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Viewpoints

Neuroscience and sex/gender: Looking back and looking forward

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Neuroscience and sex/gender: Looking back and looking forward

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Abstract

In 1959, Phoenix, Goy, Gerall & Young reported that treating pregnant guinea pigs with testosterone had enduring effects on the sex-related behavior of their female offspring. Since then, similar enduring effects of early testosterone exposure have been found in other species, including humans, and for other behaviors that show average sex differences. In humans, the affected outcomes include gender identity, sexual orientation, and children's sex-typical play behavior. The evidence linking early testosterone exposure to sex-typed play is particularly robust, and sex-typed play is also influenced by many other factors, including socialization by parents and peers and self-socialization, based on cognitive understanding of gender. In addition to influencing behavior, testosterone, and hormones produced from testosterone, affect mammalian brain structure. Studies using human autopsy material have found some sex differences in the human brain similar to those seen in other species, and have reported that some brain sex differences correlate with sexual orientation or gender identity, although the causes of these brain/behavior relationships are unclear. Studies that have imaged the living human brain have found only a small number of sex differences, and these differences are generally small in magnitude. In addition, they have not been linked to robust psychological or behavioral sex differences. Future research might benefit from improved imaging technology, and attention to other brain characteristics. In addition, it might usefully explore how different types of factors, such as early testosterone exposure and parental socialization, work together in the developmental system that produces sex/gender differences in human brain and behavior.

The Society for Neuroscience was founded in 1969, during a time of great change, not least in the roles of men and women in society. From the beginning, neuroscience was interested in these changes, and in the causes of variability in sex-related behavior more broadly.

In 1959, Phoenix, Goy, Gerall & Young reported that treating pregnant guinea pigs with testosterone masculinized the reproductive behavior of their female offspring (Phoenix, Goy, Gerall, & Young, 1959). They called these early influences of testosterone on later behaviour organizational effects, suggesting that, during early development, testosterone had influenced the organization of the brain. They also contrasted these enduring, organizational influences with what they called activational influences of hormones in adulthood – effects that waxed and waned as hormone concentrations rose and fell. They speculated that the organizational effects of testosterone on the brain were likely to be subtle "reflected in function rather than visible structure" (Phoenix et al., 1959, p. 381).

Subsequently, however, researchers identified sex differences in rodent brain structure that also were influenced by testosterone during early development. For example, female rats were found to have more non-amygdaloid synapses on dendritic spines in the preoptic area (POA) than male rats, and treating female animals with testosterone during early development reduced spine numbers, whereas castrating male animals at birth increased them (Raisman & Field, 1973).

Soon thereafter, researchers found dramatic sex differences in the brains of canaries and zebra finches, species where males sing and females do not (Nottebohm & Arnold, 1976). Volumes of three brain nuclei known to be involved in song were larger in male than in female birds. A fourth region was more developed in male than in female canaries, and, although well-developed in male zebra finches, was not recognizable in females.

Several reports of volumetric sex differences in the rodent brain followed. The first, and perhaps best known, is in the POA of the rat brain (Gorski, Gordon, Shryne, & Southam, 1978; Gorski, Harlan, Jacobson, Shryne, & Southam, 1980). This region, called the sexually dimorphic nucleus of the POA (SDN-POA), is several times larger in male than female rats, and the sex difference is so dramatic that it can be seen with the naked eye in Nissl-stained sections. Testosterone was also found to influence development of the SDN-POA. Treating females with testosterone early in life increased SDN-POA volume, and removing testosterone from males reduced it (Dohler et al., 1984; Jacobson, Csernus, Shryne, & Gorski, 1981). Subsequent research identified similar sex differences and hormonal influences in the POA of other species, including gerbils, ferrets, guinea pigs, sheep and rhesus macaques (Byne, 1998; Commins & Yahr, 1984; Hines, Davis, Coquelin, Goy, & Gorski, 1985; Roselli, Larkin, Resko, Stellflug, & Stromshak, 2004; Tobet, Zahniser, & Baum, 1986), as well as in other brain regions, including the encapsulated and medial anterior regions of the bed nucleus of the stria terminalis (BST), the posterodorsal region of the medial amygdala, and the anteroventral and parastrial regions of the POA (del Abril, Segovia, & Guillamon, 1990; Hines, Allen, & Gorski, 1992; McCarthy, Schlenker, & Pfaff, 1993; Murakami & Arai, 1989; Sanchis-Segura et al., 2019). These sex differences also are influenced by early testosterone manipulations (Hines, 2004). In addition, among sheep, animals of particular interest, because about 8% of rams prefer male sexual partners, the volume of the SDN-POA has been found to be larger in rams who prefer female partners than in rams who prefer male partners (Roselli et al., 2004).

Reports of dramatic sex differences in brain structure were exciting, in part, because they promised to allow identification of the mechanisms underlying the enduring influences of early testosterone exposure on the brain. Subsequent research has returned on this promise. For example, testosterone, and its metabolites, have been found to influence cell survival, and neurite outgrowth (Hines, 2004). More recent research has documented a varied array of molecular mechanisms involved in these structural changes that differ from one brain region to another

(McCarthy, Pickett, VanRyzin, & Kight, 2015). This variety and regional specificity has led to the conclusion that brains are unlikely to be uniformly male or female (Joel & McCarthy, 2016).

Research in rodents also suggests that experience can influence brain structure, and that these influences can interact with sex. For example, rats reared in complex environments, with cage mates and objects that are changed daily, show different patterns of neural sex differences than those reared in simple environments, with no cage mates and no objects. Female rats reared in complex environments, but not those raised in simple environments, have more myelinated axons in the posterior fifth of the corpus callosum than males (Juraska & Kopcik, 1988). Similar environmental manipulations have been found to influence patterns of sex differences in dendritic growth in hippocampus and visual cortex as well (Juraska, 1991, 1998). More recent studies have found that stress can change patterns of sex differences in spine density and density of cannabinoid receptors in the hippocampus of the rodent brain (Reich, Taylor, & McCarthy, 2009; Shors, Chua, & Falduto, 2001).

Although early conceptions of hormonal influences on sex differences in the rodent brain assumed that testosterone influenced brain structure only very early in life, we now know that brain structure can change later in life to a much greater extent than was thought in 1969. In addition to the changes in myelination, dendritic growth, spine density and density of cannabinoid receptors, mentioned above, neurogenesis and gliogenesis continue into adulthood in some brain regions (Frisen, 2016) and pubertal hormones have been found to cause new cells, including neurons, to be born in the SDN-POA and other brain regions that show sex differences in rats (Ahmed et al., 2008). Puberty seems to be an additional critical period for gonadal steroids to influence sexual behavior in rodents (Schulz & Sisk, 2016).

Translating research from non-human mammals to humans

The influences of testosterone on brain structure and later behaviour occur during critical periods of development. Consequently, testosterone must be present during a particular

developmental window to have its effects, and the effects persist after the hormone is gone. In mammals, the critical periods correspond to times when testosterone is higher in male than female animals. These periods begin prenatally, when the *SRY* gene on the Y chromosome causes the gonads to differentiate as testes, and they begin to produce testosterone. In humans, the fetal testes become active at about week 7 of gestation, and testosterone is markedly higher in male than in female fetuses from about gestational weeks 8 to 16 or 24 (Smail, Reyes, Winter, & Faiman, 1981). After birth, there is a second surge of testosterone that is larger in male than in female infants, particularly from about weeks 4 to 12 postnatal (see Fig. 1) (Forest, Cathiard, & Bertrand, 1973; Kuiri-Hanninen et al., 2011). These two periods are the presumed critical periods when testosterone might influence the development of sex differences in human brain and behavior.

Insert Fig. 1 about here

The behaviors that are affected by early testosterone exposure in non-human mammals include reproductive behaviors, as well as other behaviors that differ on average for male and female animals. For example, male and female rats differ on average in juvenile sex-typed play, physical aggression, parenting behaviour, and performance in spatial mazes, and all of these behaviors have been found to be influenced by early manipulations of testosterone (Hines, 2004) . Similarly, the female offspring of rhesus macaques who were treated with testosterone during pregnancy show more male-typical patterns of juvenile play, as well as reproductive behaviors (Wallen, 2005).

Because early testosterone exposure influences characteristics that show sex differences, it was important to identify human behaviors that differ by sex. It also was important to determine the sizes of the differences, because larger differences would be more likely to show effects of testosterone exposure than smaller differences. The metric typically used for size is Cohen's "*d*" -- the difference between mean values for males and females divided by the standard deviation (Cohen, 1988). Conventionally, *d* values of about 0.2 are considered small, those of 0.5 medium, and

those of 0.8 large. Decades of research, often using meta-analytic techniques that allow results of numerous studies to be combined to get reliable estimates of effect sizes, suggests that most sex differences in human behavior/psychology are small to negligible in size (Hyde, 2005). However, large sex differences in human behavior ($d \geq 0.8$) have been documented for some characteristics. These include scores on some specific measures of empathy (higher in females), physical aggression, and social dominance, and for performance on a specific 3-dimensional (3D) mental rotation task (all three higher in males). The sizes of these sex differences, in standard deviation units (*d*) (Cohen, 1988) are shown in Fig. 2. This figure also shows the size of the sex difference in height ($d = 2.0$), providing a familiar comparator. Although these differences are large by Cohen's standards, they are only about half the size of the sex difference in height.

Insert Fig. 2 about here

Figure 2 also shows the effect sizes for other behaviors that show sex differences as large, or larger, than that in height. For example, a population study using a questionnaire measure of children's gender-related play found a sex difference of $d = 2.8$ (Golombok et al., 2008; Hines et al., 2002). Children's sex-typed toy preferences, e.g., for dolls versus vehicles, also show large sex differences ($d \geq 1.8$). In addition, sexual orientation and gender identity show large sex differences, with males more interested in female sexual partners and more likely to identify as men or boys, compared to females. Both of these sex differences are very large ($d > 6.0$ and $d > 10.0$, respectively) (Hines, 2015).

All three of the human psychological/behavioral characteristics that show particularly large sex differences, childhood sex-typed play, sexual orientation, and gender identity, have been found to relate to early testosterone exposure. Sex-typed play has been studied more extensively than any other human behavior in this context, and at least ten independent research groups have reported a link to prenatal testosterone exposure (Hines, 2015). For example, girls with classic congenital adrenal hyperplasia (CAH), a genetic disorder, experience elevated testosterone exposure prenatally,

and they have consistently been found to show increased male-typical, and reduced female-typical, play. These findings have been reported in studies that observed toy choices in a play room and in studies that used questionnaires or interviews. They also have been found in studies using unaffected female relatives as controls and in studies using matched controls. In addition, the severity of the CAH disorder in terms of either phenotype or genotype predicts the degree of behavioral change. Studies of the children of women who were treated with hormones during pregnancy also suggest androgenic influences on sex-typed play. Similarly, XY individuals with complete androgen insensitivity syndrome (CAIS) have functioning testes but a cellular inability to respond to testosterone, and they show female-typical play patterns. Thus, although this evidence comes from studies of clinical populations, rather than experiments involving random assignment to testosterone or placebo treatment, the findings converge on the conclusion that prenatal testosterone concentrations influence children's sex-typed play behavior. Regarding sexual orientation and gender identity, findings similarly suggest increased male-typical outcomes in females with CAH and reduced male-typical outcomes in XY females with CAIS. Similar evidence regarding other characteristics that show sex differences, including performance on mental rotation tasks, has been largely inconsistent, perhaps because these measures show smaller sex differences.

Although it is well established that children's sex-typed play is influenced by early testosterone exposure, there also is extensive evidence that the social environment and children's cognitive understanding of gender play a role in the same outcomes (Hines, 2015). For instance, parents, peers, teachers and strangers reward children for playing with sex-typical toys and engaging in sex-typical activities. In addition, after children learn that they are girls or boys, they value engaging in the activities that they identify as appropriate for their sex, and they self-socialize based on social information as to what is sex-appropriate behavior.

These findings raise the question of whether external socialization or self-socialization are altered for girls with CAH. When they are observed in a laboratory playroom, with access to a range

192 of sex-typed and sex-neutral toys, parents of girls with CAH encourage them to engage with female-
193 stereotyped toys, such as dolls, more than they do their unaffected daughters (V. L. Pasterski et al.,
194 2005). Parents of girls with CAH also report, however, that they encourage them to engage in male-
195 typical activities in their day-to-day life (Wong, Pasterski, Hindmarsh, Geffner, & Hines, 2012). This
196 probably occurs because parents tend to encourage their children to engage in the activities the
197 children enjoy, and girls with CAH like male-typical activities. Nevertheless, this encouragement may
198 further masculinize the girls' behavior. Self-socialization also has been found to be altered in girls
199 with CAH. They are less likely than other girls to model the behavior of other females, and they are
200 less likely to engage with toys that they have been taught are "for girls" (Hines et al., 2016). These
201 findings suggest that prenatal androgen exposure may influence children's toy preferences not only
202 by influencing brain development prenatally, but also by changes in postnatal socialization, including
203 parental socialization, and children's self-socialization, of sex-typed behavior.

204 Developmental systems perspective

205 Developmental scientists view outcomes such as gender-related behavior as the product of a
206 developmental system that involves numerous factors interacting over time to produce stability or
207 change. In the case of gender, the influences of interest include sex chromosome genes, early
208 testosterone exposure, socialization by external forces (such as parents and broader society), and
209 self-socialization based on cognitive understanding of gender (Hines, 2015). Some current research
210 aims to evaluate how different elements of the system related to sex/gender differences in brain
211 and behavior interact. The studies of parental socialization and self-socialization in girls with CAH are
212 examples of this approach. Individuals with CAH are not numerous, however, and it would be useful
213 to assess individual variability in early testosterone exposure in typically-developing individuals. For
214 instance, some studies have related testosterone during the early postnatal surge, sometimes called
215 mini-puberty, to later behavior. Mini-puberty is of particular interest, because it provides an

opportunity to take repeated measures directly from developing individuals, at a time of rapid brain development, as well as social influence.

One study found that testosterone measured in repeated urine samples obtained approximately monthly across the first six months postnatal (during mini-puberty) predicted later sex-typed play (Lamminmaki et al., 2012). Another study found that penile growth during the first six months postnatal, which correlates with testosterone during mini-puberty, predicted later sex-typed play in boys, even when prenatal testosterone exposure, as measured by ano-genital distance at birth, was controlled (V. Pasterski et al., 2015). Testosterone during mini-puberty has also been found to relate negatively to sex-typed language development in boys and in girls (Kung, Browne, Constantinescu, Noorderhaven, & Hines, 2016), and positively to 3D mental rotation performance in boys (Constantinescu, Moore, Johnson, & Hines, 2018). In contrast, parental attitudes to gender, specifically disapproval of cross-gendered behavior, negatively predicted 3D mental rotation performance in girls (Constantinescu et al., 2018). If independently replicated, these findings could provide a foundation for studies of interactions between hormonal influences and socialization during early development as they operate within the developmental system that shapes human sex/gender development.

Sex/gender and the human brain

Sex differences in behavior necessitate sex differences in the brain. Perhaps the most obvious sex difference in the human brain involves its size. Men's brains are about 11% larger, on average, than women's brains (Luders et al., 2005; Pakkenberg & Voigt, 1964; Ruigrok et al., 2014). As noted above, men are also taller than women, however, and a larger brain may be needed to run a larger body.

Based on the findings regarding the SDN-POA in rodents, researchers also looked for a similar sex difference in the human brain, using autopsy material. Several independent research groups reported that the third interstitial nucleus of the anterior hypothalamus (INAH-3) was larger

241 in men than in women and appeared to be the human equivalent of the SDN-POA (Allen, Hines,
242 Shryne, & Gorski, 1989; Byne et al., 2000; Byne et al., 2001; LeVay, 1991). INAH-3 also is larger in
243 heterosexual men than in men who are not heterosexual, a finding that also has been independently
244 replicated (Byne et al., 2001; LeVay, 1991).

245 Similarly, the central region of the BST (BSTc) has been reported to show a sex difference
246 and has been related to gender identity. An initial study reported that BSTc was smaller in control
247 women and in a group of six trans women than in control men (Zhou, Hofman, Gooren, & Swaab,
248 1995). A subsequent study evaluated the BSTc across the lifespan, including during fetal
249 development (Chung, De Vries, & Swaab, 2002). Surprisingly, the sex difference did not appear until
250 after puberty. In contrast, animal models of sex differences, such as the SDN-POA, find that the
251 differences are present from early in life. In addition, most trans people report thinking from
252 childhood that they were assigned to the wrong sex. The findings may suggest, therefore, that the
253 sex and gender identity related differences in the human BSTc result from experience, rather than
254 causing variability in gender identity. Alternatively, the sex difference may not have been evident in
255 the brains of children, because of small sample sizes.

256 The emergence and growing access to technologies that allow visualization of the living
257 human brain shifted researchers' focus from sub-cortical structures, such as INAH-3 and BSTc, to
258 structures that could be visualized using these technologies. These studies are less closely connected
259 to the animal models of testosterone and brain organization than are studies of INAH-3 or the BST.
260 The broad hypothesis that testosterone influences brain structures that differ by sex, however,
261 contributed to a search for reliable sex differences in the living human brain. One difficulty in
262 attempting to identify such sex differences relates to the sex difference in brain size. The studies that
263 identified INAH-3 as the human equivalent of the rodent SDN-POA adjusted INAH-3 volume by
264 calculating its size as a ratio to total brain volume. This ratio procedure mitigated the possibility that
265 INAH-3 was larger in males only because it was scaled to the larger male than female brain. This

ratio procedure worked in this case, where a brain region remained larger in males after adjusting for their larger overall brain size. It can be problematic, however, because it can cause brain regions to appear larger in female than male brains, simply because of adjusting for the larger male brain by calculating a ratio. Currently, researchers generally agree that it is appropriate to adjust for the sex difference in brain size, as well as to report unadjusted values. They have, however, found adjustment procedures, such as including total brain volume as a covariate, that do not create spurious differences (Sanchis-Segura et al., 2019).

In addition to the problem of brain size, early research on sex differences in the human brain was hampered by the use of small samples and a tendency to publish significant, but not non-significant, results. Recent years have seen larger and more systematic studies, however, involving hundreds or even thousands of participants and examining sex differences across the whole brain, using non-distorting procedures to adjust for brain size. These studies have generally reported that most brain regions are similar in size in males and females, and that, where differences are seen, they tend to be small.

For example, the largest of these studies to date (Ritchie et al., 2018), involved several thousand participants, ages 45 to 75 years. Like previous studies, it reported a large (d = 1.41) sex difference in total brain volume. After using non-distorting procedures to adjust for the larger male brain, sex differences in gray matter volume, area, and cortical thickness were observed in some of 68 sub-regions examined. These differences sometimes favored females and sometimes favored males, and they were generally small, though significant in this large sample. Regarding white matter, the study reported that, for all 22 fiber tracts evaluated, men averaged higher values for fractional anisotropy (FA) (average d = 0.19) a measure that is thought to relate to white matter integrity, whereas females averaged higher values for orientation dispersion (OD) (average d = 0.30), a measure thought to relate to white matter complexity. The greater values for FA in men were reduced by adjustment for total brain volume, and this adjustment produced a significantly higher

291 FA score for women in one white matter tract. In contrast, the brain volume adjustment produced
292 few changes to the sizes of the OD differences. They continued to be of moderate size in the 22
293 tracts.

294 The use of larger sample sizes, more complete reporting of results, and non-distorting
295 procedures to adjust for brain size represents progress. Other issues could be addressed too. One
296 problem that has received relatively little attention is sampling bias, particularly related to the sex
297 difference in volunteering: women are more likely than men to volunteer to participate in research
298 (Rosnow & Rosenthal, 1976; Senn & Desmarais, 2001). Other factors, including education and
299 socioeconomic status also relate positively to volunteering for research (Rosnow & Rosenthal, 1976).
300 Thus, somewhat different selection biases may lead men versus women to participate. For instance,
301 male volunteers may be more educated or intelligent than female volunteers. Consistent with this
302 possibility, Ritchie et al. (2018) found, to their surprise, that their male participants averaged higher
303 scores than their female participants on a measure of verbal-numerical reasoning. Although this sex
304 difference was small (d = 0.18), it is similar in size to the brain differences observed. It also suggests
305 that the differences observed in this study, and perhaps others that did not measure or match for
306 intelligence, could relate to intelligence differences instead of sex differences. One approach to this
307 problem, particularly when looking at sex differences in the cerebral cortex, might be to match male
308 and female participants for intelligence.

309 Studies of children are likely to be less affected by the associations between sex and
310 volunteering. Because parents volunteer their children to participate, and because they have male
311 and female children, they are probably equally likely to volunteer boys and girls. Several studies have
312 examined sex differences in the brains of children younger than 2 years. An initial study of 74 infants
313 found that the male brain is already larger than the female brain in the first few weeks postnatal (*d* =
314 0.75), a difference similar in size to the sex difference in birth weight (*d* = 0.71) (Gilmore et al., 2007).
315 Another study of a larger sample of neonates also found a similar sex difference in intracranial

316 volume (ICV) and body weight, and a positive correlation between ICV and body weight (Knickmeyer
317 et al., 2017). A review of sex differences in the brain across childhood, adolescence and young
318 adulthood concluded that patterns of sex differences are not static, but appear to differ at different
319 ages, often in a non-linear manner (Kaczkurkin, Raznahan, & Satterthwaite, 2018).

320 Other research suggests that, in regard to brain characteristics that show sex differences,
321 the adult human brain is not uniformly male-typical or female-typical. As suggested by research in
322 rodents, where numerous tissue-specific molecular mechanisms are involved in masculinization of
323 different brain regions, most people have brains that are a mixture of characteristics that are
324 generally more typical of men and characteristics that are generally more typical of women. For
325 example, a study of over 1400 adult brains found that for the brain regions that showed the largest
326 sex differences, most brains did not show a consistent sex-typical pattern in all regions (Joel et al.,
327 2015). Similarly, the same study found that brain connectivity was never consistently male-typical or
328 female-typical in any of the brains. They concluded that brains are not simply male brains or female
329 brains.

330 In addition to studying brain structure, researchers have used imaging technologies to
331 explore sex differences in brain function. These functional studies add new dimensions that
332 complicate interpretation of results, such as whether participants are resting or completing a task
333 while their brains are imaged. In addition, it has been suggested that functional studies have not
334 used sufficiently similar procedures to allow for replication of specific findings (Carp, 2012). Another
335 challenge relates to interpretation of outcomes. For example, an early study found that male and
336 female brains functioned differently when engaging in a language task (Shaywitz et al., 1995). Male
337 and female performance on the language task was similar, however, suggesting that male and
338 female brains may function differently to produce similar behavior. This finding coincides with a
339 suggestion that sex differences in the brain exist to make male and female behavior more similar,
340 rather than different, given the different hormone milieus of men and women (De Vries & Boyle,

1998). In other words, the brain sex differences may compensate for sex differences caused by gonadal steroids.

Why study sex/gender and the brain

Why do we care about sex differences in the brain? Many researchers in this area want to identify sex differences in the human brain, and their relations to sex differences in behavior, to increase understanding of why men and women differ behaviorally, and why many psychiatric diagnoses show unequal sex ratios. For example, autistic spectrum disorder, attention deficit/hyperactivity disorder, and conduct disorder are more commonly diagnosed in males, whereas eating disorders, major depressive disorder and generalized anxiety disorder are more commonly diagnosed in females (American Psychiatric Association, 2013). Many neurological disorders (Hanamsagar & Bilbo, 2016; Sohrabji, Welsh, & Reddy, 2016) and chronic pain disorders (Fillingim, King, Riberio-Dasilva, Rahim-Williams, & Riley, 2009; Mogil, 2012) are also more common in one sex than the other. Perhaps knowledge of how male and female brains differ could provide insights that would help people with these disorders. Others want to know if there are brain differences that relate to behavioral sex differences, such as those in 3D mental rotation performance. The ultimate aim is to use understanding of links between sex differences in behavior and sex differences in the brain to facilitate changes in behavior and reduce psychopathology, as well as other types of disorders, related to the brain. In this context, it is important to note that the sex differences that have been reported in the living human brain, with the exception of that in overall brain size, appear to be substantially smaller than the sex differences in some human behaviors, including those in gender identity, sexual orientation, or children's play. In addition, although the sub-cortical regions INAH-3 and BSTc have been linked to sexual orientation and gender identity, the causes of these links are not known. Also, no other sex differences in brain structure have been linked to human behaviors or psychological characteristics that show reliable and robust sex differences.

Some researchers, and members of society more broadly, may also think that the existence of sex differences in the human brain suggests that sex differences in behavior are inborn, and therefore resistant to change. This perspective reflects a misunderstanding, however. Although we now know that there are sex differences in human brain structure, we do not know what causes them. The existence of sex differences in behavior necessitates sex differences in the brain, and the factors that influence sex differences in behavioral development are likely to also influence sex differences in the brain. So, genes on the sex chromosomes; hormones prenatally, during mini-puberty or at adolescent puberty; socialization by parents, peers and others; and self-socialization, based on cognitive understanding of gender, are all likely to contribute to sex differences both in behavior and the brain.

Where do we go from here?

Research on sex and the brain has come a long way since 1969. The 1959 report of permanent influences of early androgen exposure on later behavior, as well as reports of dramatic sex differences in the avian and rodent brain, corresponded to a historical time when the social roles of men and women differed dramatically. The narratives of that time often referenced inborn systems and hard wiring of the brain. More recent research has demonstrated greater neural plasticity than was imagined 50 years ago, and interactions between hormones and environmental factors in shaping the human brain and behavior. In addition, developmental scientists have moved on from discussions of nature versus nurture to developmental systems formulations that conceptualize sex/gender development as involving many types of influences interacting over time.

Future research is likely to be aided by improved methods of visualizing the living human brain. Research in non-human mammals reported sex differences in cell groups, often of only a few hundred cells, that cannot be visualized in the living brain using current technologies. The small magnitudes of sex differences reported to date in the living human brain may partly reflect an inability to look more closely, particularly at small subcortical cell groups. In addition, thus far,

391 human research has looked largely at relatively gross characteristics, such as regional volumes and
392 fiber tracts. Animal research, however, has found that many more subtle characteristics, such as
393 neurochemical phenotypes, dendritic branching, and synaptic densities, to name just a few, show
394 sex differences (McCarthy et al., 2015). Analyzing these types of characteristics may increase the
395 explanatory power of human research. In addition, researchers developing animal models and those
396 studying humans have not always communicated as well as they might have. It would be interesting
397 to know, for instance, which of the small sex differences that have been seen consistently in the
398 living human brain are also seen consistently in other species, and if these differences in other
399 species are influenced by early androgen exposure. Similarly, researchers examining early hormone
400 effects on human brain and behavior might benefit from closer attention to the specific hypotheses
401 suggested by the large body of relevant research in other species.

402 Recent work has benefitted from attention to issues of reliability, to sample size, and to
403 statistical procedures. Additional attention is needed to sampling biases as well. More information
404 on sex differences over the life span, beginning at birth, would also be useful. Early life is a time
405 when interventions may have maximal impact. Future research might also explore how different
406 types of factors, such as early testosterone exposure and parental socialization, work together in the
407 developmental system that produces sex/gender differences in brain and behavior. Measures of
408 testosterone and parental behaviors during the first few months of infancy (mini-puberty) might be
409 useful in pursuing this goal.

410

411 Figure captions

412 Fig. 1. Concentrations of serum testosterone in boys and men from conception to the onset
413 of puberty.

414 Fig. 2. Effect size (*d*) values for human behaviors/psychological traits that show large sex
415 differences. The effect size for the sex difference in height (*d* = 2.0) is included as a familiar
416 comparator.

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