Review

The Complex Relationships between Sex and the Brain

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Abstract

In the past decennia, our understanding of the sexual differentiation of the mammalian brain has dramatically changed. The simple model according to which testosterone masculinizes the brain of males away from a default female form, was replaced with a complex scenario, according to which sex effects on the brain of both females and males are exerted by genetic, hormonal, and environmental factors. These factors act via multiple partly independent mechanisms that may vary according to internal and external factors. These observations led to the "mosaic" hypothesis—the expectation of high variability in the degree of "maleness"/"femaleness" of different features within a single brain. Here, we briefly review animal data that form the basis of current understanding of sexual differentiation; present, in this context, the results of co-analyses of human brain measures obtained by magnetic resonance imaging or postmortem; discuss criticisms and controversies of the mosaic hypothesis and implications for research; and conclude that co-analysis of several (preferably, many) features and going back from the group level to that of the individual would advance our understanding of the relations between sex and the brain in health and disease.

Keywords

gender differences, sex differences, sexual differentiation, intermediate nucleus, sexually dimorphic nucleus, transgender

New View of the Sexual Differentiation of the Brain

Over 50 years have passed since it was proposed that in early development, testosterone would irreversibly masculinize the brain of males away from a default female form. In the past two decades, this concept has been replaced by a more complex scenario. According to this new view, sex effects on the brain are exerted in both females and males throughout life by several steroid hormones (including testosterone, estradiol, and progesterone) as well as by genetic and environmental factors. These effects are exerted via multiple partly independent mechanisms and may vary according to internal and external factors (for review, see Arnold 2012; Forger 2018; Grgurevic and Majdic 2016; McCarthy 2016; McCarthy and others 2018; McEwen and Milner 2017; Sekido 2014).

Most of the evidence supporting this new understanding of sex effects on the brain derives from studies in laboratory animals and tissue cultures. In vitro studies of neuronal cultures obtained from embryos prior to the development of the gonads revealed some differences between XX and XY neurons in the absence of sex-related hormones (for a recent review, see Grgurevic and Majdic 2016). For example, dopamine content and uptake (but not neuron number) were higher in cultures obtained from the embryonic mesencephalon (but not diencephalon) of female compared with male NMRI mice (Sibug and others 1996). It is noteworthy that the same study found no differences in these measures in cultures from two other mouse strains (Sibug and others 1996), highlighting the dependency of sex effects on other factors, in this case most likely genetic background.

In vivo studies in mice, in which the genetic and gonadal components of sex were dissociated, support the results of the in vitro studies in providing examples for a direct effect of sex-related genes independent of sex-related hormones on some brain measures (for review, see Arnold 2012; Arnold and Chen 2009; Ngun and others 2011; Sekido 2014). For example, the vasopressin innervation of the lateral septum is denser in males compared with females in many mammalian species (De

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Vries and others 1983; for a recent review and references, see Bredewold and Veenema 2018). Using the four-core genotype model, Gatewood and colleagues (2006) found that regardless of the type of gonads, mice with a Y chromosome had a larger area of vasopressin immunoreactivity in the lateral septum compared to mice without a Y chromosome. This region was also larger in mice with testes compared to mice with ovaries, regardless of the composition of the sex chromosome complement (Fig. 1a). It is noteworthy that although in that specific study the effects of chromosomal sex were quantitatively similar to the effects of gonadal sex (see Fig. 1a), most evidence to date reveals a much larger contribution of sex-related hormones compared to sex-related genes to the sexual differentiation of the rodent brain and behavior (for review, see Arnold 2012; Arnold and Chen 2009; Ngun and others 2011).

There is also evidence that sex-related environmental factors play a role in the sexual differentiation of certain features of the central nervous system (for review, see McCarthy and Arnold 2011). For example, part of the sex difference in the number (Moore and others 1992) and morphology (Lenz and Sengelaub 2006) of motor neurons in the spinal nucleus of the bulbocavernosus (which is much larger in males compared with females, Breedlove and Arnold 1981), depends on the more extensive maternal anogenital licking of male compared with female pups. The sex difference in maternal anogenital licking also plays a major role in the sex difference in the methylation and gene expression of the estrogen receptor-α promoter in the preoptic area (Kurian and others 2010).

Probably the most prevalent studies are those in which the level of sex-related hormones is manipulated in early development or later in life. Such studies revealed that feminization and masculinization are independent processes rather than two poles of a continuum (for reviews, see Bakker and Baum 2008; Grgurevic and Majdic 2016; McCarthy and Arnold 2011; McEwen and Milner 2017). For example, rats that underwent ovariectomy on postpartum day 20 to 22, that is, before puberty, had a higher number of glial cells in the upper layers of the medial prefrontal cortex compared with sham-operated females (Fig. 1b). The number of these cells in males that underwent castration or sham-operation at the same age was similar to that in ovariectomized females. Together these findings suggest that the sex difference in this measure is the result of an ovary-derived substance that is acting to reduce the number of these cells in females, rather than a testis-derived substance acting to increase the number of cells in males (Koss and others 2015). Similarly, studies in prepubertal ovariectomized rats and in aromatase knockout female mice suggest that the postnatal sex difference in the number of progesterone receptors in the medial preoptic area of mice and rats depends on estradiol acting in females (Bakker and Baum 2008; Quadros and others 2002).

Other studies revealed the multiplicity of mechanisms by which sex-related steroid hormones affect the brain (for review, see McCarthy and Arnold 2011; McEwen and Milner 2017). For example, different mechanisms are responsible for the postnatal cell death of GABAergic and dopaminergic neurons in the male rat's anteroventral periventricular nucleus of the hypothalamus (Krishnan and others 2009; Waters and Simerly 2009)—cell death, which is responsible for this nucleus being much larger in females compared with males.

Studies in which sex differences were assessed in animals that were exposed to different environmental conditions in early development or later in life revealed that the effects of sex may be different, and even opposite, under different environmental conditions. Moreover, these sex-by-environment interactions may be different for different neurobiological characteristics, even within the same neuron (for review and references, see Joel 2011, 2012). For example, in the hippocampal pyramidal neurons of rats that were kept in standard laboratory conditions, there was a sex difference in spine density of the apical dendrites, but not of the basal dendrites (Fig. 1c; Shors and others 2001; 2004). Exposure to 30 minutes of stress in adulthood resulted in the reversal of the sex difference in the apical dendrites and the emergence of a sex difference in the basal dendrites (Shors and others 2001; 2004). Similarly, exposure of adult rats to three weeks of mild chronic stress resulted in the reversal of a sex difference in the density of CB1 cannabinoid receptors in the dorsal hippocampus and abolition of a sex difference in the density of these receptors in the ventral hippocampus (Reich and others 2009).

The Mosaic Hypothesis: Evidence from Human Brain Data

The multiplicity of mechanisms by which sex affects the brain combined with the sensitivity of at least some of these mechanisms to internal and external influences led to the "mosaic" hypothesis—the expectation that the degree of "maleness"/"femaleness" of different features within a single brain would be highly variable (Joel 2011, 2012; Joel and McCarthy 2017). This hypothesis was supported by a study that assessed internal consistency in the degree of "maleness"/"femaleness" in human brain structure, as revealed by magnetic resonance imaging (MRI; Joel and others 2015). Analyzing only brain regions showing large sex/gender differences, that study found that brains in which the volume of all regions was toward the female-end of the distribution or the volume of all regions was toward the

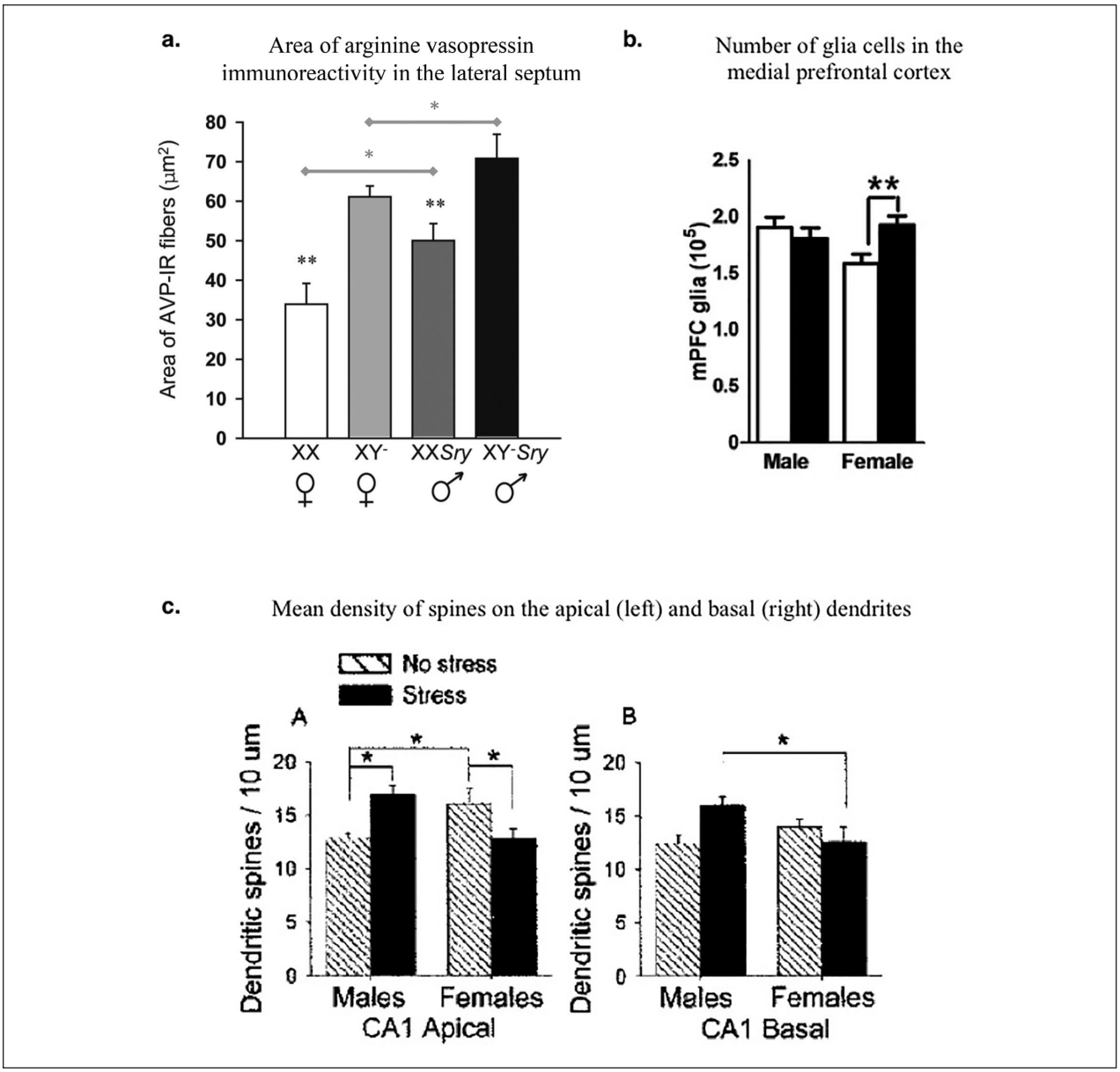

Figure 1. (a) Sex-related genes and hormones affect the brain. Mean and standard error of the mean area of arginine-vasopressin immunoreactivity in the lateral septum of mice from each of the four genotypes: genetically intact female mice, which have ovaries (XX); XY mice in which *Sry* was deleted from the Y chromosome, leading to the differentiation of the gonads into ovaries instead of testes (XY-); XX mice in which *Sry* was added in the form of a transgenic copy on an autosome, leading to the differentiation of the gonads into testes (XX*Sry*); and XY- mice in which *Sry* was replaced by a transgenic copy on an autosome, leading to the differentiation of the gonads into testes (XY-*Sry*). *Signifies a significant difference between mice with the same chromosomal complement but either testes or ovaries. **Signifies a significant difference between mice with the same type of gonads, but with XX or XY (except *Sry*) chromosomal complement (reproduced with permission from figure 6 in Gatewood and others 2006). This graph demonstrates the quantitatively similar effects of chromosomal sex and gonadal sex on arginine-vasopressin immunoreactivity in the lateral septum. (b) Sex differences may reflect processes acting in females. Mean and standard error of the mean number of glial cells in the medial prefrontal cortex of female and male rats that underwent gonadectomy (black) or sham operation (white) (reproduced with permission from figure 2 in Koss and others 2015). The results presented in this graph suggest that the sex difference in the number of glial cells in the medial prefrontal cortex reflects an ovary-derived substance that is acting to reduce the number of these cells in females, rather than a testis-derived substance acting to increase the number of cells in males. (c) Sex effects on the brain may be different under different environmental conditions. Mean and standard error of the mean density of spines on the apical (left) and basal (right) dendrites of pyramidal neurons in area CA1 of the hippocampus, in control female and male rats (white bars) and in female and male rats that underwent exposure to 30 minutes of intermittent tail-shock 24 hours earlier (black bars) (reproduced with permission from figure 4 in Shors and others 2001). These graphs illustrate a reversal of a sex difference in the apical dendrites and abolition of a sex difference in the basal dendrites.

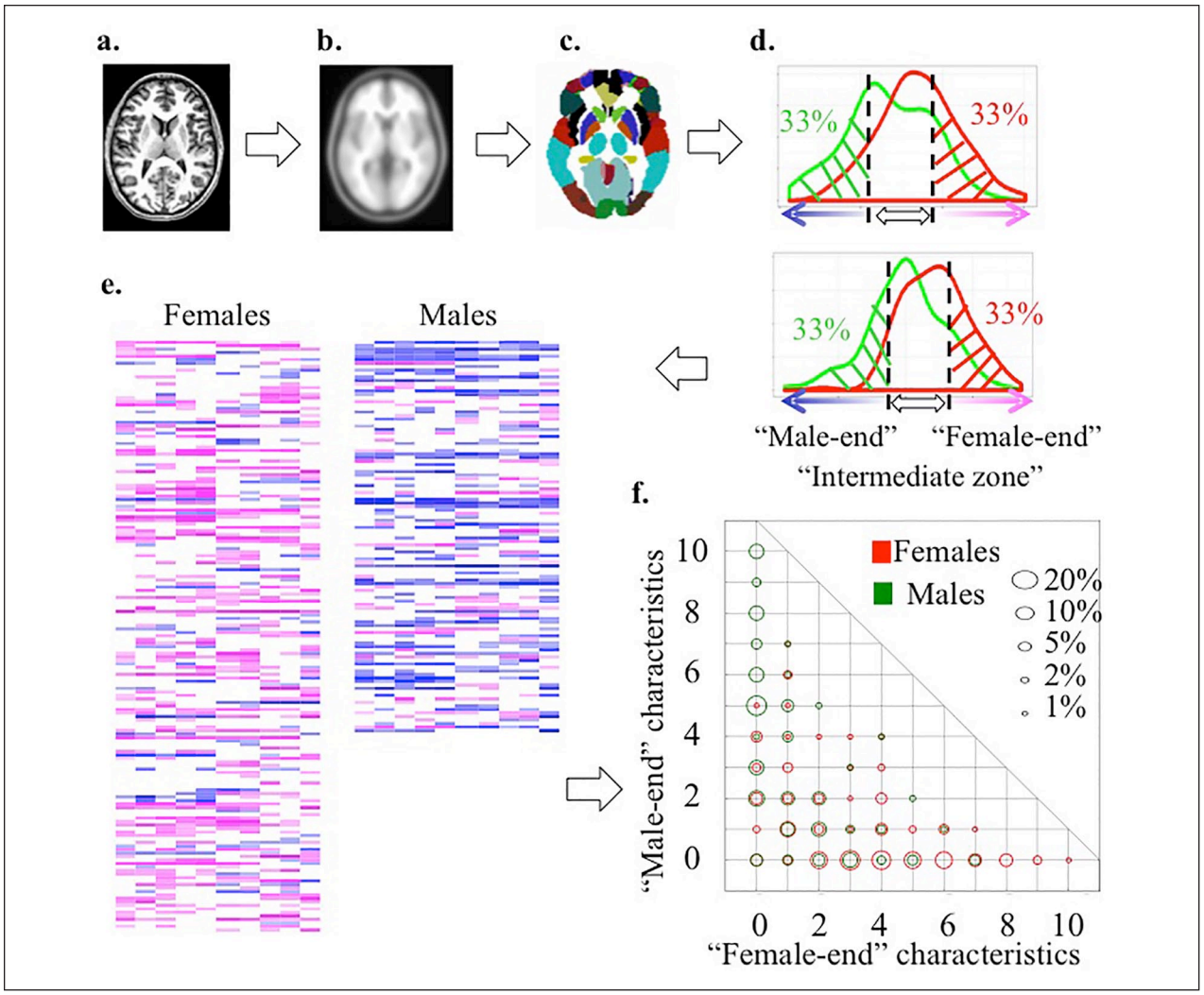

Figure 2. Assessing internal consistency and mosaicism in the human brain. (a-c) Voxel-based morphometry (VBM) of grey matter volume from an Israeli sample of 169 women and 112 men. (a) T1-weighted images were normalized and segmented using (b) the Montreal Neurological Institute (MNI) template. (c) Voxels were mapped into 116 regions according to the Automated Anatomical Labeling (AAL) atlas. (d) The frequency distribution of the gray matter volume in women (red) and men (green) of two of the regions showing the largest sex/gender differences (left hippocampus [top, Cohen's $d = 0.74$, $P < 0.0001$] and left caudate [bottom, Cohen's $d = 0.84$, $P < 0.0001$]). A continuous color representation of the degree of "maleness" and "femaleness" was created separately for each of the 10 regions showing the largest sex/gender differences ($0.70 < d \leq 0.84$, all $Ps < 0.0001$). Volumes falling in the "intermediate" zone are colored in white; volumes in the "male-end" and in the "female-end" zones are colored using continuous blue-white and pink-white scales, respectively. (e) The degree of "maleness-femaleness" of each region for each of the women (left) and men (right). Each horizontal line represents the brain of one individual and each column represents a single brain region. (f) A bivariate scatter gram of the number of regions at the "female-end" (*X* axis) and at the "male-end" (*Y* axis) in women (red) and men (green). The number of regions at the "intermediate" zone is not depicted because the number of "male-end," "intermediate," and "female-end" features always adds up to 10. The size of each circle is proportional to the percent of individuals from the same sex/gender category with an identical score on the two measures. (Created with permission on the basis of figures 1 and 2 in Joel and others 2015. For details of the samples, imaging methods, and data analyses, see Joel and others 2015.) Figures e and f reveal the group level differences in brain structure (as expected by the definition of female- and male-end zones, there are more regions at the female-end at the women's table, and more regions at the male-end at the men's table) together with the mixing-up of female-end and male-end features in many individual brains. In contrast, brains with only female-end features or only male-end features are rare.

male-end of the distribution ("internally consistent" brains) were rare. Much more prevalent were brains in which the volume of at least one region was toward the female-end of the distribution and the volume of at least one other region

Box 1. Mosaic in Human Brain Structure and Connectivity as Revealed by Magnetic Resonance Imaging.

Joel and colleagues (2015) analyzed regional volume (Fig. 2), cortical thickness, or connectivity obtained from processing magnetic resonance images of over 1400 human brains from four different datasets. In each dataset, only a few (7-12) features showing the largest sex/gender differences were analyzed. Yet the degree of overlap between women and men for these features was too large to distinguish between a range of scores typical of women and a range of scores typical of men (see Fig. 2d, which presents the distribution of men and women for two brain regions showing the largest sex/gender differences in one of the samples). Instead, the authors defined a "female-end" and a "male-end" zones, corresponding, respectively, to the range of scores that were more common in women compared with men, and the range of scores that were more common in men compared with women (Joel and others 2015). In the four datasets, the number of internally consistent brains, that is, brains in which all features are at the "female-end" zone or all features are at the "male-end" zone, was low (0% to 8%) and much lower than the number of mosaic brains (23% to 53%), that is, brains in which at least one feature was at the "female-end" zone and one feature was at the "male-end" zone (Joel and others 2015, Fig. 2).

was toward the male-end of the distribution ("mosaic" brains, Joel and others 2015; for more details see Fig. 2 and Box 1; for criticisms of this study, see below). Joel and colleagues' (2015) study was the first to co-analyze several measures in individual brains. Their finding that different features within a brain could vary greatly in their degree of "maleness"/"femaleness" is in line with the conclusion from animal studies that sexual differentiation of different brain features progresses largely independently.

Applying principles discovered in animals regarding sex effects on the brain to humans is difficult for various reasons. In contrast to studies in animals, which mainly assess the microstructure of the brain (e.g., number of neurons, dendritic morphology, neurotransmitter content, receptor density), most studies of sex differences in the human brain assess the brain's macrostructure (e.g., regional volume, cortical thickness) using MRI. Human studies also differ from animal studies in that there are many more factors on which human females and males differ. In both humans and animals, sex differences can result from multiple variables, from sex-related genes and hormones, to physiological (e.g., body weight) and environmental variables (e.g., individual vs. group housing). In humans, in which sex category is imbued with social meaning, differences between women and men could reflect in addition multiple gender-related variables, from gender identity and gender roles to type of education and socioeconomic status (Fausto-Sterling 2000; Fine 2010; Joel and Fausto-Sterling 2016; Joel and McCarthy 2017; Jordan-Young and Rumiati 2012; Kaiser 2012; Maney 2015; Rippon and others 2014), most of which cannot be modeled in animals. Thus, although studies often report differences between women and men in brain structure, they do not reveal the mechanisms by which sex affects the human brain (for this reason, we refer to differences between women and men as sex/gender differences). Some insight into these mechanisms may be derived from studying individuals with specific conditions (Bao and Swaab 2010), although also in these individuals it is difficult to disentangle the contributions of the specific condition and various other internal and external factors. For example, humans with an atypical number of X or Y chromosomes (e.g., XXX, XXY, XYY, XXYY, XXXXY) also often have an atypical hormonal profile (e.g., individuals with 47,XXY, 48,XXXY or 49,XXXXY also have lower levels of testosterone; Tartaglia and others 2011). Another example is individuals from sexuality or gender minority groups (e.g., homosexual, transgender) who often receive atypical social reactions. Studies revealed that they are being the target of bullying and harassment to a much greater extent than is typical of individuals with "normative" sexuality and gender identity (i.e., heterosexual and cisgender [cisgender is a term used for individuals whose gender self-labeling is the same as their birth-assigned gender category]) (e.g., Cohen and others 2016; Reisner and others 2015).

The work of Dick Swaab and colleagues on postmortem human brains provides a partial bridge between animal and human studies. These studies assessed the volume, neuron number and neuropeptide content of several hypothalamic and other subcortical nuclei, which cannot be visualized by MRI (Fig. 3a). Very large sex/gender differences were observed in some of these nuclei, such as, the bed nucleus of the stria terminalis (BST), the interstitial nucleus of the anterior hypothalamus, subdivision 1 (INAH1, also called intermediate nucleus or sexually dimorphic nucleus of the preoptic area), the INAH3 (a subnucleus of the uncinate nucleus), and the infundibular nucleus (Garcia-Falgueras and others 2011; Garcia-Falgueras and Swaab 2008; Kruijver and others 2000; Taziaux and others 2016; Zhou and others 1995). Importantly, some of these studies obtained several measures from the same brains, enabling the assessment of the degree of in/dependence between the mechanisms underlying sex/gender differences in the different measures (Box 2, Fig. 4).

The co-analysis of measures from the INAH1 and INAH3 of 11 cisgender women and 14 cisgender men revealed that three out of the four correlations between measures were low (Fig. 4), in line with the conclusion derived from laboratory animal studies that sexual differentiation of different brain features progresses largely independently. The co-analysis also revealed that while the

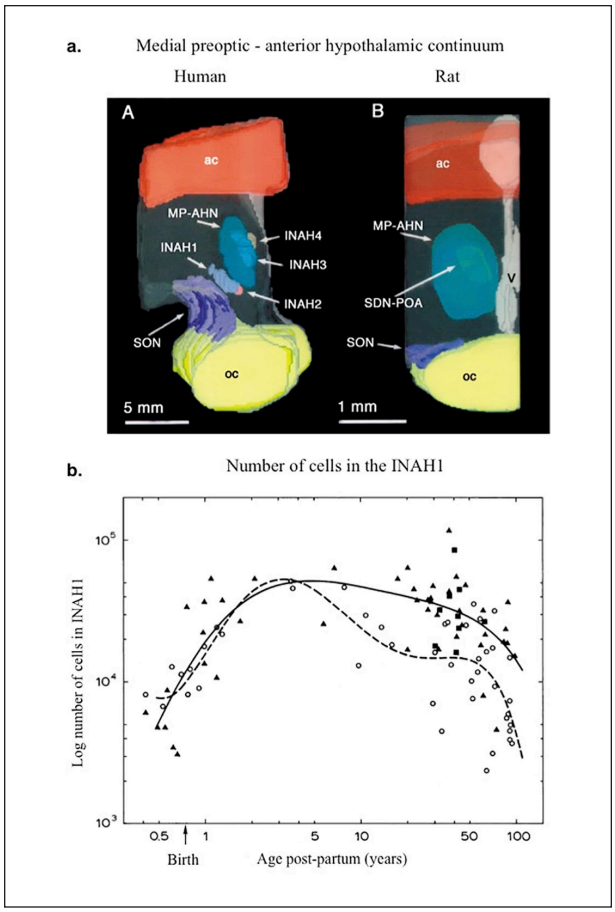

Figure 3. (a) Three-dimensional reconstructions, prepared from thionin-stained serial sections, of the medial preoptic–anterior hypothalamic continuum (MP-AHN) of the human (A) and rat (B). ac, anterior commissure; INAH, interstitial nucleus of the anterior hypothalamus; MP-AHN, medial preoptic-anterior hypothalamic nucleus; oc, optic chiasm; SDN-POA, sexually dimorphic nucleus of the preoptic area; SON, supraoptic nucleus; V, third ventricle. In human INAH1 is also called SDN-POA (reproduced with permission from figure 2 in Byne and others, 2001). (b) The (log) number of cells in the INAH1 in 99 humans as a function of their (log) age. The curves are quintic polynomial functions fitted to the original data for males (full line) and females (dashed line) (adapted with permission from figure 1 in Swaab and Hofman, 1988). Please note that the following description is derived from comparing individuals sampled at different developmental stages, and not from assessing the same individuals throughout life. At the moment of birth, the INAH1 is equally small in females (empty circles) and males (filled triangles and squares; the symbols refer to heterosexual and homosexual men, respectively; there were no differences between the two groups; Swaab and Hofman 1990) and contains about 20% of the maximum cell number, which is achieved at 2 to 4 years of age. The number of cells remains approximately unchanged in men up to the age of 50 years, when it starts to decline, whereas in women, the decline seems to start around puberty, leading to a large sex/gender difference.

brains of some cisgender individuals were internally consistent (i.e., all measures were in the male-typical range or all were in the female-typical range), the brains of other cisgender individuals were mosaic of male-typical and female-typical scores (for details, see Box 2). The three types of brain (only male-typical, only female-typical, and mosaic) were also observed in a group of 10 transgender women (i.e., individuals who were assigned male at birth but self-identify as women; Box 2, Figure 4d and e).

Possible Scenarios for the Emergence of Sex/Gender Differences in the Two Hypothalamic Nuclei

As explained above (see also Box 3), in itself, the existence of differences between females and males does not reveal their source. In particular, it neither reveals whether the differences are a result of biological or sociocultural factors associated with being male or female, nor shows whether the differences reflect a sex-/gender-related process in males, in females or in both. Yet the observations in the hypothalamic data are in line with several of the scenarios, reviewed above, previously described in laboratory animals.

The observation that cisgender women occupy in Figure 4d and e a smaller part of space compared with cisgender men, is in line with the possibility that a sex/gender-related factor(s) is acting in males to drive them away from a default female form. Yet the strong negative correlation observed in women between the number of neurons in INAH1 and INAH3 suggests that in women, a common factor plays an important role in determining the number of neurons in the two nuclei, albeit in opposite directions. This is in line with studies in laboratory animals revealing that sex differences may also reflect processes that drive females away from a default male form (Bakker and Baum 2008; Koss and others 2015; Quadros and others 2002). Similarly, in humans, the large sex/gender difference in the total number of neurons in the INAH1 found in adulthood seems to reflect a gradual decrease in cell number in women rather than an increase in men (Swaab and Hofman 1988; Fig. 3b).

Except for the high correlation between the number of neurons in INAH1 and in INAH3 in cisgender women, all other correlations assessed were low (Fig. 4d-e). Several scenarios could theoretically lead to the relative independence of different measures that show large sex/gender differences: Different sex/gender-related factors (e.g., genetic, hormonal, environmental) may be mediating sex/gender effects on each of the measures; The same sex/gender-related factor may affect several measures, but its effects on each measure may be differently influenced by other factors or overshadowed by the effects of other factors. These other factors may or may not be correlated with sex category (e.g., variations in the genome

Box 2. Co-analysis of Several Hypothalamic Measures.

Figure 4a-c presents the original figures from Garcia-Falgueras and Swaab (2008) and Garcia-Falgueras and others (2011), which depict the total number of neurons, as revealed by thionin staining, in the INAH3 (Fig. 4a) and INAH1 (Fig. 4b), and the number of galanin-stained neurons in the INAH1 (Fig. 4c). The figures reveal the large group level difference on the three measures (the median in women was about half the median in men, Mann-Whitney *U* test, tied *P*s are 0.002, 0.006, and 0.042, respectively) as well as the overlap between cisgender women and men on each measure. The "common language effect size," that is, the probability that a man picked at random will have a higher score than a woman picked at random (Del Giudice 2019), for these three measures is 0.88, 0.82, and 0.74, respectively. Figure 4d and e co-present the scores of cisgender women (n = 11, red bullets), cisgender men (n = 14, blue bullets) and transgender women (n = 10, green bullets, all of them received estrogen treatment, and eight of them also underwent castration; for demographic and clinical details of the three groups, see Garcia-Falgueras and Swaab, 2008 and Garcia-Falgueras and others 2011) on two measures at a time (Fig. 4d: number of neurons in INAH3 and INAH1; Fig. 4e: number of galanin-stained neurons and non-galanin neurons in the INAH1; all the data appear in the appendix). The co-analysis of measures reveals low correlations between measures within each of the gender groups (Fig. 4d and e), with the only exception being a large negative correlation between the number of INAH1 and INAH3 neurons in cisgender women ($r_s = -0.63$, $P < 0.05$). (We would like to note that the important point here is not that the correlations were not significant—they would most probably have been with a larger n—but that the number of one type of neurons explains very little (less than 8%) of the variance in the number of another type of neurons).

Mosaic in the human hypothalamus

The combination of overlap between women and men in each measure and a low correlation between the measures results in mosaic brains, that is, brains in which the score on one measure is in the female-typical range (i.e., the range of scores most common in women) and the score on another measure is in the male-typical range. For illustration purposes only, we drew arbitrary borders between male-typical (M) and female-typical (F) ranges for each of the four measures (Fig. 4d and e). Clearly, with other definitions of male-typical and female-typical zones, the exact number of mosaic brains would be different. But the main point this illustration attempts to stress is that mosaicism results from the combination of overlap *and* low dependence. This is because overlap combined with high correlation would have resulted in some individuals who have sex-atypical scores on both measures rather than being sex-typical on one measure and sex-atypical on the other. For example, if the number of galanin and non-galanin neurons in the INAH1 were highly correlated, then we should have expected nine cisgender women with an FF number of neurons, one with MM and only one with FM, and nine cisgender men with an MM number of neurons, four with FF, and only one with FM—a number of mosaic brains that is significantly lower than the one actually observed in the data (three cisgender women and five cisgender men had a mosaic brain $\chi^2 = 4{\cdot}5$, $P = 0.036$). With more brain measures being considered, the "mixing" of male-typical and female-typical features becomes more pronounced. Thus, if we add to the co-analysis of the number of galanin and non-galanin neurons in the INAH1 the number of neurons in INAH3, the number of individuals with a mosaic brain increases, from five to seven in cisgender men, from three to five in cisgender women, and from five to nine in transgender women. As mentioned above, the analysis of human MRI data, which included the co-analysis of 7-12 brain features, revealed that mosaic brains were much more common than internally consistent brains (Box 1, Joel and others 2015).

outside of the sex chromosomes, variations in the environment not correlated with sex category).

Note that independence between different brain characteristics may be a common phenomenon in the brain. What the analysis presented in Box 2 reveals is that this is evident also for brain measures that show large sex/gender differences, supporting previous observations in animals that sex is one of many factors that interact to determine brain structure (reviewed in Joel 2011, 2012; Joel and McCarthy 2017).

Group-Level Sex Differences and Co-analysis of Features within Individual Brains

Sex differences are discovered by comparing groups of females and males, are mostly studied in isolation—considering each sex difference separately—but are often implicitly assumed to "add up" in functionally meaningful ways within the brains of individuals. De Vries and colleagues noted that sex differences sometimes act to compensate for other sex differences—an outcome that can only be recognized by considering several sex differences together. One example is X inactivation—silencing one of the copies of the X chromosome in most cells in the body of mammalian females. By itself, this may seem as a huge sex difference—46 active chromosomes in males and only 45 in females. But considered together with another sex difference—that males' chromosome Y is much smaller than the X chromosome, the conclusion is that the sex difference in chromosome inactivation compensates for the sex difference in chromosome complement (De Vries 2004). Another example relates to the sex difference in the arginine vasopressin innervation of the lateral septum mentioned earlier. This difference is particularly large in prairie voles. Yet in this species, this difference compensates for the sex difference in exposure to hormones before one has offspring and ensures very similar parental behavior (except nursing)

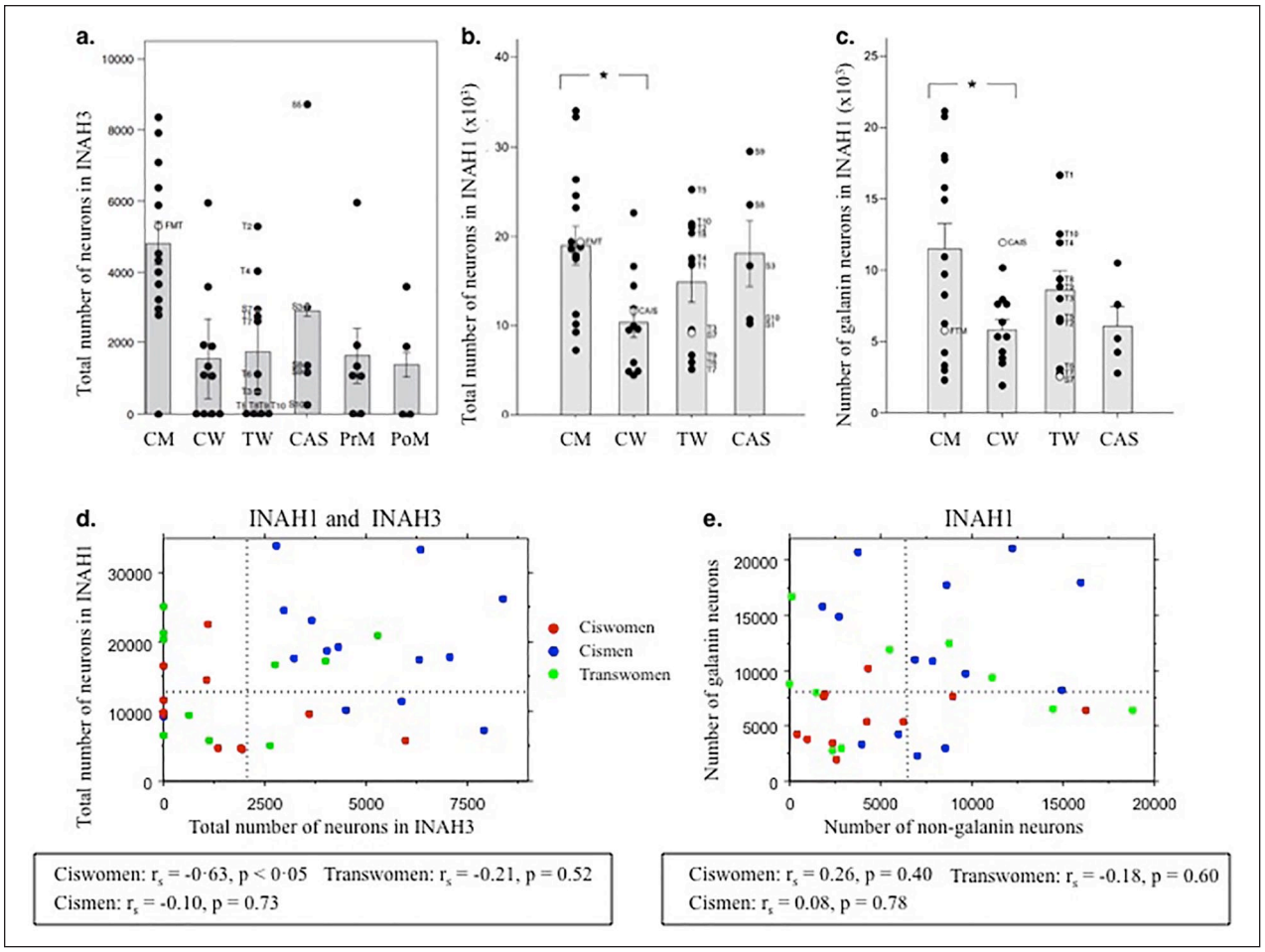

Figure 4. Co-analysis of hypothalamic measures in the postmortem human brain. (a-c). Number of neurons, as revealed by thionin staining, in the interstitial nucleus of the anterior hypothalamus-3 (INAH3) (a) and INAH1 (b), and the number of galanin-stained neurons in the INAH1 (c) in cisgender men (CM) and women (CW; the data of the women are also presented separately for women before and after menopause [PrM, PoM]), transgender women (TW), and cisgender elderly men who underwent castration because of prostate cancer (CAS). Bars represent means and SEM. (The figures were adapted with permission from figure 6 in Garcia-Falgueras and Swaab 2008, and figures 1 and 2 in Garcia-Falgueras and others 2011. For details on specific patients, see the original publications.) (d) Number of neurons, as revealed by thionin staining, in INAH3 (*X* axis) and INAH1 (*Y* axis) in cisgender women (n = 11, red bullets), cisgender men (n = 14, blue bullets), and transgender women (*n* = 10, green bullets). The dashed lines mark, for illustration purposes only, arbitrary borders between "female-typical" and "male-typical" zones for each measure. For the number of neurons in the INAH1, the arbitrary border was set at 12,000, as 8 out of the 11 cisgender women had fewer than 12,000 neurons, and 10 out of the 14 cisgender men had more than 12,000 neurons. In the INAH3, 2000 neurons were set as the arbitrary border, as 9 out of the 11 cisgender women had fewer than 2000 neurons and all but one cisgender man had more than 2000 neurons. The box below the graph presents the Spearman correlation coefficient of the two measures in the cismen, ciswomen, and transwomen groups. (e) Same as (d) for the number of galanin-stained neurons (*Y* axis) and non-galanin neurons (computed by subtracting the number of galanin-stained neurons from the number of thionin-stained neurons, *X* axis) in the INAH1. The dashed lines mark, for illustration purposes only, arbitrary borders between "female-typical" and "male-typical" zones for each measure. For the number of galanin neurons, the arbitrary border was set at 8000 neurons, as all but one cisgender woman had fewer than 8,000 neurons, and ten out of the 14 cisgender men had more than 8000 neurons. For the non-galanin neurons, 6500 neurons were set as the border, as 9 out of the 11 cisgender women had fewer than 6500 neurons and 9 out of the 14 cisgender men had more than 6500. The box below the graph presents the Spearman correlation coefficient of the two measures in the cismen, ciswomen, and transwomen groups. Figures (d) and (e) illustrate the large group level differences on each measure together with the mixing-up of female-typical and male-typical measures in some individuals.

in female and male voles (De Vries 2004; De Vries and Boyle 1998). De Vries and colleagues have therefore suggested that the existence of sex differences does not necessarily mean the existence of functionally distinct systems (e.g., De Vries 2004; De Vries and Boyle 1998; De Vries and Södersten 2009).

Box 3. Some Common Misunderstandings and Open Questions.

Sex differences versus sexual dimorphism

Sex differences, that is, a statistically significant difference between a group of females and a group of males, are often refered to as sexual dimorphism. Dimorphism literaly means having two forms, and the use of sexual dimorphism as a synonym for sex difference creates the impression that all females are different from all males. There are, however, only few examples of real sexual dimorphism. The most sexually dimorphic organ in the human body is the gonad, as the occurrence of ovotestis is exteremly rare (~1:200,000, Blackless and others 2000). Dimorphism is very rare in the brains of laboratory animals (one example is the sexually dimorphic nucleus of the preoptic area [SDN], which, depending on species, is several times larger in male compared with female rodents; Döhler and others 1982). In humans, most of the group-level sex/gender differences in the brain are small, with considerable overlap between women and men (e.g., Fig. 2d). Even in subcortical regions showing large sex/gender differences there is overlap between individual females and males (e.g., Fig. 4a-c), so these regions are not truly dimorphic (although it is possible to distinguish, at the groups level, between a male-typical and a female-typical form, as we did in Box 2). Overlap between individuals is also true for brain measures showing differnces between other groups, such as heterosexual and homosexual or cisgender and transgender (Garcia-Falgueras and others 2011; Garcia-Falgueras and Swaab 2008; Kruijver and others 2000; Zhou and others 1995; Swaab and Hofman 1990). Since two individuals with different gender or sexual identity may have the same brain measure, it is impossible to use these brain measures for diagnosis (e.g., of gender dysphoria).

Biology, nature, and nurture

Finding a difference between females and males in a biological variable (such as brain structure) is sometimes taken as evidence for a 'natural' difference between the two sexes, where "natural" means genetic, inevitable and preprogrammed. However, finding such a difference in adults (as is often the case in human studies) reveals no information regarding whether the difference is preprogrammed or results from the life experiences of the participants. Note, however, that the absence of a difference between males and females early in life (as for example in the INAH1, where the difference emerges only in adulthood; Swaab and Hofman 1988) cannot be taken as evidence that the difference reflects nurture rather than nature, because some maturational processes are preprogrameed to occur later in life (e.g., the emergence of breasts in girls in adolescence). Not confusing between a difference and its source is particularly important in discussing differences between women and men in the brain, because the brain is a plastic organ, which continues to change throughtout life.
This is also true for differnces in brain measures between additonal groups, such as homosexual and heterosexual or transgender and cisgender. It is impossible to determine whether the differences between the groups reflect the different life experiences of individuals with different identities, or precedeed these experiences. It is also impossible to determine whether differences in specific brain structures are responsible for the different identities. These questions of cause and effect are further complicated by the observation that brain functions are generally not localized in one particular brain structure but distributed over circuits of large numbers of interacting brain areas.

Evolution, inheritence, genes, and the environment

Finding a sex difference is often a trigger for evolutionary explanations, which present the observed difference as a product of sexual selection over milenia, imprinted in the genes. As we explain above, a sex difference does not reveal whether it is acquired or preprogrammed. Moreover, a sex-related trait may be evolved, but its reliable reproduction every generation does not necessarily mean that it is biologically (genetically or epigenetically) imprinted (Griffiths 2002). New thinking in evolutionary theory has highlighted the role of additional vehicles of inheritence, one of which is the environment (Griffiths 2002; Jablonka and Lamb 2014). Specifically, each organism inherits not just genes but also specific enviornmental conditions (which may range from gravity, through a particular ecology, to the precense of peers and parents). As Fine and others (2017) suggest, different environmental conditions that males and females encounter (from more urogenital licking of male compared to female pups to a gendered social structure), may be responsible for the reproduction of some sex/gender differnces across generations.

Joel (2011) noted that the degree of "femaleness" and "maleness" of different characteristics within an individual brain is rarely consistent—again, something that can only be appreciated by considering several measures that show a sex difference in the same brain. Joel therefore claimed that the existence of differences between groups of females and males does not necessarily mean the existence of structurally distinct brain types—one typical of females and another typical of males (Joel 2011; Joel and others 2015). This claim has been recently supported by a study in which Joel and others (2018) applied several unsupervised analysis approaches to MRI brain images from over 2000 humans. These analyses revealed that the brain "types" typical (i.e., common) of women are also typical of men, and vice versa, and that large sex/gender differences are found only in the prevalence of some rare (i.e., found in only a small group of humans) brain types (Joel and others 2018).

Criticism of the Mosaic Hypothesis and the Question of Prediction

Common misunderstandings and open questions regarding sex differences are discussed in Box 3. Here, we focus

on some of the issues specifically related to the mosaic hypothesis. One type of criticism relates to the method used by Joel and others (2015) to test the mosaic hypothesis. Del Giudice and others (2015) argued that Joel and colleagues' approach was inappropriate because it analyzed only the variables showing the largest sex/gender differences, discarded "most of the information in those variables by reducing them to three categories" (male-end, female-end, and intermediate), and employed "an unrealistically strict criterion for "internal consistency" coupled with a lax criterion for "substantial variability" (Del Giudice and others 2015, p. 2).

These criticisms were dealt with in Joel and colleagues' (2018) study, in which the different analytical approaches were applied to the entire dataset (i.e., regardless of whether variables showed a sex/gender difference) and without dividing scores into "zones." As described above, this study concluded that the brain "types" common in men are also common in women, and vice versa (Joel and others 2018).

Another major criticism of the conclusion that brains of women and men do not belong to two distinct categories, was based on the observation that brain structure can be used to predict with accuracy above chance whether a brain's owner is female or male (Chekroud and others 2016; Del Giudice and others 2016; Rosenblatt 2016). As Joel (2011) has claimed, and later showed (Joel and others 2016, 2018), the existence of group-level sex/gender differences in brain structure is sufficient to predict, with about 80% accuracy, one's sex category on the basis of one's brain structure. Others have obtained similar prediction accuracy using brain structure or function (e.g., Chekroud and others 2016; Del Giudice and others 2016; Rosenblatt 2016; van Putten and others 2018). Yet the fact that sex-related variance in brain structure can be used to predict the sex category of the brain's owner does not necessarily mean that sex category is a major determinant of brain structure. Moreover, because of the low internal consistency in the form of different features within a single brain, knowing one's sex category is not even sufficient to predict the unique mosaic of one's sex-related brain features (for further discussion, see Joel 2011; Joel and Fausto-Sterling 2016; Joel and others 2016, 2018).

This latter statement can be demonstrated in the hypothalamic data mentioned here (Box 2). It is possible to predict with great accuracy one's sex category on the basis of three measures: the number of neurons in INAH3, the number of galanin-stained neurons in the INAH1, and the number of non-galanin neurons in the INAH1. For instance, with a simple criterion based on the number of male- and female-typical features (i.e., if there are more female-typical than male-typical features, predict female, else predict male), one can achieve prediction accuracy of 88%—all brains from the 11 cisgender women would be predicted as such, and 11 out of the 14 brains from cisgender men would be predicted to belong to a male. However, although one can predict, with the exact same accuracy, that a brain of a woman would have more female-typical than male-typical features, and the reverse for a man, knowing that a person is male or female is not sufficient to predict the exact number of female- and male-typical features in this person's brain, nor does it predict which feature would be in which form. For example, it is highly likely that a man would have more male-typical features than female-typical features, but it is not possible to predict whether all three regions are in the male-typical form (M) or two are in the male-typical form and one in the female-typical form (F)—in which case three possible combinations are possible (MMF, MFM, or FMM brain). Yet it is the specific composition of features, rather than the number of female- and male-typical features, that determines whether a brain is similar or different from another. For example, an MMF brain, which has more male-typical features, is more similar to an MFF brain, which has more female-typical features, than to an FMM brain, even though the latter also has more male-typical features (because in the MMF and MFF brains, the first and third measures are in the same form, whereas in the MMF and FMM brains, only the second measure has the same form in the two brains). Indeed, Joel and colleagues' (2018) analysis of the structure of the entire brain revealed that the chances that a woman and a man would have the same brain architecture were very similar to the chances that two women or two men would have the same brain architecture. This was true, even though it was possible to use brain architecture to predict whether the brain's owner is female or male with accuracy of ~80% (Joel and others 2018).

Implications for Research

The observations from the co-analysis of measures of the human hypothalamus (Box 2) are in line with the complex non-linear model of sex effects on the brain described in laboratory animals, according to which sex-related mechanisms are active in both males and females and exert independent effects on different measures. Thus, sex seems to be one of the factors that drive variability in the brain, as is also evident by the prevalence of mosaicism in analysis of the entire human brain (Joel and others 2015).

Sex as an engine of brain variability seems opposite of its role in the differentiation of the reproductive system into male or female types, but is in agreement with the view that the evolutionary advantage of sexual reproduction lies in the huge increase in variation between individuals—variation that results from the combination of maternal and paternal DNA and subsequent developmental processes. Variation in the brain, and consequently in behavior, seems

as just one additional aspect of sexual reproduction as a source of variability (Fine and others 2017).

It is therefore clear that studies of humans and animals should include both females and males to capture the entire variability of a species. The question is how best to analyze the data—that is, whether to use sex category as a variable (Joel and Fausto-Sterling 2016). The current focus on sex category has several problems. It may detract the attention of the research community from other, more important variables. Whereas sex is obviously an important factor in determining the number of neurons in the INAH1 and INAH3, its effects on other brain measures is much smaller, as revealed by the small effect sizes most often found in MRI studies of the human brain, especially when brain size is taken into account (Gilmore and others 2018; Joel and others 2015; Ritchie and others 2018; Ruigrok and others 2014). Moreover, as the present review highlights, even when sex effects are large, they often interact with other variables, which are yet to be identified.

The focus on sex category often means that finding a difference between females and males is the end of the scientific endeavor, where in fact it should be the beginning. This is because the category of one's genitals (which is used to divide animals and humans into female or male) is rarely the variable responsible for sex differences. More often it is current or past (e.g., in utero) differences in gene expression, hormones, or other internal and external factors that correlate with sex category, that are responsible for the observed difference (Joel and Fausto-Sterling 2016; Joel and McCarthy 2017). While the possible contribution of some of these variables (in particular, sex-related genes and hormones) is sometimes suggested, the variables themselves are seldom directly measured. It would surely advance our scientific understanding of whatever phenomenon under study if we measure these factors in the relevant stage of life instead of using the category of genitals as a proxy (Joel and Fausto-Sterling 2016; Maney 2016).

A third problem with the binary view of sex is that even when the wealth of sex-related variables is appreciated, it is often implicitly assumed that these variables add-up consistently in individuals to create male and female physiology. Appreciating the variability in these measures and the complex ways in which they mix-up in individuals could greatly advance our understanding of health and disease.

Concluding Comments

The change in our understanding of the relations between sex and the brain from a simple linear model to a complex interplay between multiple factors should be accompanied by a change in the methods used to study these relations. The present review demonstrates the value of co-analysis of several (preferably, many) features, and the importance of going back from the group level to that of the individual. The challenge for the future is to relate the structure of individual brains to function and dysfunction.

Appendix

Patient	Symbol	Group	Estrogen	Age	Thionin INAH3	Thionin INAH1	Galanin INAH1	Non-Galanin INAH1
98188Thi	c	Male		66	3669.6761910	23209.361280	8269.111	14940.250
98012Thi	c	Male		47	4326.5150720	19328.947300	9693.527	9635.420
98299THI	c	Male		49	4020.0286570	18799.800340	10919.123	7880.677
98014Thi	c	Male		50	4505.9079100	10150.267370	4197.922	5952.345
88092THI	c	Male		61	0.0000000	9283.725915	2293.033	6990.693
81093thi	c	Male		42	5877.1727230	11480.064430	2970.394	8509.670
98-326	c	Male		81	7897.8948070	7208.474841	3272.237	3936.237
97406Thi	c	Male		33	2788.3380940	33946.154000	17957.745	15988.409
246Thio	c	Male		25	2964.5379570	24547.375200	20769.097	3778.279
97194thi	c	Male		55	6338.5879730	33325.602850	21127.287	12198.316
97096Thi	c	Male		33	8366.3554580	26340.473070	17763.067	8577.406
00185thi	c	Male		70	7076.3564360	17793.506150	10946.240	6847.266
98095NPY	c	Male		58	3213.2651930	17654.964800	14921.493	2733.472
97-398th	c	Male		54	6317.5125270	17588.007220	15784.040	1803.967
97047thi	c	Female		58	1913.8196100	4676.865626	4264.329	412.537
97233Thi	c	Female		43	0.0000000	11610.374000	5333.985	6276.389
1132Thio	c	Female		32	1341.6666450	4783.372300	3783.392	999.981
1137Thio	c	Female		25	1937.6756300	4476.375677	1916.762	2559.613
91082THI	c	Female		36	5956.5299270	5841.345500	3482.783	2358.562
00320thi	c	Female		82	0.0000000	9619.745592	5378.166	4241.579

(continued)

Appendix (continued)

Patient	Symbol	Group	Estrogen	Age	Thionin INAH3	Thionin INAH1	Galanin INAH1	Non-Galanin INAH1
98-016th	c	Female		72	0.0000000	9879.904350	7909.792	1970.112
00287THI	c	Female		21	1066.3219500	14495.793400	10163.173	4332.620
98231Thi	c	Female		58	3595.8696670	9603.635958	7704.852	1898.784
84258THI	c	Female		23	1090.6911070	22618.419900	6361.605	16256.815
01041Thi	c	Female		46	0.0000000	16632.348090	7641.496	8990.852
07021thi	T9	MtF_Castrated	Stopped	58	0.0000000	6594.131490	8862.357	0.0000000
88228thi	T3	MtF_Castrated	Stopped	43	628.1496657	9469.387739	8004.751	1464.637
84227thi	T1	MtF_Castrated	Continued	50	2745.6308120	16836.654020	16679.693	156.961
98251thi	T10	MtF_Castrated	Continued	74	0.0000000	21294.313800	12529.669	8764.645
98334t26	T7	MtF_Castrated	Continued	26	2630.0401760	5043.937466	2709.010	2334.927
95058thi	T6	MtF_Castrated	Continued	48	1132.2019740	5849.572498	3027.752	2821.821
84347thi	T2	MtF_Castrated	Stopped	44	5286.4257120	20979.385330	6567.265	14412.121
93070thi	T5	MtF_Castrated	Stopped	53	0.0000000	25237.647700	6449.867	18787.781
04074t55	T8	MtF	Continued	55	0.0000000	20444.848050	9340.363	11104.485
93042t36	T4	MtF	Continued	36	4010.8225730	17394.760100	11915.097	5479.663

Authors' Note

The datasets discussed in Box 1 are available through the original publication. The dataset analyzed in Box 2 is included in the appendix.

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