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Surprising origins of sex differences in the brain

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ABSTRACT

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Discerning the biologic origins of neuroanatomical sex differences has been of interest since they were first reported in the late 60's and early 70's. The centrality of gonadal hormone exposure during a developmental critical window cannot be denied but hormones are indirect agents of change, acting to induce gene transcription or modulate membrane bound signaling cascades. Sex differences in the brain include regional volume differences due to differential cell death, neuronal and glial genesis, dendritic branching and synaptic patterning. Early emphasis on mechanism therefore focused on neurotransmitters and neural growth factors, but by and large these endpoints failed to explain the origins of neural sex differences. More recently evidence has accumulated in favor of inflammatory mediators and immune cells as principle regulators of brain sexual differentiation and reveal that the establishment of dimorphic circuits is not cell autonomous but instead requires extensive cell-to-cell communication including cells of non-neuronal origin. Despite the multiplicity of cells involved the nature of the sex differences in the neuroanatomical endpoints suggests canalization, a process that explains the robustness of individuals in the face of intrinsic and extrinsic variability. We propose that some neuroanatomical endpoints are canalized to enhance sex differences in the brain by reducing variability within one sex while also preventing the sexes from diverging too greatly. We further propose mechanisms by which such canalization could occur and discuss what relevance this may have to sex differences in behavior.

Introduction

The origins of behavioral differences between men and women, boys and girls, males and females, have been a topic of fascination since the dawn of consciousness. In contrast, the idea that the brain is the principle means by which males and females behave differently is strikingly modern, having only been accepted as a possibility following a Battle of the Titans in the 1950's when Frank Beach famously argued that the critical variable that determined how males behaved versus females was the type of genitalia one possessed, either intromitting or receiving (Beach, 1974; Phoenix et al, 1959). This viewpoint was eventually overturned beginning with an iconic paper published in 1959. Using guinea pigs as a model William C. Young and colleagues convincingly demonstrated that prenatal hormones were capable of sex reversing the behavior of females when adults (Phoenix et al, 1959). While this and other studies succeeded in ending the debate about what organ in the body was controlling sex differences in behavior, it also generated a degree of tunnel vision as the next 2–3 decades were dominated by studies of reproductive behavior and physiology leading to the widespread belief that sex differences in the brain are narrow in both their scope and significance, being limited to control of the anterior pituitary gland, courtship, copulation and parenting.

Hormonal modulation of neural plasticity opened the gateway for sex differences outside the context of reproduction. This can largely be traced to the seminal finding of the McEwen lab that dendritic spine density on hippocampal pyramidal neurons varied by almost 30% across the few days of the estrus cycle in female rats (Woolley and McEwen, 1992). This was viewed as an astonishing level of plasticity at that time and initial reports were met with skepticism. But again, an irrefutable march of data led to the general acceptance that indeed hormones are powerful regulators of neuronal function outside of the diencephalon and outside the context of reproduction. Importantly, however, modulation of adult neural function or behavior by hormones is not the same as sex differences in neural function or behavior. Investigation into whether adult functions known to be impacted by steroids in a modulatory manner are sexually differentiated is actually relatively few. For instance, estradiol alters synaptic physiology and cognitive function in adult females, but are these endpoints also subject to sexual differentiation? Answering this question is actually surprisingly difficult precisely because of the hormonal modulation in adulthood. What does one compare? An estrous female to an intact male or maybe both sexes should be gonadectomized and hormone replaced to standardize their endocrinology? But whose endocrine profile do you choose? It quickly becomes a Gordian Knot of possibilities and scares off even the bravest

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of the curious. Further complicating the picture is the impact of a lifetime of experience and environment which can vary in profound and significant ways between males and females. These difficulties can be avoided, however, by studying the origins of sex differences in development, which while not immune to environment and experience, are at least somewhat buffered from them simply by not having had as much time to have accumulated an effect.

Historical perspective on sex differences in the brain

The first robust sex difference discovered in the healthy mammalian brain was aptly named the sexually dimorphic nucleus of the preoptic area (SDN-POA) (Gorski et al, 1978, Gorski et al, 1980) and is one of, if not the most, extensively studied sex differences in the brain. It is located under the anterior commissure, above the optic chiasm, lateral to the third ventricle, and anterior to the hypothalamus (Fig. 1). Neurogenesis in the SDN begins at embryonic day 14 (E14) and ends on E18. The sensitive period during which the size of the SDN is influenced by gonadal steroids is from E18 to postnatal day 4 (PN4) (Jacobson and Gorski, 1981; Orikasa et al, 2010; Rhees et al, 1990a, Rhees et al, 1990b). Although first described as a densely-packed bundle of neurons revealed with a simple Nissl stain (Gorski et al, 1978, Gorski et al, 1980), the SDN has since been more clearly (and reliably) defined by a subset of cells that are immunopositive for the expression of Calbindin-D28k (a calcium binding protein expressed in GABAergic neurons and implicated in neuroprotection) and is thus now referred to as the CALB-SDN (Kato et al, 2012; Sickel and McCarthy, 2000). Sex differences in the SDN and the CALB-SDN, are of the same magnitude and both arise after PN4. During development, the SDN of males and females have similar numbers of neurons but due to higher levels of apoptotic cells undergoing DNA-fragmentation in females between PN6 and PN9, this region becomes markedly smaller in females (Davis et al, 1996). Other studies support the role of apoptosis in sexual differentiation of the SDN by demonstrating that the male SDN has higher expression of anti-apoptotic Bcl-2 (at the protein but not mRNA level) while the female SDN contains higher expression of pro-apoptotic Bax from PN5–PN7. Apoptosis via caspase-3 activation occurs at higher rates in the female SDN and this corresponds with the decrease in size of the female SDN. Sex differences in pro-apoptotic Bax and Bcl-2 are abolished in response to treatment of females with estradiol but the mechanism by which estradiol modulates Bcl-2 and Bax in the SDN is not yet clear (Tsukahara et al, 2008, Tsukahara et al, 2006). Interestingly, when sex differences in the CALB-SDN were investigated in a Bax knockout mouse, there were no significant increases in the size of the CALB-SDN as compared to their wild type counterparts, suggesting that Bax expression is not the primary mechanism by which cells are dying off in females (Gilmore et al, 2012). A role for estradiol up regulation of calbindin and calretinin expression in males has been considered but not clearly demonstrated. Elevated levels of these calcium binding proteins may protect cells from toxicity that can result from excessive neuronal excitation in males compared to females. Although testosterone treatment can up regulate calbindin and calretinin expression in the hypothalamus (Brager et al., 2000; Watson et al, 1998), this has not been demonstrated specifically in the SDN. Thus, despite much speculation over mechanisms by which estradiol regulates volume of the SDN, no consensus has been reached and the origins of this iconic sex difference remain a mystery.

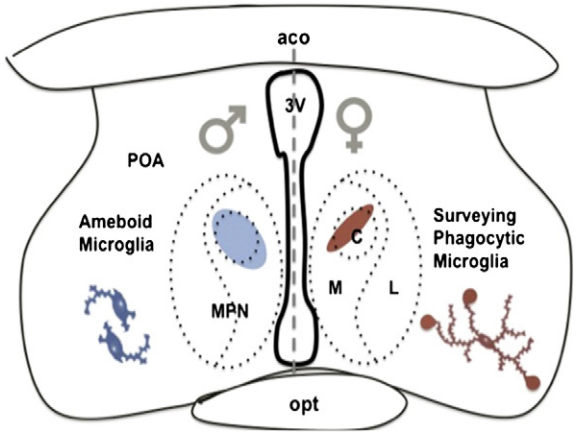

Fig. 1. The sexually dimorphic nucleus of the preoptic area. A collection of Nissl dense cells located in and around the Medial Preoptic Nucleus centralis (MPNc) constitutes the SDN. The volume of this nucleus is 3–5 times larger in males than females. Microglia morphology is also different in males and females in this brain region, being more rounded and ameboid-like in males and more ramified or "surveying" in females. POA = preoptic area, MPN = medial preoptic nucleus, C = central, M = medial, L = lateral, opt = optic chiasm, aco = anterior commissure, 3 V = third ventricle.

Subsequent to the discovery of the SDN in the rodent, analogous structures were reported in the ferret (Baum et al, 1996; Park et al, 1996), sheep (Roselli et al, 2004) and primates, including humans (Hofman and Swaab, 1989; LeVay, 1991; Swaab and Hofman, 1995). Moreover, several more brain regions were found to be larger in one sex, with the majority being male dominant. Second to the SDN, the most intensely studied volumetric sex difference is that of the anteroventral periventricular (AVPV) nucleus which like the SDN is also just a collection of cells that are distinguished by Nissl, but unlike the SDN in this case there are more in the female than the male (Simerly et al, 1985). Also unlike the SDN, there is a clear functional role for the AVPV in reproductive physiology. Neurons in this region project directly to the GnRH neurons that control the release of LH from the pituitary, which is under distinct control in males and females, showing a continuous pulsatile pattern in males versus a cyclic pattern marked by a large surge in females (Simerly, 2002). A combination of GABAergic and dopaminergic neurons make up this nucleus and evidence suggests that the relative survival of each is mediated by distinct mechanisms (Krishnan et al, 2009; Waters and Simerly, 2009; Zup et al, 2003). Moreover, the principal nucleus of the bed nucleus of the stria terminalis is also different in volume in males and females as a result of cell death in females, and there may be yet another distinct mechanism here involving epigenetic programming (Murray et al, 2009). But it is hard to say with complete confidence that there are indeed multiple unique mechanisms as each incidence has been discovered and studied by a different group and thus no systematic comparisons have been made.

The aforementioned hormonal modulation of dendritic spine synapses in hippocampal pyramidal neurons sparked interest in the potential for sex differences in dendritic morphology and synaptic patterning, and indeed several differences were found (Amateau and McCarthy, 2002a; Mong et al, 2001; Schwarz et al, 2008). When considering the sources of variability in synaptic patterning the obvious candidates to consider are the neurotransmitters that traffic at those same synapses. Indeed the notion that direct hormonal modulation of neurotransmitters and/or their cognate receptors must be the source of sex differences in the brain and behavior preceded the discovery of many neuroanatomical sex differences. The number of studies exploring virtually every neurotransmitter or neuromodulator as a candidate target for hormonally mediated sexual differentiation is too numerous to review but can largely be summarized as demonstrating that too much or too little of any particular transmitter system was sufficient to disrupt normal sexual differentiation, but none of them were capable of substituting for the hormone. In other words, none of them were both sufficient AND necessary. While never clearly articulated, there was a general consensus that the process of sexual differentiation of the brain was so fundamental to reproductive fitness that it was likely to be highly redundantly organized so that the loss of no one system was capable of derailing the entire process, or directing it.