Sex differences in molecular neuroscience: from fruit flies to humans

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Abstract | A plethora of discoveries relating to sex influences on brain function is rapidly moving this field into the spotlight for most areas of neuroscience. The domain of molecular or genetic neuroscience is no exception. The goal of this article is to highlight key developments concerning sex-based dimorphisms in molecular neuroscience, describe control mechanisms regulating these differences, address the implications of these dimorphisms for normal and abnormal brain function and discuss what these advances mean for future work in the field. The overriding conclusion is that, as for neuroscience in general, molecular neuroscience has to take into account potential sex influences that might modify signalling pathways.

Epigenetics
Changes in phenotype caused by mechanisms other than changes in the underlying DNA sequence (hence the name epi — 'in addition to' — genetics).

Sex bias
(Also known as sexual dimorphism.) The systematic difference in form or function between individuals of a different sex in the same species. Body features that are affected by sex bias include colour of skin or coat (fur, feathers, et cetera), size and the presence or absence of body parts or behaviours.

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Sex influences are being uncovered at an accelerating pace at all levels of neuroscience — including the molecular level, with sex differences in gene expression and epigenetic regulation. Recent progress has been made by mapping differential expression of genes according to sex in several species from worms to man[1–7]. These studies show that genes are expressed in a sex-biased manner in many tissues, including the brain. Together with substantial prior evidence of morphological and functional brain dimorphisms, this has raised awareness of the importance of sex in molecular neuroscience[8]. Little is known about the mechanisms that control these sex differences in development and in the adult vertebrate brain.

In principle, all differences are ultimately controlled by the gonadal sex determination systems in each species[9]. Clearly, among the factors controlled by the sex chromosomes, hormonal influences are highly important, and extensive reviews on gonadal hormones and their actions, in particular in the CNS, are available[10–12]. In short, testosterone is the main male hormone. It is secreted by the testes of mammals during late gestational and neonatal periods and causes significant brain sexual dimorphism. It is also produced in small amounts by the ovaries of females. Oestrogens, usually known as female hormones, have vital roles in both sexes. In females they are produced primarily by developing follicles in the ovaries, the corpus luteum and the placenta, circulate in the bloodstream and bind to oestrogen receptors in many target tissues, including the brain. Although it may seem counterintuitive, the male brain is masculinized by testosterone only after it has been transformed by the enzyme aromatase into oestrogen, which crosses the blood–brain barrier and enters the male brain. Proposed mechanisms of action of sex hormones in the brain include influences on neurogenesis, cell migration, cell differentiation, cell death, axon guidance and synaptogenesis. However, multiple lines of evidence indicate that hormone-independent mechanisms under the control of the sex chromosome complement are also important[13–20] — the extensive effects of hormones and their regulators are insufficient to explain all of the sex differences in the CNS. Exactly how the different sex chromosomes influence a myriad of somatic genes in both a tissue- and a developmental stage-specific manner remains largely unknown. We focus here on a few known sex-biased regulatory mechanisms operating in the brain independently of steroid hormones. The emerging view is that although many sex-biased genes evolved recently, others show conserved patterns of sex-biased activation. This is true not only for genes expressed in the gonads but also for genes with sex bias in the brain[5,21–32].

This Review begins by surveying some of the many, often surprising, sex differences in gene expression reported to date, including those involving sex and somatic chromosomes (FIG. 1). We then consider mechanisms that potentially underlie these differences, such as those involving gene splicing and epigenetics, and also discuss the contribution of bioinformatics to illuminating such control mechanisms. Finally, we close with a brief consideration of the vast clinical relevance of this topic.

Gonadal sex determination
The biological mechanism that induces the development of the ovaries or testes in an organism. In many species it is genetically determined by the presence of specific chromosomes called sex chromosomes.

Sex-biased brain gene expression in many species

Early studies based on the analysis of very few gene sets[33] suggested that sex bias in gene expression is large in some somatic tissues but limited in the human brain[34]. More recent genome-wide expression studies revealed extensive sex dimorphism in gene expression levels in the rodent brain[4]. This preceded gonadal differentiation[15], indicating that it is independent of hormone action. Interestingly, sex-dependent gene expression patterns differ between the gonads and non-reproductive tissues, suggesting that different control mechanisms operate in these tissues. It is now becoming clear that sex bias in gene expression is extensive in the adult brain of all mammalian species[7] and also occurs in other vertebrates and invertebrates, such as *Drosophila melanogaster*.

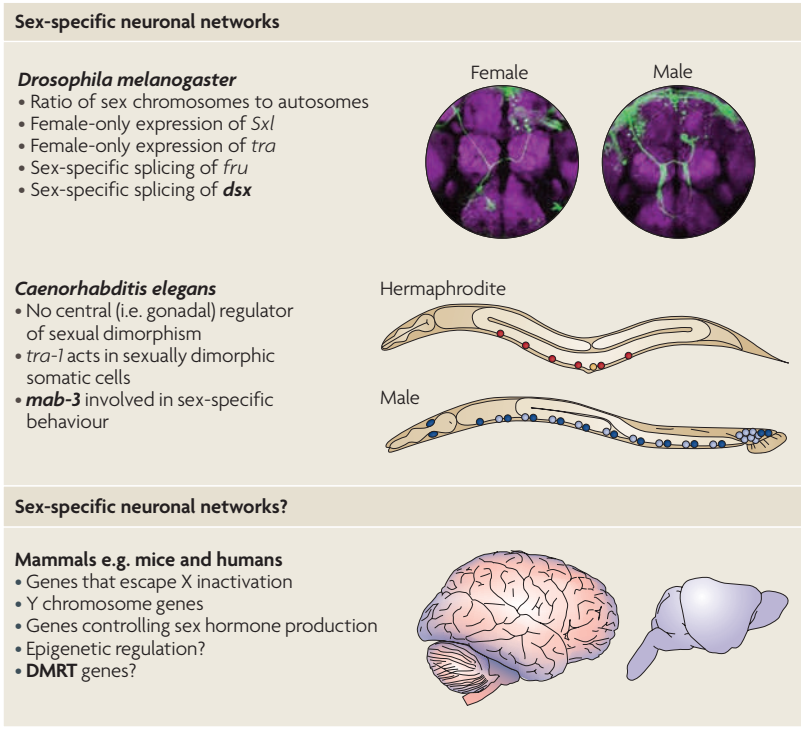


Figure 1 | **Early genetic control of sex determination in the CNS.** The top panel lists the main genetic determinants of somatic sex differences in the brains of *Drosophila melanogaster* and *Caenorhabditis elegans*. In both cases, a cascade of expression of regulatory genes results in the formation of male- and female-specific neuronal networks. The regulatory pathway of genes includes a homologous gene named *doublesex* in *D. melanogaster* and *mab-3* in *C. elegans*. More specific information about each of the genes, their functions and regulation can be found in the main text. The resulting sex-specific neuronal networks are labelled in green in the *D. melanogaster* brains. Sex-specific neuronal differences in *C. elegans* are indicated by red and yellow circles in the hermaphrodites and blue circles in the males (the circles represent neurons contributing to sex-specific networks). The bottom panel lists proposed early determinants of sex differences in the brains of mammals. Among them, the DMRT genes deserve special mention as they are the only genes known to be conserved during evolution from flies to mammals. Some of the members of this family have well-known functions in the sex determination of gonads. Others are exclusively expressed in the brain and may therefore be important for the sex determination of this tissue. Similar to the situation in flies and nematodes, genetic control of sex-specific brain development in mammals may result in the formation of sex-specific neuronal networks — the search for these is an important challenge for the future. *dsx*, *doublesex*; *fru*, *fruitless*; *Sxl*, *Sex lethal*; *tra*, *transformer*.

Sex differences in human and non-human primates. Influences of aging on gene expression are different in the brains of women and men[35]. One key question concerns whether these dimorphisms result from environmental differences between the genders — including, for example, food intake, drug use, education differences and other cultural influences that have been shown to affect gene expression differently in both sexes — or whether these gender differences are inherited. A recent evolutionary study showed that a 'gene signature' of sex-biased expression (defined as a pattern of two or more genes altered in a sex-specific way in two or more primates) was conserved during the evolution of primates, including humans[5]. These results not only show that genes are involved in sex bias in the brain of primates but also suggest that at least some of the mechanisms for the control of sex biases are inherited.

Sex differences in rodents. A general mechanism or regulatory cascade for controlling sex differences in gene expression in the mammalian brain is not known. However, the rapidly increasing numbers of reports of sex differences in gene expression in rodents might lead to the discovery of potential mechanisms. In the past, the extent of sex-biased effects in mammals was obscured by the fact that most investigators examined animals of only one sex (typically males)[36–41] or did not specifically mention the sex of the tested animals[42–44]. For example, in knockouts of the arginine vasopressin receptor1a, anxiety-like behaviour was initially reported only in male mice[45], and only later was it reported that the changes were sex specific, with lack of anxiety-like effects in female knockout mice[46]. It is noteworthy that there has been a recent increase in knockout mouse studies that have reported results from both sexes in separate analyses. Not uncommonly, key effects prove to be sex specific: a genetic manipulation may have an effect in only one sex or may have the opposite effect in the sexes (TABLE 1). Indeed, such sex differences are the 'elephant in the room' for knockout studies.

Drosophila studies. Recent investigations in *D. melanogaster* stimulated sex-related research efforts in other organisms. As much as 50% of the *D. melanogaster* genome exhibits sexually dimorphic expression[47]. A large sex bias remains when the gonads are removed[48], indicating that this bias is independent of hormonal control. These differences are even more prominent when alternative splicing is taken into account, as up to 22% of the alternatively spliced genes that produce multiple transcripts have sex-specific bias in the proportion of their splice variants[49]. This extreme sex divergence is one of the most important mechanisms for molecular evolution and speciation in flies[50]. As in rodents and humans, gender-biased gene expression in flies is tissue specific[48]. One caveat, however, in evaluating the importance of previous studies in *D. melanogaster* for neuroscience is that all the work was carried out using the complete head, which is mostly composed of large eyes. Future specific analysis of different brain regions will probably clarify this point and might provide exciting insights.

Gonadal hormones
(Also called sex steroids or sex hormones.) Hormones produced in the gonads, including oestrogen and testosterone. These hormones interact with oestrogen or androgen receptors.

Gonad
The organ that makes gametes, the germ cells used for fertilization. The gonads in males are the testes or testicles and the gonads in females are the ovaries.

Hormone dependent or independent?

In most of the examples in TABLE 1 it is not known whether the control of sex differences is independent of sex hormones. Recently, the evaluation of hormone-dependent versus -independent effects in the mouse brain has been greatly facilitated by genetic manipulations of the *SRY* gene that led to the generation of XX males and XY females[20,51]. *SRY* is the key regulator for the formation of the testes in mammals. When the testes are formed, testosterone is produced, with well-known masculinizing consequences for all somatic tissues, including the brain. In *Sry*-manipulated animals, the sex of the gonads is separated from the chromosomal sex (BOX 1). Recent experiments using *Sry*-manipulated mice have revealed multiple sex chromosome influences that are independent of hormonal effects, including on protein translation[52], nociception[19] and apoptosis as well as behaviours such as aggression[53] and habit formation[18] (BOX 1). Therefore, future studies should systematically examine and report potential sex biases, including the lack of an effect of a genetic manipulation in, for example, females, as these results are equally important for the overall understanding of gene–function relationships.

X chromosome inactivated genes

There is large diversity in the genetic mechanisms that determine gonadal sex in different species. In many animals, such as flies, birds and mammals, gender is

Table 1 | **Some sex-specific effects in knockout mice**

Gene symbol	Protein name	Mouse type	Effect	Sex	Refs
Htr1b	Serotonin receptor 1B	KO	Enhanced aggressive behaviour	Male	101
Htr1b	Serotonin receptor 1B	KO	Increased body weight from birth Increased body weight after 8 weeks of age	Male Female	102
Acvr2a	Activin receptor type II	KO	Deficit in reproductive behaviour	Male*	103
Apoe	Apolipoprotein E		Impairments in learning a water maze task and in vertical exploratory behaviour	Female > male	104
App	Amyloid precursor protein	MP	Lower Cu^{2+} and higher Mn^{2+} levels	Female	105
App	Amyloid precursor protein	MP	Higher levels of $AB_{1–40}$ and higher levels of lipid peroxidation products	Female	106
Ar	Androgen receptor gene	NM	Better perfomance in the water maze	Female	107
Bdnf	Brain-derived neurotrophic factor	CON-forebrain	Hyperactivity Increased depression-like behaviour	Male Female	98
Camkk2	Calcium/calmodulin-dependent protein kinase kinase 2, beta	KO	Long-term memory impairment and regulation of alternative splicing factors	Male	76
Cb1 (also known as *Cnr1*)	Cannabinoid receptor type 1	KO	Increased struggling in the forced-swim test	Males	108
Er (also known as *Sfn*)	Oestrogen receptor	Luc-ERE	Oestrogen receptor activity in CNS before gonad formation	Female	109
Gabrd	$GABA_A$ receptor, delta subunit	KO	Enhanced acquisition of tone and context fear	Female	110
M1 (also known as *Chrm1*)	Muscarinic receptor 1	KO	Decreased corticosterone response to muscarinic agonist	Female	111
M2 (also known as *Chrm2*)	Muscarinic receptor 2	KO	Increased ACTH responses to muscarinic agonist	Male	111
Mc1r	Melanocortin 1 receptor	KO	κ-Opioid analgesia	Female	112
Npas2	Neuronal PAS domain protein 2	KO	No rebound in NREM sleep after sleep deprivation	Male	113
Park2	Parkin 2	KO	Increased density of cannabinoid receptors in substantia nigra	Female	114
Sert (also known as *Slc6a4*)	Serotonin transporter	KO	Increased serotonin synthesis	Female > male	115
Th	Tyrosine hydroxylase	LacZ	Increased TH expression after gonadectomy Decreased TH expression	Males Female	116
Trp2	tRNA proline 2	KO	Unable to recognize the sexual identity of their conspecifics	Males*	117
Not applicable	Not applicable	Ts65Dn	Lower ACTH and higher corticosterone levels after predator exposure	Female	118
V1ar (also known as *Avpr1a*)	Arg vasopressin receptor 1a	KO	Impaired social recognition and reduced anxiety-like behaviour	Male	46

ACTH, adrenocorticotropic hormone; CON, conditional knockout; ERE, estrogen-responsive element; KO, knockout; LacZ, LacZ-transgenic mice containing the LacZ gene under the control of the promoter of the gene under study; Luc, mice containing a luciferase reporter; MP, transgenic animals with a mutated protein; NM, natural mutant; NREM, non-rapid eye movement; Ts65Dn, mice with trisomy in 136 genes used as a model for Down syndrome. *No females were tested.

determined ultimately by the presence of sex chromosomes in the fertilized egg, whereas other organisms, such as many reptiles, fish and amphibians, display temperature- or behaviour-dependent sex determination[54]. Among the organisms with sex chromosomes, different complements have evolved. For example, whereas it is the male flies, rodents and humans that have two different sex chromosomes (X and Y), it is the female birds (with Z and W) that are hemizygous[9].

Genes on the sex chromosomes could potentially control brain development and brain function in many ways, should they function in a sexually dimorphic manner during brain development. Well-tuned control systems ensure that many of the X chromosome genes are equally expressed in both sexes despite being present at the ratio 2/1 in females and males. Compensation can be achieved by increasing or decreasing gene expression, and such mechanisms are known to occur in nematodes[55,56], *D. melanogaster*[57,58], birds[59] and mammals[60]. In mammals, for example, gene expression is tuned down through the inactivation of one of the X chromosomes in females, a process that is initiated by a gene named *XIST*. The neuroscientific significance of these compensation mechanisms is illustrated by the fact that individuals with greater or fewer copies of the sex chromosomes exhibit multiple neurodevelopmental and behavioural abnormalities[61,62]. However, it is also now apparent that many genes that are located on the X chromosome are expressed at different levels in females and males[63], escaping the regulatory compensation system. This is called 'escape of X inactivation'. For example, six X-linked genes, which are paralogues of Y-encoded genes (*Usp9x*, *Ube1x* (also known as *Uba1*), *Smcx* (also known as *Kdm5c*), *Eif2s3x*, *Utx* (also known as *Kmd6a*) and *Dbx1*), are expressed in the adult female mouse brain at significantly higher levels than in the brains of males[64].

In addition, a disproportionately high number of genes on the X chromosome are involved with mental function[65]. Future studies of X chromosome genes that escape inactivation during brain development will be required for a complete understanding of the genetically based control of sex differences in brain function and cognition.

Regulation of Y and X chromosome paralogues

As mentioned above, some of the genes encoded on the Y chromosome are paralogues for X chromosome genes. It has been suggested that the expression of these genes might compensate for differences in gene dosage due to escape from X inactivation. However, in several cases the two paralogues are regulated in different ways and therefore may have different functions as they may not be expressed in the same cells at the same time.

One example is the protocadherins (PCDHs), members of the cadherin superfamily that are involved in cell–cell interactions during the development of the CNS[31]. In humans the X-linked (PCDHX) and Y-linked (PCDHY) genes share 98.3% amino acid identity, but the small differences in amino acid sequence result in a longer signal peptide (that may contribute to differences in processing and export) and a shorter cytoplasmic domain (that may result in the activation of different intracellular signalling pathways) in the Y paralogue[31]. Furthermore, PCDHX escapes from X inactivation[66] and although both the PCDHX and the PCDHY genes are mainly expressed in the brain, the promoters of the two paralogues are different and there is regional specificity, for example PCDHX is predominantly expressed in the cerebellum.

Another example of X and Y chromosome paralogues that potentially operate in a different manner in the brain are the histone demethylases UTX and UTY[67]. In mice UTX escapes X inactivation, and UTX and UTY have different expression patterns in the brain, particularly in the hypothalamus and amygdala. These differences may result in altered demethylation of histone 3 and therefore in differences in the epigenetic regulation of gene expression between the sexes (see the section on epigenetic regulation below).

The Y chromosome encodes SRY, which acts as the key regulator for the formation of the testes in mammals

Genome-wide expression analysis
Examination of RNA expression variation across the human genome, designed to identify associations with observable traits.

Alternative splicing
(Also known as differential splicing.) Variations of the splicing mechanism in which the exons of the primary gene transcript are separated and reconnected so as to produce alternative ribonucleotide arrangements.

SRY
(Sex-determining region Y). A gene encoded on the Y chromosome in many placental mammals. It encodes a transcription factor that initiates the formation of the testicles in males.

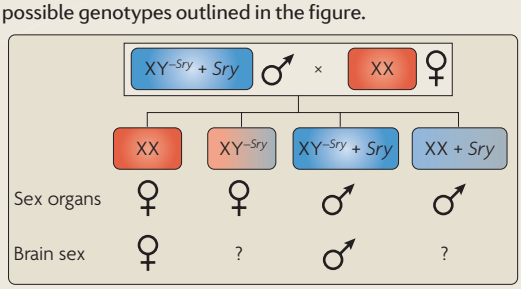
X inactivation
The process by which one of the two X chromosomes in female mammals is not expressed. Inactivation occurs at random in each cell, resulting in a mosaic of expression in each XX individual.

Paralogues
One type of homologous gene. Homologous genes are those that have a common ancestor. Paralogous genes were separated during evolution by a gene duplication event.

Box 1 | **Addressing the dependence of sex differences on sex hormones in the brain**

An ideal way to evaluate whether a sex bias in the brain is controlled by sex hormones or by the chromosome complement is to dissociate the production of sex hormones from the presence of a Y chromosome. To accomplish this, transgenic mice were first constructed so that the sex determination gene *Sry* was removed from the Y chromosome. The resulting heterochromosomal individuals were called 'XY^{-Sry}' to indicate the absence of *Sry*. In the absence of this gene, no testicles are formed and no testosterone is produced. In a second set of constructs, the *Sry* gene was reinserted into the genome, but this time on one of the autosomes. These 'XY^{-Sry} + *Sry*' individuals develop as normal males. When one of these males is crossed with a regular female, the progeny has any of the four possible genotypes outlined in the figure.

A gene with sexual dimorphism in the brain of normal females and males would present a female phenotype in the XY^{-Sry} individuals if the dimorphism were controlled by sex hormones but a male phenotype if it were controlled by the presence of a Y chromosome. Similarly, gonadal control would produce a male phenotype in 'XX + *Sry*' brains, whereas Y chromosome-mediated control would result in a female phenotype. This 'four core genotype' model thus provides a powerful new way to disentangle hormonal and chromosomal effects on brain development and function.



(discussed above). SRY is also expressed in the human brain[13,68,69], but its precise role during brain development remains unknown.

Differential splicing of somatic genes with sex bias

The male-specific expression of sex-biased alternative splice variants in the brain has recently been observed in multiple species. At present, the expression of different splice variants of the somatic gene *fruitless* (*fru*) in *D. melanogaster* neurons is the most elegant example of sex-biased differential gene splicing that is necessary for phenotypic sex differences in the brain[70]. In this case, the expression of FRUM induces the formation of a male-specific neuronal network in the brain that is involved in male mating behaviour.

The cascade responsible for the sex-specific splicing pattern of *fru* transcripts is well known (FIG. 1). Briefly, the ratio of sex chromosomes to autosomes activates in females the expression of the gene *Sex lethal* (*Sxl*). *Sxl* regulates the splicing of the *transformer* (*tra*) gene transcripts, resulting in female-only expression of TRA. TRA, together with TRA2 (expressed in both sexes), controls the splicing of *fru* transcripts, producing female- and male-specific isoforms of a transcription factor (FRUF and FRUM, respectively). FRUM is specifically expressed in a subset of neurons that promote male-specific behaviour, including courtship[71]. The neuronal network that these neurons constitute is stimulated by a male-specific pheromone called *cis*-vaccenyl acetate (cVA) that binds to the odorant receptor OR67D, which is present in one set of FRUM-expressing cells[72].

This well-established example in flies suggests that differential splicing of somatic genes may also be a key mechanism for the control of sex differences in the brains of other species. For example, gender-biased differential alternative-splicing patterns of the transcriptional cofactor CA150 are observed in the parasitic worm *Schistosoma mansoni*[73]. This could generate a cascading effect, influencing the transcription and differential splicing of several mRNAs, including those involved in egg laying. Interestingly, the human homologue of the CA150 protein, which is expressed in the brain particularly in striatal neurons, has been described as a transcriptional cofactor that influences the alternative splicing of genes *in vitro*.

In teleost fish, the transcription factor KISSPEPTIN-1 receptor, which has been implicated in the onset of puberty, was recently shown to be spliced in a sex-dependent and developmental-stage-dependent manner in the brain[74]. Moreover, the sex bias of the splice variants in the brain was different from that observed in the gonads, suggesting that there are different functions and control mechanisms in these two tissues.

Sex bias of differential splicing in the brain has recently been observed in multiple species. Interestingly, the Indian mugger crocodile's sex is determined by the incubation temperature of the developing embryos[75]. In these reptiles, a temperature-sensitive promoter and temperature-sensitive splicing factors may regulate the transcription factor Dmrt1, which is encoded by a somatic gene. This gene's transcripts also undergo sex-biased differential splicing in mammals, but the control mechanisms as well as the function of each splice variant remain to be elucidated.

In mammals, one example of sex-biased alternative splicing in the brain involves upregulation of the splicing factors PTB-associated splicing factor (PSF; also known as SFPQ) and SRP20 (also known as SFRS3)[76], both of which are expressed at higher levels in the male rodent hippocampus. In humans, Ca^{2+} channels — in particular voltage-gated Ca^{2+} channels, which have key roles in neurotransmitter release — are extensively spliced in an age- and gender-biased manner[77].

Although genome-wide approaches to search for differential expression in mammals are not yet well developed, recent results indicate widespread sex-biased expression of splice variants in mouse liver[78]; genome-wide studies investigating brain tissue are pending. These analyses are likely to reveal the extent and importance of sex-biased differential splicing in the brain.

Lessons from nematodes

Nematodes are a classical model used to study the development and composition of all cells in the CNS. Moreover, the process of sexual differentiation of the CNS has been elucidated in detail only in *Caenorhabditis elegans*. The hermaphrodites and the males of *C. elegans* share 294 neurons, but both sexes also have sex-specific neurons — 8 in hermaphrodites and 89 in males — that are essential for sex-specific behaviours. These differences, as well as all somatic differences, are ultimately controlled by the *tra-1* gene, a master regulator of *C. elegans* sexual differentiation (for a more detailed review on sex differences in *C. elegans*, including in the nervous system, see REF. 79). This gene encodes a transcriptional repressor that blocks the expression of male-specific genes in hermaphrodites. The terminal effector of the TRA-1-regulated pathway in CNS development in *C. elegans* is the transcription factor MAB-3, which belongs to a family of somatic transcription factors called the DM-domain genes. This family is the only known conserved molecular link between sex determination systems in metazoans. In fact, these genes are homologous to *doublesex* in *Drosophila* spp. and to the DMRT family in vertebrates[23,26,80–84]. They encode putative transcription factors and have undergone frequent independent events of gene duplication during the course of evolution, resulting in variable numbers of isoforms between phyla. Interestingly, in vertebrates some members of this gene family have lost their expression in the gonads and are expressed only in the brain[85], suggesting that they have acquired functions related to CNS development or regulation.

Forming a complete understanding of normal brain development in vertebrates will probably involve elucidating the interplay between factors encoded by the sex chromosomes, such as gonad-determining factors, brain-specific proteins and hormonally controlled mechanisms that collectively control somatic sex determination and sex biases in brain function and behaviour (FIGS 1,2). There are probably other, as yet unknown, sex determination factors active during brain development

Genomic imprinting
Different expression of a gene, depending on the sex of the parent who transmits it. One of the alleles is imprinted or marked to be silenced, for example by methylation.

that will be identified in this relatively simple CNS, and their discovery might initiate parallel studies in more complex animals.

Beyond genetics: inherited epigenetic control

Genomic imprinting affects the development of several mental disorders in a sexually dimorphic manner[86], and there is accumulating evidence for effects of other inherited epigenetic mechanisms, including DNA methylation, histone modifications, nucleosome repositioning, higher-order chromatin remodelling, mechanisms involving non-coding RNA, and RNA and DNA editing[87,88]. Recent experiments suggest that inherited epigenetic control should be added to the list of hormone-independent mechanisms that regulate sex differences in the brain. For example, histone 3 modifications are sexually dimorphic in the developing mouse brain independently of testosterone treatment[89]. Moreover, as indicated above, histone demethylases encoded on the X and Y chromosomes[67] also contribute to epigenetic mechanisms that regulate sex differences. It is likely that epigenetic mechanisms constitute an important, although largely unexplored, part of genetically based sex influences on the brain.

Control of sex differences in the primate brain

Specific regulators acting early during brain development, before the gonads mature, and resulting in the numerous sex differences found in the adult primate brain have not yet been identified. However, indirect regulators acting after the formation of the gonads, such as oestrogens and androgens, are well-known factors for sex differences in the brain. Oestrogen signalling is primarily conveyed by the oestrogen receptors alpha and beta. These receptors bind to specific DNA sequences — estrogen-responsive elements (EREs) — resulting in the transcriptional activation of genes[90] (FIG. 2). Similar to oestrogens, androgens exert their function by binding to androgen receptors, which in turn cause the expression of target genes by binding to different sets of androgen-responsive elements (AREs). Recent advances using computational methods make it possible to identify different types of AREs, called classical and specific AREs, in mammals[91].

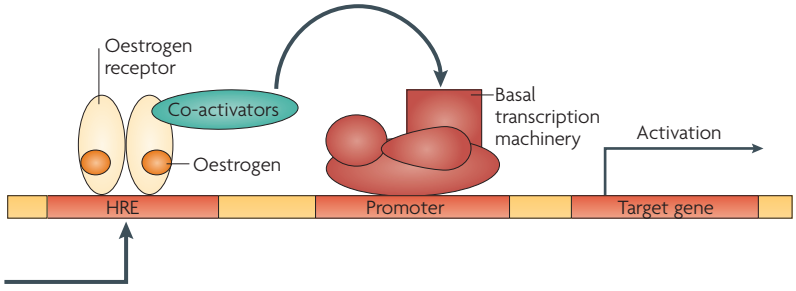


Figure 2 | **Regulation of gene expression by hormone receptors.** Oestrogen molecules form a complex with oestrogen receptors (ERs). This complex can then bind a hormone-responsive element (HRE) (in this case an oestrogen-responsive element (ERE)). The ERE–ER complex interacts through co-activators with the basal transcription machinery to increase the transcription of target genes in a hormone-dependent manner.

More research is required to elucidate the role of these recently identified AREs in the establishment of brain-specific gender differences. The presence of direct regulators acting independently of sex hormones is suggested by systematic bioinformatic searches of sex-biased genes in primate brains, which have demonstrated that many of these genes do not contain any known AREs or EREs[5]. The absence of hormone-responsive elements is not by itself proof of the presence of hormone-independent regulators. However, this fact, together with the demonstration that some sex biases in the brain are controlled in many species by sex chromosomes independently of hormones[13–20], strongly suggests the existence of somatic regulators in the brain. These additional regulators should share several characteristics. First, they should be expressed in brain tissues, possibly during development. Second, they should show some form of sexual dimorphism in expression and/or function. Third, their expression in one sex should be finally controlled by the sex chromosome complement. Somatic regulators of sex differences in the brain probably act in a cascade of events similar to those described above for flies and nematodes.

As is the case for rodents, the first candidates in a search for genes that regulate sex determination in somatic tissues in primates are genes encoded on the sex chromosomes. The gene that encodes the transcription factor SRY (discussed above) has been named as potentially the first regulatory gene to have a role during sex-specific brain development in primates, based on its expression in specific cells in the adult CNS[92,93]. However, although *SRY* is known to be expressed in specific cells in the adult human brain[68], its potential brain-specific functions remain to be elucidated. Moreover, in a recent study using brains from human embryos, although one-third of the Y chromosome-encoded genes are highly expressed in the brain before birth, *SRY* expression could not be detected in any of the regions analysed. This suggests that the main controller of sex bias in the brain is different from the one that controls the formation of the testicles[94].

Many other genes encoded in the sex chromosomes are candidates and may act early in regulatory cascades that determine sex differences. The current version of the database Ensembl for *Homo sapiens* includes 1,155 genes on the X chromosome, of which 39 are transcription factors, and 130 genes on the Y chromosome, of which 5 (including *SRY*) are transcription factors. Although many genes on the X chromosome are expressed in the developing brain, only a fraction of those escape X inactivation[95]. In some cases, X inactivation starts at very early stages of embryonic development, well before CNS formation, when the embryos are composed of only a few cells[95]. Of the genes that escape X inactivation, only those expressed later in development, during the period of brain formation, are potential regulators of sex bias in the brain. A systematic search of sex-biased genes expressed in the primate brain during development will be needed to identify early somatic determinants of dimorphic brain development.

Clinical consequences of sex-biased genes

Attention to sex differences that are caused directly or indirectly by changes in the sex chromosome complement has many implications beyond the understanding of the basic physiology of the brain. In fact, many of the genes associated with animal models of several neurological diseases, such as Huntington's disease[96], cerebral ischaemia[97], depression-related phenotypes[98] and Alzheimer's disease, as well as with cognitive ability models, such as mice lacking functional APOE[99], have sex biases in disease development, pathological processes and recovery mechanisms. However, precisely which control mechanisms for sex-biased gene expression are affected in these disorders is not known. Understanding the mechanisms that underlie the diseases will be essential for the development of new gender-specific therapies[100].

However, caution should be exercised when translating research from mice to humans, particularly with regard to hormone-dependent effects. Alterations in sex hormone levels can result directly from genetic modification (for example of hormone receptors or their regulators) or from environmental influences, such as the use of contraceptives, diet, hormone therapy and other drugs that modify steroid levels. Both types of influence will have important (although different) implications for understanding and treating clinical disorders. Clearly, more attention needs to be paid to the potential clinical implications of genetically based sex differences, whether direct or indirect.

Conclusions

It is now clear that genetically controlled sex differences are pronounced in the brains of many species, with consequences for normal and abnormal brain physiology. These sex differences result in differences in neuronal activation and network activity and may ultimately influence behavioural traits.

Contrary to well-known pathways for sex determination in the gonads, the main regulators of somatic sex differentiation in most species are not known. Among the mechanisms that control sex-biased gene expression are hormone, sex chromosome, alternative splicing and epigenetic control mechanisms. The lessons learned from *D. melanogaster* and nematodes, together with current research focusing on sex differentiation of the mammalian brain, will help to identify a cascade of somatic regulators involved in the sex differentiation of the brain during development.

Although the identity of the main controllers of brain sex differences is not known in mammals, the sex chromosomes are good candidates for being the first step of the controlling molecular pathways. In this respect, genes on the X chromosome that escape dosage-related compensation of expression levels, as well as genes on the Y chromosome that are expressed during early brain development, are good candidates for mediating the early phase of establishing sex differences. Sex chromosome genes may in turn control regulatory cascades relating to the expression of other genes encoded in the autosomes. At least some of the sex-biased controlling genes, such as *SRY*, are conserved during evolution, and therefore evolutionary studies might provide a new means of searching for the mechanisms that control sex differences in the brain.

Regardless of the current lack of answers to many important mechanistic questions, most notably those concerning sex-biased expression of somatic genes, there is overwhelming evidence for genetically based sex influences on brain function, clearly indicating that increased awareness of, and systematic investigation into, molecular-level sex influences will yield tremendous dividends for both basic and clinically applied brain science.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
Acvr2a | *Apoe* | *App* | *Ar* | *Bdnf* | *Camkk2* | *Cb1* | *Dbx1* | *doublesex* | *Eif2s3x* | *Er* | *fru* | *Gabrd* | *Htr1b* | *M1* | *M2* | *Mc1r* | *Sert* | *Smcx* | SRP20 | *SRY* | *Sxl* | *Th* | *tra* | *Trp2* | *Ube1x* | *Usp9x* | *Utx* | *V1ar* | *XIST*
OMIM: http://www.ncbi.nlm.nih.gov/omim
Alzheimer's disease | Huntington's disease
UniProtKB: http://www.uniprot.org
OR67D | PSE | TRA2

FURTHER INFORMATION
Elena Jazin's homepage: http://www.fu.uu.se/devbiol/lazin/jazin_index.html
Larry Cahill's homepage: http://cahill.bio.uci.edu/
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EREs in human and mouse: http://www.mapageweb.umontreal.ca/maders/eredatabase/index.html
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