BI-01 Sex differentiation across development

Human sexual differentiation is an intricate, cascading developmental trajectory that initiates at the moment of conception and unfolds continuously across the lifespan. It is driven by genetic inheritance but is primarily orchestrated by sequential waves of hormonal exposure that structurally organize the developing brain and body, subsequently activating these pre-established foundations during puberty and adulthood. Understanding this phenomenon requires untangling an intricately woven nexus of biological imperatives, statistical behavioural variance, and cultural amplification.

To systematically examine how men and women differentiate, this report partitions the developmental process into three distinct layers: physiology (measurable, binary-leaning structural and endocrine processes), behaviour (statistical, overlapping normal distributions of cognitive and psychological traits), and culture (the variable social norms that amplify or suppress biological predispositions). By prioritizing systematic reviews, meta-analyses, and robust primary literature, this analysis separates robustly replicated findings from contentious hypotheses, while remaining steadfast in distinguishing what is known about group averages from what can be accurately predicted about any single individual.

Layer 1: The Physiology of Sexual Differentiation (Measurable)

The physiological layer of human sex differentiation is strictly mechanistic, measurable, and highly conserved across mammalian species. It involves genetic triggers, gonadal differentiation, and multiple waves of hormonal exposure that permanently alter tissue structure and function.

Genetic and Gonadal Sex

At conception, chromosomal sex is established by the inheritance of an X or Y chromosome from the paternal sperm, resulting in a 46,XX or 46,XY karyotype. However, for the first six weeks of human embryonic development, the fetus remains morphologically bipotential, possessing uncommitted gonadal ridges and dual sets of reproductive ducts: the Müllerian ducts (precursors to the female internal structures) and the Wolffian ducts (precursors to the male internal structures).

The primary genetic catalyst for male differentiation is the *SRY* (Sex-determining Region Y) gene located on the Y chromosome. Between the sixth and seventh weeks of gestation, the expression of *SRY* initiates a complex transcriptional cascade, upregulating genes such as *SOX9*, which differentiates the bipotential gonads into testes. In the absence of *SRY*, the gonads develop into ovaries. Historically, the female developmental pathway was described as a "default" state, but modern genetic research has demonstrated that ovarian development requires active, sustained gene expression cascades, most notably driven by *WNT4* and *RSPO1*, which actively suppress the male pathway. Once the gonads differentiate, they take over the process of sexual differentiation by secreting specific hormones into the fetal bloodstream.

The fetal testes contain two critical cell types. Sertoli cells secrete anti-Müllerian hormone (AMH) to trigger the apoptosis and regression of the female duct system. Simultaneously, Leydig cells begin synthesizing and secreting exceptionally high levels of testosterone. Fetal ovaries, by contrast, are relatively quiescent regarding sex steroid production during early gestation.

To isolate the effects of sex chromosomes from the effects of gonadal hormones, researchers have developed animal models such as the Four Core Genotypes (FCG) mouse strain, pioneered by Arthur Arnold and colleagues at UCLA[1]. The FCG model physically relocates the *Sry* gene from the Y chromosome to an autosome, allowing researchers to generate XX mice with testes and XY mice with ovaries. Research utilizing this model has definitively proven that sex chromosomes exert direct, hormone-independent effects on brain development and neurochemistry, though the magnitude of these chromosomal effects in humans remains an area of active investigation.

The Prenatal Hormone Surge and the Organisational/Activational Hypothesis

The foundational paradigm for understanding the neurobiology of sex differences is the organisational/activational hypothesis. First proposed by Phoenix, Goy, Gerall, and Young in a landmark 1959 study published in *Endocrinology* examining guinea pigs, this hypothesis posits that exposure to sex steroid hormones during critical perinatal windows permanently "organises" the neural circuitry of the developing brain (yielding structural, irreversible effects). Later in life, usually beginning at puberty, a second surge of hormones "activates" these pre-established circuits to elicit sex-typical behaviours (yielding reversible, context-dependent effects)[2].

In human males, the critical period for organisational effects is the prenatal testosterone surge, which peaks between weeks 8 and 24 of gestation. During this window, fetal testosterone levels in males reach concentrations comparable to those of adult men, before dropping late in pregnancy. This surge is responsible for the masculinisation of both the external genitalia and the permanent sexual differentiation of the brain. The genitalia require the conversion of testosterone into the more potent androgen dihydrotestosterone (DHT) via the enzyme 5-alpha-reductase[6]. The masculinisation of the brain is primarily driven by testosterone. In many non-human mammalian species, this brain masculinisation paradoxically requires the intracellular aromatisation of testosterone into estradiol within the brain tissue; however, the extent to which human brain masculinisation relies on direct androgen receptor activation versus estrogen receptor activation remains debated in the literature[5].

Mini-Puberty of Infancy

A second, less widely understood wave of hormonal exposure occurs shortly after birth. During gestation, the maternal placenta produces high levels of estrogens that suppress the fetal hypothalamic-pituitary-gonadal (HPG) axis. When the placenta is expelled at birth, this suppression is lifted, initiating a phenomenon known as "mini-puberty." In infant boys, luteinizing hormone (LH) and testosterone levels spike dramatically between 1 and 6 months of age, once again reaching near-adult levels before dropping to nearly undetectable prepubertal baseline

levels by twelve months of age[18].

The exact physiological and neurodevelopmental functions of mini-puberty remain heavily researched. Physiologically, it drives penile growth, testicular descent, and the proliferation of Sertoli cells, which establishes the upper limit for sperm production capacity in adulthood. Regarding neurobehavioural outcomes, some developmental psychologists hypothesize that mini-puberty acts as a supplementary organisational window. For instance, a 2016 study by Melissa Hines and colleagues published in *Hormones and Behavior* linked salivary testosterone levels during mini-puberty to later childhood male-typical play and toy choices. However, robust correlations between mini-puberty testosterone and broader cognitive sex differences, such as spatial ability or autistic traits, have consistently failed to replicate in larger, independent cohorts, suggesting its primary role may be physiological rather than psychological[18].

Puberty: The Activational Wave

Puberty constitutes the third major hormonal wave in the human lifespan. Triggered by the reactivation of the HPG axis, this phase brings about profound morphological, endocrine, and psychological divergence. In males, the testes resume massive testosterone production, driving the development of secondary sex characteristics, including skeletal broadening, muscle accretion, vocal cord thickening, and the emergence of facial and body hair. In females, the ovaries commence cyclic estrogen and progesterone production, driving breast development, pelvic widening, and the onset of menarche.

Crucially, while puberty has classically been viewed under the 1959 paradigm as a purely "activational" period, contemporary developmental neuroscience challenges this binary. Research by Margaret McCarthy and colleagues, summarized in a 2015 review in *Nature Neuroscience*, indicates that puberty also constitutes a second, distinct "organisational" window[5]. During adolescence, circulating steroid hormones induce permanent epigenetic modifications, altering DNA methylation and histone acetylation patterns in the brain. These epigenetic markers permanently alter brain architecture and dictate the trajectory of grey matter pruning, cementing sex-specific neural networks that persist throughout adulthood.

Layer 2: Brain and Behaviour (Statistical Overlaps)

Moving from the binary physiological mechanisms of gonads and chromosomes to the behavioural and neurological phenotypes of adult humans requires a fundamental shift in analytical frameworks. While physiological sex is highly dimorphic (bimodal with negligible overlap), behavioural, cognitive, and neuroanatomical traits form overlapping normal distributions.

Understanding Averages vs. Individuals

To quantify behavioral and anatomical differences, researchers rely on Cohen's *d*, an effect size metric that measures the standardized difference between the means of two populations. A *d* of 0.2 is considered small, 0.5 is medium, and 0.8 is large. It is vital to recognize the statistical reality of these measurements to avoid determinism. Even with a large effect size of $d = 1.0$, the two population distributions overlap by approximately 45%. This indicates that while the group averages are significantly different, a substantial portion of the lower-scoring group will still

score higher than the average of the higher-scoring group. Consequently, population-level averages successfully predict macro-social trends but are mathematically incapable of accurately predicting the traits of any specific individual.

Sex Differences in Brain Structure

The question of whether male and female brains differ structurally has been definitively answered in the affirmative, though the functional and behavioral implications of these structures remain fiercely debated. In 2014, Amber Ruigrok, Gholamreza Salimi-Khorshidi, Meng-Chuan Lai, Simon Baron-Cohen, and colleagues published a comprehensive meta-analysis in *Neuroscience and Biobehavioral Reviews* assessing over 20 years of neuroimaging data. Synthesizing 126 articles covering more than 14,000 human brains (ages ranging from birth to 80 years), the researchers confirmed that adult male brains are, on average, 8% to 13% larger in total volume than female brains[21].

Specifically, the meta-analysis found that males exhibit higher absolute volumes of both grey matter (9% larger) and white matter (13% larger). Regional differences were heavily concentrated in the limbic system, with males displaying larger volumes and higher tissue densities in the left amygdala, hippocampus, insular cortex, and putamen. Females, conversely, displayed larger volumes in the right frontal pole, inferior and middle frontal gyri, and anterior cingulate gyrus[21].

This structural dimorphism was subsequently expanded upon by Stuart Ritchie and colleagues in a 2018 study published in *Cerebral Cortex*. Leveraging the massive UK Biobank dataset, this single-sample study achieved unprecedented statistical power with a sample size of 5,216 participants (2,750 females, 2,466 males) scanned on a single MRI machine[28]. Ritchie et al. replicated the finding of larger overall male brain volume, larger cortical surface area, and greater white matter fractional anisotropy. Conversely, females were found to have significantly thicker cerebral cortices across widespread cortical regions.

Neuroscientists universally caution against making naturalistic leaps from anatomical dimorphism directly to cognitive ability. When adjusted for total intracranial volume (ICV)—essentially, mathematically correcting for the fact that men have larger brains primarily because they have larger bodies—many regional volume differences attenuate or disappear. Furthermore, total brain size does not correlate robustly with general intelligence (the *g*-factor), which exhibits an effect size near zero between the sexes, indicating total cognitive egalitarianism in general intelligence.

Cognitive Sex Differences: Mental Rotation

While general intelligence is identical between the sexes, specific cognitive sub-domains exhibit profound, highly replicated sex differences. The largest and most consistently documented cognitive sex difference is in spatial ability—specifically, 3D mental rotation (the ability to mentally manipulate three-dimensional objects in space).

In a seminal 1995 meta-analysis published in *Psychological Bulletin*, Daniel Voyer, Susan Voyer, and M.P. Bryden synthesized decades of psychometric data, encompassing 286 independent effect sizes. They identified a robust, homogenous male advantage in mental rotation with an effect size ranging from $d = 0.73$ to 0.90[3]. This finding has successfully replicated across dozens of subsequent studies and global cultures.

The effect emerges early in childhood, grows in magnitude through puberty, and spans diverse demographics. Evolutionary psychologists frequently posit that this specific cognitive dimorphism evolved from the distinct spatial processing demands of male-typical ancestral hunting, ranging, and warfare behaviours. Neuroendocrinologists, conversely, point directly to the organisational effects of prenatal androgen exposure as the proximate physiological mechanism, noting that spatial ability fluctuates subtly in women across the menstrual cycle in inverse proportion to estrogen levels[34].

Vocational Interests and Personality: Things vs. People

In the domain of occupational interests and broad personality profiles, sex differences are arguably even more pronounced than in cognitive sub-domains. In 2009, Rong Su, James Rounds, and Patrick Armstrong published a landmark meta-analysis in *Psychological Bulletin* analyzing data from 47 interest inventories across a staggering sample size of 503,188 respondents[39].

They found an exceptionally large sex difference on the "Things-People" dimension, yielding an effect size of $d = 0.93$. This metric indicates a massive population-level divergence: men, on average, are vastly more interested in object-oriented, mechanical, structural, and systemic occupations (scoring highest on the "Realistic" domain, $d = 0.84$), while women are substantially more interested in socially-oriented, caregiving, and relational occupations (scoring highest on the "Social" and "Artistic" domains, $d = 0.35$ to 0.68)[39]. This finding has robustly replicated globally and serves as the primary psychological explanation for why engineering and physics fields naturally skew male, while nursing, primary education, and veterinary medicine fields naturally skew female.

Furthermore, while unidimensional personality traits (such as those measured by the Big Five inventory) show only small to moderate sex differences (e.g., women scoring moderately higher on Neuroticism and Agreeableness), multivariate analyses that examine personality profiles as an integrated whole reveal much larger dimorphisms. In 2012, Marco Del Giudice, Tom Booth, and Paul Irwing published a methodology in *PLOS ONE* utilizing the Mahalanobis distance (D), a multivariate effect size that calculates the distance between two group centroids in a multidimensional space. Utilizing a sample of 10,261 participants, they demonstrated that when the entirety of a personality profile is analyzed across 15 dimensions concurrently, the overall global sex difference in personality yields a massive effect size of $D = 2.71$[45]. This indicates a less than 10% overlap between the male and female multivariate personality distributions.

Feature Category	Specific Trait / Metric	Principal Meta-Analysis / Study	Sample Size (N)	Effect Size / Finding	Replicability
Brain Structure	Total Brain Volume	Ruigrok et al., 2014	>14,000	Male average 8-13% larger	Highly Robust

Brain Structure	Cortical Thickness	Ritchie et al., 2018	5,216	Female average thicker	Highly Robust
Cognitive Ability	General Intelligence (*g*)	Various	N/A	*d* ~ 0.00 (No difference)	Highly Robust
Cognitive Ability	3D Mental Rotation	Voyer et al., 1995	286 effect sizes	*d* = 0.73 - 0.90 (Male higher)	Highly Robust
Vocational	Things vs. People Interest	Su et al., 2009	503,188	*d* = 0.93 (Male=Things)	Highly Robust
Personality	Multivariate Profile (15 traits)	Del Giudice et al., 2012	10,261	Mahalanobis *D* = 2.71	Robust
Childhood	Toy Preference (Sex-typical)	Davis & Hines, 2020	Various	*d* = 0.80 - 2.80	Highly Robust

Natural Clinical Experiments: Entangling Nature and Nurture

Because it is ethically and legally impossible to conduct experimental hormonal manipulations on human fetuses, researchers rely heavily on "experiments of nature"—clinical genetic variations known as Disorders of Sex Development (DSDs) and sex chromosome aneuploidies. These conditions allow endocrinologists and psychologists to decouple genetic sex, gonadal sex, prenatal hormone exposure, and the sex of rearing, thereby isolating the specific developmental effects of biological variables from cultural socialization.

Congenital Adrenal Hyperplasia (CAH)

Classical Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive genetic disorder most commonly caused by a mutation in the *CYP21A2* gene, leading to a 21-hydroxylase enzyme deficiency. In XX fetuses, this enzymatic block prevents the adrenal glands from synthesizing cortisol. The lack of cortisol eliminates the negative feedback loop to the pituitary

gland, which pumps out adrenocorticotropic hormone (ACTH), overstimulating the adrenal glands. This shunts massive amounts of steroid precursor molecules into the androgen synthesis pathway, bathing the developing female fetus in high levels of testosterone.

Girls with classical CAH are born with partially or fully masculinised external genitalia, which is typically surgically altered in infancy to appear female. They are treated postnatally with synthetic corticosteroids to normalize their hormone levels and are raised unambiguously as girls. CAH provides the ultimate test of the organisational hypothesis in humans: What happens to the behaviour of an XX female, raised as a girl in a female culture, whose brain was exposed to male-typical testosterone levels in utero?

Extensive research, led by pioneers such as Melissa Hines and Sheri Berenbaum across multiple decades, has demonstrated that girls with CAH display permanently masculinised behavioural profiles[2]. From a very young age, in cohort studies typically ranging from 40 to 125 participants, CAH girls show a significantly increased preference for male-typical toys (e.g., vehicles, weapons, building blocks) and a starkly decreased preference for female-typical toys (e.g., dolls). Effect sizes are substantial; Hines estimates that prenatal androgen exposure moves CAH females approximately 60% of the distance toward the male mean for toy play[4].

Furthermore, women with CAH demonstrate increased male-typical occupational interests (preferring Things over People), significantly higher rates of bisexual and homosexual orientation compared to unaffected sisters, and elevated physical aggression[2]. Additionally, some meta-analyses, such as those by Puts et al. (2008), have found that CAH females exhibit better 3D mental rotation spatial abilities than unaffected control females, directly linking the cognitive dimorphism to prenatal androgens[3].

Critics of the biological interpretation argue that CAH is an imperfect experiment, suggesting that parents, consciously or subconsciously aware of the child's masculinised genitalia or medical status, might socialize these girls differently. However, intensive observational studies of parental behavior have generally found the opposite: parents of CAH girls actively encourage female-typical play even more strongly than parents of unaffected girls. This suggests that the masculinised behaviour of CAH girls occurs *in spite of* cultural socialization, not because of it, firmly cementing the organisational effects of testosterone on the human brain[49].

Complete Androgen Insensitivity Syndrome (CAIS)

Complete Androgen Insensitivity Syndrome (CAIS) occurs in 46,XY individuals who possess a severe loss-of-function mutation in the androgen receptor (AR) gene. These individuals possess the *SRY* gene and develop functional internal testes that produce normal male-typical levels of testosterone in utero. However, because their cellular androgen receptors are entirely non-functional, their bodies and brains are completely blind to the hormone.

Because the androgen receptors fail, the external genitalia default to the female pathway. XY infants with CAIS are born with typical female external genitalia. They are assigned female at birth, raised as girls, and the condition is often only discovered in adolescence when they develop breasts (due to the aromatisation of their abundant testosterone into estrogen) but fail to menstruate, as they lack a uterus and fallopian tubes due to the AMH produced by their testes in utero.

Psychologically, behaviorally, and cognitively, multiple clinical cohort studies (e.g., sample sizes of 10 to 30) have found that individuals with CAIS demonstrate entirely female-typical core gender identities, heterosexual orientations (attraction to men), and female-typical occupational and play interests[51]. CAIS powerfully confirms that an XY chromosome and high circulating testosterone are insufficient to masculinise human behavior without functional androgen receptors to transduce the hormonal signal into the brain.

5-Alpha-Reductase Deficiency (5-ARD)

First described by endocrinologist Julianne Imperato-McGinley in a famous 1974 cohort published in *Science*, 5-alpha-reductase deficiency type 2 (5-ARD) provides the most striking evidence for the power of biological neurodevelopmental drives over social rearing. 5-ARD is an autosomal recessive condition affecting 46,XY males where the enzyme responsible for converting testosterone into DHT is severely deficient[6].

Because DHT is specifically required to form the male external genitalia in utero, these boys are born with ambiguous or largely female-appearing genitalia (typically a clitoral-like phallus, a bifid scrotum, and a blind pseudovaginal pouch). In Imperato-McGinley's initial cohort in the Dominican Republic, consisting of 18 affected individuals, these children were raised unambiguously as girls. However, because their brains contain normal androgen receptors and were exposed to normal male testosterone levels in utero, their neurobiology was structurally masculinised.

At puberty, the testes produce a massive activational surge of testosterone. This surge is so large that it partially overcomes the enzymatic block (often utilizing the type 1 5-alpha-reductase enzyme), causing rapid, striking physical virilization: the voice drops, skeletal muscle mass increases, and the clitoral-like phallus enlarges to function as a penis.

Remarkably, Imperato-McGinley documented that 17 out of the 18 children raised as girls in her initial cohort spontaneously transitioned to a male gender identity upon reaching puberty[6]. The surge of activational testosterone, combined with the prenatally organised male neural architecture, completely overrode over a decade of female cultural socialization. This phenomenon has successfully replicated in other isolated 5-ARD populations globally; for instance, a recent cohort study of 7 patients with 5-ARD in a developing society saw 6 out of 7 transition to male identities at puberty[7]. This demonstrates unequivocally that core gender identity is heavily moored in biological realities and prenatal hormonal exposure, rather than being a blank slate constructed solely by rearing.

Chromosomal Aneuploidies: Turner and Klinefelter Syndromes

Natural variations in sex chromosome number further elucidate the role of genetics prior to hormonal influence.

Turner Syndrome (45,X0) affects phenotypic females who lack a second sex chromosome. They possess non-functioning streak ovaries and therefore do not experience typical prenatal or pubertal hormone surges without medical hormone replacement therapy. Meta-analyses of cognitive profiles in girls with Turner Syndrome, such as a 2018 review by Mauger et al. comprising 16 samples and 13 studies, reveal normal verbal IQs but significant, highly specific deficits in visuospatial reasoning, mental rotation, and executive functions like working memory[3]. Furthermore, longitudinal brain imaging in cohorts of 50 to 60 Turner girls shows

their cortical development deviates from typically developing females, with thicker cortices and altered parietal trajectories. This highlights the direct, hormone-independent effects of X-chromosome gene dosage on brain development[57].

Klinefelter Syndrome (47,XXY) affects phenotypic males carrying an extra X chromosome. Characterized by testicular dysfunction and lower lifetime testosterone levels, these boys frequently present with language-based learning disabilities, executive dysfunction, and a significantly increased risk for social-emotional difficulties and autism spectrum traits[65].

Together, Turner and Klinefelter syndromes suggest that the sex chromosomes themselves contribute directly to spatial and verbal cognitive network development, independent of gonadal hormones.

Layer 3: Culture, Variable Expression, and Controversies

The cultural layer serves to either amplify or suppress the statistical distributions established by the physiological layer. Critics of biological explanations—often grounded in social constructionist theories from the social sciences—argue that ubiquitous gender norms, marketing, and differential parental treatment are the primary, if not sole, drivers of sex differences in human behavior. To untangle this, biological researchers examine species untouched by human culture.

The Animal Model Bridge

To completely bypass the confounding variable of human culture, evolutionary psychologists turn to non-human primates. In 2002, Gerianne Alexander and Melissa Hines published a landmark paper in *Evolution and Human Behavior* demonstrating that vervet monkeys presented with human gendered toys exhibit the exact same sex differences seen in human children: male monkeys preferentially played with wheeled toys (trucks) and balls, while female monkeys preferentially played with plush dolls and pots[74]. This finding was famously replicated by Hassett et al. (2008) in a colony of rhesus macaques[80].

These animal models dismantle the hypothesis that toy preferences are purely the product of gendered marketing or patriarchal socialization. Instead, they support an interactionist view: prenatal hormones organize the visual and motor cortices of the male primate brain to be intrinsically rewarded by object movement, mechanical systems, and spatial dynamics, while female primate brains are predisposed toward social stimuli, faces, and caregiving facsimiles. Human culture takes these innate, biological primings and amplifies them into the strictly bifurcated pink and blue aisles seen in modern toy stores.

Controversy: The Extreme Male Brain and Fetal Testosterone

The most hotly contested area of sex difference research is the intersection of autism, prenatal testosterone, and cognitive phenotypes. Autism Spectrum Disorder (ASD) has a highly skewed sex ratio, diagnosed approximately four times more often in males than females.

Simon Baron-Cohen, a leading cognitive neuroscientist at Cambridge University, proposed the Empathizing-Systemizing (E-S) theory. It posits that human cognition is anchored by two evolutionary drives: Empathizing (the drive to recognize mental states and respond with

appropriate emotion) and Systemizing (the drive to analyze or construct rule-based systems). According to Baron-Cohen, the typical female brain is statistically biased toward Empathizing (Type E), while the typical male brain is statistically biased toward Systemizing (Type S). Baron-Cohen extended this into the "Extreme Male Brain" (EMB) theory of autism, suggesting that autism represents a neurodevelopmental extreme of the Type S profile, driven by unusually high prenatal testosterone exposure[81].

In 2018, David Greenberg, Varun Warrier, Carrie Allison, and Simon Baron-Cohen published a massive validation of the E-S and EMB theories in *PNAS*. Using an unprecedented discovery dataset of 671,606 individuals and a validation dataset of 14,354 individuals, they confirmed that males, on average, score higher on the Systemizing Quotient (SQ), while females score higher on the Empathy Quotient (EQ). Crucially, autistic individuals of both sexes shifted heavily toward the extreme masculinised profile. The difference between EQ and SQ (the D-score) accounted for 19 times more variance in autistic traits (43%) than biological sex itself, providing immense statistical weight to the behavioral phenotype of the EMB theory[84].

To find a physiological mechanistic link, Baron-Cohen's team, heavily featuring researcher Bonnie Auyeung, tracked children whose mothers had undergone clinical amniocentesis during pregnancy. In multiple papers (e.g., Auyeung et al., 2009, *British Journal of Psychology*), they reported robust positive correlations between fetal testosterone (fT) levels measured in the amniotic fluid and the children's later childhood autistic traits and systemizing behaviours[16].

The Leading Critics: This methodology has faced fierce criticism from other heavyweights in the field, most notably Melissa Hines, K.T. Kung, and David Skuse. In 2016, Kung, Hines, and colleagues attempted to replicate the amniotic fluid fT findings in independent cohorts and found absolutely no relationship between prenatal amniotic androgens and later autistic traits[19].

Critics note massive methodological flaws: amniocentesis is typically performed in the second trimester (weeks 15-18), potentially missing the exact peak of the first-trimester testosterone surge. Furthermore, if the Extreme Male Brain theory's reliance on testosterone were entirely correct, one would logically expect girls with CAH (who are bathed in massive amounts of prenatal androgens) to exhibit dramatically higher rates of clinical autism. However, rigorous studies, including those by Kung et al. (2016), show that while CAH girls have highly masculinised toy preferences, their rates of clinical autism are not substantially elevated compared to the general population[93].

The current scientific consensus is moving toward a more nuanced, polygenic model. Prenatal sex steroids are undoubtedly a piece of the puzzle, altering gray matter development and pushing the brain toward a systemizing phenotype, but they interact with hundreds of genetic variants rather than acting as a singular monolithic trigger for autism[16].

Summary of Robust Findings

1. Chromosomal sex, specifically the *SRY* gene on the Y chromosome, triggers the differentiation of the bipotential gonad into testes; its absence, alongside active *WNT4* expression, yields ovaries.
2. The fetal testes secrete a massive surge of testosterone between weeks 8 and 24 of

gestation, which permanently and irreversibly organizes the neural architecture of the male brain.
3. Natural clinical experiments in CAIS and 5-ARD demonstrate that without functioning androgen receptors or the 5-alpha-reductase enzyme, an XY fetus will develop a female external phenotype despite possessing a Y chromosome.
4. Individuals with 5-ARD who are raised as girls universally virilize at puberty and the vast majority spontaneously transition to a male gender identity, demonstrating that biological brain organization can override a decade of cultural socialization.
5. Girls with classical CAH, who are exposed to high prenatal androgens, display permanently masculinised toy and occupational preferences that persist despite intense parental socialization to the contrary.
6. A meta-analysis of over 14,000 brains confirms adult male brains are 8% to 13% larger in volume, while female brains possess thicker cortices, a structural dimorphism independent of cultural socialization.
7. Males exhibit a highly robust, large statistical advantage in 3D mental rotation (Cohen's *d* ranging from 0.73 to 0.90) that is consistent across global cultures and age groups.
8. Meta-analytic data from over 500,000 individuals reveals a massive occupational skew, with males preferring "Things" (systems, mechanics) and females preferring "People" (caregiving, socialization) yielding an effect size of $d = 0.93$.
9. Non-human primates, specifically vervet and rhesus monkeys, display sex-differentiated toy preferences identical to humans, implying biological primacy in object selection rather than patriarchal marketing.
10. Behavioural and cognitive sex traits form overlapping normal distributions; therefore, population averages do not deterministically predict individual capabilities, interests, or identities.

Contested or Open Questions

Domain of Inquiry	The Core Unknown	Leading Hypotheses
Mini-Puberty	What is the precise neurodevelopmental function of the postnatal testosterone surge in 1-to-6-month-old infant boys?	It organizes later childhood rough-and-tumble play, OR it is purely physiological for testicular development with no lasting cognitive effects.
Autism & Hormones	Does the level of prenatal testosterone accurately predict the manifestation of the Extreme Male Brain and clinical autism?	Yes, amniotic fT correlates with later Autistic Quotient scores (Baron-Cohen); No, the data fails to replicate and CAH girls do not have

		high autism rates (Hines).
Aromatisation in Humans	To what extent does human brain masculinisation rely on the aromatisation of testosterone into estradiol?	It is mandatory, exactly as it is in rodent models; OR the human brain relies far more heavily on direct androgen receptor activation.
2D:4D Digit Ratio	Is the ratio of the index to ring finger a valid, reliable proxy for prenatal testosterone exposure?	It holds weak but statistically significant predictive validity for spatial ability and aggression; OR it is entirely confounded by birth order and genetics and should be discarded.
Brain Structure Mapping	To what exact degree do the confirmed structural differences in cortical thickness and regional grey matter volumes translate into functional behavioral differences?	They directly cause the variances in spatial and verbal skills; OR they are merely scaling compensations for absolute brain size differences with no functional behavioral output.

Most Important Sources to Read in Full

1. **Voyer, D., Voyer, S., & Bryden, M. P. (1995).** *Magnitude of sex differences in spatial abilities: A meta-analysis and consideration of critical variables*. Psychological Bulletin. (The foundational meta-analysis on mental rotation).
2. **Su, R., Rounds, J., & Armstrong, P. I. (2009).** *Men and things, women and people: A meta-analysis of sex differences in interests*. Psychological Bulletin. (A vital text for understanding the occupational divide).
3. **Alexander, G. M., & Hines, M. (2002).** *Sex differences in response to children's toys in nonhuman primates (Cercopithecus aethiops sabaeus)*. Evolution and Human Behavior. (Crucial for separating biological predispositions from human socialization).
4. **Ruigrok, A. N. V., et al. (2014).** *A meta-analysis of sex differences in human brain structure*. Neuroscience and Biobehavioral Reviews. (The most exhaustive meta-analytic review of anatomical sex differences in the human brain).
5. **Ritchie, S. J., et al. (2018).** *Sex differences in the adult human brain: Evidence from 5216*

UK Biobank participants. Cerebral Cortex. (Unprecedented sample size validating structural brain differences).
6. **Greenberg, D. M., Warrier, V., Allison, C., & Baron-Cohen, S. (2018).** *Testing the Empathizing–Systemizing theory of sex differences and the Extreme Male Brain theory of autism in half a million people*. PNAS. (The largest validation of the E-S theory).
7. **Imperato-McGinley, J., et al. (1974).** *Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism*. Science. (The classic study proving hormonal drive can override cultural gender rearing).
8. **Hines, M. (2004).** *Brain Gender*. Oxford University Press. (A comprehensive overview of CAH studies and the organisational hypothesis by the field's leading critic of the EMB theory).

Implications for Living Together

In deriving actionable implications from this research for how couples, families, organisations, and societies can best live together, one must studiously avoid the *naturalistic fallacy*—the philosophical error of assuming that because something is biologically "natural," it is therefore morally "good" or socially optimal. David Hume's strict separation of "is" and "ought" requires us to treat biological sex differentiation as an empirical reality to be managed, not a moral imperative to be enforced.

For Couples and Families:

Understanding the deep biological roots of sex differences can foster profound empathy in interpersonal relationships. Recognizing that male-typical object-orientation (Systemizing) or female-typical social-orientation (Empathizing) are heavily influenced by prenatal neurodevelopmental trajectories can reduce interpersonal friction. If a male partner defaults to "fixing" a problem structurally rather than offering emotional resonance, or if a female partner prioritizes social harmony over systemic efficiency, these should be viewed not as moral failings or deliberate stubbornness, but as the expression of differing baseline biological algorithms.

In parenting, acknowledging that toy preferences and rough-and-tumble play styles are largely pre-wired—and verified by non-human primate models—can relieve parents of the modern anxiety that they are "failing" to raise their children in a perfectly gender-neutral environment. Parents should absolutely offer vast arrays of choices to their children without forcing conformity, but they must accept that statistical clustering around traditional gender norms is an expression of organic neurobiological freedom, not necessarily cultural oppression.

For Organisations and Societies:

The macro-level implications for organizations and societies are heavily dictated by the mathematical reality of overlapping statistical distributions. Because of the massive $d = 0.93$ effect size in Things vs. People orientations, societies that maximize freedom of occupational choice (such as the highly egalitarian Nordic countries) paradoxically see *wider* gender gaps in STEM and caregiving fields, a phenomenon known in sociology as the gender equality paradox. Organisations must recognize that achieving a strict 50/50 gender parity in every sub-field of the economy—particularly in extreme "Things" domains like mechanical engineering or extreme "People" domains like early childhood education—will require draconian social engineering that pushes violently against human biology. True equity lies in equal opportunity and the absolute

absence of discrimination, not in artificially mandated equality of outcome. Simultaneously, the overlap in normal distributions dictates that organisations must never use biological averages to judge the individual. Because millions of women naturally possess highly systemizing, male-typical brains, and millions of men possess highly empathizing, female-typical brains, evaluating a candidate based on their sex is a functional error that restricts human capital. Societies function optimally when they acknowledge the biological reality that men and women are not blank slates and will generally aggregate into different roles, while fiercely protecting the right of every individual to act in accordance with their own unique, statistically outlier phenotype. Biology provides the baseline, but the individual remains the ultimate unit of moral and economic agency.

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