candidate for numerous psychiatric disorders and endophenotypes (Tunbridge, 2010), may be modulated by sex with consequent effects on brain dopamine metabolism and behaviour (Chen et al., 2004; Gogos et al., 1998; Harrison and Tunbridge, 2008). The molecular mechanism underlying this interaction is currently unclear, but may involve differential transcription of the COMT gene modulated by estrogens in males and females i.e. an 'intermediate hormonal effect' (Harrison and Tunbridge, 2008).

Recently, Zhang and colleagues (2010) reported significant association between a risk polymorphism in *ZNF804A* (2q32.1) in females (but not males) with schizophrenia; ongoing work in our laboratory aims to

determine how the behavioural and neuroanatomical phenotypes seen in mice deficient for the *Zfp804a* protein may be modulated by gonadal and karyotypic sex. Conceptually similar studies to that of Zhang et al. in which genetic analyses at replicated candidate loci are routinely stratified by sex will undoubtedly be informative in helping to identify robust and specific mechanistic links between risk variants and sex across a variety of disorders.

An extremely hot topic in neuroscience at present is ‘psychiatric epigenetics’; this interest stems from the realisation that epigenetic modifications (i.e. molecular changes such as the addition of methyl groups or histone modifications to DNA that do not alter its sequence but do alter its transcriptional regulation) could potentially provide plausible biological mechanisms through which genetic variants and environmental influences could interact to engender vulnerability to psychiatric disorders (Ptak and Petronis, 2010).

Imprinted genes, which exhibit the unusual and tightly controlled expression patterns described above, may be regarded as the apotheosis of neural epigenetic regulation (Davies et al., 2008). Newly-developed and more affordable technologies have facilitated the identification of a large number of putative imprinted brain-expressed genes. Using a so-called ‘reciprocal cross’ mouse model, paired with Next Generation RNA sequencing methods, Gregg and colleagues have recently proposed more than 1300 novel candidate imprinted genes (a tenfold increase on current estimates), many of which were implicated in metabolism or cell adhesion pathways (Gregg et al., 2010a). Briefly, this experimental *tour de force* involved sequencing the transcriptome of two distinct strains of mice (A and B) to identify polymorphisms specific to each. Male mice of strain A were then mated to females of strain B, and the brain transcriptome of the progeny (AB) was sequenced. In parallel, male mice of strain B were mated to

females of strain A, and the brain transcriptome of their progeny (BA) was sequenced. For non-imprinted genes, transcripts indicative of A and B strains would be expected to be equally represented in both AB and BA transcriptomes. However, for paternally expressed imprinted genes one would expect that transcripts specific to the paternal strain were over-represented in the progeny transcriptome (i.e. A strain transcripts in AB mice, and B strain transcripts in BA mice); conversely, for maternally expressed genes one would expect that transcripts specific to the maternal strain were over-represented in the progeny transcriptome (i.e. B strain transcripts in AB mice, and A strain transcripts in BA mice). The experiment is summarised in **Figure 1A**. Besides hinting at a hitherto underappreciated influence of imprinted genes on mammalian brain function, the study also revealed the exciting possibility that imprinted genes solely expressed from maternally inherited alleles may be a key driver of neurodevelopment, whereas imprinted genes solely expressed from paternally inherited alleles may be more influential in adult brain function.

Of relevance to this review, Gregg and colleagues also performed a parallel study, employing identical methodology to that described above, but with an added level of complexity: the transcriptomes of two adult brain regions of AB and BA mice (medial prefrontal cortex and pre-optic area of the hypothalamus) were also analysed by sex (summarised in **Figure 1B**). The main findings were intriguing: first, the maternally inherited X chromosome appeared to be preferentially activated (and the paternally inherited X relatively silenced) in glutamatergic neurons of the cortex and in the hippocampus. Second, using a relatively relaxed criterion, besides the previously characterised maternally expressed *Xlr3b* gene, the study identified nine novel candidate X-linked imprinted genes in the hypothalamus, and three in the frontal cortex (one gene, *yipf6*, being common to both, but being maternally expressed in the hypothalamus and

paternally expressed in the cortex). It is plausible that human orthologues of these predicted X-linked imprinted genes may influence behaviour and vulnerability to psychiatric disorders in sex chromosome disorders such as Turner and Klinefelter's syndrome, as well as in healthy males and females. Third, and perhaps most importantly, the study revealed 347 possible new imprinted autosomal genes whose imprinted status was dependent upon the sex of the animal in which they were expressed; until the publication of this landmark study, it was assumed that the imprinted status of autosomal genes was independent of sex. Interestingly, females tended to show a greater tendency towards imprinted gene expression in the hypothalamus (perhaps unsurprisingly given the role of this structure in regulating sexually dimorphic reproductive and mothering behaviours previously shown to be sensitive to imprinted gene action (Curley and Mashoodh, 2010)), but there was no such sex bias in the cortex.

Given the propensity for large-scale genomic screens like this to generate false-positives (Oakey and Beechey, 2002), and a relatively relaxed definition of 'imprinting' (as defined by the degree of differential allelic expression) used in the studies described above, it will be important to verify many of the imprinted genes proposed by Gregg and co-workers empirically. If these findings are subsequently confirmed, and can be extrapolated to humans, they will represent a significant advance in our understanding of the ways in which sex (and ultimately sex-linked genetic mechanisms) may elicit spatio-temporally dynamic effects on sexually dimorphic neuroanatomy and neural function via widespread influences on the genome.

## **8. Insights from from sex-linked genetic mutant mice**

Due to our ability to delete or insert specific loci, mice have traditionally been used to explore the normal functions of brain-expressed genes. Mice deficient for the X-linked genes *Fmr1*, *Mecp2* and *Maoa* for example have

provided important insights into the neural pathways disrupted in the neurodevelopmental disorders Fragile-X syndrome (Bhogal and Jongens, 2010) and Rett syndrome (Calfa et al., 2011), and into the behavioural sequelae of monoamine oxidase deficiency (Bortolato et al., 2008) respectively. Mice in which discrete Y-linked genes are deleted cannot be readily generated and characterised due to the lack of recombination of the Y-specific region of the chromosome, to many Y-linked genes being part of larger superfamilies with some degree of redundancy, and to a high proportion of repetitive genetic sequence on the chromosome. Moreover, due to its small size and limited gene content, the Y chromosome has, until recently, been viewed as having little effect on brain function, so the impetus for attempting to create such models has not existed (Kopsida et al., 2009). In the absence of engineered Y-linked gene knockout models, information about the neural function of these genes may be obtained by undertaking neurobiological analyses in mice with small spontaneous or induced mutations (such as the XY<sup>-</sup> mouse (Lovell-Badge and Robertson, 1990)) or in Y chromosomal mutants, such as those previously used to investigate effects on male spermatogenesis and fertility (Burgoyne, 1998) and lacking brain-expressed Y-linked genes such as *Ube1y*, *Kdm5d* (formerly *Smcy*) and *Ddx3y* (formerly *Dby*) (Xu et al., 2002).

In the next few years, it is likely that rats deleted (or transgenic) for sex-linked genes will be generated (Jacob et al., 2010); given the greater existing literature on the neurobiology of the rat than the mouse, the greater availability of neuroanalytical techniques for the rat, and the rat's greater amenability to neurosurgery, studies in this new rodent model organism promise to provide even greater insights into the molecular underpinnings of sex-linked behavioural perturbations.

## 9. Conclusions

The sexes exhibit clear differences in their vulnerability to many common and disabling psychiatric and neurological disorders. Ultimately, these differences must arise from the differential sex chromosome complements in males and females, and from the sex-linked genes contained thereon. Through investigating elegant mouse models we have begun to determine the molecular and physiological substrates through which sex-linked genes may confer vulnerability to certain key disorders; however, it is important to be aware of the caveats associated with such models.

By developing new chromosomally-mutant and knockout/transgenic mouse models (notably conditional models where genes can be deleted/expressed in specific brain regions and/or at specific developmental timepoints) and equivalent rat models, and by further neuroanatomical and behavioural characterisation of existing models, we will be able to generate important new information regarding the role of sex-linked genes in modulating sexual dimorphic mammalian brain endophenotypes. The final goal will be to extensively characterise novel risk/protective sex-modulated pathways associated with brain disorders with a view to using them as targets for more effective sex-tailored therapeutics.



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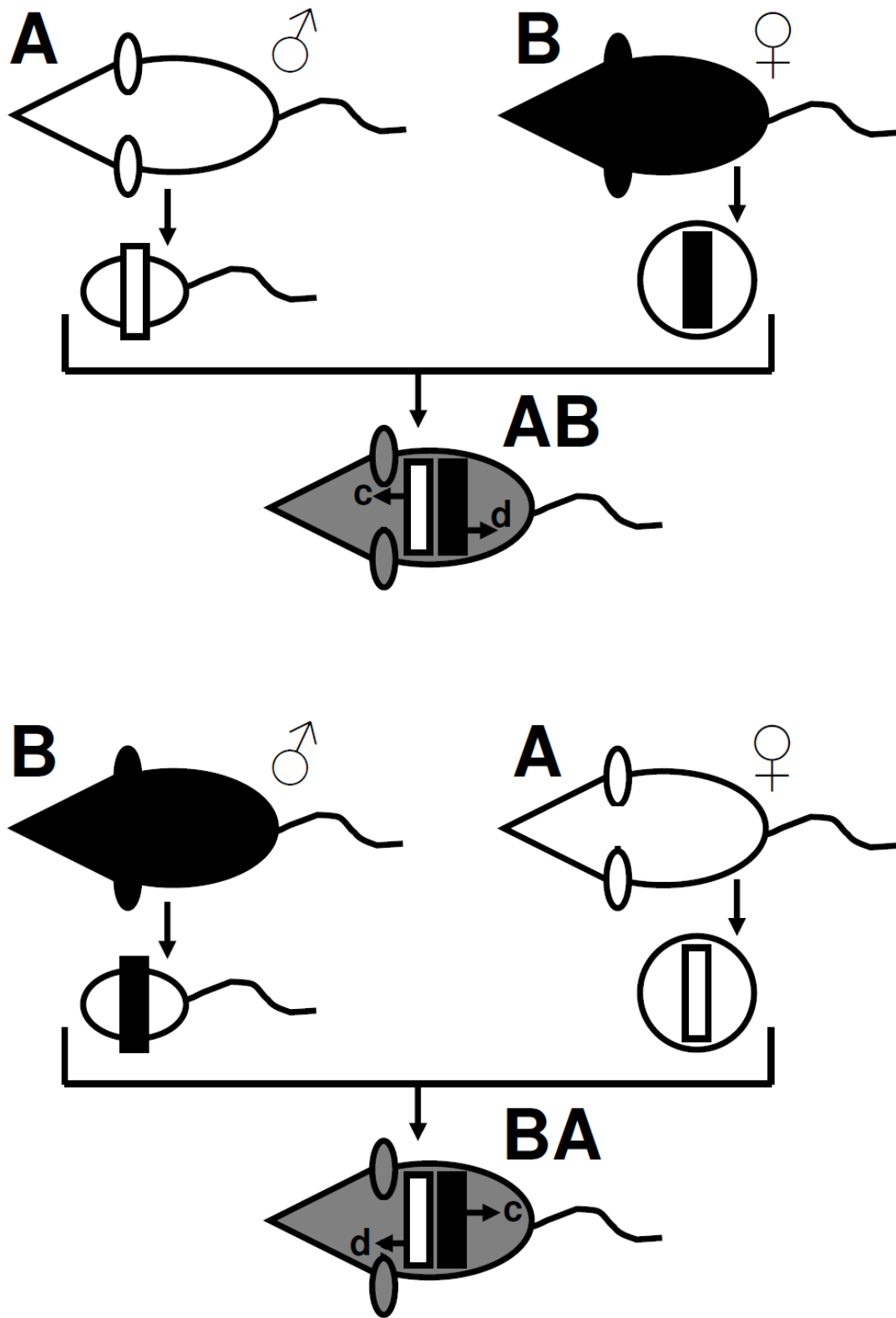
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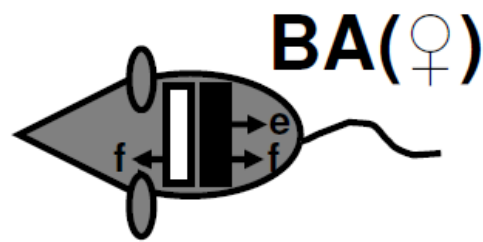
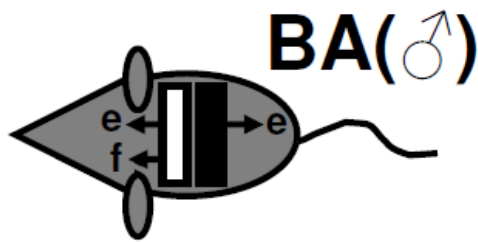
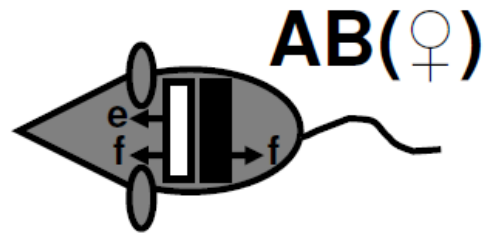
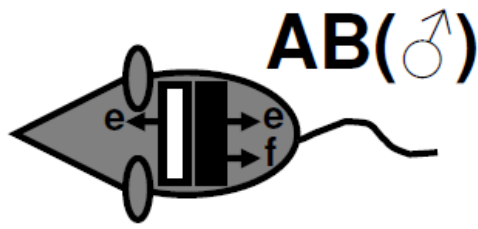
**Figure 1.** Reciprocal cross studies in mice have revealed a potentially large contribution of sex-modulated imprinted genes to brain development/function. **(A)** In the first arm of the reciprocal cross (upper panel), male mice of strain A (white) whose sperm contain chromosomes of strain A (white bar) are mated to females of strain B (black), whose eggs possess chromosomes of strain B (black bar). Upon fertilisation, strain AB mice are produced which inherit half of their chromosomes from their father (white), and half from their mother (black). In the second arm of the reciprocal cross (lower panel), male mice of strain B (black) whose sperm contain chromosomes of strain B (black bar) are mated to females of strain A (white), whose eggs possess chromosomes of strain A (white bar). Upon fertilisation, strain BA mice are produced which inherit half of their chromosomes from their father (black), and half from their mother (white). Sequencing of the brain transcriptomes of AB and BA mice may subsequently be used to identify novel imprinted genes. Paternally expressed genes such as gene c will be expressed preferentially from the chromosome of the paternal strain (transcription indicated by arrows), whereas maternally expressed genes like gene d will be preferentially expressed from the chromosome of the maternal strain. **(B)** For some autosomal genes, the sex of the reciprocal cross offspring may modulate their imprinted status; here, gene e is imprinted (paternally expressed) in female offspring, but expressed from both parentally inherited alleles in male offspring, whereas gene f is imprinted (maternally expressed) in male offspring, but biallelically expressed in female offspring.

**Table 1.** Genes known, or presumed, to escape X-inactivation to some extent in the mouse. Since *Sts* is pseudoautosomal, by definition it escapes X-inactivation, and is not therefore a classical ‘X-escapee’. The human orthologues of *Sts*, *Kdm5c*, *Eif2s3x*, *Kdm6a* and *Ddx3x* reportedly escape X-inactivation; the human orthologue of *Mid1* is subject to X-inactivation

(Greenfield et al., 1998), whilst *Enox* has no human orthologue. Expression data is taken from the Allen Brain Atlas ([www.brain-map.org](http://www.brain-map.org)).







Current gene name (previous aliases)	Associated product and function	Gene location	Degree and main sites of brain expression	References
<i>Sst</i>	Steroid sulfatase: steroid hormone-modulating enzyme	Pseudoautosomal region	Moderate: cortex, thalamus, hindbrain	Salido et al., 1996; Compagnone et al., 1997
<i>Mid1</i> ( <i>Fxy</i> , <i>Trim18</i> )	Midline 1: E3 ligase enzyme	79.19cM	Moderate: olfactory bulb, cortex, hippocampus	Dal Zotto et al., 1998
<i>Kdm5c</i> ( <i>Jarid1c</i> , <i>Smc5</i> )	Lysine (K)-specific demethylase 5C	68.46cM	High: olfactory bulb, cortex, striatum, cerebellum	Carrel et al., 1996; Lopes et al., 2010
<i>Enox</i> ( <i>Jpx</i> )	Expressed neighbour of <i>Xist</i> ; precise function unknown	46.17cM	Unknown	Johnston et al., 2002
<i>Eif2s3x</i>	Eukaryotic translation initiation factor 2, subunit 3	41.52cM	Low: widespread expression	Lopes et al., 2010
<i>Kdm6a</i> ( <i>Utx</i> )	Lysine (K)-specific demethylase 6A	13.45cM	Moderate: hippocampus, cerebellum	Greenfield et al., 1998; Lopes et al., 2010
<i>Ddx3x</i> ( <i>Ddx3</i> , <i>Fin14</i> , <i>Dbx</i> )	DEAD/H-box polypeptide 3: RNA helicase	8.17cM	Very low: undeterminable	Xu et al., 2002; Lopes et al., 2010