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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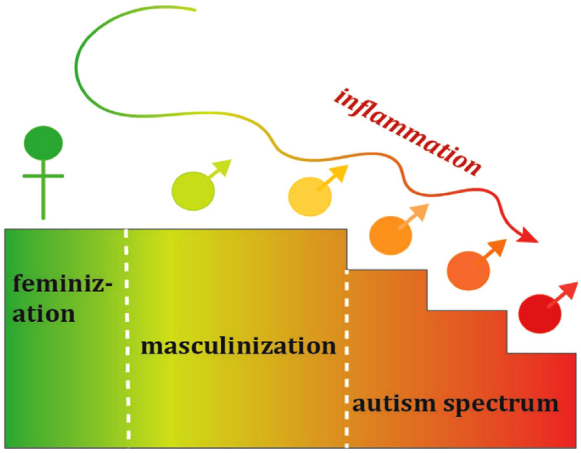

Figure 1. Masculinization of the brain converges with inflammation and enhances male vulnerability to autism spectrum disorder. Feminization and masculinization are distinct developmental processes. In rodents, normal masculinization of some brain regions involves inflammatory signaling molecules, such 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)

ASD risk genes interact with pathways that regulate normal male development (37). They landed solidly on the side of the second hypotheses after comparing gene expression in postmortem cortex and finding that gene sets naturally higher in males compared to females were also elevated in males with ASD compared to controls, based on previously published studies (38,39). By examining expression profiles in young males, they also determined that this same gene set was masculinized early in development after the prenatal surge in testosterone. While intriguing, until a dataset of sufficient numbers of males and females with and without ASD is compared, no explicit statements about sex-dependent changes in gene expression associated with ASD can be made. Nonetheless, the genes identified in the study by Werling *et al.* were largely involved in astrocyte and microglia activation, an observation that is consistent with our discovery of inflammatory mediators as fundamental regulators of male brain development. In vivo imaging of adults with ASD reveals excessive microglial activation in multiple brain regions (40), and transcriptomic analyses finds dysregulation in neuroimmune gene sets from autistic individuals (39). Converging evidence from both clinical and preclinical studies points to the neuroimmune system as being critical to normal male brain development and the risk of ASD.

THE POA IS SEXUALLY DIMORPHIC AND IMPACTS MULTIPLE BEHAVIORS WITH RELEVANCE TO ASD

The POA is a ventral region rostral to the optic chiasm, hence its name, and is notable for the presence of multiple and robust neuroanatomical sex differences. The embryonic origins are at the border of the di- and telencephalon, and distinct patterns of gene expression separate it from the closely associated hypothalamus (41). The first sex differences reported in the mammalian brain were in the POA and ranged from subtle differences in dendritic organization (42) to a large difference in the size of a particular subnucleus, called the sexually dimorphic nucleus of the POA (SDN-POA), which is three to five times larger in males (43). Converging evidence from studies of the analogous nucleus in humans and homosexual rams suggests a role in partner preference (44). Perhaps the most important contribution of the SDN to our understanding of brain masculinization is the establishment of the first mechanistic principle by which male and female brains come to differ: differential cell death. The number of neurons in the SDN is the same in males and females early in the sensitive period, but cells rapidly die off during the first week of life in females because of a lack of the trophic action of gonadal steroids, which are found at high levels in males at this time (45). This same principle, with variations on the theme, is also the basis for sex differences in the size of at least two other hypothalamic nuclei, the anteroperiventricular nucleus and the bed nucleus of the stria terminalis (BNST), contributing to differential compositions of cell types within the nuclei and the sizes of projections between key components of neural circuits regulating complex behaviors (46). There is also a significantly higher density of dendritic spine synapses on male POA neurons compared to females (47). The increase in synapses in males is established during the critical period and endures across the lifespan (48). Parallel changes in the morphology of astrocytes (49) and microglia (50) highlight the importance of cell-to-cell communication and the essential role of the neuroimmune system.

The POA is central to motivated behaviors, including male sexual behavior and parental behaviors. Lesions of the POA eliminate all sexual drive in males, while stimulating it has the opposite effect (51). Likewise, females show no nurturing behavior toward their offspring following POA lesions (52). Little or no interest in social interactions is a core symptom of ASD, but the potential involvement of the POA has not been considered. Lack of motivation because of insufficient reward in response to social interactions is speculated as an underlying source of the social deficits symptomatic of ASD. The neuropeptide oxytocin is a key component of the social reward circuit (53), and perturbations in the oxytocin system have been implicated both as a source of ASD (54) and a potential therapeutic (55). Oxytocin neurons reside within the POA, and this neuropeptide is central to maternal care (56), mother/infant bonding (57), social bonding (58), and, as most recently discovered, consoling and empathy behavior (59). For these reasons, oxytocin and its cognate receptor have long been of interest as both a source of and cure for the core social deficits characteristic of ASD, but clinical success remains elusive (60). Sleep regulatory neurons reside in the POA (61), as do the fever-producing cells that respond to inflammatory signals (62). Sleep disorders are a prominent feature of ASD (63), and for reasons that remain mysterious, in a small study some children with ASD showed marked behavioral improvement during fever (64).

The BNST is physically and functionally aligned with the POA, and together they form a key node in neural circuits regulating social, affiliative, and fear behaviors. In rodents, the BNST is also sexually dimorphic in both cell number and the size of afferent connections to other regions (46), as well as activation threshold to socially salient stimuli, such as olfaction and novelty (65). The BNST regulates innate fear responses and social play (66). Both the POA and the BNST have distinct reciprocal connections with the amygdala (67).

The organization of the human POA is functionally and neurochemically analogous to that of the rat (68). Connectivity of the human BNST has only recently been explored, but the same strong connections with the amygdala and accumbens are present and complemented by two novel connections to the temporal pole and paracingulate gyrus (69,70). Dysregulation of the developing POA and closely associated BNST can therefore have enduring impacts on cognitive brain regions by altering cortical, hippocampal, and amygdala development as well as directly impacting numerous social, affiliative, and fear responses (Figure 2).

PROSTAGLANDINS INDUCE MASCULINIZATION OF THE POA

Prostaglandins are membrane-derived fast-acting signaling molecules generated by the cyclization of arachidonic acid by cyclooxygenase-1 (COX-1) and COX-2 enzymes. Both enzymes are ubiquitously expressed throughout the body and brain, with COX-1 generally constitutively active while COX-2 is induced in response to inflammation or injury (71). Prostaglandin E2 (PGE2) is considered the most proinflammatory of the prostanoids.

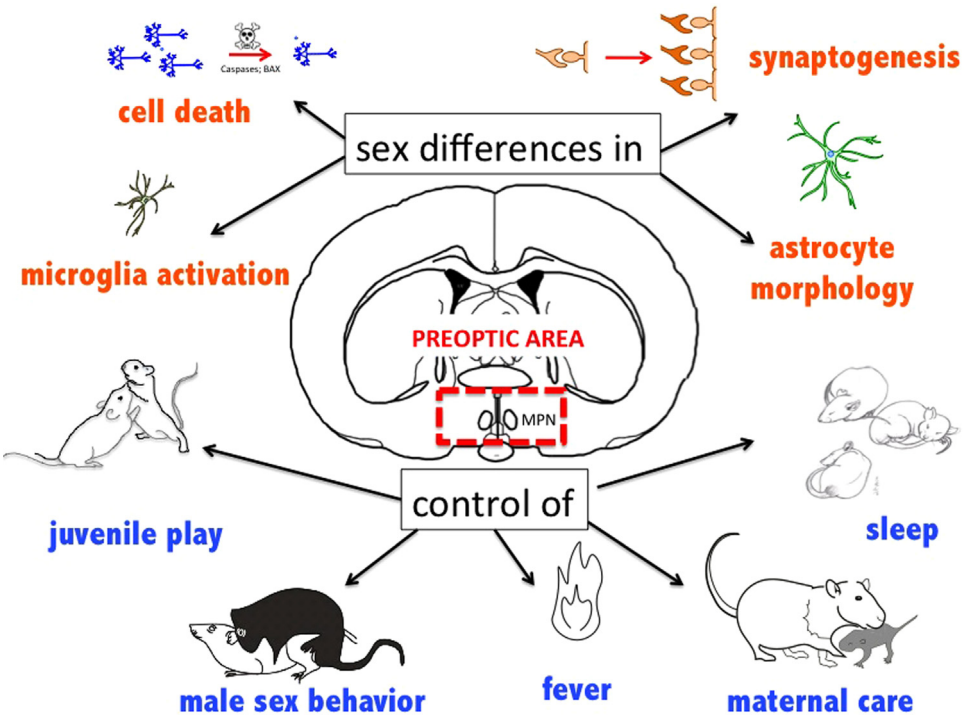

Figure 2. The preoptic area is sexually dimorphic and impacts multiple behaviors with relevance to autism spectrum disorder. The preoptic area (red box) is a small but complex brain region that contains the medial preoptic nucleus (MPN), hosts multiple neuroanatomical sex differences, and is central to the control of motivated, affiliative, and nurturing behaviors and sleep and fever generation, all of which are central to the phenotype of autism spectrum disorder. The preoptic area is also the embryonic source of gamma-aminobutyric acid neurons to the amygdala, hippocampus, and prefrontal cortex, and shares reciprocal connections with components of the reward pathway and those areas regulating fear and social behaviors.

Receptors for PGE2 are the G protein–coupled EPs 1 through 4, with EPs 2, 3, and 4 being linked to cyclic adenosine monophosphate production in various fashions. The EP4 receptor is heavily expressed by microglia and is a pivot point in a positive feedback loop, such that it can be either neuroprotective (72) or neurodamaging (73).

A prostaglandin seems an unlikely candidate for mediating a process as fundamental to species propagation as brain masculinization. Nonetheless, males have more endogenous PGE2 as a result of increased COX-1 and COX-2 levels induced by the elevated steroids of the male brain. Treatment of newborn males with COX inhibitors, including nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin, blocks the naturally occurring process of brain masculinization and prevents the expression of male sexual behavior in adulthood. Conversely, PGE2 administered directly into the POA of newborn female rat pups fully masculinizes both the synaptic profile and adult sexual behavior (47,48,74).

Localizing the cellular source of prostaglandins is challenged by both the broad distribution of the enzymes and the potential for feedforward positive regulation of PGE2 production. A low level of PGE2 production in one cell type is therefore capable of stimulating new or increased production in another cell type (50,72). We found that a single injection of PGE2 directly into the brains of newborn females was as effective at inducing neural and behavioral masculinization as treatment with steroids to mimic the hormonal milieu of males. The discovery that gonadal steroids upregulate COX-1 and COX-2 and that the masculinizing effects of estrogens was blocked by COX inhibitors confirmed that PGE2 is the downstream mediator of steroid-induced masculinization (75). It was the effectiveness of a single brief exposure to PGE2, however, that we found both shocking and puzzling. How could such an ephemeral signaling molecule as PGE2 have such a profound and enduring effect? By initiating a positive feedback response, we reasoned, and additional evidence suggests a role for microglia in this process.

MICROGLIA AND ASTROCYTES ARE ESSENTIAL CELLULAR PARTNERS IN POA MASCULINIZATION

Microglia are the brain's resident immune cells. Derived from macrophages in the yolk sac, they migrate into the nervous system early in development (embryonic day 9–10), proliferate, distribute widely, and take up permanent residence (76,77). Until recently, microglia were considered almost exclusively in the context of injury because one of their primary functions is to migrate to sites of inflammation and engulf debris from dead or dying cells (78). This narrow view has expanded dramatically with an emerging role as critical partners in healthy neural development (77). Microglia regulate synapse formation (50) and elimination (79), neurogenesis (80,81), and neuronal survival (82), but also actively kill select cell types (83). We determined that microglia are a source of positive feedback production of PGE2 in the developing POA and that this plays a critical role in establishing the sex-specific synaptic patterning (50). There are overall more microglia and they are in a more activated state in the male POA, thereby producing more PGE2 than in females. If a female is treated with PGE2, the microglia convert to an activated state and produce still further prostaglandin, leading to masculinization. The process involves activation of protein kinase A after binding to the EP2 and EP4 receptors on neurons. These receptors are adenylate cyclase–linked and protein kinase A–mediated phosphorylation of the GluR1 subunit of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

receptor induces trafficking to the membrane and clustering at the site of the nascent synapse (84). Activation of the membrane-clustered alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors is speculated to occur via PGE2-induced release of glutamate from the neighboring astrocytes (85). In males, the astrocytes of the POA are far more complex than in females, with longer and more frequently bifurcated processes (49) that can provide highly localized glutamate release and other forms of synaptic support. This multidimensional, hormonally mediated synaptogenesis requires cell-to-cell communication involving neurons, astrocytes, microglia, and prostaglandin-induced release of glutamate.

THE CEREBELLUM AND SEX DIFFERENCES IN ASD

The cerebellum was named "little brain" in Latin based on its appearance, but the name proved prescient as we have come to appreciate its wide-ranging importance (86,87). A primary fissure separates the anterior and posterior lobes, which are further divided into the vermis and lateral hemispheres. The vermis and hemispheres are further subdivided into lobules numbered I through X. Different lobules have distinct functions. Lobules IV and V, for example, are most active during working memory tasks (88), whereas stimulation to lobules VI and VII produces the strongest modulation of both sensation (89) and emotion (90). The posterior vermis has been referred to as the limbic cerebellum because of its role in affect and cognition (91). There are multiple closed loop circuits between the cerebellum and the cerebral cortex, allowing for efficient control of action, error correction, and motor learning (92). An emerging view is that these loops integrate emotional and cognitive responses originating in cortical regions that are then modified by the cerebellum as a "general purpose coprocessor" (93).

Developmentally, the cerebellum is notable for its early emergence but late maturation, in particular that of the principal cells, the Purkinje neurons (94), making it particularly vulnerable to perturbations of both intrinsic and extrinsic origin. The existence of closed loops between the cerebellum and cerebral cortex yokes the development of each region to the other and widens the impact of pathology in either.

Evidence from postmortem histology, genetics, animal models, imaging, and clinical studies of ASD implicate the cerebellum in both the etiology and manifestation of the behavioral phenotype (95). The severity of pathologies are correlated with ASD severity in monozygotic twins (96), and functional magnetic resonance imaging reveals profound activational deficits during spatial attention tasks in subjects on the autism spectrum (97). Other noted pathologies include hypertrophy, disordered circuitry (98), and neuroinflammation (99). Damage to the cerebellum in infancy is among the highest risk factors for developing ASD [an estimated fortyfold increase (100)], second only to having an identical twin with autism, with some arguing that damage to the cerebellum can lead directly to a diagnosis of ASD (95). But the damage must occur early, suggesting that there is a sensitive period during which the cerebellum is developing critical capacities that, if

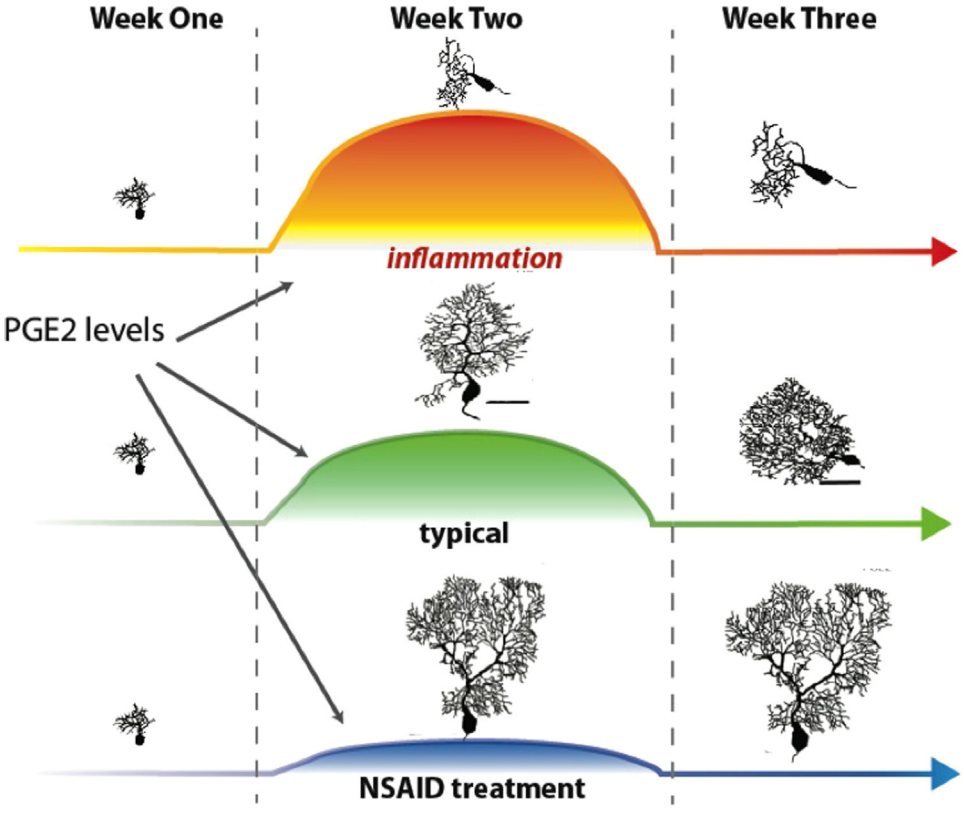

Figure 3. Purkinje neuron development is regulated by prostaglandin E2 (PGE2) during a sensitive window. The principal neurons of the cerebellum show dramatic growth and differentiation during the first 3 postnatal weeks of life in the laboratory rat. Endogenous PGE2 is elevated during postnatal week 2 and is a critical regulator of normal Purkinje neuron growth. If PGE2 production is increased by inflammation during that week, the Purkinje neuron growth is stunted. If PGE2 production is decreased by treatment with nonsteroidal anti-inflammatory drug (NSAID) inhibitors of cyclooxygenase, the Purkinje neurons show excessive growth of the dendritic tree. Changes in PGE2 levels in either the first or third postnatal week have no impact on Purkinje neuron growth, and this appears to be because of an intrinsic gene expression program in which the transducers of the PGE2 signal are upregulated during the second week but expressed only at low levels during the first and third weeks. There is a narrowly defined sensitive window during which inflammation or treatment with NSAIDs dysregulates cerebellar development.

disrupted, will have a lifelong impact. The two most prominent neuropsychiatric disorders associated with cerebellar pathology, ASD and schizophrenia, exhibit a male bias in prevalence or symptomology (101,102).

Prostaglandins Regulate Cerebellar Development

Given the strong inference of a critical role for the cerebellum in ASD, we reasoned that developmental steroids might also stimulate prostaglandin synthesis there as in the POA. To our surprise, we discovered exactly the opposite: prostaglandins in the cerebellum stimulate the aromatase enzyme and local estradiol production (103). As part of a naturally occurring developmental progression, cerebellar PGE2 synthesis rises during the second postnatal week in the rodent and drives a parallel increase in local estradiol production. Deviation in either direction of the levels of PGE2 or estradiol has deleterious consequences for the developing Purkinje neurons (Figure 3). Too much PGE2 stunts the growth of the dendritic tree, resulting in an overall reduction in excitatory synapses. Inhibition of PGE2 production by COX inhibitors has the opposite effect, an overall sprouting of the dendritic tree and increase in excitatory synapses (104).

Inflammation in the Cerebellum During a Sensitive Window Impairs Later Social Behavior Only in Males

Once PGE2 was identified as an important component of cerebellar development, the next step was to determine the impact of inflammation and associated increases in prostaglandins. By administering the inflammatory agent lipopolysaccharide peripherally and COX inhibitors or PGE2 directly into the cerebellum of rat pups, the contribution of inflammation in this region to later behavioral changes was determined. Remarkably, either lipopolysaccharide or intracerebellar PGE2 had deleterious effects only during the second week of life and not the first or the third (105). The boundaries of this sensitive window are set by an intrinsic gene expression profile in which the enzymes for both arachidonic acid and estradiol production peak during the second week and then plummet during the third. As a result, neither PGE2 nor estradiol increase in response to an inflammatory insult during the third week. This remarkably scripted pattern of gene expression presumably subserves the unique developmental demands of the cerebellum. In particular, there must be events during the second postnatal week, such as pruning of climbing fiber input to the Purkinje neurons or growth of the Purkinje dendritic tree, that requires estrogen action, but precisely what that process is remains unknown. Nevertheless, a consequence of this narrowly constrained critical period is an equally constrained sensitive period, highlighting the importance of timing on the impact of developmental insults.

NSAIDs target the cyclooxygenases COX-1 and COX-2 to block prostaglandin production and reduce inflammation and the associated fever and pain. An unintended consequence of NSAID therapy could be oversuppression of an important development signal, as in the case of PGE2. In classic Goldilocks fashion, PGE levels during the second postnatal week need to be neither too high nor too low but instead just right.

Reciprocal social behavior varies along a continuum of which children with ASD are at an extreme end (106,107). One form of reciprocal social behavior that can be modeled in rats is rough-and-tumble play, which involves chasing, pinning, and boxing between same-aged cage- or littermates. The frequency of play is highly stable across days, but can be modulated by early life experiences, such as stress, isolation, or rearing environment. Social behavior in children with ASD can also be modulated by training, but there have been calls for more controlled trials and the establishment of consistent parameters in order to assess the success of these interventions (108). Across all species that exhibit rough-and-tumble play, including humans, males engage in more frequent and more intense physical contact (109). We found that dysregulation of cerebellar PGE2 production during the second postnatal week by either inflammation or NSAID administration impairs later play behavior, but only in males (104). Females

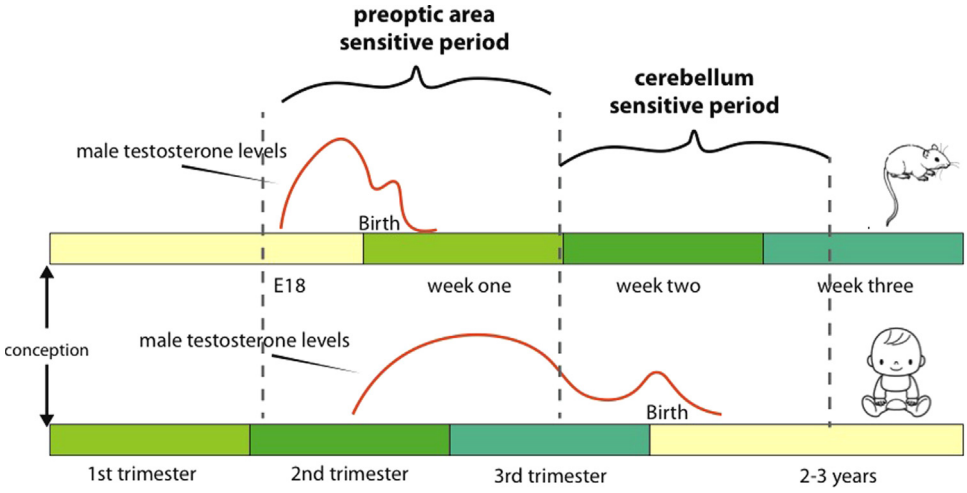

Figure 4. Different sensitive periods at different life stages of rats and humans. The developmental profile of the rat is shifted from humans in that a newborn pup is roughly equivalent to a mid- to late-gestation human fetus. The sensitive period for sexual differentiation of the preoptic area in the rat is operationally defined by the onset of testicular androgen production in male fetuses on embryonic day 18 and the loss of sensitivity of females to exogenous hormone treatment by the end of the first postnatal week. In humans, the sensitive period for the preoptic area begins during the second trimester with fetal androgen production and probably ends before birth, although this conclusion is constrained by a lack of experimental data. The sensitive period we have identified in cerebellar development is during the second postnatal week in the rat, which corresponds to the peripartum period in the human. The factors constraining the sensitive period in the rat are the onset and offset of gene expression profiles. Whether a similar profile exists in humans is currently unknown. E18, embryonic day 18.

naturally play less, but their level of play activity can still be reduced (110), indicating that the sex difference is not a floor effect. Determining why only males are negatively impacted by this modest insult to the developing cerebellum is an important future goal.

CONCLUSIONS

Sex differences in diagnosis of disease can occur for a multitude of reasons that include sex differences in presentation or implicit bias by the physician that a condition is more likely in one sex versus the other. Determining a biological basis for the male preponderance of ASD diagnosis will provide new avenues for therapeutic intervention and prevention, whereas identifying a cultural basis may require a change in diagnostic criteria (4). Teasing apart the impact of biological variables from social and cultural influences requires animal models in which the latter influences are negligible. However, sensitive periods for modulation by endogenous and exogenous variables vary in important ways in rodents and humans (Figure 4). Identifying the natural processes by which sex differences are established provides a backdrop against which dysregulated processes can be fully understood. The value of this approach is evident in the conversion of findings that implicate inflammatory signaling molecules and immune cells as critical contributors to male brain development and the role of inflammation in either the etiology or manifestation of ASD symptoms. The next step is to determine why the genes regulating normal development in males become dysregulated and identifying therapeutic approaches for reversing the deleterious effects.

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ARTICLE INFORMATION

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