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Review article

Beyond the binary: Rethinking sex and the brain

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ABSTRACT

The paper reviews the relations between sex and brain in light of the binary conceptualization of these relations and the challenges posed to it by the 'mosaic' hypothesis. Recent formulations of the binary framework range from arguing that the typical male brain is different from the typical female brain to claiming that brains are typically male or female because brain structure can be used to predict the sex category (female/male) of the brain's owner. These formulations are challenged by evidence that sex effects on the brain may be opposite under different conditions, that human brains are comprised of mosaics of female-typical and male-typical features, and that sex category explains only a small part of the variability in human brain structure. These findings led to a new, non-binary, framework, according to which mosaic brains reside in a multi-dimensional space that cannot meaningfully be reduced to a male-female continuum or to a binary variable. This framework may also apply to sex-related variables and has implications for research.

1. Background: how the binary framework affects the conceptualization of the relations between sex and the brain

> "*The problem with the sex binary is that there has never been a hypothesis or a theory to test— it is an epistemological framework that runs behind, above, and beyond particular theories and research projects*" Sanz (2017, p. 20).

When we talk about female and male genitalia, we have quite a clear and agreed-upon understanding of what this means – two distinct sets of organs, one comprised of only genital organs with a form typical (i.e., common) of females, and the other comprised of only genital organs with a form typical of males. Genitalia that do not fall into one of these distinct sets, because of having either one or more genital organs with a form intermediate between the female- and male-typical forms, or some genital organs with the female-typical form and others with the male-typical form, are termed intersex, rather than 'male' or 'female'. Estimates of the prevalence of humans with intersex genitalia typically do not exceed 0.2 % (on the basis of Table 8 in Blackless et al., 2000).

This is clearly not the case in the human brain, in which, if we were to apply the terminology used to describe genitals, most brains would be 'intersex'. This is because there is overlap between the distributions of females and of males on all currently known measures of the human brain that show sex/gender differences (i.e., these measures do not appear in distinct female and male forms, reviewed in Joel, 2011). Yet, in spite of the fact that most scientists nowadays acknowledge this overlap and would not argue that brains of males and females belong to two distinct types, the binary framework still dominates thinking about the relations between sex and the brain, and the 'male brain - female brain' or 'typical male brain - typical female brain' terminology still prevails. These terms, however, may have different meaning for different scientists.

Some scientists hold that there is a typical male brain which is distinct from the typical female brain. This is often evidenced in phrases of the sort - *male brains are like this, female brains are like that* – as in: "During developmental periods, male brains tend to be structured to facilitate within-lobe and within-hemisphere connectivity… In contrast, female brains tend to have better interhemispheric connectivity and better cross-hemispheric participation…" (Tyan et al., 2017, p. 380). Other scientists assume that human brains are aligned along a male-female continuum, yet still hold that the typical female brain is different from the typical male brain. This hypothesis underlies, for example, the extreme male brain theory of autism (e.g., Baron-Cohen, 2002), as evident in this citation: "to examine the probability of autism spectrum disorder along a normative phenotypic axis ranging from the characteristic female to male brain phenotype" (Ecker et al., 2017, p.

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330).

Common to these descriptions of the typical female and male brain is the implicit assumption that different features within a single brain would be similarly located along each feature's male-female continuum (i.e., all features would be located at the male-end[1] of their distribution, or all would be located at the female-end, or all would be located in-between the two extremes, Fig. 1a). If this were the case, then indeed brains would be aligned along a female-male continuum, with the typical female brain different from the typical male brain (Fig. 1b). Yet, in 2015 we found that 'mosaic' brains – that is, brains consisting of a mixture of features – some located at the male-end of their distribution and others located at the female-end, are much more common than internally consistent brains consisting of only one type of features[2] (Fig. 1c–d; see Section 5 for more details on this analysis). On the basis of this finding we concluded that brains of women and of men do not belong to two distinct categories nor aligned along a female-male continuum (Joel et al., 2015).

To illustrate the importance of the internal consistency assumption for the binary formulations of the relations between sex and the brain described above, consider two studies of human brain connectivity. Both studies reported the existence of sex/gender differences in some connections (Ingalhalikar et al., 2014; Joel et al., 2015), but only one explicitly assessed the presence (or lack of) internal consistency (Joel et al., 2015). The first study (Ingalhalikar et al., 2014) concluded that "This analysis revealed conspicuous and significant sex differences that *suggest fundamentally different connectivity patterns in males and females*" (p. 824, emphasis added) and pointed the reader to Fig. 2 (reproduced here as Fig. 1e). This figure depicts in the upper pair of brain images, all the connections that were significantly stronger in men compared to women, and in the lower pair of brain images, all the connections that were significantly stronger in women compared to men (blue here stands for intra-hemispheric connections, and orange for inter-hemispheric connections). Although this was never explicitly stated, this type of presentation and the accompanying conclusion suggest that the authors' underlying assumption is that the differences consistently add up within individual brains, so that the upper images represent the connectivity pattern typical of males - having male-typical connections, while the lower images represent the connectivity pattern typical of females - having female-typical connections. While this may seem to be a reasonable assumption (and is true of genitalia), it was refuted by an analysis of internal consistency (Joel et al., 2015). An analysis of the seven connections showing the largest sex/gender differences (out of over 4000 connections assessed), revealed that none of the brains was internally consistent (i.e., had all seven connections in the female-end range, or all in the male-end range, both defined with the 33 % cutoff) but about half of the brains were mosaic (had at least one connection, out of the seven analyzed, in the female-end range, and at least one connection in the male-end range; for the results with additional cutoffs see Table S2 in Joel et al., 2015). Thus, the brain images in Ingalhalikar et al. (2014) do not represent the connectivity patterns typical of males and females, but rather connectivity patterns that are nonexistent or extremely rare. Instead, the connectivity pattern common in both women and men is a mosaic of blue connections (which are more common in men than in women) and orange connections (which are more common in women than in men).

In response to Joel and colleagues (2015), a new version of the binary view of the human brain was formulated - one that does not depend on the existence of internal consistency. According to this new formulation, "brains are indeed typically male or typically female" (Rosenblatt, 2016, p. E1966) because brain structure can be used to predict whether the brain's owner is female or male (Chekroud et al., 2016; Del Giudice et al., 2016; Rosenblatt, 2016). As I explain in Section 6.3, this definition of a typical male and female brain is very different from the previous ones and from our understanding of male and female genitalia.

Below I shortly summarize the results of animal studies that led to the mosaic hypothesis and human studies that support it. I then discuss the validity and usefulness of the different formulations of the binary view in light of the evidence. Next, I suggest a new, non-binary, framework for thinking about human brains, according to which mosaic brains reside in a multi-dimensional space that cannot meaningfully be reduced to a male-female continuum or to a binary variable (male and female brain). Last, I shortly discuss the mosaic nature of sex-related variables, suggest that these too reside in a multi-dimensional space that cannot meaningfully be reduced to a binary variable, and discuss the implications of these conclusions for research.

2. Evidence leading to the mosaic hypothesis: sex effects on brain structure may be opposite under different conditions

According to the classical view of sex effects on the brain, the brain undergoes sexual differentiation, with testosterone, secreted by the fetus' testes, masculinizing the brain of the male away from the default female form. According to this view, all the features within a single brain that are affected by testosterone are expected to be similarly located along their female-male continuum. Moreover, brains are expected to be located along a female-male continuum, depending on each brain's testosterone levels during development.

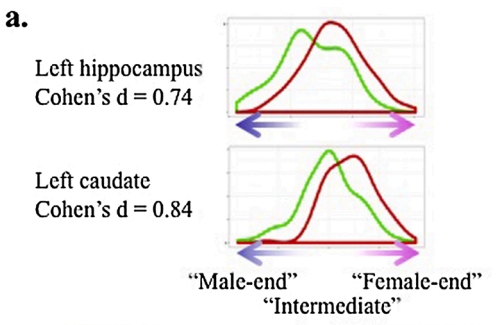
This scenario, however, is highly unlikely given what is currently known about sex effects on the developing (and mature) brain (for a detailed review and examples, see Joel et al., 2020). Thus, while animal studies provided plenty of evidence that testosterone affects multiple aspects of brain structure, brain structure is also influenced, in both males and females, by other sex-related hormones and by sex-related genes (for reviews see Arnold, 2012; Arnold and Chen, 2009; Grgurevic and Majdic, 2016; McCarthy and Arnold, 2011; McEwen and Milner, 2017; Ngun et al., 2011; Sekido, 2014). This is expected to lead to higher variability in the 'femaleness-maleness' of different features within a single brain than the one expected in the case of a single factor. Moreover, sex-related hormones, including testosterone, act on different brain features via multiple independent mechanisms, so that even features affected by the same hormone may vary considerably in their location along their female-male continuum (for review see Joel and McCarthy, 2017; McCarthy and Arnold, 2011; McEwen and Milner, 2017). Finally, at least some of the effects of sex-related genes and hormones may be *opposite* under different external conditions, suggesting that within a single brain, some features may not only be poorly correlated in their 'femaleness-maleness', but located at opposing ends of each feature's female-male continuum (for review and references see Joel, 2011, 2012; Joel et al., 2020).

At the ***group level***, the result of this multitude of mechanisms and interactions is that the form[3] of a particular brain feature that is typical of females under one set of conditions (e.g., individual housing) may be typical of males under another set of conditions (e.g., group housing), and vice versa (i.e., the form typical of males under the first set of conditions may be typical of females under the second set of conditions).

[1] As shown in Fig. 1a, the female-end and the male-end correspond to the two extremes of the distribution, where there are large differences between the frequencies of females and males. Data reviewed below relates to an operational definition of the female- and male-end zones as the scores of the 33% most extreme females and males, respectively.

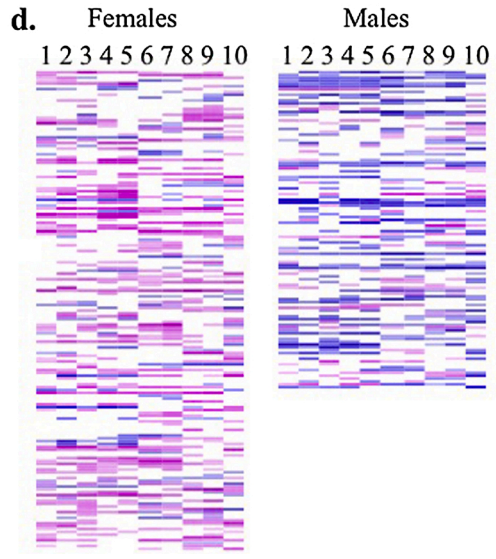
[2] This conclusion was true over operational definitions of the female- and male-end zones with cutoffs of 10%, 20%, 33%, and 50%.

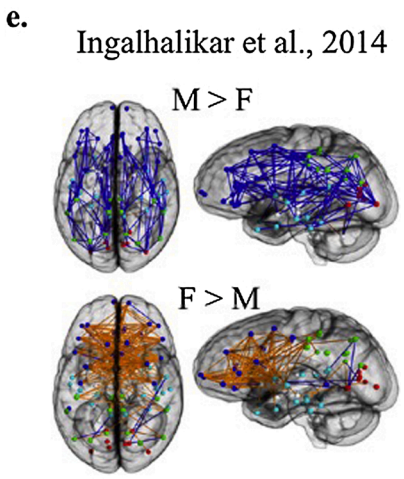
[3] 'Form' here may relate to the size of a brain region, the morphology of neurons, the density of receptors, or any other measure of brain structure. If, for example, a region is larger, on average, in males compared to females, then the form typical of males would correspond to a volume-range in which more males than females fall, and the form typical of females would correspond to another volume-range, in which more females than males fall.



c.

	Brain region	Cohen's d
1.	Left superior frontal gyrus, orbital part	0.81
2.	Right superior frontal gyrus, orbital part	0.84
3.	Left superior frontal gyrus, medial orbital	0.77
4.	Left gyrus rectus	0.83
5.	Right gyrus rectus	0.75
6.	Left hippocampus	0.74
7.	Right hippocampus	0.72
8.	Left caudate nucleus	0.84
9.	Right caudate nucleus	0.71
10.	Vermic lobule X	0.8





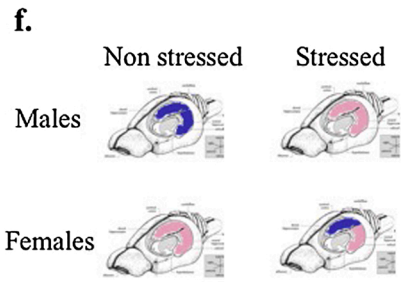


Fig. 1. Internal consistency versus mosaic. a. Grey matter volume of 116 regions in the brains of 169 women and 112 men were assessed using voxel-based morphometry (VBM). The figure presents the frequency distribution of the grey matter volume in women (red) and men (green) of two of the regions showing the largest sex/gender differences in this sample (left hippocampus [top, Cohen's d = 0.74, $p < 0.0001$] and left caudate [bottom, Cohen's d = 0.84, $p < 0.0001$]). b. A schematic representation of internal consistency – if all regions in a brain are similarly located at each feature's female-male continuum, then brains would be similarly located along a female-male continuum, ranging from a brain in which all features are at the female-end (pink) to a brain in which all features are at the male-end (blue). c. Ten regions from the data set described in 1a, that showed the largest sex/gender differences ($0.70 < d \leq 0.84$, all p's < 0.0001) were chosen for the mosaic analysis. d. A continuous color representation of each region's location along its male-female continuum was created separately for each of the 10 regions. Volumes falling in the "male-end" and in the "female-end" zones (defined as the scores of the 33 % most extreme males and females, respectively) are colored using continuous blue-white and pink-white scales, respectively; Volumes falling in the "intermediate" zone are colored in white. In the tables, each horizontal line represents the brain of one individual and each column represents a single brain region. The number above each column corresponds to the region's number in 1c. (a and d were created with permission on the basis of Fig. 1 in Joel et al., 2015; For details of the sample, imaging methods, and data analyses see Joel et al., 2015). e. A summary diagram of sex/gender differences in brain connectivity. The top pair of brain images depicts connections that were significantly stronger in men compared to women, and the bottom pair of brain images depicts connections that were significantly stronger in women compared to men. Intra-hemispheric connections are shown in blue, and inter-hemispheric connections are shown in orange. (Reproduced with permission from Fig. 2 in Ingalhalikar et al., 2014). f. A schematic summary of the results of Reich et al (2009). The density of CB1 receptors that is typical of females and males that are kept in standard laboratory conditions is marked in pink and blue, respectively. In male rats that were exposed to chronic stress, the density of the receptors in both the ventral and dorsal hippocampus is the one typical of non-stressed females. In female rats that were exposed to chronic stress, the density of the receptors in the ventral hippocampus is the one typical of non-stressed females, whereas the density of receptors in the dorsal hippocampus is the one typical of non-stressed males.

At the ***individual level***, the multiplicity of mechanisms and interactions is expected to result in brains comprised of unique combinations of features that greatly vary in their location along each feature's female-male continuum, leading to brains consisting of a mosaic of both female-end and male-end features. Moreover, these mosaic brains would not be meaningfully aligned along a male-female continuum (Joel, 2011, see also Section 6.2).

The general principles described above may be illustrated by the results of a single study, which assessed the effects of three weeks of mild stress on the density of CB1 cannabinoid receptors in the rat hippocampus (Reich et al., 2009): In rats kept under standard laboratory conditions, the density of CB1 receptors was on average 3–4 times higher in males compared to females in both the ventral and dorsal hippocampus. Following the stress exposure, the effects of sex in the

a.

	Brain region	Typical change in females (F) and males (M)
1.	Right inferiorparietal cortex	F - M ↓
2.	Left inferiorparietal cortex	F - M ↓
3.	Right pericalcarine cortex	F - M ↑
4.	Left pericalcarine cortex	F - M ↑
5.	Corpus callosum - Central	F↓ M ↑
6.	Corpus callosum - Mid Anterior	F↓ M -
7.	Corpus callosum - Anterior	F↓ M -

b.

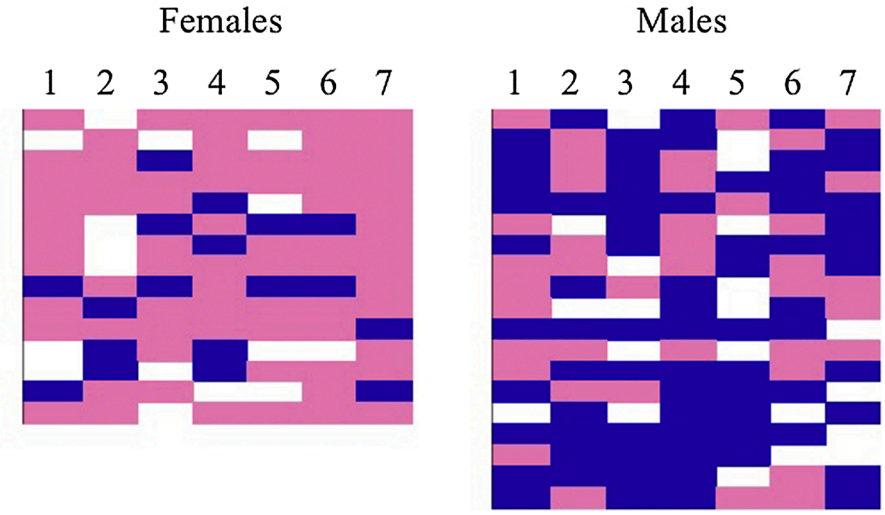


Fig. 2. Mosaic in the human brain response to stress. The volume of 68 cortical regions and 26 subcortical structures was assessed in 34 participants (15 women) using surface-based analysis. Each participant was scanned twice: Once during their first week of a premilitary paramedic preparation course, and again 36 months later. During their military service, all participants experienced at least one highly stressful event, which was similarly accompanied in women and men by intense negative emotions and an increase in stress-related symptoms. a. Seven regions, in which the most commonly observed (i.e., mode) change (increase, decrease, or no change) in volume in women was different from the most commonly observed change in men, *and* the Sex x Time interaction for the region was significant. b. The tables present for each women (left) and each men (right) whether the change in each brain region was the one typical of women (pink), of men (blue) or of neither (white). Each horizontal line represents the brain of one individual and each column represents a single brain region. The number above each column corresponds to the region's number in 2a. (Created with permission on the basis of Fig. 1 and Table 2 in Shalev et al., 2020; For more details of the sample, imaging methods, and data analyses see Shalev et al., 2020).

dorsal hippocampus were reversed – the average receptor density of the stressed female rats was as high as that observed in non-stressed males, whereas the average receptor density of the stressed males was as low as that found in non-stressed females. In the ventral hippocampus the effects of sex on CB1 receptor density were again reversed in males, but were unaffected in females (leading to the disappearance of the group-level sex difference that was observed under the no-stress condition) (Reich et al., 2009).

Thus, at the ***group level***, for both the ventral and dorsal hippocampus, the density of CB1 receptors that is typical of females and of males depends on an external factor (exposure to stress). Moreover, considering the density of CB1 cannabinoid receptors in the dorsal and ventral hippocampus together, the hippocampus could be found in one of three forms: low receptor density in both the ventral and dorsal hippocampus (in non-stressed females and in stressed males); high receptor density in both the ventral and dorsal hippocampus (in non-stressed males); and high receptor density in the dorsal hippocampus and low receptor density in the ventral hippocampus (in stressed females). These three forms of the hippocampus cannot be meaningfully sorted into a male-typical and a female-typical form, nor be meaningfully aligned along a female-male continuum.

This example also demonstrates how the interactions between sex and other factors may lead to the formation of a mosaic brain at the ***individual level***. Consider for example a sample of rats, all of which are kept under standard laboratory conditions. Most males would exhibit high CB1 receptor density in the dorsal and ventral hippocampus, whereas most females would exhibit low receptor density in the two hippocampal regions (Fig. 1f). A few rats in the sample may be exposed to stress (because, for instance, they were unintentionally housed with a dominant and aggressive rat). These rats would exhibit sex-atypical features - in males, the density of CB1 receptors in both the ventral and dorsal hippocampus would be in the range typical of females in this sample. In females, receptor density in the dorsal hippocampus would be in the male-typical range whereas receptor density in the ventral hippocampus would be in the female-typical range. Thus, in terms of the density of CB1 receptors in the hippocampus, these females would exhibit a mosaic of female-typical and male-typical features (Fig. 1f).

Taking into account that interactions between sex and other factors have been reported for additional brain measures (e.g., spine density, number of neurons), brain regions (e.g., amygdala, cortex, cerebellum), and types of manipulation (e.g., housing conditions, drug exposure, for review see Joel, 2011, 2012, 2020) and moving from considering two brain features and two environmental conditions to considering the entire brain and the huge complexity of the environment from the moment of conception throughout life, it is difficult to imagine that brains would be internally consistent in the 'sex-typicality' of their different features. Instead, the mosaic hypothesis holds that most brains would consist of unique mosaics of features - some in the form typical of the females in that sample and others in the form typical of the males in that sample - and that these mosaics would not fall into two distinct types nor be meaningfully aligned along a male-female continuum (Joel, 2011).

I would like to stress that the mosaic hypothesis does *not* hold that sex does not affect the brain or that there are no group-level sex differences in specific brain features. Rather, the mosaic hypothesis holds that the multiplicity of mechanisms by which sex affects the brain combined with the repeated observation that sex-related effects depend on other factors, result in brains with features that greatly vary in their location along each feature's male-female continuum.

One prediction of the mosaic hypothesis would therefore be that mosaicism would be greater under conditions of greater genetic and environmental variability. Thus, little mosaicism is expected in an

inbred strain of laboratory animals kept under the same external conditions from utero, more mosaicism is expected in an out-bred strain of laboratory animals kept under different sets of external conditions, and a lot of mosaicism is expected in wild-type animals living in the wild.

A note on mosaic and variance. Variance in biological systems is always expected. There is large variability in the form of genital organs within females and within males. But variance in sex effects on the brain differs from variance in sex effects on the genitalia, in that the former may be so large as to lead to the existence of female-typical and male-typical features in the same brain - a situation that is very rarely observed in human genitalia. It is evidence for the existence of this type of variance that led to the formulation of the mosaic hypothesis and to the construction of methods to test it. (See more on mosaic versus noise in Section 5.2.1).

3. Mosaic in the brain's response to external events

The observation that various manipulations alter how sex affects the brain means that the effects of these manipulations on the brain are different in females and males (e.g., stress decreased the density of CB1 receptors in the dorsal hippocampus in males, but increased it in females, Reich et al., 2009). Indeed, most studies that reported interactions between sex and other factors framed their results as sex differences in the effects of the other factor (e.g., the title of the study by Reich et al. (2009) described above is: "Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats"). Could it thus be that the brains of females and males are distinct not in their structure but rather in their response to environmental conditions (e.g., there's a female-typical and a male-typical neural response to stress)? This would be the case if all the features in a single brain responded to an environmental event (such as stress) in the way typical of females or all responded in the way typical of males. This would not be the case, however, if in an individual brain, some features would change in the way typical of males while others would change in the way typical of females – that is, if the response of each brain consisted of a mosaic of female-typical and male-typical changes. Such 'mixing' of responses would occur if the way in which a brain feature responds to an environmental event depends not only on sex, but on an interaction between sex and other factors.

Animal studies are seldom designed in a way suitable for answering this question - that is, they rarely test the effects of a specific manipulation (e.g., stress) under different conditions (e.g., individual versus group housing) in females and males. The results of one study, which was designed this way, suggest that the effects of a manipulation on females and males may depend on other factors. Horovitz et al. (2014) assessed the behavioral effects of stress experienced in adulthood in male and female rats that were either exposed to stress early in life or not. Thus, it was possible to appreciate whether sex differences in the response to stress experienced in adulthood depend on other factors – in this case, early exposure to stress. Horovitz et al. (2014) found that at the ***group level***, the early exposure to stress interacted with sex to determine the average response to stress experienced in adulthood. At the ***individual level***, while the behavioral response to adulthood stress that was typical (i.e., common) of females exposed to early stress was different from the one typical of males exposed to early stress, there were some females and males that exhibited the response typical of the other sex. These observations suggest that additional factors, that were not measured or manipulated in Horovitz et al's (2014) study, interacted with sex to determine an individual's response to stress, and that mosaicism may also occur in sex-related responses to stress.

The possibility that mosaicism may also be seen in sex-related responses to stress was recently supported by a small-scale magnetic resonance imaging (MRI) study in humans exposed to real-life extreme stress (Shalev et al., 2020). Considering seven regions (listed in Fig. 2) in which the change in volume that was most common in women (increase, decrease or no change) was different from the change most common in men, we found that 25 out of the 34 participants exhibited a mosaic of female-typical and male-typical structural changes, whereas in only one participant all changes were of the same type (Fig. 2, created with permission on the basis of Fig. 1 and Table 2 in Shalev et al., 2020).

4. A note on sex effects and sex differences

Studies in which sex-related genes or hormones are directly manipulated demonstrate that sex affects brain structure and function (for reviews see Arnold, 2012; Arnold and Chen, 2009; Grgurevic and Majdic, 2016; McCarthy and Arnold, 2011; McEwen and Milner, 2017; Ngun et al., 2011; Sekido, 2014). Yet, most of the evidence for sex effects on the brain derives from studies reporting a difference between a group of females and a group of males on some endpoint(s) (e.g., regional volume, receptor density). While such studies show that sex-related variables affect the endpoint, they do not suffice to identify the variable(s) responsible for this effect, nor even to provide information on whether these variables are part of "sex itself" (i.e., sex-related genes and hormones, Richardson, 2013), are affected by sex-related genes or hormones (e.g., body size), or are correlated with sex category (e.g., single versus group housing) (for a detailed discussion of the direct and indirect effects of sex, see Joel and McCarthy, 2017). This problem is intensified in studies of the human brain, as many more variables (environmental, psychological and social) correlate with sex category (e.g., Fausto-Sterling, 2000; Fine, 2010; Joel and Fausto-Sterling, 2016; Joel and McCarthy, 2017; Jordan-Young and Rumiati, 2012; Kaiser, 2012; Maney, 2015; Rippon et al., 2014). I therefore refrain from using the term 'sex effects' when discussing the human brain, and use instead 'sex/gender differences'.

5. Mosaic in human brain structure

To assess whether sex differences add up consistently or 'mix' to create mosaics, one has to consider at least two measures showing sex differences for each brain. Below I describe two studies that tested the mosaic hypothesis in the human brain – one used postmortem data of the type often assessed in laboratory animals (namely, the number of neurons in two hypothalamic nuclei, Joel et al., 2020); the second used different types of measures obtained from MRI studies of the entire brain (Joel et al., 2015).

5.1. Mosaic in the human hypothalamus: analysing post-mortem data

We (Joel et al., 2020) have recently co-analyzed three hypothalamic measures that show large sex/gender differences - differences that are amongst the largest known to date in the human brain. Specifically, we assessed mosaicism in the total number of neurons in the interstitial nucleus of the anterior hypothalamus, subdivision 3 (INAH3, a sub-nucleus of the uncinate nucleus), and in the number of galanin-stained and non-galanin stained neurons in the INAH1 (also called sexually dimorphic nucleus or intermediate nucleus) (Garcia--Falgueras et al., 2011; Garcia-Falgueras and Swaab, 2008). There was relatively little overlap between the distribution of scores for women and for men in each of the three measures - the probability that a man picked at random will have a higher score than a woman picked at random (Del Giudice, 2019) was 0.88, 0.74 and 0.73, respectively. This allowed the delineation of a male-typical and a female-typical range of scores (a range of scores which are very common in men but rare in women, or are very common in women but rare in men, respectively[4]), and subsequently the assessment of mosaicism within each brain. We

[4] For example, in the INAH3, the female-typical and the male-typical range of scores were defined as scores below and above 2,000 neurons, as 82% of the women in the sample had fewer than 2,000 neurons and 93% of the men had more than 2,000 neurons (Joel et al., 2020).

found that even when considering only three brain measures each showing a large sex/gender difference, about half the brains contained a mixture of female-typical and male-typical measures – a proportion significantly higher than expected if brains were internally consistent (Joel et al., 2020).

5.2. Mosaic in the human brain: analyzing MRI data

We analyzed MRI data of over 1400 brains from four datasets (the analysis of one dataset is described in Fig. 1). Because most MRI-derived brain measures show no or only small sex differences, we analyzed only a few (7–12) brain measures in each dataset - those showing the largest sex/gender differences (for example, in the analysis of the dataset described in Fig. 1, the Cohen's d of the sex difference in the regions included in the analysis ranged between 0.70 to 0.84). In addition, because the overlap between women and men in brain measures obtained from MRI data is much greater than that observed in the human hypothalamic measures described above, a female-typical and a male-typical range of scores could not be defined even for the measures showing the largest sex/gender differences (because the scores common in women are also common in men, and vice versa, e.g., Fig. 1a). We therefore defined for each of these measures a female-end and a male-end range of scores, each at one extreme of the distribution, where there are large differences between the frequencies of females and males (Fig. 1a). For example, with the female-end and the male-end ranges defined as the scores of the 33 % most extreme females and males, respectively, the average percent of males with a female-end score and of females with a male-end score was 17 % (thus, the chances of falling at the end zone of the other sex were half the chances of falling at the end zone of one's sex; Joel et al., 2015). We then assessed whether brains were internally consistent (i.e., all the measures fell in the male-end zone, or all fell in the female-end zone) or mosaic (at least one measure fell in the male-end zone and at least one measure fell in the female-end zone, Fig. 1d).

We found that regardless of the sample, the MRI-derived measure analyzed (volume, cortical thickness or connectivity), or the male-end - female-end cutoff (50 %, 33 %, 20 % or 10 %), mosaic brains were more common than internally consistent brains (depending on the sample, with a cutoff of 33 %, the percent of mosaic brains ranged between 23 and 53, and the percent of internally consistent brains ranged between 0 and 8.2; the remaining brains were comprised either of male-end and intermediate features, or of female-end and intermediate features, Joel et al., 2015). (The results with cutoffs of 10 %, 20 % and 50 % can be found in Table S2 in Joel et al., 2015).

Clearly, the number of internally consistent and mosaic brains depends on the choice of cutoff. The more lenient the cutoff (i.e., more participants are included in the male- and female-end ranges), the higher the number of both internally consistent and mosaic brains. Therefore the number of internally consistent brains or of mosaic brains by itself is meaningless; it is the comparison between the two that is important. The mosaic hypothesis is supported when mosaic brains are more prevalent than internally consistent brains, whereas the reverse scenario suggests the existence of two distinct types (Joel et al., 2015, 2016). Indeed, a higher number of internally consistent faces than of mosaic faces was found in an analysis of the facial morphology of three primate species (i.e., distinct types, Del Guidice et al., 2016). (For a summary and discussion of the criticism of the mosaic analysis, see Joel, 2020; Joel et al., 2020).

5.2.1. Mosaic versus noise

I want to stress that with the above definition of a mosaic, a brain is considered a mosaic only if it shows large variability in the location of its features on each feature's male-female continuum. For example, with a cutoff of 33 %, a brain of a male would be considered a mosaic only if at least one brain measure (of the 7–12 analyzed in that dataset) fell at the male-end zone (where 33 % of males fall), and at least one measure fell at the other extreme, namely, the female-end zone (where, on average, only 17 % of males fall). Smaller variance that results in some features falling at the male-end (or female-end) and all others falling at the intermediate range (where, on average, ~50 % of males and females fall, Fig. 1a), would not be classified as mosaic (nor as internally consistent).

I want to reiterate that the mosaic hypothesis was built on the basis of the observation that sex effects on brain features may be opposite under different conditions, and that the interactions of sex with other variables may be different for different brain features. The mosaic analysis was specifically constructed to detect this type of variability – created by features located at opposite ends of their male-female continuum – and ignore variability due to random noise. Using simulations, we have shown that the pattern of results (i.e., the number of internally consistent brains and of mosaic brains) obtained by the mosaic analysis of human brain measures is different from the one expected were these measures internally consistent but noisy (see Fig. S1 in Joel et al., 2015).

Finally, I would like to point out that the mosaic analysis is more sensitive than correlation coefficients in detecting internal consistency and in differentiating between mosaicism and noise. Clearly, if the correlation coefficient between two variables is very low (as was the case for most correlations between the hypothalamic measures described above, Joel et al., 2020), then the two variables are not internally consistent (and a mosaic analysis would reveal many mosaic brains and very few internally consistent brains, see, Fig. S1E in Joel et al., 2015). Similarly, if the correlation coefficient between two variables is near 1, then the two variables are internally consistent (and a mosaic analysis would reveal some internally consistent brains and no mosaic brains, see, Fig. S1A in Joel et al., 2015). However, high correlation coefficients between variables (i.e., in the range of 0.7−0.8) may reflect either an internally consistent system with some degree of random noise (Fig. S1B and S1C in Joel et al., 2015) or a system with no underlying internal consistency (the simulated data in Del Giudice et al., 2016). The mosaic analysis can differentiate between the two possibilities – in the former case there would be more internally consistent brains than mosaic brains, whereas in the second case, the opposite would be true (for further discussion see Joel et al., 2016 and the Supplementary Material of Joel et al., 2015).

6. The validity and usefulness of the different formulations of the binary view of the human brain

That human brains do not belong to two distinct types, the way human genitalia do, stems from the observation that mosaic brains are common whereas internally consistent brains are rare (in contrast to human genitalia, where the opposite is true).

6.1. Is the typical female brain different from the typical male brain?

But does the prevalence of mosaic brains also contradict the view that the typical female brain is different from the typical male brain? We have recently tested this question using MRI data of over 2100 brains from two datasets, and concluded that brain architectures typical of women are also typical of men, and vice versa (Joel et al., 2018; Note that in these analyses we used all brain measures, not only the ones showing the largest sex/gender differences, as we had done in the mosaic analysis). Specifically, if the typical female brain were different from the typical male brain, we should expect an anomaly detection algorithm that was trained on women's brains to mark many more brains of men as anomalous compared to brains of women. Instead, the anomaly detection algorithm marked very similar numbers of men's and women's brains as anomalous, suggesting that the brain architectures typical of women are also common in men (Joel et al., 2018). Training the algorithm on men's brains and then testing it on men's and women's brains yielded the same result - the brain architectures typical of men are also common in women (Joel et al., 2018). An unsupervised cluster analysis supported this conclusion by showing that large clusters –

which represent common human brain architectures, include a similar number of brains from women and from men. Large sex/gender differences were found only in some of the small clusters, which represent rare brain architectures (Joel et al., 2018). (Although this has not been tested, these small clusters could potentially account for sex/gender differences in the prevalence of some neuro/psychiatric conditions, such as autism, which are rare in the population but show large sex/gender differences.)

The conclusion that the brain architectures typical of women are also common in men, and vice versa, is consistent with the observation that when total brain size it taken into account, there are only few and mostly small sex/gender differences in MRI-derived brain measures (e.g., Jancke et al., 2015; Sanchis-Segura et al., 2019) and sex/gender category accounts for less than 2% of the variance in human brain structure (Eliot, 2020).

Note that whereas the lack of large differences in the proportion of women and men in the large clusters indicates that sex category is less important than other variables (such as age, Jancke et al., 2015) in explaining human variability in brain structure, it does not indicate that there are no sex/gender differences in the brain or that these differences cannot be used to cluster brains according to sex category (Joel, 2011; Joel et al., 2016, 2018, see also Section 6.3).

6.2. *From a male-female continuum to considering mosaic brains in a multi-dimensional space*

The view that emerges from the two studies (Joel et al., 2015, 2018) is that, when human brains are described by the vector of their feature values (e.g., the volume of 116 regions of grey matter, Fig. 3a), human brains constitute a cloud of points in a multi-dimensional space, with women and men sharing quite equally the dense central part, and differing in some of the sparser periphery. Even in the bivariate scatterplot of the two principal components that differentiate most between women and men (Fig. 3b), the overlap is all encompassing.

I suggest that this new multi-dimensional description should replace the image of brains aligned along a male-female continuum (e.g., Baron-Cohen, 2002; Ecker et al., 2017; Phillips et al., 2019). The male-female continuum may be useful for describing the distributions of

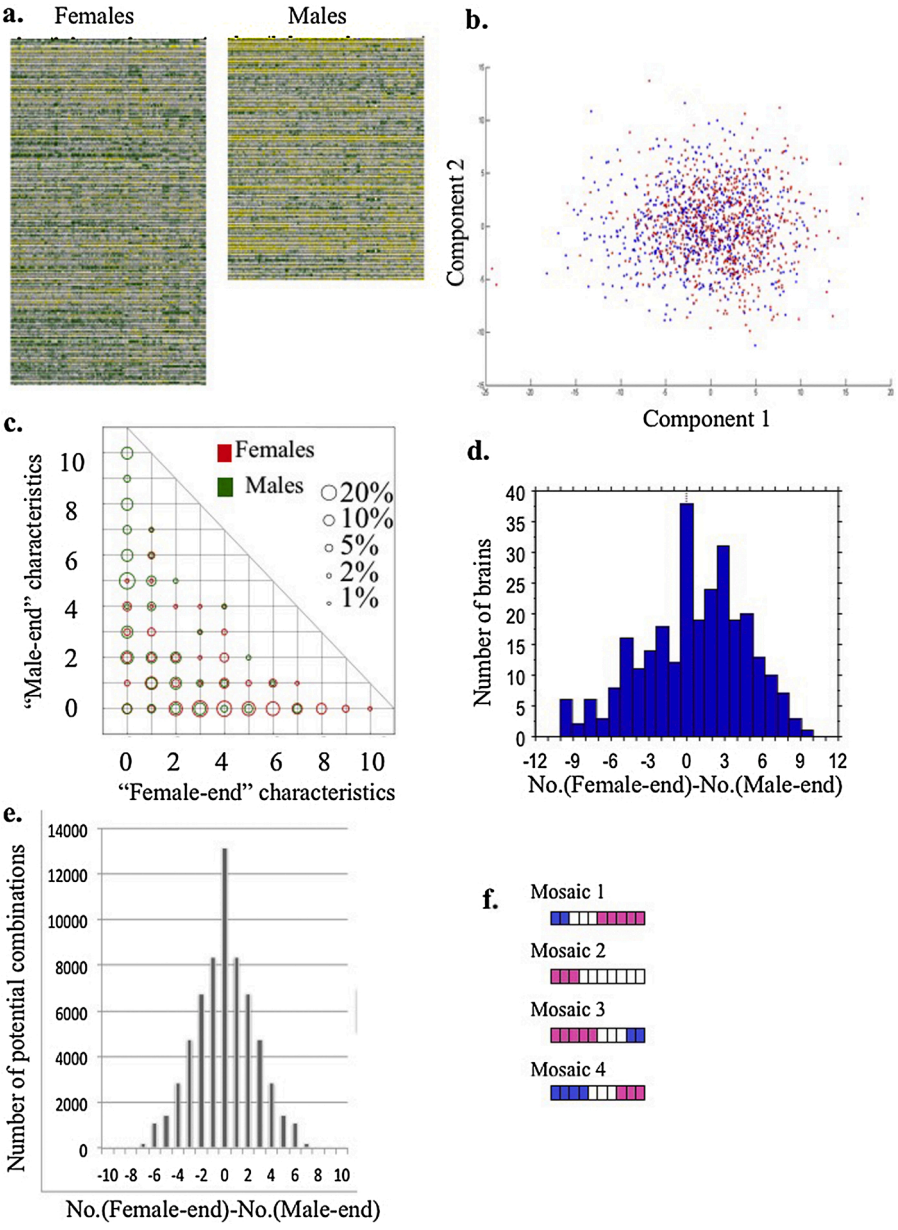


Fig. 3. From a male-female continuum to a multi-dimension space. a. The grey matter volume of all 116 regions of grey matter in females (left) and in males (right) from the sample described in Fig. 1a,c,d is represented using a continuous green (large) – white – yellow (small) scale. Each horizontal line represents the brain of one individual and each column represents a single brain region. The continuous large-small scale represents the volume of a brain region in a given brain relative to the volume of this brain region in all other brains (of both females and males). (Created with permission on the basis of Fig. 3 in Joel et al., 2015). b. A principal component analysis of the dataset presented in (a) was conducted and the two principal components that differentiate most between women and men were selected. The graph presents the bivariate scatter gram of the scores of brains of females (red) and males (blue) on these two components. c. A bivariate scatter gram of the number of regions (out of the ten regions listed in Fig. 1c) at the "female-end" (x axis) and at the "male-end" (y axis) in females (red) and males (green) in that sample (the actual mosaics can be found in Fig. 1d). d. The difference between the number of female-end characteristics and the number of male-end characteristics was computed for each brain, and is presented in a histogram. e. A histogram of the number of all possible combinations of female-end, intermediate and male-end characteristics for each "female-end minus male-end characteristics score". f. Hypothetical brains with different combinations of "male-end" (blue), "intermediate" (white), and "female-end" (pink) features.

women and men on a single brain feature (e.g., Fig. 1a), but fails to account for the observations obtained when several brain features (or the brain as a whole) are considered together (Joel, 2020; Joel et al., 2015, 2018, 2020). Moreover, I claim that although it is mathematically possible to align brains on a male-female continuum (there are many mathematical ways to achieve this, e.g., Phillips et al., 2019), such an alignment would carry little information.

To illustrate this, take for example the set of brains shown in Fig. 3a. A mosaic analysis of the ten brain regions showing the largest sex/gender differences in this dataset (listed in Fig. 1c) reveals that most women have more female-end features than male-end features, whereas the opposite is true for men (Figs. 1d, 3 c). We can thus align the brains on a female-male continuum by assigning each brain a score calculated as the difference between the number of its female-end and male-end characteristics (Fig. 3d; a similar method has been used by Baron-Cohen and colleagues to align humans along a systemizer-empathizer continuum, e.g., Greenberg et al., 2018). On this continuum, brains with only female-end characteristics would be at one pole (+10), and brains with only male-end characteristics would be at the other pole (-10). The two poles are thus well defined, having either all regions in the female-end form or all regions in the male-end form. Note that these poles do not represent typical male and female brains, but rather brain types that are extremely rare (Fig. 3c,d). In contrast, scores along the rest of the continuum, where most brains reside, are ill defined as they may include very different brain mosaics. Continuing with the ten regions example, a brain with a score of +3 may be comprised of any of the following four combinations of female-end, intermediate and male-end characteristics – [3,7,0], [4,5,1], [5,3,2], [6,1,3] – and each of these combinations potentially includes many different mosaics, depending on which of the ten regions is in which form. For example, the number of potential mosaic brains with five female-end characteristics, three intermediate characteristics and two male-end characteristics is 2520 (Fig. 3e; the actual mosaics observed among the 169 women and 112 men of this dataset are depicted in Fig. 1c). Thus, two mosaics with the same female-end - male-end score (e.g., Mosaics 1 and 2 in Fig. 3f) or even the same number of female-end, intermediate and male-end characteristics (e.g., Mosaics 1 and 3 in Fig. 3f) may be very different from one another, and more similar to other mosaics with a different female-end - male-end score (e.g., Mosaics 1 and 4 in Fig. 3f). More generally, it is the specific composition of a brain, not the difference between the number of its female-end and male-end features, that determines whether it is similar to or different from other brains. Indeed, the unsupervised cluster analysis described above revealed that the chances of a woman and a man to be in the same cluster are very similar to the chances of two women or two men to be in the same cluster (Joel et al., 2018).

The above example demonstrates the type of information that is being lost when information residing in a ten-dimension space (1c) is reduced to a single dimension (Fig. 3d). Moreover, brains have many features in addition to those showing sex/gender differences (e.g., Fig. 3a), and information about these features is also being lost when brains are aligned along a male-female continuum. Given that sex/gender accounts for a very small part of the variability in human brain structure (Eliot, 2020) and probably also function (e.g., Kersey et al., 2019; Mitricheva et al., 2019) it is clear why even though sex/gender differences may be used to align brains on a male-female continuum, such alignment carries little information about an individual's brain structure.

6.3. The "prediction" version of the binary view of human brains

This discussion brings us to the question of prediction, and specifically to how does this new multi-dimensional view of human brains reconcile with the repeated observation that the structure and function of the brain can be used to predict with high accuracy (often 80 % or higher) whether the brain's owner is female or male (e.g., Anderson et al., 2019; Chekroud et al., 2016; Del Giudice et al., 2016; Joel et al., 2016, 2018; Rosenblatt, 2016; van Putten et al., 2018v; Zhang et al., 2018; Note, however, that Sanchis-Segura et al., 2020 showed that the accuracy of sex prediction on the basis of brain structure drops to around ~60 % when total brain size is properly controlled for). Here, instead of reducing the information in the multi-dimensional space into a single dimension (a female-male continuum), it is reduced into a binary variable – female or male. Clearly, this binary variable carries very little information about a person's specific brain mosaic. It merely assures us that had we known the structure of this person's brain, we could have guessed her/his sex category with high accuracy. Yet, it is a person's sex category that led us to predict that s/he has a female or a male brain in the first place, so what kind of information have we gained from the "prediction" definition of a male and a female brain? Knowing that someone is, say, male, gives you much more information about the form of his genital organs than that you're very likely to conclude he is male had you seen his genitalia. The latter is of course true, but knowing that someone is male allows you in addition to very safely predict that he has a penis, scrotum, prostate and vas deference and surely does not have a clitoris, minor and major labia, vagina, fallopian tubes and uterus. The studies cited in the present review clearly demonstrate that not only such a prediction is not possible for brain structure (because most brains are unique mosaics of female-typical and male-typical features), sex category provides little information about the structure of an individual's brain.

Is it then worth maintaining the "prediction" formulation of the male and female brain only for the sake of preserving a binary view of the human brain?

I do not think so.

6.4. "Costs" of the binary view of the human brain

Maintaining the binary framework interferes with our efforts to understand the human brain because it diverts us from studying other variables, which may be more important in understanding the human brain in health and disease (e.g., Mitricheva et al., 2019). In addition, the focus on sex differences often leads researchers and readers to overestimate their importance. The title of too many studies declares that females and males differ in brain structure, function or connectivity, whereas careful reading of the Methods and Results sections reveals that of the hundreds or even thousands of variables assessed, a significant sex difference was found in only a few. For example, a recent study of functional connectivity in utero was titled "Sex differences in functional connectivity during fetal brain development", even though there were no sex differences in connectivity patterns, and of the 128 correlations between sex and age that were assessed, there were significant differences in only three (Wheelock et al., 2019).

The binary view of the human brain and the accompanying practice of looking for sex differences may also send researchers chasing false positive results. As I explain elsewhere (e.g., Joel, 2011, 2020; Joel and Fausto-Sterling, 2016), when the population is highly heterogeneous and the samples are relatively small (as, for example, in functional MRI studies), comparing two samples from this population (one of females and the other of males) is likely to yield some significant differences. But these would not reflect genuine sex differences worth pursuing, but rather false-positive errors. The results of a recent study support this claim. David et al. (2018) assessed the relations between sample size and the number of significant sex/gender differences in human functional MRI studies. If the "prediction", or any other definition, of the typical male and female brain were meaningful, then larger samples, which have greater power, should have discovered more sex/gender differences. Yet no correlation was found between sample size and the number of significant sex/gender differences (David et al., 2018). The authors concluded: "The extremely high prevalence of "positive" results and the lack of the expected relationship between sample size and the number of discovered foci reflect probable reporting bias and excess significance

bias in this literature" (David et al., 2018).

7. Mosaics of sex-related variables?

The above discussion reveals that, contrary to the privileged position and coordinated action of sex-related variables in determining the form of the genitalia, these variables are a fragment of a large array of variables that interact to determine brain structure. These interactions result in poor correlations between sex effects on different brain measures, and as a result, sex category provides little information about a brain's specific structure.

As mentioned above (Section 4), there are many variables that correlate with sex category (i.e., variables on which females and males differ at the group level) and many of these may affect brain structure and function. These sex-related variables include aspects of "sex itself", that is, sex-related genes and hormones, as well as many physiological (e.g., body size), psychological (e.g., empathy), and social/environmental (e.g., status) variables (e.g., Fausto-Sterling, 2000; Fine, 2010; Joel and Fausto-Sterling, 2016; Joel and McCarthy, 2017; Jordan-Young and Rumiati, 2012; Kaiser, 2012; Maney, 2015; Rippon et al., 2014; Ritz et al., 2014). These variables may be affected by sex itself, by gender (that is, the social construction of the sex categories), by both, and by many other variables (e.g., one's height also depends on genetic and environmental (e.g., nutrition) variation not related to sex) (e.g., Joel and McCarthy, 2017; Joel et al., 2020).

As noted by Maney (2016), one's sex/gender category provides very little information about a person's specific values on all of these sex-related variables. This is particularly problematic because only the Y chromosome, the gonads and the genitalia appear in a binary form (e.g., present or absent for the Y chromosome; ovaries or testes for the gonads). For all other sex-related variables, including sex-related hormones (for a recent review see, Hyde et al., 2019), there is overlap between the values observed in females and in males – overlap that is often considerable (e.g., Hyde, 2005, 2014; Hyde et al., 2019; Zell et al., 2015). Moreover, there is little reason to believe that the many sex-related variables are highly correlated. For example, there is no a priori reason to believe that muscle to fat ratio is strongly correlated with empathy or socioeconomic status, or even with height. Therefore, specifically because men and women differ on average on many variables, it is highly likely that most humans possess a mosaic of values on these variables, with some values falling on their male-end of the distribution and others on their female-end.

If sex-related variables reside in a multi-dimensional space that cannot meaningfully be reduced into a binary variable (female, male), then the current practice of studying sex mostly in the context of sex differences should be replaced with the measurement of sex-related variables and the assessment of their associations with the phenomenon under study.

7.1. Implications for research and diagnosis: considering sex as a biological variable

The exclusion of females from clinical trials as well as from many areas of basic research harmed not only the health of women, but also the advance of science and medicine (for several examples, see https://genderedinnovations.stanford.edu). The requests of the National Institute of Health (NIH) and other funding agencies to include women in clinical trials (NIH revitalization Act, 1993) and later to consider sex as a biological variable in basic research (e.g., Clayton and Collins, 2014) were necessary steps to correct this situation. The problem is that the justified call to include both sexes in research is often followed by a binary conceptualization of the physiology of females and males. This binary conceptualization is most evident in the common understanding of the request to consider sex as a biological variable as a request to study sex differences (see Ritz et al. (2014) for a similar warning against a simplistic binary approach to sex and sex differences).

The most obvious implication of the conclusion of the previous section, that sex-related variables reside in a multi-dimensional space that cannot meaningfully be reduced into a binary variable (female, male), is replacing the common practice of comparing a group of females to a group of males with an attempt to associate sex-related variables with the phenomenon under study. As has been previously noted (e.g., Ritz et al., 2014), finding a difference between females and males may provide the first clue that sex-related variables are relevant, but it is only a first step in understanding the relations between sex and this phenomenon. Subsequent steps should attempt to detect the sex-related variable(s) that affect the studied phenomenon – an endeavour that would require the assessment of many sex-related variables at the individual level and the use of statistical tools that allow the detection of complex interactions between variables (Joel and Fausto-Sterling, 2016; Joel et al., 2020; Ritz et al., 2014).

Practically, I suggest always including females and males in a sample, to capture the entire variability of the studied species, human or non-human (Joel, 2015; Joel and Fausto-Sterling, 2016; Joel and McCarthy, 2017). This is important in both basic and clinical research and should be done regardless of whether sex differences in the studied endpoint(s) have previously been reported. Prior knowledge of the existence or lack of sex differences should, however, direct the researcher in designing the study and in deciding whether to use sex category as a variable in the analysis of the results. There are three possible scenarios: there are no or only a few prior studies on sex differences in the studied phenomenon; there is strong evidence for the lack of sex differences; there is strong evidence for the existence of sex differences.

In the first scenario - no or only few relevant studies - one should assess sex differences as a crude way to evaluate the possible involvement of sex-related variables, which could be assessed in subsequent studies. In studies in which sex category is used as a variable, one should be careful with generalizations of the results across environmental conditions, strains and species, because sex effects may be different under different genetic, developmental, or environmental conditions (Joel and Fausto-Sterling, 2016; Joel and McCarthy, 2017). Special caution is required in generalizing the results of animal studies, in which the variability in these other variables is often very limited.

In the second scenario - strong evidence for the lack of sex differences - it may be best not to include sex category as a variable when analysing the results. This is because adding a variable that does not account for variability in the endpoint(s) detracts from the study's power to detect differences on other variables (because of the reduction in the degrees of freedom).

In the third scenario - strong evidence for the existence of sex differences - it would be wise to collect data on sex-related variables that may be relevant for the phenomenon under study, because simply finding (again) a sex difference would not advance much our understanding of the phenomenon nor its relations to sex. A famous example for the importance of going beyond sex differences to consider sex-related variables is the case of zolpidem. The sex difference in the drug's clearance, which probably contributes to the higher rate of adverse side effects in women, became non-significant when participants' weight was taken into account (Greenblatt et al., 2014). Another example is the cardiovascular system, which is affected by variables that correlate with sex category, such as smoking, height and physical activity. It is clearly better to ask a patient whether they smoke and how much, then rely on their sex category and the average difference between women and men in smoking to predict outcome or assign treatment.

Recent years have seen a welcome increase in the number of studies that assess sex/gender-related variables in addition to sex category. Unfortunately, instead of using the powerful tools of deep learning to uncover the probably complex relations between these variables and specific endpoint(s) (e.g., disease outcome), many of these studies reduce the data to a single continuum – the probability that the participant is a woman (or a man) – and then assess the correlations of this

variable with the endpoints (e.g., Ballering et al., 2020; Norris et al., 2017; Pelletier et al., 2015; Smith and Koehoorn, 2016).

The binary framework and the focus on sex differences have implications also for clinical and diagnostic settings. Clearly, one's sex category provides crucial information for diagnosis in some situations – for example, a patient presenting with acute pelvic pain – and should surely be recorded, together with other data such as age, blood pressure, chronic disease, etc., in any medical encounter. But, as the examples above demonstrate, providing good medicine, and surely providing personalized medicine, requires much more than a person's sex category – it requires gathering information on a variety of variables that are related to sex and gender (in addition to many other variables).

Sex category is not only dull in the information it provides, the current dominance of the binary framework may lead to misdiagnosis when a patient is suffering from a condition or is presenting with symptoms that are associated with the other gender. One example is depression, and especially postpartum depression, which is underdiagnosed in men, even though it is not as rare as commonly believed. For example, a recent study revealed a 10:9 ratio of mothers to fathers suffering from postpartum depression (Cheng et al., 2018). Another example is heart disease, which is considered a men's disease, even though it is the number one killer of women in the United States (Canto and Kiefe, 2014). The association between heart disease and men leads to a gender bias in referrals for both diagnostic and therapeutic procedures, which may result in poorer outcomes (e.g., Eberly et al., 2019; Humphries et al., 2017).

8. Summary

The sex binary is a powerful framework through which we understand not only the social world but also physiology. However, current data suggest that this framework is not appropriate for understanding sex effects on the brain or even sex itself. Moreover, the dominance of the binary framework interferes with the scientific endeavor to understand sex, the brain, and the relations between them. The challenge for the future is to develop new analytical methods that take human variability on all measures, including sex-related ones, into account (Joel, 2014; Joel and Fausto-Sterling, 2016).

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