Male Predominance in Autism: Neuroendocrine Influences on Arousal and Social Anxiety

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We offer a neurobiologic theory based on animal work that helps account for the conspicuous male predominance in autism spectrum disorders (ASD). In young male animals, testosterone (TST) binds to androgen receptors (AR) in brainstem neurons responsible for enhancing brain arousal. As a consequence, arousal-related neurotransmitters bombard the amygdala hypersensitized by TST acting though AR. Arousal-related inputs are known to prime amygdaloid mechanisms for fear and anxiety, with resultant social avoidance. We hypothesize that similar mechanisms contribute to autism's male predominance and to its defining impaired social skills. The theory rests on two key interacting factors: the molecular effects of TST in genetically vulnerable boys in combination with environmental stresses they experienced in utero, neonatally, or during the first years. We postulate that higher TST levels and, therefore, higher amounts of arousal-related inputs to the amygdala sensitize these genetically vulnerable male infants to very early stresses. In sharp contrast to boys, girls not only do not have high levels of TST-facilitated arousal-causing inputs to the amygdala but they also enjoy the protection afforded by estrogenic hormones, oxytocin, and the oxytocin receptor. This theory suggests that novel technologies applied to the molecular endocrinology of TST's actions through AR will offer new avenues of enquiry into ASD. Since the high male preponderance in autism is important yet understudied, we offer our theory, which is based on detailed neurobehavioral research with animals, to stimulate basic and clinical research in animals and humans and hopefully help develop novel more effective medical treatments for autism. *Autism Res* 2011,4:xxx–xxx. © 2011 International Society for Autism Research, Wiley Periodicals, Inc.

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Introduction

One of the major unexplained, yet best documented, features of autism is its skewed sex distribution. The complementary perspectives of a neuroscientist, a child neurologist, and a developmental psychologist considering jointly this feature of autism prompted them to propose that the extensive data on the neuroendocrinologic basis of social anxiety in animals might help explain the core social deficits of the autism spectrum disorders (ASDs—autism for short). Anxiety is pervasive and prevalent in individuals with autism [Skokauskas & Gallagher, 2010] without being one of its defining diagnostic criteria in the DSM IV-TR [American Psychiatric Association, 2000] or specific to it. If the findings on the biology of sex-linked social anxiety in animals turn out to apply to humans, they will provide strong support for a theory that will help explain the remarkable male preponderance in autism. They might also spur investigators to assess virtually unstudied sex-linked effects on specific symptoms of autism [Knickmeyer, Baron-Cohen, Raggatt, & Taylor, 2005].

The hypothesis developed in this paper grew out of Pfaff and colleagues' extensive neuroendocrine experiments in rodents that demonstrate the important influence of androgens (male sex hormones) on generalized central nervous system (CNS) arousal [Pfaff, 2006] and of androgen receptors (ARs) in the brain on social anxiety and social avoidance, both key deficits in autism. We propose that heightened generalized arousal coupled with stresses very early in life engender both enhanced anxiety and impaired sociability in male—but not female—mice. We hypothesize that androgens bound to ARs most likely play a similar contributory role to social anxiety in humans, which has barely started to be investigated.

We do not suggest that rodent behaviors constitute veridical models of the ASDs in all their complexities, of course. Yet it stands to reason that neuroendocrine effects on the immature brain must contribute in some way to the incontrovertible male preponderance in autism [Auyeung et al., 2009a] and some other developmental disorders. We offer here detailed evidence, inferences, and theoretical suggestions in the service of extending

research in autism in a direction that is important but relatively neglected.

Sex-Hormone Influences on the Generalized CNS Arousal System

An organism's fluctuations in arousal are entrained by the diurnal light/dark cycle's influence on specialized hypothalamic neurons, so-called "biological clocks" that are connected to the generalized CNS arousal system. These fluctuations represent adaptive adjustments to endogenous (body) inputs, on-going organismic priorities, and predictable and unpredictable environmental sensory inputs [Corbett, Schupp, Levine, & Mendoza, 2009]. Generalized CNS arousal is mediated by an ancient brainstem system, which originates mainly from collections of brainstem neurons located from the ventral medulla to the midbrain. Specific brainstem neurons produce specific neurotransmitters—dopamine (DA), norepinephrine/noradrenaline (NE), serotonin (5-HT), or histamine (HA)—that travel along their axons and are taken up by specialized receptors in post-synaptic neurons. These brainstem neurons' long ascending axons project to targets in the neocortex and subcortical nuclei, among them, importantly, the amygdala that is part of the limbic system. Their descending axons project to spinal cord and autonomic neurons. The ascending axons constitute discrete interconnected pathways that release specific neurotransmitters to their diverse targets (Fig. 1). These brainstem arousal neurons and their axon terminals interact extensively with other neurotransmitter inputs from sensory pathways, and also with circuitry releasing other molecules such as neuropeptides, hypothalamic, and systemic hormones—among them adrenal and sex hormones—that influence the arousal neurotransmitters to modulate brain activity [Pfaff, 2006].

These pre-synaptic molecular inputs access post-synaptic neurons through huge numbers of complex specialized receptors acting as private open or closed gates. Other molecules, including steroid hormones, gain access to the DNA in the nucleus. There, they may turn gene transcription on or off, altering temporary or long-term (epigenetically modified) cellular activity without altering the nucleotide gene sequences of the DNA. Recent epigenetic research is starting to explain how unique environmental experiences and genetics interact to sculpt brain development uniquely in each individual [Mehler, 2008].

Important for our main idea, in animals one particular steroid hormone, testosterone (TST) and its derivative dihydrotestosterone (DHT) (both androgens), stimulate neurotransmitter synthesis in the neurons of the CNS arousal-system and the transport of neurotransmitters to the subcortical *amygdala* where they are released. In turn, androgens act through ARs to increase responsiveness of amygdaloid neurons to neurotransmitters. In humans,
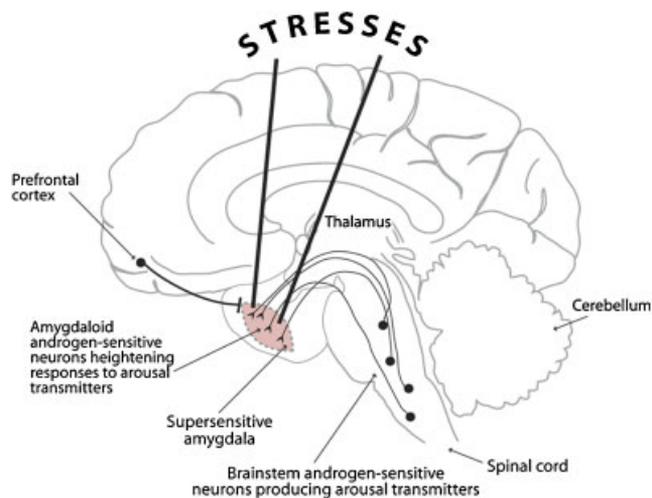

Figure 1. Brainstem arousal systems and their targets. Schematic medial view of the human right cerebral hemisphere, cerebellum, and brainstem. The axons of brainstem androgen-sensitive neurons secrete arousal-related neurotransmitters such as norepinephrine (NE), dopamine (DA), serotonin (5-hydroxytryptamine, 5HT), and others. Their widely activating effects on the cortex and subcortical nuclei, notably the amygdala, heighten alertness. In the amygdala, axonal inputs from these androgen-sensitive brainstem neurons increase sensitivity to fear-producing situations. From uterine life onwards, boys are exposed to higher levels of androgens than girls; consequently as fetuses, neonates, and throughout life they tend to react more strongly to various exogenous stresses. Among its many roles, the prefrontal cortex has inhibitory effects on the amygdala and thus mitigates excessive stress reactions.

the ARs are the product of a gene on the X-chromosome. ARs are activated by TST and DHT, the levels of which, from fetal life on, are much higher in boys than girls [Migeon & Wisniewski, 1998].

In laboratory animals, heightened CNS arousal can result in heightened social anxiety, which is reflected by decreased social interactions. Deficient social skills are at the core of autism, suggesting that these findings in animals might explain some of the gender-related features of autism, insofar as androgen-stimulated arousal translates into social anxiety and, as a consequence, decreased social interaction.

Brief Review of the Genetic Causes of Autism; Its Skewed Sex-Ratio

The ASDs are behaviorally defined neurodevelopmental disorders characterized biologically by heterogeneous causes, often genetic. Autism has a broad range of symptoms and a variable prognosis. Whereas the oft-quoted proportion of males to females in classic Kanner autism is 4/1, the severity of the disorder appears to influence this dramatic skewness. The ratio in Asperger syndrome (AS), the least severe ASD and least likely to be

associated with other evidence of brain malfunction, is reported to be 7/1 [Scott, Baron-Cohen, Bolton, & Brayne, 2002]. As far as we are aware, no study has measured anxiety across the autism severity spectrum, which would provide evidence relevant to the hypothesis proposed in this paper. In contrast, the ratio of boys to girls approaches equality at the more severe end of the autism spectrum [Wing, 1981], a potential explanations being that autosomal gene mutations and exogenous exposures harmful to the brain are unrelated to the sex of the developing child, which dilutes any intrinsic effect of maleness.

One of the most prevalent postnatal potentially deleterious influences on brain development is epilepsy, be it genetically or environmentally determined. By adolescence, epilepsy (at least 2 unprovoked seizures of any type) occurs in some 30% of children with autism [Tuchman & Rapin, 2002]. Unless it is genetic, the severity of epilepsy is a reasonably reliable sign of underlying brain damage or dysfunction. Studies of epilepsy in autism support reports of increased prevalence of more severe brain dysfunction among girls than boys, in that a meta-analysis of 14 studies revealed that the boy/girl ratio decreased significantly from 3.5/1 in individuals without epilepsy to 2/1 in those with epilepsy [Amiet et al., 2008].

Genetics is responsible for or plays a major—but not exclusive—role in the etiology of the ASDs. Surprisingly among genetic causes of autism, mutations and rearrangements of genes on the X chromosome are not over-represented, compared to those on autosomes. Two notable exceptions are fragile-X, which affects mostly boys, and Rett syndrome, mostly girls, both of which may fulfill behavioral criteria for an ASD in a considerable proportion of affected children. We note that the AR gene is also on the X chromosome; it needs to be explored thoroughly for polymorphisms that could be associated with autism. There is a rapidly increasing number of reports of disease-causing single Mendelian, and some mitochondrial, gene mutations, as well as cytogenetic chromosomal abnormalities and single DNA strand copy number variations (CNVs) associated with the autism behavioral phenotype [Abrahams & Geschwind, 2008]. Almost all of the many known genetic causes of autism are individually rare so that, in the aggregate, they account for only a fraction, perhaps 10–20%, of affected individuals. The genetics of autism are extremely complex because by no means are all the carriers of these mutations or CNVs on the broader autism spectrum or even symptomatic, and because, generally, the severity of the autism varies substantially among individual carriers of the same gene mutation [Weiss et al., 2008]. Much higher concordance in identical than fraternal twins, an elevated prevalence of the ASDs in siblings, and less severe but related developmental disorders in family members are well documented [Rosenberg et al., 2009]. The inescapable conclusion is that other genetic, epigenetic,

or environmental factors influence phenotypic expression [van Vliet, Oates, & Whitelaw, 2007]. Currently, most investigators subscribe to the hypothesis that polygenic background effects play a preponderant etiologic role in ASD, but without explaining the dramatic excess of affected males [Anderson, 2008].

A few investigations of the possible effect of parental age on the risk of autism have been carried out. Advanced paternal—but not maternal—age has been incriminated for the skewed sex distribution in a recent study of 393 families with two or more children with ASD, in which the overall 4/1 male/female ratio tended to decrease from 6/1 in fathers under age 30 to 1/1 in those above age 45 [Anello et al., 2009]. Several investigators [Durkin et al., 2008] have documented that advanced parental age at conception (above 35 for mothers, above 40 for fathers) increases the risk of a first child being on the autism spectrum, but unfortunately these studies do not provide data on the sex ratio of the affected offspring.

Sex Differences in Autism
Sex Differences in Autistic Symptomatology

Although many studies document the higher prevalence of ASDs in boys, few studies address sex differences in ASD symptomatology and there is poor consensus among those that do. Sex-related discrepancies can be due to sampling issues. According to the Asperger ratio just mentioned, selection of high functioning experimental subjects implies a higher ratio of males than low functioning individuals characterized by a more even sex ratio [Lord & Schopler, 1985; McLennan, Lord, & Schopler, 1993]. Caution is also required in the interpretation of studies reporting more severe social and communication impairment in girls [Hartley & Sikora, 2009]. Because girls tend to be more socially driven than boys, some mildly affected girls may go unrecognized. Those girls who are detected may be considered more severely impaired than boys owing to the cultural bias toward higher social expectations for girls, which may in turn influence behavioral ratings. As to neuropsychological profiles reporting a tendency for lower IQs and more visuo-spatial weaknesses in girls with ASD, these too remain controversial [Baron-Cohen, Knickmeyer, & Belmonte, 2005; Jolliffe & Baron-Cohen, 1997; Volkmar, Szatmari, & Sparrow, 1993].

Early-Life Exposure of the Developing Brain to Sex-Hormones and Stress

A convincing factor for gender differences in the ASDs is the consistently differential exposure of the immature brain to male and female sex hormones. Boys are exposed to higher intrauterine TST levels than girls, owing to the addition of the TST fetal boys secrete to the androgens the mother's adrenal provides to fetuses of either sex.

Levels in boys are especially elevated during the first few weeks after birth due to additive stimulation of residual maternal androgens and gonadotrophins to boys' own androgen production. Androgens continue to be higher throughout childhood in boys, albeit at more modest levels, until the pubertal surge [Gilmartin, 1987]. Baron-Cohen and colleagues characterized AS as ''the extreme male brain'' [Baron-Cohen, 2005; Baron-Cohen & Belmonte, 2005]. In typically developing children, Auyeung et al. [2009b; Auyeung, Taylor, Hackett, & Baron-Cohen, 2010] and others [Bergman, Glover, Sarkar, Abbott, & O'Connor, 2010] linked heightened amniotic fluid TST levels to later behavioral characteristics potentially relevant to autism (see below). We emphasize that the effects of androgen hormones on nerve cells with ARs in the brainstem and those with ARs in the amygdala are potentially multiplicative and thus liable to enhance androgen effects on the brain.

Animal models discussed earlier support our theory that elevated androgen-dependent CNS arousal systems in the brains of affected boys may render them more vulnerable than girls to both biologic and social stresses. By far the most provocative evidence for a putative environmental postnatal precipitating factor in autism is the unexplained regression or stagnation of behavior and language development at ~15–30 months reported by some third of parents of toddlers [Kurita, 1985; Rogers, 2004; Wilson, Djukic, Shinnar, Dharmani, & Rapin, 2003]. There is sparse information about sex ratio among these children, although one study reports that the proportion of boys was marginally higher (90%) in those who regressed than in those who did not (84%) [Wiggins, Rice, & Baio, 2009]. If regression is indeed more prevalent in boys and is in some way environmentally–influenced, which has not been documented, it might indicate that some genetically susceptible boys are more vulnerable than girls to some environmental factors such as common-place potentially triggering events like infections or immunologic factors. Although there are suggestions that autoimmune factors may play a role in autism [Jyonouchi, Geng, Cushing-Ruby, & Quraishi, 2008; Zimmerman et al., 2007], this remains a disputed issue and studies are typically underpowered to tease out excess vulnerability of boys. Population studies thoroughly discredit a direct role of live vaccines and mercury (thimerosal) toxicity as causes of autism [Fombonne, 2008], even though this does not exclude their role in rare genetically susceptible children. Other types of factors need consideration because, when asked specifically, some 15% of parents report that regression followed on the heels of a psychologically stressful change in the child's family or environment [Kurita, Kita, & Miyake, 1992; Wilson et al., 2003].

The timing and nature of environmental stresses together with the genetic background of the victim are relevant to their consequences. But this has not been studied in the ASDs. Stresses affecting brain development in embryonic life, during fetal life, during the birth process and immediate post-natal period, and at various postnatal ages have vastly different consequences. The prevalence of perinatal stresses is reportedly higher in autism than in unaffected controls [Steffenburg et al., 1989], but their importance has been downplayed in the more recent genetic era [Gardener, Spiegelman, & Buka, 2009]. It remains to be determined whether these early stresses have more severe consequences in male than female infants, leading to young boys with ASD, because there is some evidence of selective male vulnerability considered in the section below.

Anxiety as a Prevalent Symptom in Autism

In his original paper Kanner [1943] noted that many of the 11 children he was describing were excessively anxious. Anxiety is frequent and often severe in autism, but it is not required for the diagnosis. Contemporary investigators are likely to consider anxiety co-morbid, that is, meeting criteria for a separate DSM IV disorder [American Psychiatric Association, 2000]. A study of co-morbid symptoms in 44 children with ASD (age range 5–11 years) states that 84% met the full criteria for at least one type of anxiety disorder defined on the basis of DSM III-R criteria [Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998]. Because the number of children diagnosed with ASDs is growing (for a number of reasons not discussed here [Fombonne, 2007]), the number being treated for anxiety has ballooned. A recent review of anxiety in ASDs quotes prevalences that range from 11 to 84% [White, Oswald, Ollendick, & Scahill, 2009], which points to significant sampling differences among studies. Verbal children with autism report fears similar to those of typically developing children and of children with other developmental disorders. Simple phobia (e.g., of animals, darkness) is most common, with generalized anxiety, separation anxiety, obsessive–compulsive disorder, and social phobia somewhat less common [White et al., 2009], each of which is observed across all cognitive levels.

In young and very impaired nonverbal children with ASDs, anxiety has to be inferred from their behaviors. Verbal children and adolescents are more likely to be self-aware and thus more likely to be cognizant of their anxieties. Indeed it is the brighter but more socially impaired children who report the most severe anxieties [Sukhodolsky et al., 2008]. This means that studies limited to higher functioning older children capable of answering questionnaires run the danger of over-sampling anxious individuals, and that questionnaires may not capture the range of anxiety in autism because self-awareness and cognitive abilities vary so widely. DSM IV does not list social phobia and obsessive–compulsive disorder as distinct

co-morbidities in autism, despite their frequency. Perhaps DSM IV considers social phobia a feature of autism's social impairment, and obsessive–compulsive disorder as one of the restricted interests and repetitive behaviors (stereotypies) that define it. Clinical observation suggests that stereotypies, like essentially all movement disorders that implicate dysfunction of basal ganglia circuitry, tend to increase with anxiety in ASD, although scientifically based evidence to document this observation is lacking.

A number of factors may influence the level of anxiety and its manifestations in autism. The severities of the anxiety and social impairment might be correlated. The existence of a mutually amplifying feedback loop between anxiety and social stress has been suggested, but with limited objective evidence [Hofmann & Bitran, 2007; Phan, Fitzgerald, Nathan, & Tancer, 2006]. In many affected individuals, bland sensory stimuli are perceived as aversive and are associated with both enhanced arousal and anxiety [Green & Hollander, 2010]. Age appears to have little influence on these notoriously aberrant sensory experiences and on the severity of the anxiety they arouse. Yet the combined influence of cognitive ability, age, and intensity of sensory experiences appears to modulate how children with ASDs express their anxiety [Pfeiffer, Kinnealey, Reed, & Herzberg, 2005].

Information about the exact neurobiologic basis of anxiety in humans is sparse. A neuroimaging study focusing on the limbic system reported a correlation between symptoms of anxiety/depression and enlargement of the right amygdala in 42 low-functioning children with autism [Juranek et al., 2006]. A family study indicated that social anxiety disorders are frequent in the parents of children with ASDs [Piven & Palmer, 1999]. Unfortunately, most studies did not include sex as a variable, thus we lack information on how sex relates to anxiety in autism. Judging from animal studies, social anxiety might be regulated differently from anxiety caused by physical attributes of the environment like unexpected loud sounds, but this has not been documented in humans [Kuusikko et al., 2008; South et al., 2008].

In animal models, social anxiety arises from ''negative'' non-affiliative, aggressive interactions. In rats and other species [Alleva & Santucci, 2001; Kollack-Walker, Watson, & Akil, 1997; Sgoifo, Koolhaas, Musso, & De Boer, 1999] findings suggest that social stress is regulated differently from other types of stresses such as those generated by certain sounds, pain, immunological response to infection, etc. Moreover, the impact of social anxiety was shown to be worse in male than in female animals [Westenbroek, Den Boer, & Ter Horst, 2003]. Social anxiety may potentially culminate in ''persistent social inhibition'' [Truitt et al., 2007]. It may be correlated with detectable anatomic or functional changes in the amygdala [Etkin & Wager, 2007]. In male rats, social anxiety has to do with the ''combat and social dominance''

relationships involved in the establishment and maintenance of a territory, whereas the ''roles of female mice were usually passive and their involvement in combats occurred only through mistaken identity'' [Anderson & Hill, 1965; p. 1755]. There are provocative parallels between these sexually divergent reactions in rodents with the lesser involvement of girls than boys in antagonistic social interactions.

Essentially all infants, murine or human, experience many challenging experiences. Early life experiences have a lot to do with the generation of an anxious temperament in both infants and mice [Fox, Halpern, Ryan, & Lowe, 2010; Lo Iacono & Gross, 2008], that is, with a tendency to interpret ambiguous situations as threatening or dangerous. A recent study in over 100 typically developing infants at an average age of 17 months indicates that higher levels of amniotic fluid TST, but not cortisol, was a correlate of fearfulness in boys but not girls [Bergman et al., 2010]. Neither hormone was associated with joy or pleasure in either sex. If duplicated, this study provides strong support for the human relevance of TST to anxiety in male rodents. We propose that boys' heightened arousal and anxiety attributable to their higher TST levels contribute to making them more susceptible than girls to the development of autistic symptoms, be they social withdrawal or disproportionately aggressive responses.

Neurobiological and Hormonal Basis for Sex-Related Symptoms of Social Anxiety
The Amygdala

The importance of the amygdala for the expression [Adolphs et al., 2005] and, presumably, the experience of fear established in laboratory rodents holds true for higher primates, including humans [Hariri et al., 2002a; Kalin, Shelton, & Davidson, 2004; Paton, Belova, Morrison, & Salzman, 2006; Phelps & LeDoux, 2005]. Responses to fear are adaptive if an organism adopts appropriate fight-or-flight strategies to save itself from danger, but maladaptive if responses are inappropriate to the severity of the threat. If signals from stimuli conditioned to elicit fear do not reach the amygdala, conditioned fear responses do not occur [reviewed in LeDoux, 2000]. Examples of maladaptive responses, which involve amygdaloid circuitry, include excessive anxiety, fight-or-flight responses to non-threatening situations, and paralysis when challenged by actual threats.

Two types of data from animals—non-human primates as well as rodents—and humans emphasize the role of the amygdala in sex-related differences in social behavior. The first type pertains to structural and functional alterations in the amygdala of individuals with autism and of behaviorally relevant animal models; the second focuses on the general arousal system and the role in

arousal of the neurotransmitters dopamine, norepinephr-ine, and serotonin, all of which are involved in amygdaloid function and all of which are implicated in stress, anxiety, and autism [reviewed in Penn, 2006].

Several studies document structural abnormalities and important functional involvement of the amygdala in individuals with autism [Aylward et al., 1999; Schumann et al., 2004; Sparks et al., 2002]. Substantial damage to the amygdala in humans reduces eye contact during con-versations [Spezio, Huang, Castelli, & Adolphs, 2007], and destruction of the amygdala affects the ability to respond to others' expressions of fear [Adolphs et al., 2005]. Yet removal of the amygdala in neonatal monkeys, while it enhanced fear, had little effect on social behaviors [Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004], for reasons we do not yet understand.

Fear depends on arousal-signaling inputs to the amyg-dala from the aminergic cell groups of the brainstem arousal-activating system discussed earlier (Fig. 1). Infus-ing DA into the amygdala of rodents enhances fear and anxiety behaviors and releases it from prefrontal inhibitory influences [Rosenkranz & Grace, 2001, 2002]. CNS-arousing inputs are necessary in order for frightening situations, emotions, and emotional memories to stimu-late amygdaloid mechanisms required for biologically adaptive fear responses. These adaptive responses involve production of corticotrophin releasing factor/hormone (CRF) in amygdaloid neurons [Roozendaal, Schelling, & McGaugh, 2008] Conversely, infusing a dopamine recep-tor (DAR) *antagonist* into the lateral amygdala blunted the previously well-learned fear response of rats trained to avoid returning to a place where their feet had been shocked [Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006b; Roozendaal, Okuda, de Quervain, & McGaugh, 2006a]. Adaptive responses to fear depend on synaptic release in the amygdala not only of the arousal neurotransmitter DA but, most importantly, of NE [Roo-zendaal et al., 2008] released by axon terminals of neurons located in the pontine locus coeruleus to the limbic forebrain [reviewed in Hamson, Jones, & Watson, 2004; Pfaff, 2006]. The NE work is especially impressive because of NE's proven role in behavioral responses to stress [Morilak et al., 2005].

Serotonin (5HT), another arousal-supporting neuro-transmitter, is also involved in the production of anxiety and fear. Serotonin-containing fibers reach the amygdala through axonal projections from the median and dorsal raphe nuclei of the midbrain which innervate neurons in the lateral amygdala [McEwen et al., 1984; Muller, Mascagni, & McDonald, 2007]. Serotonergic influences have long been implicated in abnormal brain develop-ment in autism [Cook et al., 1997]. They are likely contributors to social anxiety and thus, very likely, to autism in view of long-known abnormally high serotonin levels in the platelets of a significant proportion of individuals with ASDs [Mulder et al., 2004; Schain & Freedman, 1961].

Hormones, neurotransmitters, and their transporters and receptors are under the control of multiple genes. Exciting work on genetic contributions to fear and anxiety has focused on mutations of the serotonin transporter (5HTT) found in some individuals with autism [Anderson et al., 2002]. This transporter is responsible for the re-uptake after release of serotonin into the synaptic cleft. The gene encoding 5-HTT is, in some individuals, regulated in a manner that affects fear and anxiety through pathophysiologic mechanisms that include neurons in the amygdala [Furmark et al., 2004; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002b; Heinz et al., 2005].

The Role of TST in Gender-Related Findings and Responses to Stress in Autism

The androgen theory of autism proposes that exposure to high levels of fetal TST is positively correlated with several autistic traits and is inversely correlated with social development and empathy [Baron-Cohen, Auyeung, Ashwin, & Knickmeyer, 2009]. The present theory, which rests in large part on animal work, goes further and adds pathophysiologic detail. It posits that there are two factors converging on the amygdala that in concert contribute to the risk of an ASD in genetically susceptible individuals: exposure of boys to high levels of TST *and* androgen-mediated prenatal, perinatal, or early life stresses enhancing the general arousal system.

This dual hypothesis is bolstered by extensive animal work. For example, the male pups of dams stressed early in pregnancy by exposure to a variety of chronic stressors (e.g., noxious odor, constant light, noise, movement restraint) exhibited maladaptive behaviors and stress responses [Mueller & Bale, 2008]. These studies address the important issue of timing of stresses and the sex-related vulnerability to maternal perturbations. Effects of stress, be it social or biologic, on amygdaloid function in animals are strongly sex-dependent. Prior stress *enhances* stress-evoked eye blink conditioning in male rats, whereas it *retards* these responses in females [Waddell, Bangasser, & Shors, 2008]. Laboratory experiments indicate that, typically, anticipation of social interaction is more likely to cause male than female mammals to respond with aggression, although anxiety may result in avoidance of social interaction in both sexes [Amaral, Bauman, & Schumann, 2003; Prather et al., 2001]. Bauman and Amaral [2005] emphasize the powerful influence of serotonergic inputs to the monkey's amyg-dala and its CRF neurons, an influence fully concordant with its role in arousal-related inputs, social anxiety, and social avoidance in mice.

As stated earlier, androgens act through numerous ARs both in brain stem arousal nuclei [Simerlym Chang, Muramatsu, & Swanson, 1990] that release excitatory neurotransmitters (DA, NE, and 5HT) to the amygdala, and in the amygdala itself (Fig. 1). Levels of ARs, regulated by TST [McAbee & DonCarlos, 1999], are higher in the developing forebrains of males than females, in parallel with males' exposure to higher levels of TST and DHT. Heightened general arousal leading to anxiety receives further support from strong AR expression in the locus coeruleus [Hamson et al., 2004] which sends massive NE arousal-related projections to the amygdala and limbic forebrain involved in the expression of emotion [reviewed in Pfaff, 2006]. That is, the AR-dependent arousal-related systems have multiple androgen-dependent targets in the brain.

Higher androgen levels may make susceptible unborn boys more vulnerable than girls to environmental stressors experienced by their pregnant mothers such as infections [Meyer, Yee, & Feldon, 2007], hurricanes [Kinney, Miller, Crowley, Huang, & Gerber, 2008a], or deaths of relatives [James, 2008]. These and various other gestational abnormalities affecting neural, immune, and endocrine systems of the mother have been implicated for their potential indirect fetal effects [Glasson et al., 2004], although, mostly, small numbers of subjects and lack of replication weaken the relevance of their claims [Gardener et al., 2009].

The combination of high androgen levels and one or more environmental or endogeneous stressors may influence responses to challenging social events in a multiplicative manner, the androgens exacerbating the disruption of normal social development caused by the stressors [Kinney, Munir, Crowley, & Miller, 2008b]. *Key point*: If under the influence of androgenic hormones, excessive quantities of arousal-related neurotransmitters reach the amygdala, they are more likely to promote fearful or anxious reactions to even non-threatening social signals, especially if little boys have already endured substantial stress.

That human sex hormones influence specific (but not all) sexually dimorphic aspects of cognition and behavior has long been taken for granted. What the Baron–Cohen group showed recently is that the effect starts prenatally. They found a relationship between TST levels in routinely collected amniotic fluid and mothers' descriptors of male-typical play in 212 unselected boys and girls at a mean age of 8.6 years [Auyeung et al., 2009b]. They also gave a behavioral questionnaire to the parents of 129 ostensibly typical toddlers of both sexes and demonstrated that higher amniotic androgen levels were associated with responses to some questions meant to elicit possibly autistic traits, with boys having significantly more of them than girls. Even more germane to the thesis of this paper, the previously quoted study by

Bergman et al. [2010] linked amniotic fluid TST levels to observed heightened fearfulness in 108 typically developing toddler boys but not girls. They found no relationship between estrogens and behavior in either sex. If duplicated, this study provides strong support for the relevance of TST to anxiety in boys, ostensibly making them more susceptible than girls to the development of social withdrawal or disproportionately aggressive responses so common in autism.

In summary, TST and DHT work through ARs in brainstem arousal systems and in the amygdala of experimental animals to raise the level of arousal-related signaling (Fig. 2). The consequence is that androgens potentiate amygdaloid activity associated with anxiety and fear, thus increasing the likelihood of autism in males.

Another contribution of sex hormones to the skewed sex ratio in autism is that, whereas androgens heighten vulnerability of males, estrogens and oxytocin protect females.

Estrogens and Oxytocin

Estrogens (such as estradiol) and androgens (TST and DHT) are closely related steroid hormones that share a common metabolic pathway, in which TST is aromatized to estrogen. Both are produced and have effects in both sexes but in vastly differing amounts. Chemical and behavioral evidence indicates that, besides the amygdala, the preoptic area of the hypothalamus is another important site of androgen action.

The effects on social behavior and anxiety of estrogens (E) and oxytocin (OXT) are reciprocal to those of androgens. Estrogenic hormones work in tight synergy with oxytocin (OXT), a peptide expressed by neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SO) of the hypothalamus. Females are exposed in utero and post-natally to higher levels of E and OXT and to lower levels of androgens than males because they are protected from androgens by both lacking testes and by the P450 aromatase in the placenta and ovaries that metabolizes maternal and fetal adrenal androgens to estrogens [Meinhardt & Mullis, 2002]. In addition to its effects on sex behavior, OXT is one of the important contributors to the support of pro-social behaviors [reviewed in Zingg, 2002]. A presumed strong contributor to male susceptibility to autism is that males, besides the burden of higher androgen and AR arousal signaling, just discussed, have less of the ameliorating effects of E and OXT on sociability and damping of anxiety.

Work from the Pfaff laboratory and others established that E up-regulates activity in OXT systems. This was shown both in molecular terms in the amygdala, which is rich in oxytocin receptors (OTR) [Elands, Beetsma, Barberis, & de Kloet, 1988; Quinones-Jenab et al., 1997] and in electrophysiological terms in neurons of the paraventricular nucleus (PVN) of the hypothalamus
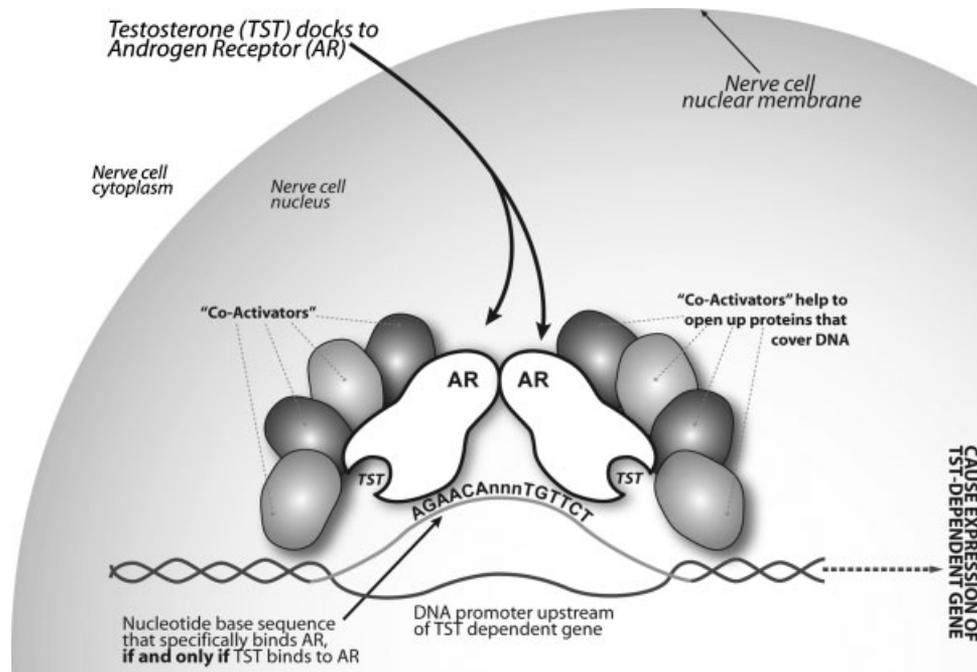
Figure 2. Effect of testosterone (TST) on DNA transcription in TST susceptible neurons. Schematic diagram of the role of TST on the DNA of neurons in the brainstem and amygdala (see Fig. 1), not to mention other neurons and cells in somatic tissues. TST enters the nucleus from the neuronal cytoplasm after binding to the androgen receptor (AR). Only when TST is bound to the AR do the co-activator proteins that cover the coiled DNA open up the chromatin, uncovering and uncoiling the DNA to allow gene transcription. Uncoiling enables the TST-carrying AR to interact with the DNA nucleotide base sequence of the promoter that commands the downstream expression of TST-dependent genes ("downstream" is off the right side of the diagram). We propose that differences among individuals in their co-activator chemistry likely account for differences among very young boys' responses to TST. We hypothesize that mechanisms illustrated here contribute to some aspects of sex-related individual differences in social anxiety and the severity of social withdrawal in ASD. We do not propose a direct role of these neuroendocrine effects in other core deficits or associated features of autism.

[Akaishi & Sakuma, 1985; Kow, Johnson, Ogawa, & Pfaff, 1991]. There is evidence in the mouse that OXT reduces anxiety, but only in the presence of E, probably by facilitating transcription of OTR proteins [McCarthy, McDonald, Brooks, & Goldman, 1996].

In mice OXT's involvement in maternal behavior, sociability, and reduction of anxiety is well documented [e.g., Ross et al., 2009]. OXT reduces anxiety in rats bred for the production of high or low levels of anxiety-related behaviors [Bosch, Meddle, Beiderbeck, Douglas, & Neumann, 2005]. In female mice, groups of E and OXT neurons in the amygdala contribute to the reduction of social anxiety and to increases in social interactions and social memory [reviewed in Ferguson, Young, & Insel, 2002]. OXT can reverse deficits in social behavior linked to early life stresses [Lee, Brady, Shapiro, Dorsa, & Koenig, 2007]. In rhesus monkeys [Winslow, Noble, Lyons, Sterk, & Insel, 2003] and humans [Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Guastella, Mitchell, & Mathews, 2008], OXT is implicated in affiliative social behaviors, a finding that supports the hypothesis of the link between social anxiety and brain arousal in autism proposed in this paper, OXT is involved in social bonding in both animals and humans in whom it sustains romantic and maternal love [Bartels & Zeki, 2004; Loup, Tribollet, Dubois-Dauphin, & Dreifuss, 1991].

Thus, estrogens and oxytocin work synergistically in rodents to foster social recognition and approach [Choleris et al., 2003, 2006; Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009], elementary components of pro-social behaviors that are severely deficient in many individuals with autism. In the social prairie vole, the anxiolytic action of OXT required for normal social behaviors [Cushing & Carter, 2000] is strongest in the presence of estrogen [McCarthy, Chung, Ogawa, Kow, & Pfaff, 1991; McCarthy et al., 1996]. This requirement for E is likely due to the strong influence of E on the *OTR* gene transcription. In fact, in female mice, blocking OTR activity in the brain increases anxiety-like behaviors in a manner that depends on the hormonal state of the female [Neumann, Torner, & Wigger, 2000]. In the male, T-dependent sexual activity can be followed by the post-ejaculation reduction of anxiety due, at least in part, to the release of OXT within the forebrain [Waldherr & Neumann, 2007]. Thus, in males as well as females, OXT can help to reduce anxiety.

Several genetic association studies have linked mutations in the human *OTR* gene to autism [e.g., Lerer et al., 2008; Wu et al., 2005]. Low plasma OXT levels were

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found in children with autism [Green et al., 2001; Modahl et al., 1998]. The exciting news is that several small studies report that OXT may ameliorate sociability and decrease repetitive movements in high functioning autism/AS [Andari et al., 2010; Green & Hollander, 2010; Hollander et al., 2003]. Kirsch et al. [2005] found with fMRI that OXT reduced the coupling of the amygdala to brainstem nuclei activated by fear. Inactivation of the *OTR* gene was discovered in an individual with autism and his unaffected mother but not in his affected brother [Gregory et al., 2009]; hence the relevance of this mutation to autism is not straightforward, although epigenetic silencing of the *OTR* gene was found in brain tissue of eight unrelated individuals with autism.

In summary, estrogens, acting in the amygdala together with OXT, reduce social anxiety, thus fostering higher levels of social interaction. In doing so, they apparently oppose the influence of TST-dependent social arousal and anxiety.

Perspectives, Limitations, and Outlook

The extensive evidence in mice from the neuroendocrine field on the environmentally triggered arousal- and anxiety-producing effects of androgens on both the ascending brainstem pathways and directly and indirectly on the amygdala is undeniable. The reciprocal prosocial and affiliative effects of estrogens and OXT on the amygdala are equally strong.

However, we acknowledge, again, the limitations of using animal models to address questions about human social behaviors and their pathologies. We are aware that neuroscientific animal models are tools for studying specific behavioral symptoms and their pathophysiology, but not the ASD's in all their clinical complexities. The goal of the animal experiments we presented has been to focus attention on certain sex-related aspects of human neuroendocrinology and neurophysiology relevant to autism. That said, the recent enormous progress in autism research is a tribute to the insistence of parents who urged basic scientists and clinicians to collaborate.

In this paper we have advanced, in detail, the hypothesis that human males, like male laboratory animals, are especially susceptible to pre- and neonatal stress, partly as a result of androgenic hormone action. Our review of the human literature yielded only wisps of evidence for this androgenic excitatory effect. What the field needs now is specifically to demonstrate that baby boys are more vulnerable to prenatal maternal and other perinatal stresses than baby girls and in fact show more instances of behaviors suggesting anxiety than baby girls, as has been shown in laboratory animals.

The literature contains a number of studies describing adverse events and exposures during pregnancy [Bilder,

Pinborough-Zimmerman, Miller, & McMahon, 2009; Dodds et al., 2010; Kinney et al., 2008a] and early life of children on the autism spectrum. Most are retrospective, and inadequately documented and need extension and replication. We are aware that more systematic efforts at collecting this type of information on larger samples are in progress and look forward to their findings. Recent work on the effect of prenatal maternal stress [Davis, Glynn, Waffarn, & Sandman, 2010], depression [Field, Diego, & Hernandez-Reif, 2006], shows that amniotic fluid levels at the time of amniocentesis reflect maternal blood cortisol [Davis & Sandman, 2010; Glover, Bergman, Sarkar, & O'Connor, 2009; Sarkar, Bergman, Fisk, O'Connor, & Glover, 2007]. Studies on behavioral consequences of elevated cortisol exposure in utero for anxiety and other health issues are just starting and in our opinion must be pursued vigorously.

This type of information is of course highly relevant to autism, if the goal is to support, or refute, our androgen hypothesis of heightened anxiety and reactivity to stress. It will require the development of new objective methodologies to measure stress applicable to infants from birth onward, methodologies we intend to develop. It will be necessary to expand observational instruments [e.g., Brazelton & Nugent, 1995; Gunnar, 1989] in order to quantify anxiety threshold (i.e., tolerance) and expression (i.e., intensity) of anxiety in babies of both sexes. Baseline responses to particular stressors and self-regulation will need to be assessed with both behavioral [Derryberry & Rothbart, 1988] and physiological tools. We predict that longitudinal studies will show that heightened anxiety and stress responses in early infancy are often precursors of maladaptive socio-emotional responses, as has been shown in animals. Such tools should especially be applied to studies of high-risk siblings, in a manner that will lead to earlier more targeted interventions for specific behavioral ASD symptoms.

Acknowledgments

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