Review

Biological Psychiatry

Convergence of Sex Differences and the Neuroimmune System in Autism Spectrum Disorder

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ABSTRACT

The male bias in autism spectrum disorder incidence is among the most extreme of all neuropsychiatric disorders, yet the origins of the sex difference remain obscure. Developmentally, males are exposed to high levels of testosterone and its byproduct, estradiol. Together these steroids modify the course of brain development by altering neurogenesis, cell death, migration, differentiation, dendritic and axonal growth, synaptogenesis, and synaptic pruning, all of which can be deleteriously impacted during the course of developmental neuropsychiatric disorders. Elucidating the cellular mechanisms by which steroids modulate brain development provides valuable insights into how these processes may go awry. An emerging theme is the role of inflammatory signaling molecules and the innate immune system in directing brain masculinization, the evidence for which we review here. Evidence is also emerging that the neuroimmune system is overactivated in individuals with autism spectrum disorder. These combined observations lead us to propose that the natural process of brain masculinization puts males at risk by moving them closer to a vulnerability threshold that could more easily be breached by inflammation during critical periods of brain development. Two brain regions are highlighted: the preoptic area and the cerebellum. Both are developmentally regulated by the inflammatory prostaglandin E2, but in different ways. Microglia, innate immune cells of the brain, and astrocytes are also critical contributors to masculinization and illustrate the importance of nonneuronal cells to the health of the developing brain.

Keywords: Androgens, Autism spectrum disorder, Cerebellum, Estrogens, Masculinization, Microglia, Preoptic area, Prostaglandins

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The profound gender bias in the frequency and presentation of autism spectrum disorder (ASD) requires our attention. Boys are diagnosed with ASD four to five times more frequently than girls, but the origins of the male prevalence remain incompletely understood (1–7). Equally mysterious but equally compelling is the accumulating evidence that inflammation may contribute to or be a consequence of ASD (8–12). This review focuses on the convergence of the biological risk factor of being male with the environmental risk factor of inflammation and proposes that the cellular mechanisms mediating brain masculinization enhance vulnerability for ASD (Figure 1).

GENETICS OF AUTISM AND SEX DIFFERENCES IN THE BRAIN

Advances in the genetics of autism highlight the impact of small de novo mutations in individual genes associated with synaptic functioning, transcriptional regulation, or epigenetic modifications of the genome (13–15). Hundreds of genes have been implicated as risk factors, with varying degrees of confidence (16,17). With the exception of rare syndromic conditions, there is no clear gender bias in the identified risk genes (13,15), confirming that males are not at greater risk from a unique genetic source. Indeed, the contribution of de novo mutations to the frequency of ASD appears to be overall higher for girls than boys (13), leaving unexplained the increased vulnerability of males.

There is now sufficient understanding of the genetics of ASD to conclude that there is a preponderance of genes associated with neurogenesis and synaptic activity across all forms of ASD (16,18), which may lead to dysfunctional homeostatic feedback loops (19). Likewise, sufficient observational studies on the role of maternal inflammation in ASD allow for meta-analyses in the hopes of resolving conflicting reports and clarifying issues about timing and source of infection (20).

A parallel increase in understanding of cellular mechanisms mediating brain sexual differentiation provides a similar opportunity to propose working models and unifying hypotheses [for review, see (21)]. When comparing variables essential to enduring sex differences in brain and behavior with those implicated in ASD, several commonalities emerge. Many neuroanatomical sex differences are established early, beginning in utero and extending to the postnatal period. The principal driver is an increase in androgens and estrogens in the brains of developing males as a result of steroidogenesis

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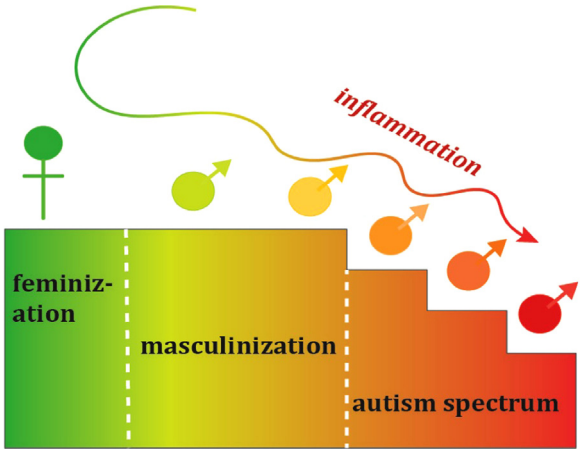

Figure 1. Masculinization of the brain converges with inflammation and enhances male vulnerability to autism spectrum disorder. Feminization and masculinization are distinct developmental processes. In rodents, normal masculinization of some brain regions involves inflammatory signaling molecules, such as prostaglandins, which are derived from activated innate immune cells, the microglia, and reactive astrocytes. Inflammation during pregnancy in humans is a putative risk factor for the development of autism spectrum disorder, with evidence that the greater the inflammation the more severe the disorder (20). Whether in utero inflammation increases the risk for autism spectrum disorder disproportionately in males is unknown. Based on research in rodents and correlational evidence in humans (37), we propose that females have low levels of inflammatory signaling in the brain (green), while the natural process of masculinization increases inflammation in males (yellow-orange) and pushes males closer to a threshold of vulnerability that can be more easily breached if inflammation occurs during a sensitive developmental period (orange-red).

by the fetal testis. Steroids modulate neurogenesis, synaptogenesis, and cell differentiation by inducing or repressing the expression of genes associated with excitation/inhibition, management of calcium, and regulators of transcription [for review, see (22)], all of which are dysregulated in ASD. Moreover, the normal process of brain masculinization is mediated by cells and signaling molecules normally associated with inflammation (23), a striking overlap with an environmental risk factor for ASD.

This review will focus on two regions in which the neuroimmune system is a critical contributor to normal development: the preoptic area (POA) and the cerebellum. These two regions differ in that the POA is highly sexually dimorphic whereas the cerebellum generally is not. They also differ in that the cerebellum is strongly implicated in ASD whereas the POA is not, but we contend that this is a function of not being properly considered, and we make that case here.

UNRAVELING THE MYSTERY OF HIGHER ASD RATES IN BOYS COMPARED WITH GIRLS

There are two sides to the coin of higher rates of ASD in boys. One is the possibility that males carry inherent risk factors that make them more vulnerable to genetic mutation or environmental insult. The other is that girls are inherently protected from the same. Studies that explore a biological origin of the sex difference in ASD emphasize circulating gonadal steroid levels in utero (6,24,25) or cumulative genetic risk factors that have differential penetrance in boys compared with girls (1,5). The extreme male brain theory postulates that many autistic traits are maleness pushed to the point of dysfunction (i.e., excessive systematizing, low empathy, poor bonding, and a lack of social skills) (26). If true, measures of the normal male brain should be exaggerated in an autistic brain or animal model thereof. Voxel-based brain morphometry using magnetic resonance imaging does not support this view, but does suggest a move toward the masculine phenotype in girls with ASD (27). In contrast, several studies support the contention that females carry a higher load of genetic mutation before succumbing to ASD, suggesting that they are protected (1,2,5,13,28). A third and currently untested possibility is that females are actually more sensitive to genetic anomalies impacting brain development and disproportionately die in utero.

The multifactorial nature of the gender bias in ASD led to a conceptual four-level framework proposed by Lai *et al.* (29) and summarized in these questions: 1) How is ASD defined and diagnosed as a function of sex?, 2) What is similar and what is different in boys and girls with ASD?, 3) How does sex/gender contribute to liability for ASD?, and 4) What aspects of normal development in boys and girls goes awry in ASD? The last two questions are amenable to experimental approaches using animal models. Determining how sex contributes to liability for ASD can be achieved by looking for sex differences in the impact of deletions or mutations of ASD risk genes, as has been done in many mouse models (30) and limited rat models (31,32). For instance, *Nrxn1* is a strong candidate ASD risk gene. There are social impairments in the homozygous *Nrxn1* knockout mouse (33) and a male-specific effect on novelty (34). Preliminary analyses of *Nrxn1* knockout rats found hyperactivity and cognitive impairments, some of which are specific to males (32). There are relatively few other examples of an ASD candidate risk gene explored in the context of sex differences. Identifying essential regulators of normal development for potential sex differences is another approach. For example, loss of the gene for cell death regulator caspase-3 results in devastating impairments in social behavior in male but not female mice, a circumstance reminiscent of ASD (35). Finally, the gender bias can be indirectly addressed by treating animals with exogenous substances that are suspected or have been proposed as risk factors for ASD, such as inflammatory agents, neurotoxins, and endocrine disrupters, and determining if males and females differ in sensitivity. While animal studies cannot identify risk factors for ASD, they can highlight sources of potential biological variability in humans. For example, the ingestion of heavy metals severely impairs social behavior in male prairie voles but has no effect on females (36).

UNDERSTANDING THE MALE BIAS IN ASD FREQUENCY REQUIRES UNDERSTANDING BRAIN MASCULINIZATION

Determining what aspects of normal development go awry in ASD requires an in-depth understanding of the processes of masculinization and feminization of the brain. Werling *et al.* codified this in the form of two hypotheses: 1) ASD risk genes are expressed differently in males and females, versus 2)