Why sex matters for neuroscience

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Abstract | A rapidly burgeoning literature documents copious sex influences on brain anatomy, chemistry and function. This article highlights some of the more intriguing recent discoveries and their implications. Consideration of the effects of sex can help to explain seemingly contradictory findings. Research into sex influences is mandatory to fully understand a host of brain disorders with sex differences in their incidence and/or nature. The striking quantity and diversity of sex-related influences on brain function indicate that the still widespread assumption that sex influences are negligible cannot be justified, and probably retards progress in our field.

In his 1966 article in *Scientific American* entitled 'Sex differences in the brain'[1] Seymour Levine summarized the current knowledge on the topic. He described different mating behaviours in male and female rats, and evidence indicating how sex hormones influence these behaviours. Levine mentioned only one brain region, the hypothalamus, known by then to be a crucial regulator of hormone action. For the neuroscience mainstream, 'sex differences in the brain' came to refer exclusively to sex behaviours, sex hormones and the hypothalamus.

Abundant research since Levine's article disproves this idea. In fact, the past 5–10 years have witnessed a surge of findings from animals and humans concerning sex influences on many areas of brain and behaviour, including emotion, memory, vision, hearing, processing faces, pain perception, navigation, neurotransmitter levels, stress hormone action on the brain and disease states. Even otoacoustic emissions (audible 'clicks' made by the inner ear) differ reliably between the sexes, being both louder and more frequent in female than male adults, children and infants[2]. The advent of human brain-imaging techniques such as positron emission tomography (PET) and functional MRI (fMRI) has heightened awareness of sex differences by revealing sex influences on brain functions for which the sex of participants was previously assumed to matter little, if at all. Concurrently, animal research has increasingly documented new, often surprising, sex influences on the brain.

This article highlights some of the more important recent investigations and their implications. I begin by addressing some widely held misconceptions regarding sex differences in the brain. Next, I focus on particular sex difference findings, such as those concerning the hippocampus and amygdala, which are likely to be of broad general interest to the field. Finally, I discuss ways in which the issue of sex differences affects our understanding of disease states. Reviews covering classic sex influences on, for example, verbal and spatial behaviours, and covering well-known anatomical differences (such as those involving the corpus callosum) can be found elsewhere[3–6].

Some common misconceptions

To best appreciate the evidence for sex influences on the brain, it is helpful to first consider some common misconceptions about the topic. Although not often stated in print, investigators commonly encounter these misconceptions in their neuroscience colleagues. The first misconception is that sex influences are small and unreliable. Although there is some evidence for, and some against, this view when purely behavioural studies are considered, there is no evidence to support it regarding sex differences in the brain[3]. No evidence of which I am aware suggests that the average effect size in the domain of sex influences on brain function differs from the average effect size found in other domains of neuroscience[3].

A second, and related, misconception holds that average differences between the sexes result from a few extreme cases in a distribution. Again, I know of no evidence to support this general conclusion.

A third, also related, misconception holds that the differences within a sex are much more substantial than those between the sexes, the implication being that sex influences can therefore be dismissed as trivial. It is ironic that this view is advanced so often by scientists who use the statistical analyses (for example, ANOVA) that reveal sex influences and that specifically compare within versus between group variance to detect significant differences.

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Box 1 | **The menstrual cycle**

The effects of circulating sex hormones cannot fully account for all sex differences observed in the adult brain, as many sex differences persist even in the absence of these hormones. That said, hormonal influences due to the menstrual cycle can be detected on an impressively wide array of behaviours. For example, substantial evidence indicates that sex hormones influence learning and memory processes[83], and interact with stress hormones to do so. In rats, the stage in the oestrus cycle has been shown to interact with the level of stress in a learning situation to influence memory for that situation[84]. Similarly in humans, the stage in the menstrual cycle significantly influences performance on both verbal and spatial tasks[85], and modulates the neural circuitry associated with arousal[86]. Menstrual cycle influences have even been detected on the degree of hemispheric asymmetry associated with various cognitive tasks[87]. As a final example, menstrual cycle influences exist on brain responsiveness to addictive drugs such as cocaine[88] and amphetamines[89], factors that will probably help to explain sex differences in addictive processes[90].

A fourth widespread misconception is that all sex differences, once established, can be completely explained by the action of sex hormones, typically oestrogen. The unstated assumption underlying this view is that male and female brains are identical except for fluctuating (and unnecessarily complicating) sex hormone influences. Sex hormones are crucial for many sex differences (BOX 1), but, equally, cannot explain all observed sex differences. For example, a recent study reported several sex differences in cocaine-seeking behaviour in rats and, in addition, found that these differences were unaffected by oestrus state[7]. The view that circulating sex hormones in adult animals fully account for all sex differences in the brain also ignores the pronounced organizational effects of these hormones on brain development, as well as rapidly growing evidence for genetic mechanisms that induce sex differences in the brain independently of hormone action (reviewed in REFS 8,9).

A final misconception holds that if no sex difference exists in a particular behaviour, it can be assumed that the neural substrates underlying that behaviour are identical for both sexes. However, numerous studies report sex differences in neural activity despite no behavioural difference between the sexes. For example, Piefke and colleagues[10] examined the neural correlates of retrieval of emotional, autobiographical memories in men and women. Memory performance did not differ between the sexes, nor did the degree of emotion induced by retrieval. However, brain regions associated with retrieval in the two sexes differed significantly. As a second example, Grabowski *et al.* examined the neural correlates of naming images[11]. Men and women performed the task equally well, but the patterns of brain activity associated with their performance differed significantly. Findings such as these indicate that isomorphic performance between the sexes does not necessitate isomorphic neural mechanisms. Indeed, as De Vries[12] has effectively argued, neural sex differences can, in some cases, create behavioural sex differences, but might, in other cases, prevent them (when, for instance, they would be maladaptive) by compensating for sex differences in other physiological conditions, such as sex hormone levels.

Voxel-based morphometry (VBM). A computational approach to neuroanatomy that measures differences in local concentrations of brain tissue through a voxel-wise comparison of multiple brain images. The value of VBM is that it allows for comprehensive measurement of differences, not just in specific structures, but throughout the entire brain.

Functional and structural dimorphisms

Sex differences exist in every brain lobe, including in many 'cognitive' regions such as the hippocampus, amygdala and neocortex[13] (FIG. 1). Sex differences can also be relatively global in nature. For example, widespread areas of the cortical mantle are significantly thicker in women than in men[14]. Ratios of grey to white matter also differ significantly between the sexes in diverse regions of the human cortex[15]. In many cases, the differences are not evident in overt anatomical structure, but in some type of functional dimension (hence the distinction above between 'functional' and 'structural' dimorphisms). For example, a region may differ between the sexes in aspects of its neurotransmitter function, or in its genetic or metabolic response to experience. Furthermore, new methodological approaches — from gene modification in mice to voxel-based morphometry analyses of human imaging data — are revealing previously undetected sexual dimorphisms[16–18]. It seems that the sexual dimorphisms uncovered so far, abundant as they may be, represent only a fraction of the sexual dimorphisms that are likely to exist in the brain.

The hippocampus. One region that is evidently sexually dimorphic in its structure and function is the hippocampus, a region perhaps most associated with learning and memory. Extensive evidence demonstrates that male and female hippocampi differ significantly in their anatomical structure, their neurochemical make-up and

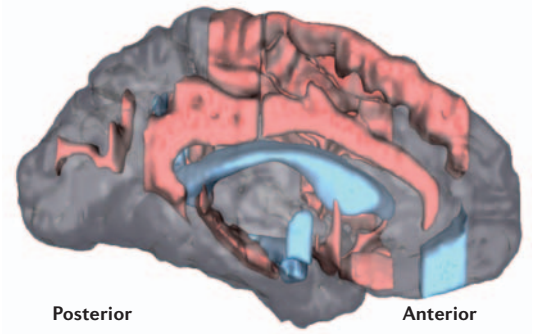

Figure 1 | **An illustration of sex differences in the size of various human brain regions.** Goldstein *et al.*[20] measured the volume of 45 brain structures taken from MRI scans in a sample of male ($n = 27$) and female ($n = 21$) subjects. For both sexes, the size of each region was determined relative to volume of the cerebrum. As shown here, significant differences between the sexes were detected in widespread brain regions. The authors also found that the size of the sex differences were related to the presence of sex steroid receptors in homologous brain regions during critical developmental periods, as determined in animal studies, suggesting that sex differences in the adult stem from sex hormone influences on brain development. Data from REF. 20.

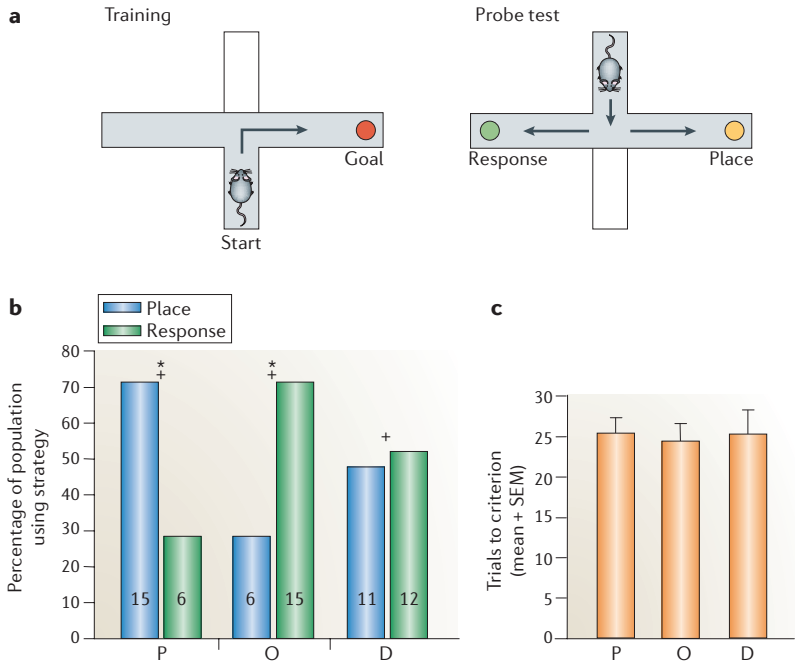

Figure 2 | **Demonstration of oestrus cycle influence on maze learning strategy in rats. a** | Korol and colleagues[31] trained rats over several trials to find food in a goal arm of a T-maze. A probe trial with the maze orientation reversed allowed the investigators to determine whether each rat was using a 'place' strategy to find the food (the rat goes to the place in the room where the food should be) or a 'response' strategy (the rat simply moves left or right at the maze choice point). The key finding was that whether the rats used a 'place' or 'response' strategy depended heavily on the state of the oestrus cycle. **b** | Percentage of rats in proestrus (P), oestrus (O) or dioestrus (D) choosing a place or response strategy during the probe trial. Interestingly, the magnitude of these behavioural effects is similar to those found in previous studies using this task and intracranial infusions of drugs into relevant brain regions, such as the hippocampus and caudate nucleus[94]. Numbers on bars indicate the number of rats tested in each condition. Crosses indicate a significant difference between the cycle stages, and asterisks indicate significant difference within a stage. **c** | The number of trials needed in each group to reach criterion performance before the probe test, indicating that hormonal status did not affect learning speed despite its pronounced effects on strategy. Modified, with permission, from REF. 31 © (2004) Elsevier Science.

their reactivity to stressful situations[19]. Imaging studies consistently show, for example, that the hippocampus is larger in women than in men when adjusted for total brain size[20]. Animal research reveals a plethora of additional differences. For example, both the volume of the CA1 region and the number of pyramidal cells it contains are significantly larger in male than in female rats, as is the density of neurons in the dentate gyrus[19]. Evidence also exists for sex differences in many neurotransmitter systems within the hippocampus, including the adrenergic, serotonergic, cholinergic, corticosterone, benzodiazepine and cholecystokinin systems[19]. For example, receptor affinity of glucocorticoids in females is half that in males, a difference that does not depend on circulating sex hormones[21]. In addition, sex hormones such as oestrogen can alter the excitability of hippocampal cells[22], strongly influence their dendritic structure[23] and augment NMDA (*N*-methyl-D-aspartate) receptor binding[24]. Intrahippocampal oestrogen infusions modulate memory processes[25]. Finally, sex differences exist in hippocampal long-term potentiation[26], a phenomenon that is widely viewed to be related to memory processes.

Such evidence indicates that sex should influence the role of the hippocampus in learning, and there is growing evidence to support this conclusion. For example, Ruecker and colleagues[27] found that avoidance learning affected hippocampal enzymatic activity that was related to memory consolidation in markedly different ways in male and female rats, despite similar behavioural performance of the two sexes. Other investigators have uncovered differing behavioural and hippocampal responses to learning. Shors and colleagues[28] have shown that a brief exposure to a stressful learning situation (for example, exposure to a series of tail shocks) increases the density of dendritic spines in male rats, but decreases spine density in female rats. They found similarly opposing effects of stress on Pavlovian conditioning performance in males and females, with stress enhancing performance in males, but impairing it in females. Parallel results were recently reported in a study of Pavlovian conditioning in men and women[29].

Juraska and colleagues[30] reported opposing effects of early experience on the dendritic structure of dentate gyrus cells: female rats that were raised in an enriched environment displayed increased dendritic bushiness (or degree of branching) relative to males raised in the same environment. By contrast, the bushiness of the dendritic structure was decreased in females compared with males that were raised in standard housing conditions. The hippocampal relationship to other structures in learning is also proving to be sexually dimorphic. Korol and colleagues[31] recently found that changes in the balance of sex hormones seem to shift the balance between hippocampal and striatal learning strategies in female rats (FIG. 2). Whether and how sex influences hippocampal function in humans has not yet been systematically examined, but should be given the evidence from animal research.

An intriguing but relatively unknown hippocampal sex difference is the reaction to chronic stress. In both rats and monkeys, chronic stress causes damage to the hippocampus in males, but does so far less, if at all, in females[32]. Chronic stress damage in males is widely known among neuroscientists, whereas the effect in females is much less well known. Logically, however, both are equally important in understanding how chronic stress affects the hippocampus. Indeed, the susceptibility of hippocampal cells to chronic stress has been suggested to have a role in two debilitating disorders — post-traumatic stress disorder (PTSD) and clinical depression[32]. Both disorders disproportionately affect women, but animal models for these disorders continue to use male subjects almost exclusively. Clearly, the relative resistance of female hippocampal cells to stress-induced damage demands consideration by anyone attempting to link stress-induced cell death to disease states such as depression and PTSD.

The amygdala. The medial nucleus of the amygdala has long been known to be sexually dimorphic, a fact that is easily accepted given its role in reproductive behaviour[33]. However, it is now clear that sexual dimorphism encompasses most, if not all, of the amygdaloid nuclei.

Long-term potentiation (LTP). An enduring increase in the amplitude of excitatory postsynaptic potentials as a result of high-frequency (tetanic) stimulation of afferent pathways. It is measured as an increase in the amplitude of excitatory postsynaptic potentials or in the magnitude of the postsynaptic cell population spike. LTP is most frequently studied in the hippocampus and is often considered to be part of the cellular basis of learning and memory in vertebrates.

An example of the sexually dimorphic function in other amygdala nuclei comes from a recent study by Braun and colleagues[34]. Pups of a rodent species known as *Octodon degus* were exposed to the stress of temporary separation from their mother. They found that hearing the mother's call during the stress of separation increased the number of serotonin receptors in the basomedial amygdala of male pups, but decreased serotonin receptor concentrations in female pups (opposing effects that are reminiscent of those described above for the hippocampus).

A rapidly growing body of evidence also documents the sexually dimorphic nature of the human amygdala[35,36]. For example, it is significantly larger in men than in women (adjusted for total brain size)[20]. Sex differences also exist in its structural relationship with the rest of the brain. In a study of a large sample of men and women, the patterns of covariance in the size of many brain structures were 'remarkably consistent' between men and women, with one exception — the amygdala (in particular, the left hemisphere amygdala), which showed several marked sex differences[18].

Several studies now report sex influences on amygdala function, including in the context of its well-known role in memory for emotional events. Extensive evidence from animal research documents that the amygdala can modulate the storage of memory for emotional events, and does so through interactions with endogenous stress hormones released during stressful events[37]. This amygdala/stress hormone mechanism provides an evolutionarily adaptive way to create memory strength that is, in general, proportional to memory importance (BOX 2). Both lesion and imaging studies have confirmed this conclusion in humans[38]. However, imaging studies have also revealed a sex-related hemispheric lateralization of amygdala function in relation to memory for emotional material. Specifically, the studies consistently indicate a preferential involvement of the left amygdala in memory for emotional material (generally visual images) in women, but a preferential involvement of the right amygdala in memory for the same material in men[39–41] (FIG. 3). In an intriguing parallel with the studies in humans, Lalumiere and McGaugh[42] recently reported that stimulation of the right but not the left hemisphere amygdala modulates memory storage in male rats.

Sex-related hemispheric lateralities of human amygdala function have also been reported in other circumstances; for example, Killgore and Yurgelun-Todd[43] examined amygdala reactivity to emotional facial expressions in men and women using fMRI. Most strikingly, they found an interaction of sex and hemisphere on amygdala responses to happy faces: the left amygdala was significantly more active in response to happy faces in females than in males, whereas the opposite pattern occurred for the right amygdala. Another, more recent study[44] used fMRI to examine amygdala responses to fearful faces in men and women. This study also reported significantly different patterns of amygdala responsiveness depending both on the sex of the subjects and on whether the right or left hemisphere amygdala was being studied.

Sex-related hemispheric differences in the amygdala do not occur only in response to emotional stimulation, a fact made clear by a recent study involving amygdala functional activity. Kilpatrick and colleagues[45] examined the functional covariance of the right and left hemisphere amygdalae with the rest of the brain in a large sample of men and women who received blood flow PET scans while simply resting with their eyes closed. The results revealed a striking hemispheric lateralization of function: activity of the right hemisphere amygdala covaried with that of other brain regions to a much greater extent in men than it did in women, whereas the reverse was true for left hemisphere amygdala activity. This laterality, 'women left, men right', parallels that described above from studies of the amygdala relationship to memory for emotional material[39–41], indicating that the laterality occurring in response to emotional stimulation stems from a baseline that is already differentially 'tilted' between the sexes at rest.

There are also intriguing parallels between this functional lateralization of amygdala function in healthy individuals and amygdala dysfunction in certain disease states. For example, women with Turner syndrome (who lack an X chromosome) show reduced responsiveness of the left hemisphere amygdala to emotional material[46], whereas mainly female samples of patients with depression show heightened left hemisphere amygdala activity[47], as do women with irritable bowel syndrome[48].

In summary, the available evidence indicates that studies of amygdala function risk conclusions that are incomplete at best, and wrong at worst, if they fail to address potential influences of both sex and hemisphere.

Other sex and hemisphere influences. The amygdala is not the only brain region with a function that seems to be influenced by both the variables of sex and hemisphere. Sex-by-hemisphere interactions in brain function even occur in the hypothalamus in rats[49]. Although a full treatment of this topic is well outside the scope of

Box 2 | **Evolutionary explanations for sex differences in the brain**

What evolutionary explanations might be offered to account for widespread sex influences on brain function? In some cases, they seem obvious. For example, Kazuhito Tomizawa and his colleagues[91] recently found that oxytocin, a hormone that is necessary for mammalian labour and lactation, improves both spatial memory and memory-related neurochemistry in the hippocampus of female mice that have had litters. The improved spatial memory has clear advantages, allowing a mother to wander further afield to find and recall locations of food and water and thereby better ensure the development and survival of her offspring.

In more general terms, the best developed idea concerns sexual selection, a concept originally proposed by Charles Darwin and developed more recently by David Geary[92]. Sexual selection refers to the competition for mates that occurs both within and between sexes. Extensive evidence from many species makes it clear that males and females have evolved different behavioural strategies to optimize their chances of successful mating. Females tend to compete with other females more subtly, in ways that may depend more heavily on the processing of finer details; for example, of social cues. Such evolutionary accounts may help to explain the heightened recall of detailed information in females found in several studies of human memory so far[93].

Regardless of the ultimate evolutionary explanations, it seems incontrovertible that males and females evolved under some similar, and some very different pressures. We should therefore expect *a priori* that their brain organization will be both similar in some respects, and markedly different in others. This is precisely the situation suggested by the sex difference literature.

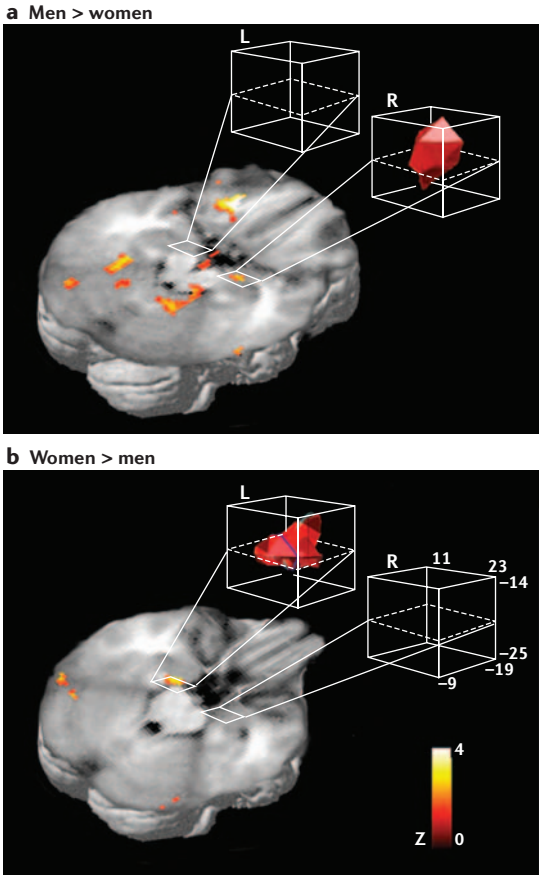

Figure 3 | **Sex differences in the relationship between amygdala activity during emotional experiences and memory for those experiences.** Extensive evidence indicates that the amygdala modulates memory storage for emotionally arousing events. However, findings from several studies now demonstrate sex differences in the relationship between amygdala activity during emotional experiences and memory for those experiences. As illustrated here, activity in the right hemisphere amygdala while viewing emotionally arousing images is more significantly related to subsequent memory for the images in men than it is in women (**a**), whereas the converse is true for the left hemisphere amygdala (**b**). The reasons for this hemispheric laterality, and what it means for the qualities of memories for emotional events in men and women, are now important areas of study. Reproduced, with permission, from REF. 41 © (2004) Cold Spring Harbour Laboratory Press.

this review (see REF. 50 for a more complete discussion), facts relating to various brain regions are highlighted in this review.

There have been reports of sex-related hemispheric differences in the brain for many years, but often these reports do not seem to have been developed by additional work. For example, in 1964, Lansdell[51] observed opposite patterns of myelination in the hemispheres of male and female brains, and suggested that structure–function relationships might be clarified "if observations on cerebral asymmetry were analysed separately for each sex", a prescient observation in light of recent studies of the prefrontal cortex (PFC, discussed below.).

The PFC is rich in sex hormone receptors, and has among the highest concentration of oestrogen receptors in the human brain[52]. Sex differences in the neural substrate for working memory, a function thought to depend on the PFC, have been reported[53,54]. The PFC is also associated with sex differences in its response to stress[55,56], and might develop at different rates in males and females[57].

One function that is thought to involve the PFC is decision making. In recent years, a number of studies have reported deficits in a decision-making task after PFC lesions. However, the literature is somewhat inconsistent on this point. Considering the evidence, Tranel and colleagues[58] note that "the message from the literature is that in studies that report deficits associated with unilateral [PFC] damage, the participants are almost entirely men and the lesions are mainly right sided." Tranel *et al.* present evidence that right hemisphere PFC lesions impair performance on this decision-making task in men but not women, whereas left hemisphere lesions impair performance in women but not men. Converging evidence for this conclusion came from an earlier brain imaging study of PFC function in normal subjects performing the decision-making task[59]. Given this evidence, Tranel and colleagues[58] surmise that inconsistencies in the literature connecting the PFC to decision making may well have resulted from a failure of investigators to account for the sex of the subjects, and the hemisphere damaged.

Neurochemical sexual dimorphisms

Sexual dimorphisms occur in a wide array of neurotransmitter systems, including serotonin, GABA (γ-aminobutyric acid), acetylcholine, vasopressin, opioids and monoamines[19,60]. Again, as a full treatment of this topic is outside the scope of this review, I briefly highlight a few salient findings.

An early study identified sex differences in monoamine content in the human brain[61]. Levels of monoamine oxidase were significantly higher in several brain regions in women than in men. A more recent study found a striking sex difference in rats in the response of the monoamine-rich locus coeruleus to stress: the stress-related hormone CRF was up to 30 times more potent in activating locus coeruleus neurons in female than in male rats[62].

Several studies have documented sex differences in the serotonin system (FIG. 4). Sex differences have been reported in the rate of serotonin synthesis in the healthy human brain[63], in the levels of serotonin metabolites in post-mortem tissue[64] and in the number of cells in the human raphe nucleus[65]. Many studies have also documented sex differences in opioid peptides, and in their analgesic effectiveness (reviewed in REF. 60). A PET scan investigation revealed significantly different levels of opioid receptor binding in several brain regions in men versus women, including the amygdala and thalamus[66]. Finally, some neurochemical sex differences arise during development. For example, GABA-mediated stimulation of cells from the substantia nigra of rat pups produces depolarization in males, but hyperpolarizes these same cells in female pups[67]. These examples show that sex differences in brain neurochemistry are proving to be much more pervasive than has been assumed by many.

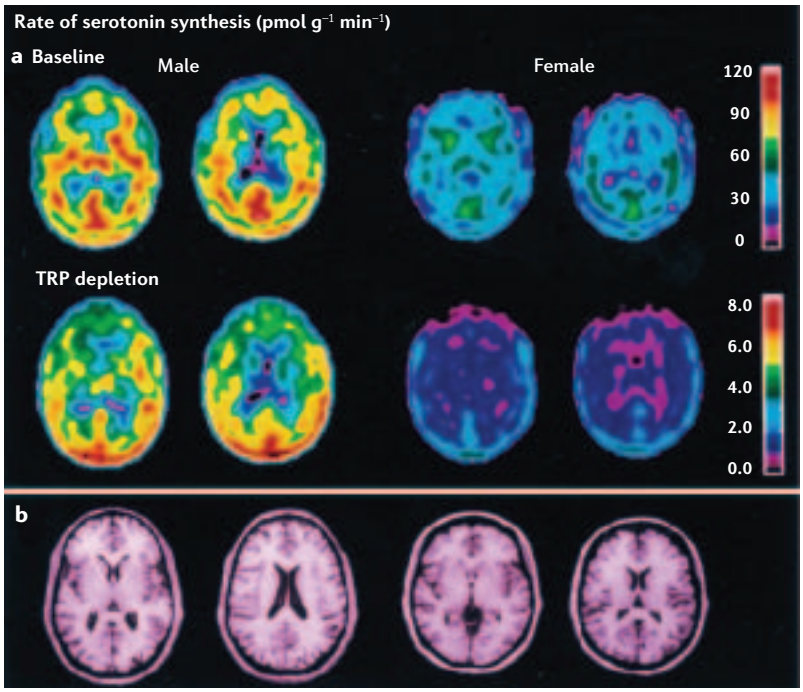

Figure 4 | **Rates of serotonin synthesis in men and women.** Nishizawa and colleagues[63] used positron emission tomography (PET) to assess serotonin synthesis rates in healthy men and women. **a** | Images show PET scans taken from a representative male and female subject. Images are shown before and after depletion of plasma tryptophan. The mean rate of synthesis was found to be 52% higher in males than in females. **b** | Magnetic resonance images for reference taken from the same level as the PET images. The results may help to explain why some disorders (such as unipolar depression) that involve serotonin dysfunction do not equally affect men and women. Reproduced, with permission, from REF. 63 © (1997) National Academy of Sciences.

Implications for understanding diseases

The implications of sex influences for understanding and treating disease states are considerable[68]. Many CNS-related disorders show sex differences in their incidence and/or nature. These diseases include, but are not limited to, Alzheimer's disease (AD), PTSD and other anxiety disorders, schizophrenia, stroke, multiple sclerosis, autism, addiction, fibromyalgia, attention deficit disorder, irritable bowel syndrome, Tourette's syndrome and eating disorders[3,28,68]. The mere existence of sex differences in the incidence and/or nature of a disorder requires us to examine sex influences in both our basic and clinical research to fully understand, and treat, the disorder. AD, schizophrenia and addiction are considered in more depth below.

The results of some studies suggest that AD disproportionately affects women[69]. Regardless of whether the incidence of the disease differs between men and women, there are growing indications that the disease pathology, and the relationship between pathology and behavioural disturbance, differs significantly between the sexes. Let us first consider AD-related pathology. AD-related neurofibrillary pathology associated with abnormally phosphorylated tau protein differs in the hypothalamus of men and women: up to 90% of older men show this pathology, whereas it is found in only 8–10% of age-matched women. An opposite sex difference occurs in the nucleus basalis of Meynert, the major source of cholinergic innervation to the neocortex. Here, the percentage of neurons containing pretangles with hyperphosphorylated tau protein is significantly higher in women than in men[70].

Other evidence indicates that the relationship of AD pathology to behavioural disruption also differs between the sexes. The presence of a single *APOE*E4* allele (an allele of a gene associated with an increased risk of AD) has been linked with significantly greater hippocampal atrophy and memory disruption in women than in men[71]. As another example, symptoms of depression significantly increase the risk of developing AD in men, but not in women[72]. Finally, Barnes *et al.*[69] recently showed that the relationship between the presence of cortical neurofibrillary tangles and a clinical diagnosis of AD differed dramatically between men and women[69]. Using regression models, they found that each unit increase in pathology was associated with an approximately 3-fold increase in AD risk in men, but with a more than 20-fold increase in women. As Barnes *et al.*[69] note, "understanding why the association between AD pathology and dementia differs in men and women could yield important clues about the pathophysiology of AD or eventually lead to sex-specific preventative or therapeutic strategies."

Schizophrenia is another brain disease that differs in both incidence and nature between the sexes. Men and women differ on average in several clinical features of the disease, including its presentation, symptoms, age of onset, and the time course of the illness. Some patterns of brain morphology that are associated with the illness also differ between the sexes. For example, men with schizophrenia show significantly larger ventricles than do healthy men, whereas no such enlargement is seen in women with schizophrenia[73]. As another example, the ratio of the size of the amygdala to that of the orbitofrontal cortex (which is sexually dimorphic in healthy individuals) is increased in men with psychosis, but decreased in women with psychosis[74]. The results of studies from several laboratories (reviewed in REF. 75) indicate that the normal patterns of hemispheric asymmetry seen in the brains of healthy individuals are reduced in schizophrenia, and that sex interacts with the changes in asymmetry. Sex differences even occur in the facial features of patients with schizophrenia: male patients display significantly less facial hemispheric asymmetry than do male controls, whereas female patients display marked facial asymmetries that are absent in female controls[76]. With each report such as these, the conclusion that the pathology of schizophrenia differs substantially between men and women strengthens.

The same conclusion is now apparent from investigations of addiction. Here, the neurotransmitter dopamine is a key player. Becker and colleagues[77] have discovered clear sex differences in the levels of dopamine in several brain regions, as well as differences in the responsiveness of dopamine to stimulation by amphetamine and sex hormones. In humans, addiction differs between the sexes in important ways. Women, for example, are more sensitive than men to the reinforcing effects of psychostimulants (for example, amphetamine and cocaine), which may account for the more rapid progression from initial use to drug dependence in women compared with men[78].

Fibromyalgia
A chronic, painful condition, primarily occurring in women, characterized by widespread musculoskeletal pain, fatigue and tender points at defined locations.

Box 3 | **Developmental influences on sex differences in the brain**

Considerable research has been directed at understanding how sex differences in the adult brain develop. In fact, some sex differences seem to result, at least in part, from different maturational rates in males and females. This fact is illustrated in work by Waber[80], who examined various mental abilities known to be associated with sex differences in adults — such as spatial and verbal abilities — in boys and girls classified as either 'early' or 'late' maturing (on the basis of the appearance of secondary sex characteristics). For both sexes, late-maturing children performed significantly better than early-maturing children of the same sex on spatial measures. Given that boys tend to mature later than girls, this finding suggests that different maturational rates help to produce the male advantage in spatial abilities often found in adults.

Despite the evidence for sex differences in dopamine function, and despite sex differences in the nature of addiction, functional brain imaging studies of addiction have focused almost exclusively on men. Recently, however, Kilts and colleagues[79] compared brain activation in response to drug cues in male and female cocaine addicts, and found several clear differences. For example, whereas drug cues increased activity in the right amygdala of male addicts, they decreased activity in the right amygdala of female addicts. A recurring theme may strike the reader at this point: in addiction, as in so many other domains of neuroscience, investigators are increasingly realizing that they can no longer assume that essentially identical processes occur in men and women, nor that identical therapeutics will apply.

Fibromyalgia
A chronic, painfull condition, Primarily occurring in women, characterized by widespread musculoskeletal pains, fatigue and tender points at defined locations.

Concluding remarks and future directions

Many important topics such as developmental effects[80] (BOX 3) and key strategies for studying sex differences[81] have either not been addressed, or have only been alluded to in this review. Nevertheless, it is evident that there are sex influences at all levels of the nervous system, from genetic to systems to behavioural levels. The picture of brain organization that emerges is of two complex mosaics — one male and one female — that are similar in many respects but very different in others[6]. The way that information is processed though the two mosaics, and the behaviours that each produce, could be identical or strikingly different, depending on a host of parameters.

A few suggestions for future work may be worth considering. The immediate task for neuroscientists at all levels of our field is to challenge the (often implicit) assumption that sex matters little, if at all. Obviously, ignoring significant influences of sex, should they exist, can only retard progress. When consistent significant sex influences in a domain are discovered, investigators in that domain will, at the very least, need to either rationalize focusing on one sex for future studies, or develop parallel research tracts for each sex. For those now actively working on the sex differences issue, I suggest that the largest challenge at present is to begin identifying those aspects of brain organization that differ most fundamentally between males and females, and from which many of the sex differences observed so far presumably arise. This is, of course, not a simple task, but it is a necessary one if we are to fully comprehend how and why sex influences brain function in so many ways.

Despite the heightened complexity it implies, the issue of sex influences seems to be much too important, both practically and theoretically, to be ignored or marginalized any longer in our field. To quote a recent report from the medical branch of the National Academy of Sciences[82], "Sex does matter. It matters in ways that we did not expect. Undoubtedly, it matters in ways that we have not yet begun to imagine."

1. Levine, S. Sex differences in the brain. *Scientific American* **214**, 84–90 (1966).
2. McFadden, D. Masculinizing effects on otoacoustic emissions and auditory evoked potentials in women using oral contraceptives. *Hearing Research* **142**, 23–33 (2000).
3. Hines, M. *Brain Gender* (Oxford Univ. Press, New York, 2004).
4. Maccoby, E. E. & Jacklin, C. N. *The Psychology of Sex Differences* Vol. 1 (Stanford Univ. Press, Stanford, 1974).
5. Kimura, D. Sex, sexual orientation and sex hormones influence human cognitive function. *Curr. Opin. Neurobiol.* **6**, 259–263 (1996).
6. Witelson, S. F. Neural sexual mosaicism: sexual differentiation of the human temporo-parietal region for functional asymmetry. *Psychoneuroendocrinology* **16**, 131–153 (1991).
7. Fuchs, R. A., Evans, A., Mehta, R., Case, J. M. & See, R. E. Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior. *Psychopharmacology* **179**, 662–672 (2005).
8. Arnold, A. P. Sex chromosomes and brain gender. *Nature Rev. Neurosci.* **5**, 701–708 (2004).
Provides an excellent overview of striking developments in molecular neurobiology that are generating new insight into neural mechanisms behind sexual differentiation of the brain.
9. Reisert, I. & Pilgrim, C. Sexual differentiation of monoaminergic neurons — genetic or epigenetic? *Trends Neurosci.* **14**, 468–473 (1991).
10. Piefke, M., Weiss, P., Markowitsch, H. & Fink, G. Gender differences in the functional neuroanatomy of emotional episodic autobiographical memory. *Hum. Brain Mapp.* **24**, 313–324 (2005).
11. Grabowski, T. J., Damasio, H., Eichhorn, G. R. & Tranel, D. Effects of gender on blood flow correlates of naming concrete entities. *Neuroimage* **20**, 940–954 (2003).
12. De Vries, G. J. Sex differences in adult and developing brains: compensation, compensation, compensation. *Endocrinology* **145**, 1063–1068 (2004).
Excellent review of the evidence supporting an often underappreciated concept, namely, that sexual dimorphisms in the brain may exist to prevent, rather than create, sexual dimorphisms in behaviour.
13. Juraska, J. M. Sex differences in 'cognitive' regions of the rat brain. *Psychoneuroendocrinology* **16**, 105–109 (1991).
14. Luders, E. *et al.* Gender effects on cortical thickness and the influence of scaling. *Hum. Brain. Mapp.* **27**, 314–324 (2005).
15. Allen, J. S., Damasio, H., Grabowski, T. J., Bruss, J. & Zhang, W. Sexual dimorphism and asymmetries in the gray–white composition of the human cerebrum. *Neuroimage* **18**, 880–894 (2003).
16. Shah, N. M. *et al.* Visualizing sexual dimorphism in the brain. *Neuron* **43**, 313–319 (2004).
Illustration of the ability of some newer methodologies to reveal sexual dimorphisms in the brain missed by more traditional methods.
17. Bielsky, I. F., Hu, S. B. & Young, L. J. Sexual dimorphism in the vasopressin system: lack of an altered behavioral phenotype in female V1a receptor knockout mice. *Behav. Brain Res.* **164**, 132–136 (2005).
18. Mechelli, A., Friston, K. J., Frackowiak, R. S. & Price, C. J. Structural covariance in the human cortex. *J. Neurosci.* **25**, 8303–8310 (2005).
Findings suggest that the amygdala is probably an especially important locus of sex influences on brain function.
19. Madeira, M. D. & Lieberman, A. R. Sexual dimorphism in the mammalian limbic system. *Prog. Neurobiol.* **45**, 275–333 (1995).
20. Goldstein, J. M. *et al.* Normal sexual dimorphism of the adult human brain assessed by *in vivo* magnetic resonance imaging. *Cereb. Cortex* **11**, 490–497 (2001).
21. Turner, B. B. & Weaver, D. A. Sexual dimorphism of glucocorticoid binding in rat brain. *Brain Res.* **343**, 16–23 (1985).
22. Teyler, T. J., Vardaris, R. M., Lewis, D. & Rawitch, A. B. Gonadal steroids: effects on excitability of hippocampal pyramidal cells. *Science* **209**, 1017–1018 (1980).
23. Cooke, B. M. & Woolley, C. S. Gonadal hormone modulation of dendrites in the mammalian CNS. *J. Neurobiol.* **64**, 34–46 (2005).
24. Romeo, R. D., McCarthy, J. B., Wang, A., Milner, T. A. & McEwen, B. S. Sex differences in hippocampal estradiol-induced *N*-methyl-D-aspartic acid binding and ultrastructural localization of estrogen receptor-α. *Neuroendocrinology* **81**, 391–399 (2005).
25. Packard, M., Kohlmaier, J. & Alexander, G. Posttraining intra-hippocampal estradiol injections enhance spatial memory in male rats: interaction with cholinergic systems. *Behav. Neurosci.* **110**, 626–632 (1996).
26. Maren, S., De Oca, B. & Fanselow, M. S. Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. *Brain Res.* **661**, 25–34 (1994).
27. Ruecker, B. *et al.* Inhibitory avoidance task reveals differences in ectonucleotidase activities between male and female rats. *Neurochem. Res.* **29**, 2231–2237 (2004).
28. Shors, T. Opposite effects of stressful experience on memory formation in males versus females. *Dialogues Clin. Neurosci.* **4**, 139–147 (2002).

29. Jackson, E. D., Payne, J. D., Nadel, L. & Jacobs, W. J. Stress differentially modulates fear conditioning in healthy men and women. *Biol. Psychiatry* **59**, 516–522 (2005).
30. Juraska, J., Fitch, J., Henderson, C. & Rivers, N. Sex differences in the dendritic branching of dentate granule cells following differential experience. *Brain Res.* **333**, 73–80 (1985).
31. Korol, D. L. Role of estrogen in balancing contributions from multiple memory systems. *Neurobiol. Learn. Mem.* **82**, 309–323 (2004).
Summary of the recent studies demonstrating pronounced influences of sex hormones on learning strategies in rats.
32. McEwen, B. S. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* **886**, 172–189 (2000).
33. Cooke, B. M. & Woolley, C.S. Sexually dimorphic synaptic organization of the medial amygdala. *J. Neurosci.* **25**, 10759–10767 (2005).
34. Ziabreva, I., Poeggel, G., Schnabel, R. & Braun, K. Separation-induced receptor changes in the hippocampus and amygdala of *Octodon degus*: influence of maternal vocalizations. *J. Neurosci.* **23**, 5329–5336 (2003).
Demonstrates a qualitative difference in the neurochemical response to stress of an amygdala nucleus, the basomedial nucleus, not traditionally thought to be sexually dimorphic.
35. Cahill, L. Sex- and hemisphere-related influences on the neurobiology of emotionally influenced memory. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **27**, 1235–1241 (2003).
36. Hamann, S. Sex differences in the responses of the human amygdala. *Neuroscientist* **11**, 288–293 (2005).
37. McGaugh, J. L. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci.* **27**, 1–28 (2004).
38. Cahill, L. in *The Amygdala: A Functional Analysis* (ed. Aggleton, J.) 425–444 (Oxford Univ. Press, London, 2000).
39. Cahill, L. *et al.* Sex-related difference in amygdala activity during emotionally influenced memory storage. *Neurobiol. Learn. Mem.* **75**, 1–9 (2001).
40. Canli, T., Desmond, J., Zhao, Z. & Gabrieli, J. D. E. Sex differences in the neural basis of emotional memories. *Proc. Natl Acad. Sci. USA* **99**, 10789–10794 (2002).
41. Cahill, L., Uncapher, M., Kilpatrick, L., Alkire, M. T. & Turner, J. Sex-related hemispheric lateralization of amygdala function in emotionally influenced memory: an fMRI investigation. *Learn. Mem.* **11**, 261–266 (2004).
42. Lalumiere, R. T. & McGaugh, J. L. Memory enhancement induced by post-training intrabasolateral amygdala infusions of β-adrenergic or muscarinic agonists requires activation of dopamine receptors: involvement of right, but not left, basolateral amygdala. *Learn. Mem.* **12**, 527–532 (2005).
43. Killgore, W. & Yurgelun-Todd, D. Sex differences in amygdala activation during the perception of facial affect. *Neuroreport* **12**, 2543–2547 (2001).
One of the first studies to document sex differences in the function of the human amygdala.
44. Williams, L. M. *et al.* Distinct amygdala–autonomic arousal profiles in response to fear signals in healthy males and females. *Neuroimage* **28**, 618–626 (2005).
45. Kilpatrick, L. A., Zald, D. H., Pardo, J. V. & Cahill, L. F. Sex-related differences in amygdala functional connectivity during resting conditions. *Neuroimage* **30**, 452–461 (2006).
Examines the functional connectivities of the left and right hemisphere amygdalae in a large sample of men and women who received PET scans while resting with their eyes closed. Indicates that sexually dimorphic amygdala function exists in the brain independently of overt stimulation.
46. Skuse, D. H., Morris, J. S. & Dolan, R. J. Functional dissociation of amygdala-modulated arousal and cognitive appraisal, in Turner syndrome. *Brain* **128**, 2084–2096 (2005).
47. Drevets, W. Neuroimaging abnormalities in the amygdala in mood disorders. *Ann. NY Acad. Sci.* **985**, 420–444 (2003).
48. Naliboff, B. D. *et al.* Sex-related differences in IBS patients: central processing of visceral stimuli. *Gastroenterology* **124**, 1738–1747 (2003).
49. Nordeen, E. J. & Yahr, P. Hemispheric asymmetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. *Science* **218**, 391–394 (1982).
A striking demonstration of hemispheric lateralization in the effects of a sex hormone on the developing brain.
50. Wisniewski, A. B. Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. *Psychoneuroendocrinology* **23**, 519–547 (1998).
51. Lansdell, H. Sex differences in hemispheric asymmetries of the human brain. *Nature* **203**, 550 (1964).
52. Bixo, M., Backstrom, T., Winblad, B. & Andersson, A. Estradiol and testosterone in specific regions of the human female brain in different endocrine states. *J. Steroid Biochem. Mol. Biol.* **55**, 297–303 (1995).
53. Duff, S. J. & Hampson, E. A sex difference on a novel spatial working memory task in humans. *Brain Cogn.* **47**, 470–493 (2001).
54. Speck, O. *et al.* Gender differences in the functional organization of the brain for working memory. *Neuroreport* **11**, 2581–2585 (2000).
55. Bland, S. T. *et al.* Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. *Brain Res.* **1051**, 90–99 (2005).
56. Shansky, R. M. *et al.* Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Mol. Psychiatry* **9**, 531–538 (2004).
57. Goldman, P. S., Crawford, H. T., Stokes, L. P., Galkin, T. W. & Rosvold, H. E. Sex-dependent behavioral effects of cerebral cortical lesions in the developing rhesus monkey. *Science* **186**, 540–542 (1974).
58. Tranel, D., Damasio, H., Denburg, N. L. & Bechara, A. Does gender play a role in functional asymmetry of ventromedial prefrontal cortex? *Brain* **128**, 2872–2881 (2005).
59. Bolla, K. I., Eldreth, D. A., Matochik, J. A. & Cadet, J. L. Sex-related differences in a gambling task and its neurological correlates. *Cereb. Cortex* **14**, 1226–1232 (2004).
Together with reference 58, this suggests that the involvement of the prefrontal cortex in decision making is influenced both by a subject's sex and cerebral hemisphere, and suggests that attention to these variables can reconcile seemingly contradictory studies.
60. Craft, R. M. Sex differences in opioid analgesia: 'from mouse to man'. *Clin. J. Pain* **19**, 175–186 (2003).
61. Robinson, D. S. *et al.* Monoamine metabolism in human brain. *Arch. Gen. Psychiatry* **34**, 89–92 (1977).
62. Curtis, A. L., Bethea, T. & Valentino, R. J. Sexually dimorphic responses of the brain norepinephrine system to stress and corticotropin-releasing factor. *Neuropsychopharmacology* **31**, 544–554 (2005).
63. Nishizawa, S. *et al.* Differences between males and females in rates of serotonin synthesis in human brain. *Proc. Natl Acad. Sci. USA* **94**, 5308–5313 (1997).
64. Gottfries, C. G., Roos, B. E. & Winblad, B. Determination of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and homovanillic acid in brain tissue from an autopsy material. *Acta. Psychiatr. Scand.* **50,** 496–507 (1974).
65. Cordero, M. E., Rodriguez, A., Torres, R. & Valenzuela, C. Y. Human raphe magnus nucleus: a morphometric Golgi-Cox study with emphasis on sex differences. *Brain Res. Dev. Brain Res.* **131**, 85–92 (2001).
66. Zubieta, J. K., Dannals, R. & Frost, J. Gender and age influences on human brain mu-opiod receptor binding measured by PET. *Am. J. Psychiatry* **156**, 842–848 (1999).
67. Galanopoulou, A. S. GABA receptors as broadcasters of sexually differentiating signals in the brain. *Epilepsia* **46**, 107–112 (2005).
68. Klein, L. C. & Corwin, E. J. Seeing the unexpected: how sex differences in stress responses may provide a new perspective on the manifestation of psychiatric disorders. *Curr. Psychiatry Rep.* **4**, 441–448 (2002).
69. Barnes, L. L. *et al.* Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch. Gen. Psychiatry* **62**, 685–691 (2005).
70. Swaab, D. F., Chung, W. C., Kruijver, F. P., Hofman, M. A. & Ishunina, T. A. Structural and functional sex differences in the human hypothalamus. *Horm. Behav.* **40**, 93–98 (2001).
71. Fleisher, A. *et al.* Alzheimer's Disease Cooperative Study. Sex, apolipoprotein E ε 4 status, and hippocampal volume in mild cognitive impairment. *Arch. Neurol.* **62**, 953–957 (2005).
72. Dal Forno, G. *et al.* Depressive symptoms, sex, and risk for Alzheimer's disease. *Ann. Neurol.* **57**, 381–387 (2005).
73. Nopoulos, P., Flaum, M. & Andreasen, N. Sex differences in brain morphology in schizophrenia. *Am. J. Psychiatry* **154,** 1648–1654 (1997).
74. Gur, R. E. *et al.* A sexually dimorphic ratio of orbitofrontal to amygdala volume is altered in schizophrenia. *Biol. Psychiatry* **55**, 512–517 (2004).
75. Crow, T. J. Cerebral asymmetry and the lateralization of language: core deficits in schizophrenia as pointers to the gene. *Curr. Opin. Psychiatry* **17**, 96–106 (2004).
76. Hennessy, R. J. *et al.* 3D morphometrics of craniofacial dysmorphology reveals sex-specific asymmetries in schizophrenia. *Schizophr. Res.* **67**, 261–268 (2004).
77. Becker, J. B. Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol. Biochem. Behav.* **64**, 803–812 (1999).
78. Lynch, W. J., Roth, M. E. & Carroll, M. E. Biological basis of sex differences in drug abuse: preclinical and clinical studies. *Psychopharmacology* **164**, 121–137 (2002).
79. Kilts, C. D., Gross, R. E., Ely, T. D. & Drexler, K. P. The neural correlates of cue-induced craving in cocaine-dependent women. *Am. J. Psychiatry* **161**, 233–241 (2004).
80. Waber, D. P. Sex differences in mental abilities, hemispheric lateralization, and rate of physical growth in adolescence. *Dev. Psychol.* **13**, 29–38 (1977).
81. Becker, J. B. *et al.* Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* **146**, 1650–1673 (2005).
An excellent, comprehensive review by field leaders of various approaches taken to studying the issue of sex influences on the brain, including description of the pitfalls to be avoided. Should be mandatory reading for anyone entering the field.
82. Wizemann, T. M. *Exploring the Biological Contributions to Human Health: Does Sex Matter?* (ed. Pardue, M.L.) (National Academy, Washington, DC, 2001).
83. Dohanich, G. P. Gonadal steroids, learning and memory. *Hormones, Brain, and Behavior* Vol. 2 (ed. Pfaff, D.W.) 265–327 (Academic, San Diego, 2002).
84. Rubinow, M. J., Arseneau, L. M., Beverly, J. L. & Juraska, J. M. Effect of the estrous cycle on water maze acquisition depends on the temperature of the water. *Behav. Neurosci.* **118**, 863–868 (2004).
85. Halpern, D. F. & Tan, U. Stereotypes and steroids: using a psychobiosocial model to understand cognitive sex differences. *Brain Cogn.* **45**, 392–414 (2001).
86. Goldstein, J. M. *et al.* Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. *J. Neurosci.* **25**, 9309–9316 (2005).
87. Hausmann, M. Hemispheric asymmetry in spatial attention across the menstrual cycle. *Neuropsychologia* **43**, 1559–1567 (2005).
88. Kaufman, M. J. *et al.* Cocaine-induced cerebral vasoconstriction differs as a function of sex and menstrual cycle phase. *Biol. Psychiatry* **49**, 774–781 (2001).
89. Justice, A. J. & de Wit, H. Acute effects of D-amphetamine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology* **145**, 67–75 (1999).
90. Lynch, W. J., Roth, M. E. & Carroll, M. E. Biological basis of sex differences in drug abuse: preclinical and clinical studies. *Psychopharmacology* **164**, 121–137 (2002).
91. Tomizawa, K. *et al.* Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nature Neurosci.* **6**, 384–390 (2003).
92. Geary, D. C. *Male, Female: the Evolution of Human Sex Differences* (American Psychological Association, Washington DC, 1998).
93. Seidlitz, L. & Diener, E. Sex differences in the recall of affective experiences. *J. Pers. Soc. Psychol.* **74**, 262–271 (1998).
94. Packard, M. G. & McGaugh, J. L. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.* **65**, 65–72 (1996).

Competing interests statement
The author declares no competing financial interests.

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